

# **State of Technology Review – Algae Bioenergy**

**An IEA Bioenergy Inter-Task Strategic Project**



Image of climate simulating photobioreactor growing microalgae under controlled light, CO<sub>2</sub> and temperature, image by Dennis Schoeder, NREL (#25528)

**IEA Bioenergy**

## State of Technology Review – Algae Bioenergy

An IEA Bioenergy Inter-Task Strategic Project

Report coordinated by Lieve M.L. Laurens, National Renewable energy Laboratory, Golden, CO, USA, funded by IEA Bioenergy Task 39, with input from members of IEA Bioenergy tasks 34, 37, 38, 39 and 42

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## KEY MESSAGES

- Algae exhibit high photosynthetic efficiencies and yield ( $\sim 55$  tonnes  $\text{ha}^{-1}$   $\text{yr}^{-1}$ ) up to twice that of terrestrial plants and remain an attractive target for improving the sustainability of future bioenergy production
- The single biggest barrier to market deployment of algae remains the high cost of cultivating the algal biomass feedstocks, currently a factor of 10-20 too high for commodity fuel production
- A decline in the price of petroleum, coupled with on-going low prices for natural gas and absence of consistent policies on carbon pricing, causes a significant challenge in the development of cost-competitive production algae-based bioenergy products like gaseous and liquid fuels.
- Nearer term opportunities exist to use algae in an integrated biorefinery context to make higher value food, feed, nutraceutical and oleochemical bio-products, to help drive the economic development bioenergy production
- Alternative market opportunities for algal biomass, e.g. food and feed applications, will generate land use competition
- Resource (water, land, sunlight) and nutrients (N, P) remain key drivers for economic and environmental sustainability, where integration with wastewater provides near-term opportunities
- Recent technology developments facilitate the use of all algal biomass components; no longer focusing the biomass production solely on achieving high lipid production
- Numerous permutations of process operations are described in the literature; three categories are promising for future commercial development; 1) biomass conversion and fractionation into lipids, protein and carbohydrates and 2) thermochemical hydrothermal liquefaction and 3) biogas production from whole algal biomass
- Algae-based production to produce bioenergy products like liquid or gaseous fuels as primary products is not foreseen to be economically viable in the near to intermediate term and the technical, cost and sustainability barriers are reviewed
- Macroalgae have significant potential as a biogas, chemicals and biofuels crop in temperate oceanic climates in coastal areas. Their commercial exploitation also remains limited due to cost and scalability challenges
- There is a clear and urgent need for more open data sharing and harmonization of analytical approaches, from cultivation to product isolation, to TEA and LCA modelling, allowing for the identification and prioritization of barriers to low cost bioenergy production

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## **List of Abbreviations**

<b>ACCase</b>	Acetyl-CoA Carboxylase Gene
<b>ATP<sup>3</sup></b>	Algae Testbed Public Private Partnership
<b>ACC</b>	Algal Carbon Conversion
<b>ALU</b>	Algal Lipid Extraction and Upgrading
<b>ALU</b>	Algal Lipid Extraction and Upgrading
<b>AET</b>	Alternative Electron Transport
<b>ARRA</b>	American Recovery and Reinvestment Act
<b>AD</b>	Anaerobic Digestion
<b>ANL</b>	Argonne National Laboratory
<b>AzCATI</b>	Arizona Center for Algae Technology and Innovation
<b>AFDW</b>	Ash-free dry Weight
<b>BGY</b>	Billion Gallons per Year
<b>CAD</b>	Canadian Dollars
<b>C:N</b>	Carbon to Nitrogen Ratio
<b>CHG</b>	Catalytic Hydrothermal Gasification
<b>CEVA</b>	Center of Studies and Valorization of Algae
<b>COD</b>	Chemical Oxygen Demand
<b>CAP</b>	Combined Algal Processing
<b>CHP</b>	Combined Heat and Power
<b>CAB-comm</b>	Consortium for Algal Biofuels Commercialization
<b>CPI</b>	Consumer Price Index
<b>MAB3</b>	Danish MacroAlgaeBiorefinery
<b>DLUC</b>	Direct Land use Change
<b>DAF</b>	Dissolved air Flotation
<b>EISA</b>	Energy Independence and Security Act
<b>ECN</b>	Energy Research Centre of the Netherlands
<b>EROI</b>	Energy Return on Investment
<b>EC</b>	European Commission
<b>FAME</b>	Fatty Acid Methyl Ester
<b>IEA</b>	International Energy Agency
<b>FT-ICR MS</b>	Fourier Transform ion Cyclotron Resonance
<b>FFA</b>	Free Fatty Acids
<b>FDCA</b>	Furandicarboxylic Acid
<b>Gge</b>	Gallon Gasoline Equivalent
<b>GWP</b>	Global Warming Potential
<b>GHG</b>	Greenhouse Gases
<b>HDN</b>	Hydrodenitrogenation
<b>HTL</b>	Hydrothermal Liquefaction
<b>ILUC</b>	Indirect Land use Change
<b>IBR</b>	Integrated Biorefinery
<b>IMTA</b>	Integrated Multi-trophic Aquaculture
<b>IV</b>	Iodine Value
<b>LCA</b>	Life Cycle Analysis
<b>ME</b>	Major Equipment
<b>MFSP</b>	Minimum Fuel Selling Price
<b>MYPP</b>	Multiyear Program Plan
<b>NAABB</b>	National Alliance for Algal Biofuels and Bioproducts
<b>NREL</b>	National Renewable Energy Laboratory
<b>NRC</b>	National Research Council
<b>NER</b>	Net Energy Ratio
<b>NPD</b>	Nitrogen and Phosphorus Detection

<b>NPQ</b>	Non-photochemical Quenching
<b>NSTC</b>	Norwegian Seaweed Technology Center
<b>NTNU</b>	Norwegian University of Science and Technology
<b>OECD</b>	Organization for Economic Co-operation and Development
<b>PNNL</b>	Pacific Northwest National Laboratory
<b>PBR</b>	Photobioreactor
<b>PHB</b>	Poly- $\beta$ -HydroxyButyrate
<b>PHA</b>	Poly-HydroxyAlkanoates
<b>PLA</b>	Polylactic Acid
<b>PY</b>	Pyrolysis
<b>RAFT</b>	Regional Algal Feedstock Testbed
<b>RDB</b>	Renewable Diesel Blendstock
<b>RFS</b>	Renewable Fuel Standard
<b>RAFT</b>	Resource Assessment
<b>SAMS</b>	Scottish Association for Marine Sciences
<b>SES</b>	Seaweed Energy Solutions AS
<b>SLS</b>	Solid Liquid Separation
<b>SMY</b>	Specific Methane Yields
<b>SABC</b>	Sustainable Algal Biofuels Consortium
<b>DTU</b>	Technical University of Denmark
<b>TEA</b>	Techno-economic Assessments
<b>TRL</b>	Technology Readiness Level
<b>TAN</b>	Total Acid Number
<b>TCI</b>	Total Capital Investment
<b>TSCA</b>	Toxic Substances Control Act
<b>TAG</b>	Triacylglycerides
<b>TERA</b>	TSCA Environmental Release Application
<b>DOE</b>	US Department of Energy
<b>EIA</b>	US Energy Information Administration
<b>EPA</b>	US Environmental Protection Agency
<b>VS</b>	Volatile Solids
<b>WWT</b>	Wastewater Treatment

# **Executive Summary**

## **THE CHALLENGE**

Significant opportunities exist to take advantage of the high photosynthetic efficiency of algae, both macroalgae and microalgae, for bioenergy/biofuels production. Diverse algal biology and inherent cellular constraints around strain production capacity coupled with large differences in projections about production scenarios for both micro- and macroalgae production impose substantial challenges to extrapolating productivity reported in the literature to outdoor cultivation performance over the long term. The energetic considerations of algal production, which are presented in the body of this report, provide a framework to consider the maximum boundaries for areal algal biomass productivity and absolute biofuels/bio-products production potential given physical and geographical constraints. Clear economic and sustainability challenges exist to develop large-scale cost-competitive algal biomass-derived biofuels. While absolute economical considerations on biomass and fuel costs are a function of variables that vary with physical, geographic and socio-economic environments, which remain difficult to compare, there are opportunities to integrate production of algal biomass (both micro- and macroalgae) and apply a biorefinery approach to derive additional value from products coproduced along with gaseous or liquid biofuels. Considerations for successful implementation of bioenergy producing platforms from algae are summarized throughout this report.

## **THIS REPORT AND TEAMS INVOLVED**

This IEA Bioenergy report provides an international update on the status and prospects for using microalgae and macroalgae as feedstocks for producing biofuels and bioenergy products. The report's scope covers algae-based options for producing liquid and gaseous biofuels, and also algae-based bioenergy in the more general context of integrated biorefineries. The IEA Bioenergy Executive Committee supported this report's compilation and it is co-authored by members of IEA Bioenergy Tasks 34, 37, 38, 39 and 42.

This report is intended as an update to the prior IEA Bioenergy Task 39 report published in 2010.<sup>1</sup> Additions to published literature since 2010 are reviewed and used to inform a critical analysis of the state of the industry. As this report's scope expands beyond algae-based liquid biofuels to consider bioenergy options more generally, a collaborative effort across multiple IEA Bioenergy tasks was implemented to better capture the breadth of recent literature and industry information about advances in algal bioenergy production systems. The outcome is a more in depth and critical analysis of the subject intended to help inform the international community on the promises and challenges of algal biofuels and bioenergy options. Progress towards commercial bioenergy production using algae-based systems is reviewed and discussed in the context of existing fuels, chemicals and food/feed markets. This analysis is intended to help inform deeper understanding and insight into the promises and challenges for algal biofuels and bioenergy technologies to be substantial contributors to future liquid and gaseous transportation fuel supplies. This deep dive assessment of the recent literature provides a new synthesis of the opportunities and challenges to realising algae bioenergy's commercial and market potential.

The structure of the report reflects the different areas in algae bioenergy applications and studies. The primary emphasis is on microalgae routes to biofuel and bio-product applications, consistent with the much larger body of literature and research reports (and public and private funding) available related to microalgae compared to macroalgae. The state of macroalgae-based bioenergy production is reviewed at the end of the report, and the prospective use of low-cost, cast seaweed for biogas production may be a potential near-term commercial bioenergy opportunity in some regions. Finally, we include an overview of commercialized technologies and a detailed list of global research and development projects and commercially deployed algae-based production installations.

## STATE OF TECHNOLOGY

Despite tremendous progress made since 2010 in better understanding and demonstrating algae-based production, the prospects for commercial algae-based bioenergy or biofuels production are more challenging today than they were in 2010. This is primarily the result of the substantial decline in petroleum prices since August 2014. As a result of much lower petroleum prices, the economic challenge of bringing cost competitive algae-based biofuels to market has significantly increased, despite substantial improvements being made in the underlying core algal cultivation and upgrading technologies. As a consequence, companies that were leading commercial development of algae-based biofuels have been increasingly redirecting their commercial focus towards production of higher value food, feed and specialty products. At least until oil prices return to near their pre-August 2014 levels or reducing carbon emissions (GHG emissions) becomes sufficiently economically valued, primary strategies for liquid biofuels production from algae will need to rely on a biorefinery approach where the coproduction of higher value products can help promote the economical viability of algal biofuels production.

The single biggest barrier to market deployment remains the high cost of cultivating algal biomass feedstocks relative to producing terrestrial plant biomass. The relatively high cost of producing algal biomass remains the most critical barrier to commercial viability of algae-based production. Unfortunately commercial TEA models are not available and thus the sought 'current state of technology' cannot be included in the discussion here. However, there are a number of reports that, to varying degrees of detail and transparency, establish a baseline of estimated costs at a future projection, e.g. 5-year timeline. For example, projected future costs for algal biomass cultivation range from a low of \$541/tonne (\$0.54/kg) for open pond-based production in Arizona, USA<sup>2</sup> to a high of \$10,177 (€9,000)/tonne (\$10.2/kg) for photobioreactor-based cultivation in The Netherlands (projected out from 12 m<sup>2</sup> controlled conditions experiments).<sup>3</sup> The factors with the largest impact on cost are the growth productivity of the algae and type of cultivation system (e.g., open pond versus closed photobioreactor). A more typical estimate for algal biomass production cost is \$1.35/kg to \$1.8/kg (\$1,227/ton to \$1,641/ton), which was obtained integrating a detailed algae-farm engineering design with a state of technology projection of algal biomass productivity based on open pond algae cultivation testbed data scaled up to a 2023 ha (5000 acre) farm.<sup>4</sup> The U.S. Department of Energy's projected cost target for 2022 for algal biomass production is \$0.54/kg (\$491/ton), a cost which may enable cost-effective biofuel production from algal biomass.<sup>2</sup>

This report mainly describes two potential processing pathways for algal biomass-based biofuels. This is because these are the only pathways for which highly detailed process descriptions are available, including projections for scaled up processing in large-scale biorefineries. In the U.S., the National Renewable Energy Laboratory (NREL) and the Pacific Northwest National Laboratory (PNNL) published conceptual design reports in 2014 projecting algal biofuel minimum fuel selling price (MFSP) targets achievable by year 2022 for the conversion of algal biomass to biofuels either via algal lipid extraction and upgrading (ALU) or via hydrothermal liquefaction (HTL), respectively.<sup>5,6</sup> Both reports documented a set of targets for yields and processing conditions that would support a modeled MFSP of roughly \$1.1 - \$1.19/L (\$4–4.5/ gallon gasoline equivalent, GGE) for the respective conversion technology pathways, dependent on an assumed algal biomass feedstock cost of \$0.47/kg (\$430/ton) algal biomass ash-free dry weight (AFDW) following upstream dewatering to 20 wt% solids, and extrapolated to 2022. Reflecting the primacy of the cost of algal biomass production to biofuel production economics, both conversion pathways exhibit strong sensitivities to the algal biomass cost; MFSP is reduced by nearly \$1/GGE if algal production cost is reduced \$0.14/kg (\$130/ton) from the base case (i.e., to \$0.36/kg or \$300/ton), and reciprocally increases by slightly less than \$1/GGE if the algal production cost increases by \$0.13/kg (\$120/ton) to \$0.61/kg (\$550/ton), which is more in line with the updated algal biomass cost target of \$0.54/kg (\$491/ton) in the U.S. Department of Energy's 2016 algal biomass production design case.<sup>2,5,6</sup>

Recent research findings and technology development have not changed the basic promise of using algae-based systems to produce bioenergy as well as chemical and nutritional products. The meritorious sustainability attributes of phototrophic algae-based production remain valid. Algae as a class exhibit large biological diversity and metabolic plasticity compared to terrestrial plants, i.e., they are able to more widely adapt their biochemical metabolic pathways and cell wall composition in response to external physiological inputs. At least for some locations and geographies, there need not be significant competition with land used to provide existing food and feed supplies thanks to the potential of growing algae on non-arable land. Moreover, the rapid growth and high photosynthetic efficiency of algal feedstocks potentially allows for a higher fuel/energy areal yields to be achieved compared with terrestrial crops. In this context, algae remain a promising biological feedstock to research to address future energy and sustainability challenges.

The many positive aspects of algae-based production notwithstanding, however, significant economic and sustainability barriers impede commercial production of algae feedstocks for relatively low value energy and fuel market applications. Future research and commercial implementation of algae as feedstocks should provide global, economical and sustainable solutions to identified barriers, which range from effective use of biological diversity to integration of technologies at the demonstration scale. For example, the inverse relationship between productivity and lipid content may prove to be a challenge in overall process optimization.<sup>7</sup> Even though many technologies have been demonstrated at laboratory scale, most often this has been done in isolation and thus the challenge remains to fully integrate and validate the efficacy of the different technologies working together. Reducing energy, water and land use footprints of the integrated operation must be one of the key objectives of future larger scale demonstrations.<sup>8</sup> Overall potential production yields and process challenges are intimately related to specific production strains and their cultivation characteristics including geographic location. Great care should be taken in interpreting yields reported in the literature if they have not been vetted in fully integrated larger scale demonstrations.

Technological challenges to realize increased future application of algae-based systems can be categorized into the following **barriers to cost effective and sustainable algae-bioenergy deployment** (beyond the market and logistics barriers that accompany the deployment of novel technologies):<sup>7</sup>

- Biomass productivity, energy, water, nutrient (fertilizer), greenhouse gas (GHG) emissions and land use of any algae operation needs to be sustainable across the entire value chain and data needs to be collected in a consistent and scale-relevant manner to support life-cycle analysis.
- Ecological, genetic and biochemical development of algal species is needed to improve productivity and robustness of species against perturbations such as temperature, seasonality, predation, and competition.
- Physical, chemical, biological, and post-harvest physiological variations of produced algal species need to be researched and understood and integrated with biorefinery operations.
- Integration of co-located inoculation, cultivation, primary harvest, concentration, and preprocessing systems needs to be developed to maximize the economical viability of the process.
- The value of algal biomass, on-site processing or fractionation of biomass into lipids, carbohydrates, and/or proteins needs to be maximized at scales compatible with large-scale cultivation and farming
- Support of process and operations sustainability is needed to maximize the recycle of nitrogen, phosphorus, carbon and other nutrients from residual materials remaining after

preprocessing and/or residual processing to minimize fresh fertilizer input requirements in upstream cultivation.

One of the most challenging aspects for sustainable cultivation of algae for commercial production to supply commodity-scale markets is to mitigate the enormous amounts of water and nutrients required to grow and process algal feedstocks. Effective wastewater recycling is essential to minimize freshwater and chemical nutrients consumption.<sup>9,10</sup> Water usage requirements for biomass and biofuel production will vary depending on growth conditions and ultimately the lipid or biofuel yield from the biomass. For example, for a production system growing algae at approximately 1 g/L, with about 20% oil content of the biomass for biofuel applications, a total of ~5000 L of algae culture would need to be processed to generate 1 kg of biofuel (green or biodiesel). Algal biomass typically contains 45-50% carbon (C), 7.6% nitrogen (N) and 1.4% phosphorus (P). However, the elemental composition can vary dramatically based on growth conditions and species of algae used, but on average, the above approximation can be made and is consistent with the Redfield ratio (106:16:1 C:N:P) on a weight basis (40:7:1 C:N:P).<sup>11</sup> Thus, the nutrient requirements to support the same 1 kg of biofuel would be in the range of 0.38 kg N and 0.07 kg P equivalent (corresponding to 0.214 kg phosphate delivered). This is consistent with earlier reported estimates in the literature, where reports of 3000 liters of water per kg of microalgae-based biodiesel have been estimated,<sup>12</sup> and associated nutrients requirements are reported as 0.18-0.33 kg nitrogen if freshwater without any recycling is used for open pond cultivation.<sup>13,14</sup> While closed photo-bioreactors can be used to reduce water losses due to evaporation,<sup>9</sup> this imposes additional costs in installed capital equipment (CAPEX). Higher efficiency water use and wastewater recycle may further reduce water consumption, and the direct use of wastewater may provide an inexpensive and effective source of nutrients that also reduces freshwater use.<sup>15</sup>

A developing area that holds promise to be economically feasible in the nearer term is the integration of algal production and wastewater treatment (WWT) to allow both processes to achieve improved economic and environmental sustainability. The two main approaches being examined are: 1) direct WWT via algal production, with the treated effluent discharged for offsite use (i.e., the wastewater is only used once for algal production); and 2) use of treated or untreated wastewater as a cultivation medium for algal production, with the wastewater then re-treated and recycled. In the WWT application, the main products are reclaimed water, algae-based fertilizer, and algal biomass-derived products such as biofuels. However, at current prices, biofuels and fertilizers would not be economical products,<sup>16-19</sup> and it would be fees for WWT and sales of reclaimed water that would provide most of the revenue. The dedicated production of algae-based biofuels using treated or untreated wastewaters has only been investigated at small scales so far, and while economically appealing much more needs to be done to develop and demonstrate the viability of this approach for large-scale applications. For municipal wastewaters, the limiting nutrients for algal growth are typically (in sequence of limitation) inorganic carbon, nitrogen, possibly some trace metals, and phosphorus.<sup>15,20</sup> For cultivation systems using extensive water recycle, salts can build up to high enough concentrations to become inhibitory for growth, but for low salinity waters, such as municipal wastewater, organic inhibitors are more likely to be the limiting factor for water recycling.

There are many and diverse options for cultivating microalgae and maximizing the recovery of bioenergy products. Biochemical processing or other means of more extensively fractionating algal biomass into its major biochemical constituents – lipids, carbohydrates and proteins – presents opportunities to process different algal biomass components separately using a biorefinery approach, i.e., to develop specific biofuels or alternative products from each component stream of the fractionated algal biomass. This approach can potentially increase algae-based product yields to well above those possible only using the lipid fraction. Total biofuels yields from whole algal biomass using a biorefinery approach can, even with conservative assumptions, exceed the yields typically achieved using terrestrial feedstocks.<sup>21</sup> It is noteworthy that all of the process options

discussed in this report to exploit such an approach rely on biogas (methane) production by anaerobic digestion (AD) of residual algal cell mass to help power the plant. Thus, incorporating AD appears critical to both the economics and sustainability of the conversion process, since AD also provides the main route for recycling nutrients to the cultivation process.<sup>22,23</sup>

HTL is an alternative promising thermochemical conversion technology for algal biomass receiving attention for nearer-term deployment, with a number of research groups actively evaluating HTL and several companies pushing its commercialization.<sup>24</sup> Results reported to date suggest that HTL provides a robust approach for algal biomass upgrading to a liquid biocrude intermediate product, which can then be upgraded catalytically to a renewable diesel blendstock. It has been reported that algal species and cell mass biochemical composition exhibits only minimal impact on fuel/product yields. However, HTL-based upgrading for algal feedstocks remains at a relatively early stage of development and additional testing in continuous flow systems is needed as well as more characterization of product composition(s) and quality.

Algal biomass-based bio-products can provide the critically needed revenue to reduce the net cost of producing an algae-based biofuel. As such, a biorefinery approach appears essential to realize the full value of algal biomass, i.e., wherein each component of the algal biomass is used for its most profitable application to maximize the biorefinery's overall economic viability. The highly complex and specific nature of product separations and the multiple potential bio-product options that exist need to be prioritized as research topics to provide the maximum value to on-going and future work. For each of the major algal biomass biochemical fractions (i.e., lipids, carbohydrates and proteins), there are a subset of products and pathways to experimentally demonstrate the valorization approaches discussed in the main report. The alternative higher value products area closest to being experimentally demonstrated and deployed is the production of oleochemical products from algal oils.

Formalized techno-economic assessments (TEA) continue to be the main tool used to understand the market viability of algae-based systems for producing biofuels, bioenergy or other bio-based products. Literature TEAs for various algae-based production scenarios continue to report large variances in estimated process economics, making it difficult to draw definitive conclusions about "true" or "most likely" production costs. Beyond differences in financial modeling assumptions, the wide variability in projected algal biomass costs reported for a given algae-based production pathway is primarily attributed to differences in assumptions about algal growth characteristics and cultivation productivity. A TEA harmonization effort began in the U.S. in 2012 to better align and standardize underlying TEA modeling assumptions, boundary conditions and data inputs and outputs for a described process.<sup>25</sup> Expanding such a harmonization effort internationally is recommended to help improve the ability to meaningfully compare results across different production and conversion process scenarios. Greater harmonization and standardization of TEA (and life cycle analysis (LCA)) models and methodologies is needed, as well as access to relevant and reliable pilot and demonstration performance data for model validation. Unfortunately, since algae-based production is a relatively new area of inquiry, there are still no recognized authoritative databases and steps are also needed to build shared global databases. Helpfully, a body of ISO standards literature is available that provides a consistent framework for developing LCAs of different algae production and processing scenarios.

While algae-derived biofuels and bioenergy applications present an intriguing technology route towards improved future energy security and environmental sustainability, scalability and economics remain significant challenges. In general, the reliability of TEAs and LCAs of prospective algae-based production pathways suffers from their need to extrapolate large-scale production performance from more idealized laboratory or pilot scale data. For LCA, like TEA, the lack of a consistent reference framework makes side-by-side comparison of sustainability metrics for different approaches very difficult. Although LCA remains the *de facto* standard approach being used to compare the sustainability attributes of different processes, differences in assumptions

and how bio-product credits and system boundaries are handled need to be resolved or better standardized before meaningful conclusions can be drawn.

Most TEA and LCA results reported in recent years for algae-based production fall short of the high expectations placed on algae-based biofuels being able to be cost competitive with fossil fuels, i.e., when petroleum prices were remaining above \$80/barrel and trending upward and liquid fuels markets were also expanding. Similar to TEA, recently published LCA studies of microalgae-based biofuel production report a very wide range of net GHG emissions. While the overall span of results is between -2.6 and 7.3 kg CO<sub>2</sub> eq MJ<sup>-1</sup>, more than 85% of the reported results lie between -0.35 and 0.5 kg CO<sub>2</sub> eq MJ<sup>-1</sup>. As with TEA, the main causes for the relatively high variability are due to differences in LCA modeling approaches. Again, a lack of harmonizing LCA methodologies between different research groups is a significant issue. Overall, LCA sensitivity analyses indicate that the energy recovered in the main product (nominally a renewable fuel) has the largest influence on the outcome of the LCA, closely followed by nutrient use efficiency in cultivating algal biomass. Thus, any improvements in biology, productivity or conversion technologies that improve these parameters are likely to positively impact the LCA.

Relatively high parameter uncertainty remains concerning the main steps of microalgae cultivation, harvesting and oil extraction, and this reduces the overall confidence in and strength of conclusions that can be drawn from TEAs and LCAs. Many studies are based on extrapolation of data from pilot and lab-scale levels, and larger and more extended demonstration scale data for algae cultivation and upgrading remains a key need. Because of this, where parameter uncertainty persists, it is recommended that uncertainty assessments be included in order to increase the robustness and transparency of model projections and better guide research towards reducing overall uncertainty. The development of less energy-intensive technologies for microalgae cultivation and harvesting steps is critical to further reduce the life cycle GHG emissions of microalgae-based biofuels. Nevertheless, microalgae biofuel production systems are quite recent and the development of improved production technologies is still taking place. It is recommended that future TEA and LCA studies should be performed for envisioned commercial systems, both to better support and justify the selection of a particular production pathway as the "best" as well as to confirm previous results based on lab or pilot scale experiments.

In addition to microalgae, there are also a myriad of macroalgal species (seaweeds) that can be grown as biomass feedstock as well as many potential pathways to produce bioenergy or other bio-based products from seaweeds. Conversion of seaweeds to biogas using AD technologies is among the most investigated approaches. However, AD-based approaches for macroalgae may prove to be problematic in the longer term due to the potential for high salinity and sand accumulation over time. It is also unlikely that cast seaweed can be harvested at a scale sufficient to provide significant quantities of transport fuel or on a consistent enough basis to meet the continuous supply needs for a biofuel-focused biorefinery. However, colocation of conversion plants where terrestrial biomass could also be sourced and used may provide a means of achieving continuous production from an intermittent supply of macroalgae feedstock. In addition, methane (CH<sub>4</sub>) obtained from AD could be cleaned up, compressed and injected into the existing gas grid to bolster the gaseous bioenergy supply. The more likely scenario is new cultivation of seaweeds being established, more than likely associated with aquaculture. Seaweed-based production for bioenergy products (as opposed to higher value food, nutritional and chemical products, which is already commercialized to a significant extent) is at an early stage of development. It is not yet known which species would be best suited for such a bioenergy application. Numerous parameters, including the method of cultivation, species of seaweed, seaweed yield per hectare, time of harvest, method of harvest, suitability of seaweed to ensiling, gross and net energy yields of biogas, carbon balance, cost of the harvested seaweed, cost of the produced biofuel, etc. have not yet been adequately assessed. Much additional research is required.

## **OPPORTUNITIES AND OUTLOOK**

At least until oil prices return to near their pre-August 2014 levels, or carbon emissions reductions are rewarded through higher carbon pricing in a global climate disruption mitigation policy, primary strategies for liquid biofuels production from algae will need to rely on a biorefinery approach where the production of higher value products can aid the economical viability of algal biofuel production. Summarizing conclusions are that the basic promise of algae-based bioenergy applications is still valid; there does not need to be competition with existing food and feed supply thanks to the potential of growing algae on non-arable land, and, though water and nutrient availability represent real challenges, there is potential to use wastewater and to recover nutrients at each step of an integrated process to minimize the strain on limited available resources. The rapid growth and high photosynthetic efficiency of algae allows for a higher fuel/energy areal yield compared with terrestrial crops. However, the other side of the coin of potential is that there are significant barriers currently impeding commercialization and economic production of algae for relatively low value energy and fuel markets. The barriers addressed in this report range from incomplete knowledge of algae biology to the challenges associated with economical integration of technologies at the demonstration scale. Even though many algae-based technologies have been demonstrated at the laboratory scale, most often this has been done in isolation and thus the challenge remains to fully integrate algae-based processes and prove them out through extended multi-season operation. Progress in minimizing/reducing the energy, water and land use footprints of integrated algae-based operations needs to be a primary objective of future larger scale demonstrations.

## **1. Introduction**

Algae represent a diverse group of photosynthetic organisms spanning simple unicellular cyanobacteria to complex multicellular macroalgae, also known as seaweeds, that possess organized cellular structures and structurally distinctive organs and have the ability to grow to large size. Algae's diverse nature, attractive photosynthetic efficiency and metabolic plasticity, and ability to adapt and thrive in a range of different environments have made them ubiquitous across the earth, though they are most common in aquatic environments. Because of their rapid rate of adaptation to potentially challenging environments, algae are considered excellent feedstocks for future bioenergy production; algae-based biofuels and bio-product applications and their associated promises and challenges have been the subject of a number of recent literature reviews.<sup>21,26-29</sup>

This report highlights the state of technology of algal-derived biofuels, bioenergy and bio-based products from an international perspective. Its objective is to review progress in developing algal biofuel and bioenergy technologies and applications since the previous report by IEA Bioenergy Task 39 was published in 2010.<sup>1</sup> We aim to place algae production technologies in the context of an integrated biorefinery approach to combined production of biofuels and bio-products. Since the 2010 state of technology report was published, industry and academic groups have made tremendous progress in the application of algae for bioenergy production. This report provides an overview of both micro- and macroalgae as bio-based feedstocks to support future biorefineries for economical and sustainable production. The structure of this report reflects the different areas in algae bioenergy applications and studies; an emphasis on microalgae for biofuel and bio-product applications is consistent with a large body of literature and public and private funding and research. We also include a discussion of macroalgae, in particular the application of low-cost, cast seaweed for biogas production as a near-term commercial bioenergy opportunity. Finally, we include an overview of commercialized technologies and a detailed list of global research and commercially deployed algae installations.

The 2010 report sought to examine the technical and economical feasibility of generating algal biomass for the production of liquid biofuels.<sup>1</sup> Its executive summary states that with continued development algal biofuels have the potential to become economically viable alternatives to fossil fuels and to replace a significant portion of fossil diesel with a smaller environmental footprint using marginal land and saline water, placing no additional pressure on land needed for food production or on freshwater supplies.

The authors of the 2010 report concluded: *"the production of liquid transportation fuels from algal biomass is technically feasible. However there is a need for innovation in all elements of algal biofuels production to address technical inefficiencies, which represent significant challenges to the development of economically viable large-scale algal biofuels enterprises."* Furthermore, *"Algal biofuels have the potential to replace a significant portion of the total diesel used today with a smaller environmental footprint. In addition, algal biofuel production can be carried out using marginal land and saline water, placing no additional pressure on land needed for food production and freshwater supplies."<sup>1</sup>*

In 2010, several groups made commercialization and market size projections for algal biofuels and bioenergy applications. These projections were mostly based on the then-rapidly growing knowledgebase of literature and government supported pilot and demonstration projects. For example, SBI Energy among others predicted an annual algal biofuels market growth rate of 43%, estimating a \$1.6 billion total market size in 2015. Similarly, Emerging Markets Online and Pike Research estimated that 230 ML to 3,800 ML of algal biofuels, respectively, would be supplied to the markets by 2015. The IEA Bioenergy Task 39's 2010 report was less optimistic; projecting that in the next 1 to 2 decades algal biofuels would make a transition from pilot to commercial production. If we survey the market today, the earlier projected large volumes of algae-derived

biofuels are not being produced. Even though commercial production of microalgae has been established for decades (for production of higher value non-bioenergy products), the total worldwide production of algal biomass is still relatively modest (~1000 tonnes/year) and commercial applications remain primarily for higher value products. There are challenges that this industry faces beyond technological feasibility, which will be discussed in more detail below. The current significant decline in oil prices in combination with the recent global economic crisis has placed brakes on the implementation and commercialization of current algal technologies for biofuels or bioenergy applications.

Even though the industry has been plagued by significant hype in the projections on the rate and economic viability of algae technology commercialization, much of this hype can be traced back to only a few literature reports that have been repeatedly and uncritically cited; these few reports provide an arguably overly optimistic view of the biological potential of algal systems for producing biofuels. One of these reports is by Chisti and has been cited over 5000 times (since 2007), despite it including several highly optimistic, and as yet unproven, statements about the potential of algae-based biofuels production, such as "*50% of US transportation fuels could be produced on 2 million ha of land*" and "*algal biofuels can sustainably and completely replace all petroleum-derived transportation fuels*".<sup>30</sup> Wide and uncritical referencing of this paper led to an unrealistically optimistic outlook on the actual potential of the then demonstrated technologies. The highly questionable underlying assumptions in Chisti's projections are manifold and include: over 340 days per year operation; 70% oil content by mass; and areal algal biomass growth productivities of 50 - 460 g m<sup>-2</sup> day<sup>-1</sup>. This paper was included and cited in the 2010 report as the basis of the reported feasibility calculations.<sup>1</sup> A similarly highly cited report is by Hu et al.<sup>31</sup> This report, which has been cited almost 2000 times (since 2008), reviewed the biological potential of lipid accumulation in algae as reported in the literature and then related this lipid content to parameters subject to physiological manipulation. When this report published, the estimation was that enough resources were available and that the potential was there to displace a significant fraction of petroleum derived fuels with algae-derived fuels. However, the single biggest economic driver was found to be the growth rate and lipid quantity and quality of the algal biomass. This report summarized the available literature at the time, however, when translating lipid content from algae into fuel potential, it misrepresented the potential of algae-based lipid production, e.g., in assuming that all extractable lipids reported in the historical literature are equivalent to highly desirable triglyceride lipids. As a consequence, this report reinforced an unrealistically high expectation on the actual lipid content of algae and thus also greatly contributed to the recent hype (pre-2015) on the prospects for rapid development of economical algal biofuels production.

In the 6 years since the publication of the 2010 report, significant progress has been made on algae-based technologies, with many demonstration and pilot projects now installed and beginning to operate. This report reviews the current state of technology as reported in recent academic, patent and commercial literature. It also provides the background information needed to generate an informed projection about the technical feasibility as well as economical and sustainability potential of future algae bioenergy commercialization. It is understood (at the onset of writing this report) that even with the perfect location and optimum strains deployed, with a demonstrated downstream conversion process implemented, there is still a competition of biomass use for feed that negatively impact the economical feasibility of any bioenergy application. This report does not explicitly address the Technology Readiness Levels (TRLs) of the algae-based technologies being researched and developed for bioenergy, as these vary widely across the various approaches; whereas some technologies such as open pond-based production of higher value products are already commercialized and at a high TRL (albeit not yet for any bioenergy products), others such as closed photo-bioreactor based cultivation or HTL processing are at much earlier stages of development and technical readiness. Because the approaches to and markets for algal biofuels/bioenergy and other product applications are extremely diverse, the report is structured to separately address the different topics of algal biology and conversion processes, as well as related techno-economic and sustainability assessments.

## **2. International Activities Advancing Algae for Bioenergy**

Since IEA Bioenergy Task 39 published the report on the status of algal biofuels in 2010, a significant number of government and peer reviewed literature reports were published. Some of these reports have significantly impacted public perception of algal production as well as influenced general technical and economic feasibility discussions and the funding and support landscape. This section summarizes several of these reports that have particularly influenced the policy and the funding environment for algae-based bioenergy and biofuels production.

### **2.1. INFLUENTIAL REPORTS SINCE 2010**

In 2012, the United States National Research Council (NRC) commissioned a report to investigate the sustainability of algal biofuels in light of the projected resource demands on water, energy and nutrients for large-scale production. This report<sup>8</sup>, which was completed as a collaboration between members of the US National Academy of Sciences and the US Department of Energy, concluded:

*"Based on a review of literature published until the authoring of this report, the committee concluded that the scale-up of algal biofuel production sufficient to meet at least 5 percent of U.S. demand for transportation fuels would place unsustainable demands on energy, water, and nutrients with current technologies and knowledge. However, the potential to shift this dynamic through improvements in biological and engineering variables exists. Sustainable development of algal biofuels would require research, development, and demonstration of the following: i) algal strain selection and improvement to enhance desired characteristics and biofuel productivity, ii) an energy return on investment (EROI) that is comparable to other transportation fuels, or at least improving and approaching the EROIs of other transportation fuels, iii) the use of wastewater for cultivating algae for fuels or the recycling of harvest water, particularly if freshwater algae are used, iv) recycling of nutrients in algal biofuel pathways that require harvesting unless bio-products that meet an equivalent nutrient need are produced, v) a national assessment of land requirements for algae cultivation that takes into account climatic conditions; fresh water, inland and coastal saline water, and wastewater resources; sources of CO<sub>2</sub>; and land prices is needed to inform the potential amount of algal biofuels that could be produced economically in the United States."*

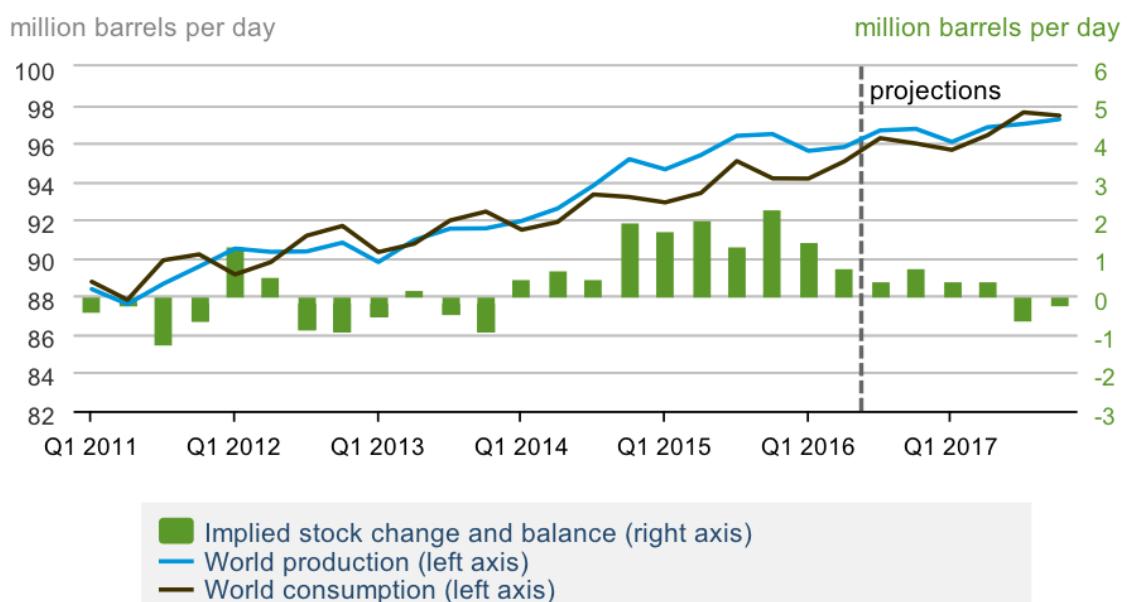
Some of the major shortcomings of this report are that its nutrient utilization criticism is based on a single reference and assumes that the nutrient requirements of algae are static and exclusively reflect the Redfield ratio; this ratio is derived from open water algae cell mass elemental composition.

### **2.2. INTERNATIONAL FUEL USE AND PETROCHEMICAL MARKETS**

The IEA projects under its New Policies Scenario that world oil demand will increase from approximately 90 million barrels per day (mb/d) in 2014 to 104 mb/d by 2040.<sup>32</sup> The US Energy Information Administration's (EIA) forecasts somewhat higher global demand growth over this same period, with total worldwide production rising to almost 120 mb/d by 2040.<sup>33</sup> However, the price of petroleum has fallen by roughly 50% since these projections were made. This has greatly increased the techno-economic hurdle to achieve cost competitive biofuel production. It has also created great uncertainty about the future petroleum market (and fossil fuel markets more generally) owing to a variety of on-going and unresolved geopolitical, technical and environmental factors. Thus, while vehicle energy efficiency technologies continue to reduce fuel use per unit distance travelled, total worldwide demand for liquid fuels is projected to continue to grow over the next several decades.

Although worldwide petroleum reserves continue to be depleted and the costs of fossil fuel use to

the global environmental continue to mount, many countries remain highly dependent upon non-renewable and often imported sources of oil (and other fossil fuels), especially for transportation fuels. The Energy Information Agency (EIA) estimates that global petroleum and other liquid fuels inventory builds averaged 1.8 million b/d in 2015. The pace of inventory builds is expected to slow to an average of 0.8 million b/d in 2016. Inventory builds are expected to continue into early 2017, and then consistent inventory draws are forecast to begin in June 2017, when there is projected to be a higher demand than production (**Figure 2.1**). The United States, for example, currently imports approximately one third of its petroleum, mostly from only a few countries around the world. The projected increased demand for energy and liquid fuels worldwide is driven by rising living standards and higher energy diets and lifestyles in more rapidly developing countries, particularly China and India. Nonetheless, the continued extraction and combustion of fossil fuels has created serious environmental concerns about increased global warming and ocean acidification due to greenhouse gases (GHG) accumulating in the atmosphere. Biofuels remain one of only a few potential options to reduce the world's dependence on using fossil fuels for transportation.



**Figure 2-1:** Projected future petroleum and liquid fuel consumption from the Organization for Economic Co-operation and Development (OECD) and Non-OECD relative to petroleum stock change and balance (EIA, Short-Term Energy Outlook, September 2016)

However, despite possessing many attractive features, biofuels also have their limitations. One concern associated with substantially increasing biofuels production is limited availability of or competition for suitable land. In particular, the GHG mitigation benefits of biofuels can be negated if land with existing high carbon intensity is cleared for the production of biofuel feedstocks. Biofuels that could be produced without large increases in arable land or reductions in tropical rainforests that obviate or greatly diminish land availability and land use change concerns remain highly attractive to develop. It is in this context that algal biomass-based-routes to biofuels have potential.

According to an earlier 2011 published roadmap for biofuels use in the transportation sector,<sup>34</sup> up to 27% of worldwide transportation fuel could be supplied by biofuels by 2050, in particular to replace diesel, kerosene and jet fuel. This projected use would avoid 2.1 Gtonnes of CO<sub>2</sub> emissions per year. To achieve this target it is necessary to create a stable long-term policy framework to increase investor and end-user confidence and allow rapid expansion of this nascent industry. There also needs to be a sustained funding and support infrastructure provided by the governing agencies globally. As part of this financial and policy support for the development of technology, it

is critical to link the performance of groups developing and implementing the technology to GHG emissions reduction and other sustainability targets. The use of incentives may help spur commercial implementation and help reach the set targets. Finally, because of the magnitude of the global biofuel and bioenergy challenge, there should be international support and collaboration to aid with building out capacity as well as increased technology transfer to promote adoption of sustainable biofuel production pathways globally. It is within this context that algal biofuels are discussed in this report: How can algal biomass play a role in the global adoption and expansion of a vibrant biofuels industry? And what are the main techno-economic barriers to commercial deployment of algae-based bioenergy production?

Across the global algae industry and research institutions, there has been government support for the development of algal biomass and algal biomass-derived biofuels and bio-products, mainly through public-private partnerships. This support is mainly motivated by a need to implement policies to i) reduce each country's dependence on importing fossil fuels (energy security and independence) and ii) mitigate GHG emissions through reduced fossil fuel usage (environmental health and security).

### 2.3. INTERNATIONAL BIOFUELS POLICY

On a global scale, each country or region has a distinct approach to the implementation of bioenergy into its national or regional fuel and energy infrastructure. It is generally accepted that on an international level, renewable energy must play a fundamental role in the transition towards a more competitive, secure and sustainable energy system. This transition will not be possible without a much larger contribution of renewable energy to our current infrastructure. The production and use of biofuels has been mainly driven by government policies in order to reduce oil dependency and in turn increase the share of renewable energy contributing to carbon dioxide (CO<sub>2</sub>) emissions mitigation. The following section lists some of the current and past international commitments to bioenergy and describes how algae fit into this approach. The main mechanisms for governments supporting biofuel policies are blending mandates and tax exemptions, however, other policies also can be used to support the development of nascent industries; e.g. grants to support the installation of production facilities, farmer premiums for the production of energy crops, and supporting research and development (R&D) funding.

The recent **European Union (EU)** energy strategy has called for a substantial transformation of Europe's energy system based on a more secure, sustainable and low-carbon economy, with the commitment to achieve, by 2030, at least 27% share of renewables and 40% greenhouse gas (GHG) emissions reduction relative to emissions in 1990.<sup>35</sup> In this context, the EU has set a cap of 7% on the final consumption of biofuels produced from agricultural crops in favor of advanced biofuels produced from non-food materials, including algae.<sup>36</sup> Under the Renewable Energy Directive (RED, 2009/28/EC)<sup>37</sup>, the European Commission (EC) promotes the use of biofuels and bioenergy to accomplish various climate and energy targets to be met in the European Union (EU) by 2020 (also known as the 20-20-20 targets). These targets include: i) a reduction in GHG emissions of at least 20% compared to 1990 levels; ii) a final energy consumption of 20% derived from renewable sources, including biofuels and bioenergy, among others; iii) a reduction in primary energy use of 20% compared with the projected levels, to be achieved by improving energy efficiency. To guarantee the sustainable use of biofuels and bioenergy, the RED established mandatory sustainability criteria.<sup>38</sup> Among them, a minimal threshold of GHG saving from the use of biofuels of 35% has to be achieved. From 2017, the GHG emissions saving from the use of biofuels must be at least 50% and, from 2018, it must be at least 60% from the use of biofuels produced in new installations.<sup>37</sup>

In the **US**, the Energy Independence and Security Act of 2007 (EISA) expanded the national Renewable Fuel Standard (RFS) by increasing and diversifying the qualifying biofuel alternatives as well as increasing the contribution of renewable fuels to the total liquid transportation fuel

mix.<sup>39</sup> In particular, EISA aimed to increase the supply of alternative fuels and set a target for the use of 136 billion liters (36 billion gallons) of renewable fuels, including advanced and cellulosic biofuels and biomass-based diesel, by 2022. EISA defines four categories of renewable fuel, with specified minimum GHG reduction thresholds that must be met to qualify under each category. EISA requires ≥ 20% GHG reduction for any renewable fuel production facility constructed after 2007, 50% reduction for advanced biofuels, 50% reduction for biomass-based diesel, and 60% reduction for cellulosic biofuels. All of these are measured against the 2005 average petroleum baseline. Having achieved significant success through 2014, primarily through expansion of conventional corn grain (starch-based) ethanol production, the majority of biofuels production growth remaining in the program is to be fulfilled by advanced biofuels, which include biomass-based diesel and cellulosic biofuels. Implementation of the US RFS requires the US Environmental Protection Agency (EPA) to estimate the life cycle GHG emissions for renewable fuels pathways to determine their eligibility for the available RFS fuel categories. EISA defines life cycle GHG emissions as "*the aggregate quantity of GHG emissions (including direct emissions and significant indirect emissions such as those from [feedstock production and any associated] land use changes), related to the full fuel life cycle, including all stages of fuel and feedstock production and distribution, from feedstock generation or extraction through the distribution and delivery and use of the finished fuel to the ultimate consumer, where the mass values for all GHGs are adjusted to account for their relative global warming potential.*"<sup>40</sup>

**China's** commitment to renewable energy is defined by its 13th Five Year Plan (FYP). The 13th FYP covers the years 2016-2020 and strengthens the 12th FYP's efforts to address China's severe environmental degradation by building the country's clean energy, green manufacturing, and environmental services sectors. Of the 25 priority targets outlined in the plan, ten are related to environment. These ten are included as part of the thirteen binding targets which must be achieved by 2020. Much of the research done on algae to bioenergy is contributed by partnerships between universities, commercial entities, and research institutes. The National Basic Research Development Program has funded collaborations to investigate energy production from microalgae, and the National Science and Technology and Support program has funded research on the cultivation and feedstock development for algae.<sup>41</sup> Microalgal oil production has been the focus of institutions like the Ocean University of China and Tsinghua University, and industry partners. The Dalian Institute of Chemical Physics has also done work on microalgal hydrogen production.<sup>42</sup> Like South Korea, China investigates using algae for fixation of CO<sub>2</sub>. Their CO<sub>2</sub>-Microalge-Fuels Project is funded by the National High-Technology Development Program.<sup>41</sup>

**South Korea** currently sponsors four major research and development projects, which will be in effect until 2019. The Marine Bioenergy Development Project and Green Growth Via Marine Algal Biomass project focus on clean energy production from marine algal biomass. Algae technology is also incorporated in the Global Frontier project, which investigates the mass cultivation of biomass, and the Carbon Capture and Sequestration 2020 project; which investigates technologies for CO<sub>2</sub> capture and storage.<sup>43</sup>

As part of the **Kyoto Protocol**, **Japan** also has a biofuels program.<sup>44</sup> The country has high standards for biofuels, especially bioethanol which must have a 50% reduction in CO<sub>2</sub> emissions compared to gasoline. Due to high food prices, however, this program is more focused on second and third generation biofuels, with feedstocks such as rice straw, woody biomass, and algae.<sup>44,45</sup> *Pseudochlorocystis* is the main algae used for technology development, and the Ministry of Agriculture, Forestry, and Fisheries is funding a joint research project with firms and universities to produce biofuel from algae. One of the main goals is to produce jet fuel from algae and commercialize by 2020.<sup>45</sup>

In 2009, **Taiwan** passed the Renewable Energy Development Act, which allocated government funds to support the generation of 650-1000 MW of renewable energy by 2030. The Bureau of Energy, monitors policies and regulates the energy market accordingly. Funding is likewise

distributed to universities, research laboratories, and industry partners. In dealing with policy, Taiwan has three main research areas of interest: selection of microalgal species, cultivation strategies, and lipid extraction. Entities like National Taiwan Ocean University research feedstock; the university currently has a seed stock for microalgae, macroalgae, prokaryotes and eukaryotes. In terms of industry, Far Easter Bio-Tec Co. partners with Taiwan's China Steel Co. to investigate emissions reduction through fixation of flue gas by algae. The Industrial Technology Research Institute's Green Energy and Environment Research Labs also studies topics relevant to production of algae biofuel, such as cell disruption and nutrient starvation.<sup>46</sup>

In **India**, a National Policy on Biofuels supports funding for research in first, second, and third generation biofuels.<sup>47</sup> This support can be seen in the creation of the Vivekananda Institute of Algal Technology, which investigates topics such as algal treatment of wastewater and biofuel production from phytoplanktonic algae (diatoms). Universities like the University of Madras, Chennai, research biogas production and algae cultivation.<sup>48</sup> Throughout 2008-2009, a National Algal Biofuels Network was launched to support algal biofuels research, however research progressed slowly and the program has since diminished in size.<sup>49</sup>

**Brazil** is known for its production of bioethanol from sugarcane, however it also supports the production of biofuels from algae. The National Research Council and National Fund for Research Projects, in conjunction with other federal organizations, state organizations, and private initiatives, finance bioenergy research. Much of the research on algae is done at universities, like the University of Campinas. The government funds research on algae growth because of its potential to mitigate CO<sub>2</sub> emissions from ethanol fermentation through FAPESP 2008/57873-8.<sup>50</sup> The Federal University of Rio Grande do Norte partners with the Petrobras Research Center (CENPES-Petrobras) to operate a pilot plant with 100 m<sup>2</sup> of open pond cultivation.<sup>51</sup>

## 2.4. NORTH AMERICAN SUPPORT FOR ALGAE TECHNOLOGY DEVELOPMENT

In the context of the RFS and EISA, there has been considerable support to study the feasibility of algal biofuel production in the US. A large part of this work was financially supported by the US Department of Energy (DOE), where a strategic multiyear program plan (MYPP)<sup>52</sup> has been established to provide a roadmap towards an economically viable algal biofuels or algal biorefinery industry. The information presented here is a summary of projects funded and installed in the US and Canada, with most of the data provided by media, websites and personal communication, rather than peer reviewed publications. All relevant weblinks and further information are provided in **Appendix B**.

Over the past 6 years, the American Recovery and Reinvestment Act (ARRA) of 2009 infused a significant amount of funding into supporting the Algae Research Community. As a leading example, in 2009 the **National Alliance for Algal Biofuels and Bioproducts (NAABB)** was funded for \$44M. Specific outcomes from this large multi-year project range from basic advances in algal biology such as genomic sequencing of production strains, development of a new open pond cultivation system, demonstrated use of low-energy harvesting technology, to the development of a new HTL conversion pathway for algal biomass upgrading. Alongside NAABB, three integrated biorefinery (IBR) demonstration plants were funded, at a combined total of almost \$97M, respectively for the algal technology developers Solazyme, Algenol and Sapphire.

**Solazyme Inc.** was awarded \$22M from DOE for an integrated pilot project in Riverside, Pennsylvania, involving heterotrophic algae that can convert cellulosic sugars to diesel fuel. This demonstration plant has a capacity to process daily 13 metric tons of dry lignocellulosic feedstocks, including switchgrass, corn stover, wheat straw, and municipal green waste, transforming it through an industrial deconstruction and fermentation process to produce algal oil,

which can then be converted into FAME biodiesel or renewable (hydrocarbon) diesel. The biofuels produced by this project target reducing lifecycle GHG emissions by 90%, with the facility having the capacity to produce 300,000 gal yr<sup>-1</sup> of purified algal oil. In 2016, Solyzme rebranded itself as TerraVia, shifting its focus to food and personal care products. **Algenol Biotech LLC.**, of Fort Meyers, Florida, was awarded \$25 million from DOE for an integrated pilot project involving photosynthesis driven algal conversion of solar energy and CO<sub>2</sub> to ethanol and the delivery of a photobioreactor system that can be economically scaled to enable commercial production. This project utilizes a hybrid cyanobacteria species to directly secrete ethanol within a closed photobioreactor. It is worth noting that =following the collapse in oil prices, Algenol has shifted their approach towards algae for food and feed, and increased emphasis on using their approach to achieve carbon capture and fresh water production until global oil prices recover. **Sapphire Energy Inc.**, was awarded \$50 million from DOE for a demonstration-scale project to construct and operate a 120 ha (300 acre) algae cultivation farm and conversion facility in Columbus, New Mexico, to produce renewable bio-crude (for subsequent upgrading to jet and diesel fuels). The target capacity of this plant is 1 million gallons per year of finished product, or 100 barrels of green crude algal oil per day. The biofuels produced are intended to achieve a 60-70% GHG reduction over the traditional fossil fuels being displaced.

Three additional consortia were funded in the US shortly thereafter: The **Consortium for Algal Biofuels Commercialization (CAB-Comm)** was a 4 year (2011-2015), \$11 million project led by the University of California, San Diego, with the main goals of improved algal feedstock protection, nutrient utilization and recycling; and genetic tools. The outcomes of this project include increasing algal biomass productivity, creating new advanced biotechnology tools, and commercializing bio-products with industrial partners. The **Sustainable Algal Biofuels Consortium (SABC)**, led by Arizona State University's Arizona Center for Algae Technology and Innovation (AzCATI) in Mesa, AZ was funded \$6M in 2010 for three years, with a main goal of developing a feedstock matrix for algal biomass species based on promising algal species and growth/process conditions; determine and characterize the biochemical composition of selected species; explore multiple biochemical routes to hydrolyze and convert untreated or pretreated whole algal biomass, oil extracts, and algal residues; and determine the acceptability of algal biofuels as replacements for petroleum-based fuels. A key outcome from this project was the development of a novel approach for fractionating algal biomass and converting each fraction to higher value fuel products. The **Cornell Marine Algal Biofuels Consortium** was a 5 year, \$9 million dollar project led by Cornell University and Cellana, Inc., focused on large-scale production of marine microalgae for biofuels and products. This consortium utilized the large-scale algae production facility operated by Cellana in Kona, Hawaii, to develop integrated design cases for the production of higher value products alongside advanced biofuel production. Highlight technical accomplishments include developing two novel algal species well suited for large-scale production and demonstrating an improved annual operating reliability of 350 days per year.

Since 2012, two additional large consortia were funded in the US, with a focus on developing user facilities for long-term cultivation trials across the country in order to support a more rapid transition from lab to outdoor production systems and thereby reduce the risk to budding commercial operations. The **Algae Testbed Public Private Partnership (ATP<sup>3</sup>)** is a 5 year (starting in 2012) \$15M project, lead by AzCATI in Arizona. The objectives of this project are to establish collaborative open testbeds that increase stakeholder access to scale up facilities as well as collect and publish high-impact data on long-term outdoor cultivation trials. These testbed sites are located at universities and companies across the southern US, specifically at ASU (Arizona), CalPoly (California), Georgia Institute of Technology (Georgia), Florida Algae (Florida) and Cellana (Hawaii). Similarly, a **Regional Algal Feedstock Testbed (RAFT)**, a \$5M project, was established in 2013 with the goal of creating long-term cultivation data to understand, de-risk and thereby promote increased algal biomass production. The RAFT's four testbeds are located in Texas, New Mexico, Washington and Arizona.

Similar to the US, the **Canadian government** has actively supported domestic algal biofuel industry activities through funding various projects. In 2008 - 2011, the Canadian government invested approximately 5M Canadian dollars (CAD) in research led by Canada's National Research Council (NRC) to produce algal fuels on a large scale in Canada under a program called the **National Bioproducts Program Algal Biofuels Initiative**. Some of the activities conducted through this program included an international collaboration between NRC Canada, NREL, Sandia National Laboratories, and PNNL on microalgae strain collection, site modeling for the bio-deployment of algal biorefineries with Canadian wastewater treatment plants, and early hydrothermal liquefaction (HTL) experiments with sugar kelp biomass collected from the east and west coasts of North America. This bilateral collaboration was conducted from 2010 – 2012 under the framework of Canada-US Clean Energy Dialogue.

The Canadian federal government also invested over 377,000 CAD in November 2010 in R&D conducted by **Solarvest (PEI), Inc.**, a subsidiary of Solarvest Bioenergy Inc., to generate hydrogen through algae production. In 2011, a large inter-institutional project, the **Algal Carbon Conversion (ACC) Flagship Program**, was established by Canada's National Research Council to mitigate carbon emissions through the commercial scale cultivation microalgae linked to large final CO<sub>2</sub> emitters. The ACC program addresses a number of factors which influence the commercial potential of ACC technologies, including identifying the most appropriate algal species for industrial deployment, increasing the productivity and reducing energy costs of photobioreactors, identifying ways to reduce energy costs for processing algal biomass and assisting in the development of high value, sustainable products from algal biomass. In the spring of 2016, NRC and its industry partners began operation of a pilot scale ACC facility linked to a cement manufacturing plant in Southern Ontario.

**Mexico's** Secretariat of Energy and its National Council for Science and Technology supply the majority of funding for algae biofuels research. Many universities in the country are involved with research on biocatalysts and feedstocks for second and third generation biodiesel production, which includes microalgae. Funding is also available for production of hydrogen from algae biomass, and algal cultivation using wastewater.<sup>53</sup> Most of these projects are still in their early stages.<sup>54</sup>

## 2.5. EUROPEAN SUPPORT FOR ALGAE TECHNOLOGY DEVELOPMENT

European support for the development of algae as bioenergy feedstocks was mainly driven by the European Commission's (EC) FP7, and this support has been continued through the Horizon 2020 program. As above, the information presented here is a summary of projects funded and installed the European Union, with most of the data provided by media, websites and personal communication rather than peer review literature. All relevant weblinks and further information are provided in **Appendix B**.

The **AQUAFUELS project** (2009) was a European Union funded action in the field of algae biomass production for the creation of energy, in this specific case for the production of biofuels. AQUAFUELS was coordinated by the European Biodiesel Board and included partners from 12 European countries. During the 18 months of work, the partners put together their efforts and their scientific, legal, industrial and technical knowledge in order to understand the real potential of algae and other aquatic biomass as feedstocks for biofuels. One of the outcomes was the creation of the European Algae Biomass Association (EABA).

Aligned with the EU's renewable energy targets, the EC is participating in the funding of three large-scale industry-led projects aimed at demonstrating the production of algal biofuels along the whole value chain at a 10 ha scale, spanning algae strain selection to cultivation and production, algal oil extraction, biofuel production and biofuel testing in transportation applications, with a minimum productivity target of 90 dry solid tonnes per hectare per year. The total cost for the

three projects is about €31 million and the corresponding EC contribution amounts to about €20 million. The three large currently funded, collaborative R&D projects under the **Algae Cluster** umbrella ([algaecluster.eu](http://algaecluster.eu)) are:

- **InteSusAI, conducted by the Centre for Process Information, UK,** strives to demonstrate an integrated approach to generate biofuels from algae in a sustainable manner on an industrial scale. This project aims to demonstrate an optimized approach to produce biofuels from algae in a sustainable manner on an industrial scale. It will integrate high quality research that has previously been undertaken at national and international levels, both publicly supported and privately financed. InteSusAI has so far built a 1 hectare pilot facility in Necton Olhão, Portugal, based on a mixture of fermenters and photobioreactors. The system concept involves the recycling of waste glycerol from the transesterification stage of biodiesel production, and the fermenters contribute CO<sub>2</sub> to the photobioreactors
- **All-Gas, coordinated by Aqualia, Spain,** targets 10 ha of microalgae cultivation and use for biofuels production lead by Aqualia, the third-largest private water and wastewater company in the world, consisting of seven partners and supported by a scientific advisory board to provide the knowledge and experience for this challenging endeavor. All-Gas is based around the concept of using a mixture of algae and bacteria to clean wastewater and produce fuel. All-Gas has built a 1 hectare pilot facility in Chiclana de la Frontera, Spain, and will soon expand to 3.5 hectare system.
- **BioFAT, coordinated by A4F, Portugal,** aims for 10 ha demonstration of cultivation and conversion to biofuels, a microalgae-to-biofuel demonstration project targeted at both biodiesel and ethanol production, and will integrate the whole algae process value chain from algae optimized for growth and starch and/or oil accumulation, to downstream biofuel production processes, including biorefinery. CO<sub>2</sub> from industrial fermentation will be used as a renewable carbon source. BioFAT has so far constructed two ½ hectare pilot facilities, one in Pataias (Portugal) and one in Camporosso (Italy). In 2016, a 10 ha facility was designed for construction in 2017.

In addition, the EU more recently initiated support for 5 more projects that seek to demonstrate the feasibility of algae-based bioenergy: **AlgaeBioGas**: aims to demonstrate algal treatment of biogas digestate; **DEMA**: aims to demonstrate a technology for direct production of ethanol from microalgae; **D-Factory**: targets demonstration of large scale (100s ha) cultivation of *Dunaliella salina*; **EnAlgae**: operates 9 pilot facilities for micro- and macroalgae cultivation; **Fuel4ME**: targets the pilot scale production of biofuels from algal lipids. Projects also focus on algae production without fuel considerations: **MIRACLES**: aimed at overcoming technological barriers preventing microalgae application in food, aquaculture, and other products; **SPLASH**: researches the potential for algae to produce polymers.

## 2.6. GLOBAL INDUSTRIAL DEVELOPMENT OF ALGAE TECHNOLOGY

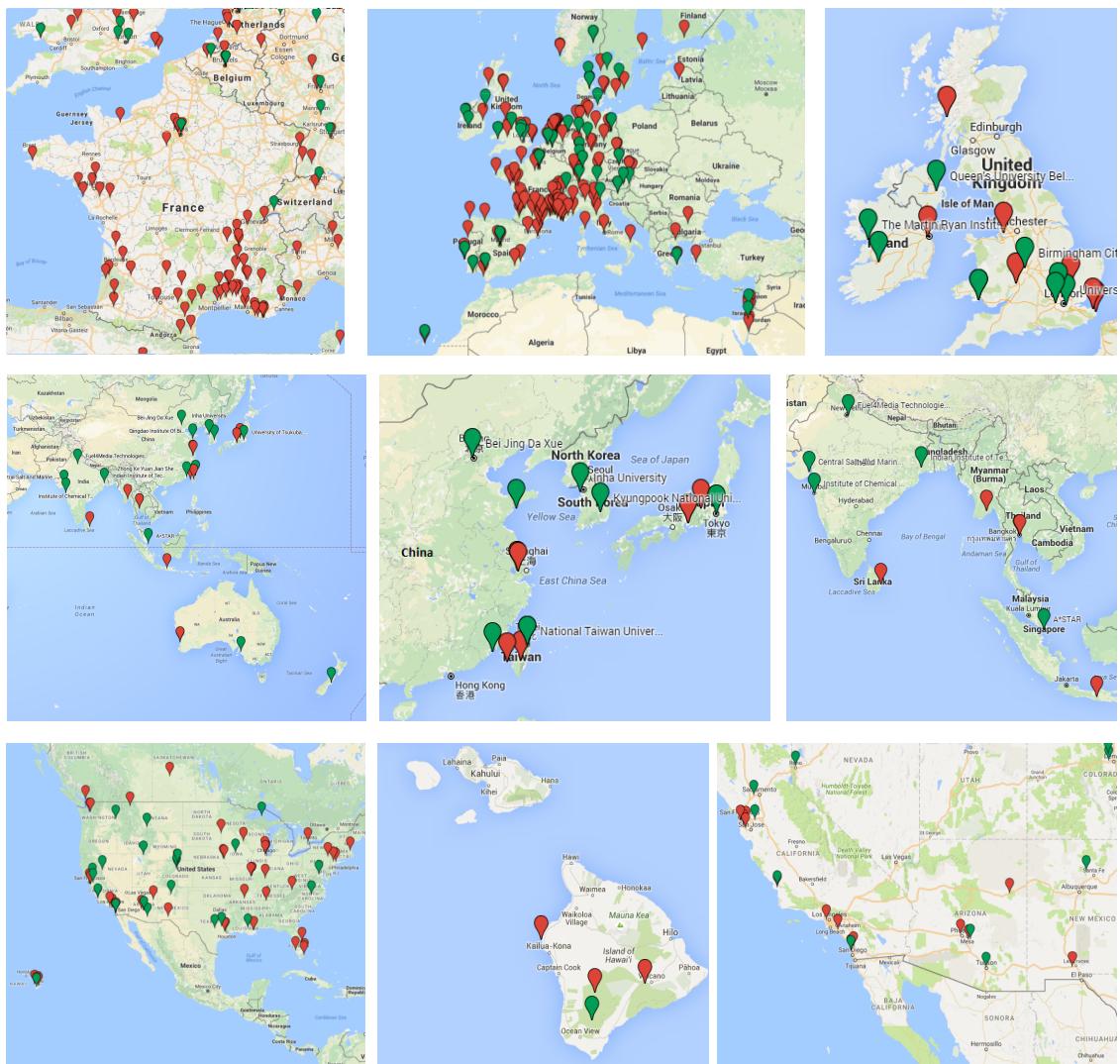
Along with numerous government-supported projects, a large number of commercial entities are supporting algae (both micro- and macroalgae) production and research. The strategies employed range widely, from open pond cultivation to photobioreactors in the area of phototrophic cultivation to large-scale aerobic fermentors for heterotrophic production of algae. Commercial facilities are either for algal biomass feedstock production, i.e., both phototrophic and heterotrophic microalgae cultivation installations, or for macroalgae production.

Similarly, at intermediate scales, there are research projects underway to support the development of a bioenergy industry based on the production of algae. A summary of currently funded research projects and commercial operations worldwide is included as **Appendix B**, separated by region, and also summarized in **Table 2-1** and visually represented in **Figure 2-2**.

They describe the state of the algae industry across several different regions. The data is divided between research and corporate entities, and gives the year of last known website update. Incorporated in company data are businesses involved in producing any algae related higher volume commercial products, including biofuels, skin care products, nutrients, and animal feedstock. Information pertaining to the cultivation process is also given in **Table 2-1**. Most of the research groups included, directly research algae characterization and the algae to biofuel process, as the companies that produce more mainstream consumer goods generally conduct their own research and don't publish their results. The research subsection incorporates government funded projects, as well as universities and national laboratories and mainly represent larger projects (>\$1M approximately). The areas studied in the cited research projects range from strain improvement strategies, cultivation improvements, as well as a large emphasis on conversion to bio-products and biofuels. Some of the research is carried out in public private partnerships that include large-scale deployment to help understand the barriers to commercialization. The compilation of this list was achieved through browser searches, and by reading company websites, where data is available. While this list is undoubtedly incomplete, it gives a good sense of general trends worldwide and within specific geographic regions. For example, the large concentration of red pins in France (**Figure 2-2**) are mostly due to *Arthrospira* sp. (*Spirulina*) farms engaging in indoor pond cultivation. In the area of algal biofuels, the North America and Europe region in the world dominates the academic publishing realm, whereas the majority of the patent applications filed worldwide are distributed between the US, the EU and China.<sup>55</sup> In contrast, many businesses in Asian countries and a large fraction of the EU commercial groups focus on the production of seaweed and microalgae as a food crop. In the coastal regions, the majority of the algae related companies focus on either the natural harvesting, or, in Asia, an emphasis on cultivation of seaweed as a food or bioenergy crop. Seaweed has a historically established place in China's economy and aquaculture industries, however a large percentage of the world's microalgae is grown in this region as well.<sup>56-58</sup> This region is underrepresented in **Table 2-1**, however the Chinese Algae Industry Association has over 600 members.<sup>59</sup>

**Table 2-1:** Summary of commercial and research operations working towards commodity algae-based (both micro- and macroalgae) products globally, separated by region and by commercial installation. Fermentation includes predominantly heterotrophic cultivation companies, Suppliers include cultivation systems, measurement and general equipment manufacturers, Research includes large government supported academic and public private partnerships projects and consortia. N/A = No information available (full list and more details of operations and focus areas are included in **Appendix B**)

	Total	Europe	North America	Asia	Oceania	Middle East
<b>Commercial</b>	306	166	105	26	2	7
<b>PBR</b>	50	32	14	1	N/A	3
<b>Raceway</b>	50	32	11	4	1	2
<b>Combined PBR and Raceway</b>	12	5	7	N/A	N/A	N/A
<b>Fermentation</b>	13	4	5	4	N/A	N/A
<b>Unknown cultivation method</b>	160	85	55	17	1	2
<b>Suppliers</b>	21	8	13	N/A	N/A	N/A
<b>Research</b>	94	50	27	9	5	3
<b>Total</b>	400	216	132	35	7	10
<b>Shut operations</b>	50	28	22	N/A	N/A	N/A



**Figure 2-2:** Overview of global commercial and research operations; red balloons represent commercial operations, green balloons research/demonstration projects

### **3. Overview of Current Technology Routes for Algae-Derived Bioenergy Products**

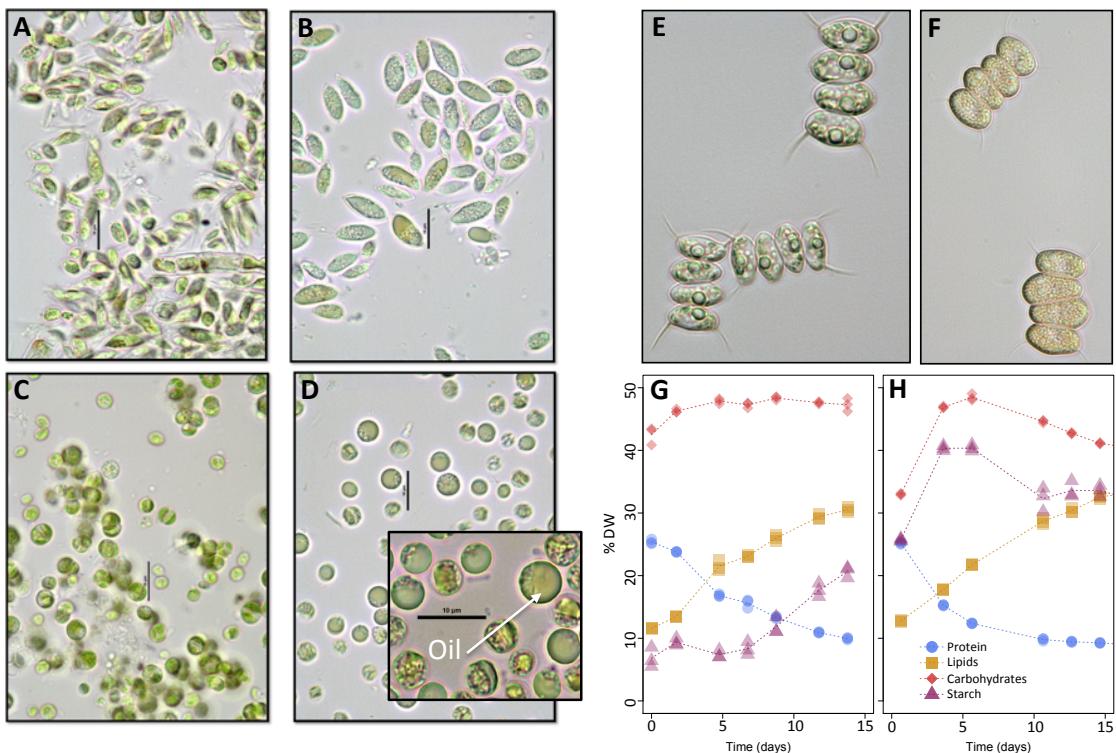
Algae comprise a large amount of biological diversity, metabolic plasticity, i.e. ability to adapt the biochemistry and cell compositional profile in response to a range of external physiological inputs, compared to terrestrial feedstocks. In this context algae are a promising biological feedstock to address future bioenergy challenges. This section provides an overview of algae, algal biology and production systems to provide the background needed to critically review the state of the art.

#### **3.1. ALGAL BIOLOGY**

This report considers both micro- and macroalgae as potential primary biomass feedstocks for bioenergy production. Microalgae dominate the discussion and research on liquid biofuels production from algae thanks to their high inherent lipid content and the direct link an algal production route provides to a lipid-based, energy-dense fuel product via a straightforward extraction process. In most of the following discussion both prokaryotic cyanobacteria and eukaryotic single celled microalgae are considered under the larger umbrella of microalgae. Macroalgae, or seaweeds, can contribute significantly to the discussion of algae-based bioenergy production, and are of great interest to countries with coastlines amenable to macroalgae cultivation. However, for macroalgae, primary bioenergy applications are more for biogas production than for liquid biofuels, even though the production of ethanol from macroalgae has been demonstrated.<sup>60,61</sup> More detailed discussion of using macroalgae in a bioenergy context is provided in **Sections 9 and 10**, with the other sections of the report primarily focused on microalgae. The vast majority of research and commercial development in the bioenergy space on algae has been directed towards microalgae, ranging from fundamental research to conversion and deployment. This report reflects this situation, with the bulk of the information presented and discussed focused on microalgae.

Microalgae are diverse single-cell organisms, capable of photosynthesis to convert inorganic carbon in the form of CO<sub>2</sub>/carbonate to organic constituents that make up the cell's composition. Their high photosynthetic productivity provides the rationale for developing algae-based bioenergy supply chains to displace significant quantities of fossil energy. Photosynthetic conversion of CO<sub>2</sub> with sunlight (or suitable artificial light) and nutrients to form lipids, carbohydrates and protein, is referred to as phototrophic growth or cultivation (using either open or closed production systems). Phototrophic growth of microalgae is mainly implemented in large-scale outdoor facilities, where the focus is on achieving high algal biomass productivity. Water and nutrient management are used to tailor the biochemical composition of the produced algal biomass towards an economically viable biofuels feedstock (**Figure 3-1**).<sup>5,21,62</sup> Alternative production scenarios include heterotrophic cultivation, where sugars and air or molecular oxygen (rather than CO<sub>2</sub> and sunlight) are fed into a fermentor to grow algae to high algal cell mass concentrations in more highly controlled conditions than are possible in outdoor systems. Heterotrophic production of microalgae is already commercialized technology thanks to the high lipid content of heterotrophically-grown algae coupled with the potential to manipulate the cell's biochemistry through metabolic engineering. However, there are economic challenges to adapting heterotrophic algae technology to biofuel or bioenergy applications, as it requires more expensive inputs, such as exogenous sugars and the provision of oxygen to support aerobic submerged cultivation. Nonetheless, many companies are already commercially producing heterotrophic algal oils for higher value product applications in the food, feed and nutraceutical markets (e.g., Roquette, Solazyme, Bunge, DSM, ADM, etc. see **Appendix B**). Because most biofuel and bioenergy applications involve lower value products to serve commodity-scale markets, most of the discussion that follows is centered around different configurations for phototrophic cultivation of algae, encompassing both open pond and closed photobioreactor systems for producing algal biomass. Even though there is technical potential for microalgae to make a large-scale contribution to future biofuels production, a number of economic challenges remain in the way of commercial deployment.<sup>10</sup> The following sections cover the

current state of technology and future development of microalgae as feedstocks for bioenergy applications.



**Figure 3-1:** Overview of cellular morphology during biochemical compositional rearrangement; changes in protein and carbohydrate content as the lipid content of the cells increases. Cells of *Scenedesmus acutus* (A-B), *Chlorella vulgaris* (C-D) and *Desmodesmus armatus* (E-F) in nutrient replete and deplete conditions respectively, alongside biochemical compositional changes in *Scenedesmus acutus* (G) and *Chlorella vulgaris* (H) with respect to protein, lipid and carbohydrate content when cells are kept for up to 15 days in nutrient deplete media. Images courtesy of N. Sweeney (NREL)

Photosynthetic conversion of inorganic CO<sub>2</sub> to metabolic energy stored in the form of carbohydrates and lipids is fundamental to algal productivity, as the resulting biochemical energy storage components provide the feedstocks for the bioenergy applications discussed below in more detail.<sup>1,21,31</sup> In brief, microalgae (and macroalgae), like terrestrial plants, grow and multiply through photosynthesis, a process whereby light energy is converted into chemical energy by assimilating atmospheric CO<sub>2</sub> by the following reaction:

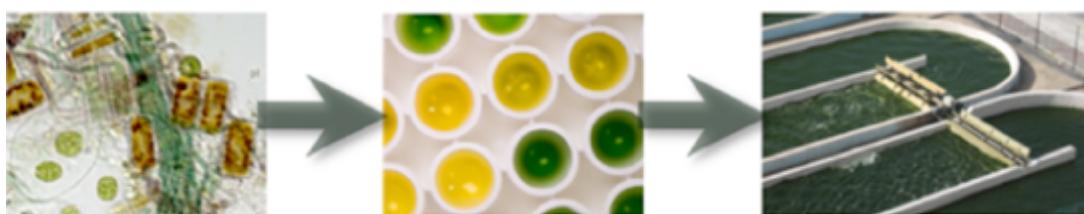


The sugars formed by photosynthesis are converted to all other cellular components (lipids, carbohydrates, and proteins) that make up the algal cell mass. The photosynthetic process in microalgae is similar to that found in terrestrial plants. However, microalgae, due to their simpler unicellular structure, are particularly efficient converters of solar energy. Because microalgae do not need to generate elaborate support and reproductive structures, they can devote more of their energy into trapping and converting light energy and CO<sub>2</sub> into cell mass. Therefore, microalgae production is estimated to require less land per unit oil produced compared to terrestrial feedstocks such as soybean, rapeseed, oil palm and jatropha, even if the growth conditions are not optimized to increase a microalgae's lipid content.<sup>63</sup>

What is important to emphasize here is the critical link between the biomass production system

and the valuation of the produced algal (or terrestrial) biomass for a given bioenergy application. There is an inherent correlation between algal growth productivity and algal cell mass composition, typically exhibiting an inverse relationship between lipid content and productivity.<sup>21,62,64</sup> It is because of these highly dynamic compositional characteristics that a review of the reported literature and data should not be carried out in isolation. It is necessary to track and review results of both production and downstream processing in an integrated fashion. Recent work has overturned previous assumptions that algal cell mass composition from phototrophic cultivation systems is relatively stable and consistent across species and growth conditions. Rather, the biochemical composition of algal cell mass varies greatly depending upon the strain, nutrient status and environmental conditions (temperature, light/dark cycle, etc).

**(Figure 3-1.G-H).**<sup>64-67</sup> Thus, timing of harvest can greatly affect overall achievable cell energetic content and ultimately fuel yields as well as influence how downstream processing is performed. The general observation that algal cells change their metabolic composition throughout their growth and in response of environmental and physiological stimuli is decades old;<sup>68</sup> however, these principles often have been overlooked when assessing prospects for developing algae-based biofuels or biorefineries.



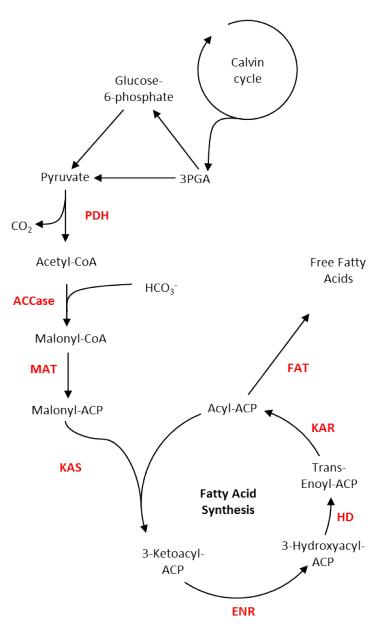
**Figure 3-2:** Flow of algae strains from outdoor native water sample through to outdoor deployment via laboratory strain development to achieve improved biofuels feedstocks

Considering the theoretical potential and challenges that currently stand in the way of improving the economics towards a cost competitive algae-based fuels scenario, there is a need to maximize the yield and composition of any given algae strain. The potential of native algal species is enormous by virtue of their large metabolic and physiological plasticity and native diversity. Nonetheless, there are opportunities to further increase the productivity or make the composition of the resulting cells more amenable to the downstream conversion process, e.g. by increasing algal cell lipid content through overcoming or altering the inverse relationship between growth rate and lipid content. Recent advances in metabolic engineering have opened up new opportunities to improve upon native algal properties such as productivity and oil content.<sup>69,70</sup> Beyond targeted engineering, classical mutagenesis and selection, alternative approaches of adaptation and external stimuli can be used to increase lipid productivities. Adaptation is an excellent way to increase large-scale productivities, and avoids introduced traits becoming lost.

Metabolic engineering targets in the literature have ranged from lipid and carotenoid biosynthesis to trophic conversion (heterotrophic cultivation), CO<sub>2</sub> assimilation (RuBisCO) and photochemistry, with thorough reviews of these topics already published.<sup>69,71</sup> In light of the current emphasis on bioenergy feedstocks, we will only cover some highlights on photochemistry and lipid metabolism. Metabolic engineering has significant challenges with being implemented for outdoor cultivation, but also initially to design and construct the right properties in an already productive strain. A lot of research has historically been performed in the model organism *Chlamydomonas reinhardtii*, with a limited amount of work performed on the transfer to more production-relevant species such as *Nannochloropsis*, *Scenedesmus* and *Phaeodactylum*. Because of the wide divergence of these species phylogenetically, e.g. there is only a 16% functional overlap in the genetic diversity,<sup>71</sup> the likelihood that knowledge is directly translatable between different species is small and caution should be exercised when interpreting findings in *C. reinhardtii*, since implementation of findings with this model strain to a real world scenario may not be feasible.

The ultimate goal of many metabolic engineering efforts is to manipulate the cell's biochemistry independent of the cell's growth mechanisms. It is still unclear whether this will be possible, but several advances have been made in engineering transcription factors.<sup>72,73</sup> The development of new genetic tools will be a major driver for metabolic engineering in eukaryotic microalgae, with better methods for nuclear genome editing allowing for more precise gene deletion and gene integration, thereby facilitating the reconfiguration of metabolic networks as well as obtaining more predictable expression levels of transgenes.<sup>74</sup>

One of the more important targets for engineering increased algal productivity is the photosynthetic light-harvesting complex (LHC). The LHCs have evolved to maximize light absorption in low-light environments. Excess energy that cannot be dissipated as heat or fluorescence usually results in direct photodamage and the production of reactive oxygen species (photoinhibition). In the first genetic engineering attempt to increase effective light utilization all twenty LHC protein isoforms in *C. reinhardtii* were silenced, resulting in a 290% higher light transmittance in the culture. Furthermore, the cells dissipated less energy through fluorescence quenching and increased their photosynthetic quantum yield. Under high-light conditions, transformed cells were less susceptible to photoinhibition and grew at a 65% faster rate; however, they did not reach a higher final cell density.<sup>75</sup> Similarly, it was shown that disrupting non-photochemical quenching (NPQ) in *Synechocystis* sp. can increase productivity by 28% despite this process' proven role in maintaining cell fitness in high, constant light conditions.<sup>76</sup>



**Figure 3-3:** Simplified overview of the microalgal lipid biosynthesis pathway. Metabolites and representative pathways are shown in black and enzymes are shown in red. Free fatty acids are synthesized in the chloroplast, while triacylglycerols (TAGs) may be assembled at the endoplasmic reticulum. ACCase, acetyl-CoA carboxylase; ACP, acyl carrier protein; CoA, coenzyme A; ENR, enoyl-ACP reductase; FAT, fatty acyl-ACP thioesterase; HD, 3-hydroxyacyl-ACP dehydratase; KAR, 3-ketoacyl-ACP reductase; KAS, 3-ketoacyl-ACP synthase; MAT, malonyl-CoA:ACP transacylase; PDH, pyruvate dehydrogenase complex. Adapted from<sup>70</sup>

Other relevant targets for algae metabolic engineering include different steps along the lipid biosynthesis pathway to increase the flux of photosynthate to lipids (**Figure 3-3**). The first metabolic engineering reported to increase fatty acid (FA) production in algae was overexpression of the acetyl-CoA carboxylase gene (ACCase) in the diatom *Cyclotella cryptica*.<sup>77</sup> ACCase codes for the enzyme that carboxylates acetyl-CoA to malonyl-CoA, the first committed step for FA synthesis. Expression vectors and transformation protocols were developed for *C. cryptica* and the diatom *Navicula saprophila*. A two-to-three-fold increase in the level of ACCase activity was reported for the transformed diatoms, but no increase in FA accumulation was detected. However, no experimental data was presented for the increase of ACCase activity.<sup>77</sup> Pyruvate can be oxidatively decarboxylated to acetyl-CoA (substrate for ACCase) catalyzed by the mitochondrial pyruvate dehydrogenase (PDH), which is deactivated through phosphorylation by pyruvate dehydrogenase kinase (PDK). In contrast, plastid PDH is not regulated by a PDK homolog. Using an antisense cDNA construct, PDK expression was knocked down in the diatom *Phaeodactylum tricornutum*, resulting in up to 82% total neutral lipid increase without changes in the lipid profile.<sup>78</sup> Malic enzyme (ME) catalyzes the decarboxylation of malate into pyruvate, producing at the same time NADH and CO<sub>2</sub>. In addition to pyruvate, NADH is an essential source of reducing power for lipogenesis. Overexpression of the endogenous ME in *P. tricornutum* resulted in a 2.5-fold increase of total lipid accumulation under nutrient-replete

*Cyclotella cryptica*.<sup>77</sup> ACCase codes for the enzyme that carboxylates acetyl-CoA to malonyl-CoA, the first committed step for FA synthesis. Expression vectors and transformation protocols were developed for *C. cryptica* and the diatom *Navicula saprophila*. A two-to-three-fold increase in the level of ACCase activity was reported for the transformed diatoms, but no increase in FA accumulation was detected. However, no experimental data was presented for the increase of ACCase activity.<sup>77</sup> Pyruvate can be oxidatively decarboxylated to acetyl-CoA (substrate for ACCase) catalyzed by the mitochondrial pyruvate dehydrogenase (PDH), which is deactivated through phosphorylation by pyruvate dehydrogenase kinase (PDK). In contrast, plastid PDH is not regulated by a PDK homolog. Using an antisense cDNA construct, PDK expression was knocked down in the diatom *Phaeodactylum tricornutum*, resulting in up to 82% total neutral lipid increase without changes in the lipid profile.<sup>78</sup> Malic enzyme (ME) catalyzes the decarboxylation of malate into pyruvate, producing at the same time NADH and CO<sub>2</sub>. In addition to pyruvate, NADH is an essential source of reducing power for lipogenesis. Overexpression of the endogenous ME in *P. tricornutum* resulted in a 2.5-fold increase of total lipid accumulation under nutrient-replete

conditions when compared to the control, without impacting growth.<sup>79</sup> Another way of enhancing lipid accumulation is preventing lipid catabolism. After analyzing transcriptomic data from the diatom *Thalassiosira pseudonana* under silicon-deplete conditions, a multifunctional lipase gene was selected as a target for knockdown experiments, which resulted in up to 3.3-fold higher total lipid content than wild-type during the exponential growth phase, again without impacting growth rate.<sup>80</sup>

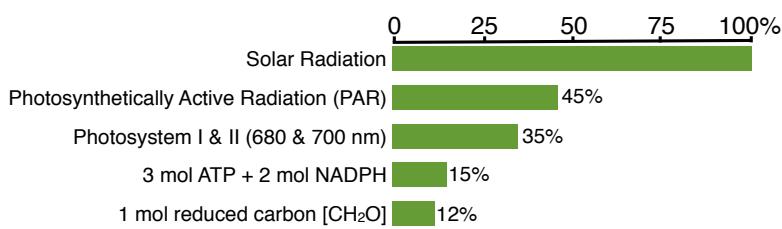
Even though the options for improving the lipid metabolic profile of algae are promising, there are significant challenges associated with the deployment of improved strains in outdoor or even large-scale cultivation. For example, in the U.S., use of genetically engineered algae for production of fuels or chemicals may fall under the Toxic Substances Control Act (TSCA) regulations administered by the EPA that governs the use of genetically modified (GM) microorganisms. Briefly, if a modified algal strain contains coding nucleic acids from more than one genus, it is considered a “new microorganism” under these regulations. Although many research and development (R&D) uses of new microorganisms are exempt from EPA oversight, R&D in open ponds would require EPA’s advance review and approval of an application called a TSCA Environmental Release Application (TERA); there has been at least one field trial of modified algae that has been conducted under an approved TERA.<sup>81</sup> Prior to deployment, a thorough investigation should be carried out of work that is planned with GM algal strains, e.g., ranging from small benchtop scale in a laboratory to demonstration scale outdoors. Safety and regulatory concerns arise when working with GM strains including the likelihood and scale of accidental release, the survivability of the GM species in the surrounding environment, its ability to reproduce, spread and compete in the natural environment, and the mechanisms and magnitude of any possible risks to the environment or human health. In brief, the key risks that are associated with GM algae deployment can be categorized as follows: 1) stability of DNA vector and introduced genes; 2) possible deleterious functions encoded by transgene(s) such as algal toxins; 3) potential for horizontal gene transfer, crossing to wild algal species; 4) potential for GM strain to be transported outside facility, survive, and compete in the environment; 5) potential persistence in the environment, e.g., in soil or water in vicinity of site of use; and 6) potential for disruption of natural ecosystems or native algae populations, creation or enhancement of harmful algal blooms or ecologically disruptive algal blooms.<sup>82,83</sup> Even though GM algae may ultimately prove to be critical to achieve the cost and sustainability goals set by different groups, their practical implementation at large-scale may take longer to prove out.

### **3.2. THEORETICAL CONSTRAINTS TO PRODUCTION AND YIELDS OF ALGAL BIOMASS**

Even though most algal biomass production development is occurring in pre-commercial and research spheres, it is important to provide some theoretical background to better inform the productivity and potential yield claims that are made throughout the industry and in some highly cited papers. All too often, productivity estimates and associated economic assessments are extrapolated from small-scale laboratory experiments that do not effectively model or represent real world scenarios or realistic outdoor productivities.

Microalgae can theoretically convert roughly 8-12% of total incident radiation into new cell biomass (**Figure 3-4**), with actual observed efficiencies more in the range of 2-3% after taking metabolic and energetic losses into account.<sup>21,84</sup> The energetic losses are categorized as either solar radiation losses or metabolic losses that occur during conversion of light energy into metabolic energy and energy storage molecules, which are highly dependent on the species and the light intensity the culture is experiencing. In mass culture of algal cells possessing large chlorophyll (Chl) antennae, cells at the surface of the reactor absorb incident sunlight (intensity of 2,500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) at rates that far exceed the capacity of the photosynthetic apparatus to utilize them (light saturation of photosynthesis occurs at less than 500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ).<sup>21</sup> The excess absorbed sunlight energy is dissipated via a process of nonphotochemical quenching to

prevent photodamage and photoinhibition phenomena at the thylakoid membrane level.<sup>85</sup> The two main processes for dissipating excess light energy (and thus reducing photon wastage) are non-photochemical quenching and alternative electron transport (AET), which “short circuits” the photosynthetic electron transport chain by donating excess electrons to oxygen to create water. These processes are necessary for maintaining algal fitness in a natural environment but are not as relevant for maintaining culture productivity in dense, light-limited cultures like those found in algal ponds or photo-bioreactors.<sup>86</sup> Algal biomass productivity losses can also occur as a result of night-time respiration (dark respiration), which is essentially a carbon wastage for the alga, though some dark respiration may be necessary to sustain growth during the dark phase of the daily solar cycle.<sup>87</sup> These sources of wastage of metabolic energy can together represent up to 23% unproductive energy loss and lead to reduced overall photosynthetic efficiency. By comparison, terrestrial crops generally have much lower photosynthetic conversion efficiencies. For example, sugar cane, which is one of the most productive terrestrial crops, has a theoretical maximum photosynthetic efficiency of ~4%, with the main difference compared to algae being attributed to higher respiration losses.<sup>88</sup> This feature – higher photosynthetic efficiency of algae – translates into algae achieving higher areal growth productivity than terrestrial plants and motivates the attraction of considering algae as potentially superior photosynthetic feedstocks for biofuel and bioenergy production.<sup>89</sup>



**Figure 3-4:** Stepwise loss of energy during photosynthetic assimilation of inorganic carbon to reduced carbohydrate.<sup>21</sup>

Improvements in photosynthetic efficiency have long been attempted, and reducing the size of the LHC of the algal photosynthetic apparatus is one approach that has been tried a number of times in the model species *Chlamydomonas* sp.<sup>84,90-93</sup> In the published studies, a significant increase in the rate of photosynthesis has been observed, and in some instances this coincided with a reduction in the rate of dark respiration.<sup>90</sup> This appears to be a promising approach to increasing algal productivity; however, such an approach also imposes a distinct disadvantage in the cell’s competitiveness (to compete for available light) in the wild or outdoor cultivation settings. This is supported by the observation that spontaneous mutants with smaller photosynthetic apparatus have not been isolated in the wild. It is argued that reduced antenna mutants have not been isolated and applied to large-scale cultivation, mainly due to the impact of the resulting photo-oxidative damage to the algae when exposed to high light environments. There is however a potential that a mutagenesis-derived, rather than a targeted engineering approach may yield the improvements in productivity that are sought. It remains to be seen whether these mutants are able to thrive in large scale production systems and ultimately able to move the needle in terms of increasing overall areal productivity. This is an area that should be investigated in future algae research and ideally the knowledge in model organisms such as *Chlamydomonas* should be transferred to large-scale-relevant species of algae. An alternative approach to improve photosynthetic efficiency is to increase the temporary carbon sink of the algal biomass, such as into storage carbohydrates rather than TAGs, to allow for a higher assimilation rate of metabolic energy from photosynthesis.

As described in the literature, the actual versus theoretical efficiency of productivity (based solely on photosynthetic efficiency) will be highly dependent on the physical constraints of a particular production facility, e.g., total available solar irradiance, which varies by latitude and the local climate.<sup>21,89,94,95</sup> A detailed description of the theoretical considerations and losses associated with

photosynthetic algae production systems has been described and is discussed here in the context of arriving at a theoretical value for maximum potential algal biomass and oil yields.<sup>21,94</sup> (Note that the ultimate biomass composition and reactor or pond geometry might influence these values). For biomass productivity, high to low latitude facilities (with insolation being the biggest driver of average daily productivity) best-case scenario estimates range from 120 to 153 dry tonnes ha<sup>-1</sup> yr<sup>-1</sup> (equivalent to 33-42 g m<sup>-2</sup> d<sup>-1</sup>), while the demonstrated yields in closed photobioreactor systems and open ponds are roughly 2-fold lower, respectively, with averages of 75 and 47 dry tonnes ha<sup>-1</sup> yr<sup>-1</sup> (equivalent to 20.7 and 13 g m<sup>-2</sup> d<sup>-1</sup>, respectively).<sup>96,97</sup> This contrasts to maximum theoretical yields of 715 dry tonnes ha<sup>-1</sup> yr<sup>-1</sup> (or 196 g m<sup>-2</sup> d<sup>-1</sup>).<sup>94</sup> The differences between the theoretical and best case scenario lies in the biomass accumulation efficiency (reflecting respiration and other metabolic losses), which is set at 100% in the theoretical case, and at 50% in the best case scenario.<sup>94</sup> In our discussion, algal oil yield has been left out deliberately to avoid the confusion around the inverse relationship between oil content and biomass productivity. In the original work on the theoretical and best case oil production estimate, the oil content was set at 50%,<sup>94</sup> which is overly optimistic and at this point in time, not feasible in conjunction with a 33 g m<sup>-2</sup> d<sup>-1</sup> productivity. As a comparison, the yields of terrestrial crops such as corn and soybean are in the range of 2 to 10-fold lower. For example, in 2015 in the U.S., annual production of corn grain averaged 9.6 tonnes/ha and soy beans averaged 3.2 tonnes/ha.<sup>98</sup> Even at a modest productivity of 8 g m<sup>-2</sup> d<sup>-1</sup>, the overall yields of algal biomass (29 tonnes/ha/year) still exceed those of current highly productive agricultural crops.

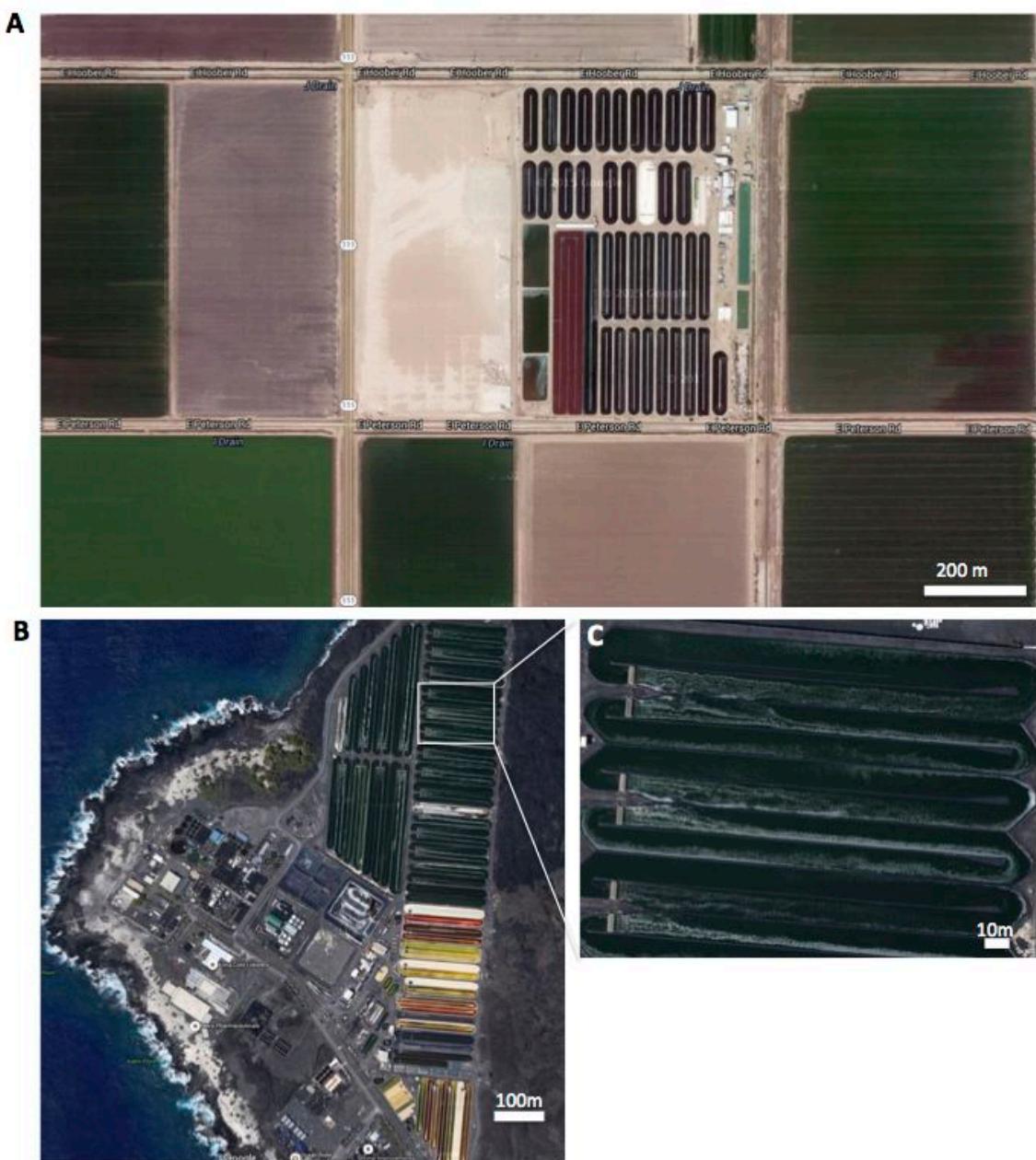
As already mentioned, there is a highly dynamic relationship between algal oil content and algal biomass growth productivity, which depends on the integration of species and the physiological conditions it is exposed to. There are opportunities to improve the productivity of algae through minimizing losses occurring during photosynthesis while avoiding impairing algal cells' robustness for outdoor deployment. This overall issue represents both one of the greatest technical opportunities and challenges to advancing microalgae-for-bioenergy deployment, and should be a major emphasis area for future research.

### 3.3. PHOTOTROPHIC CULTIVATION OF MICROALGAE

Algal cultivation must be economically advantageous and energy efficient to reach the scale of biomass commensurate with other biofuels. Phototrophic cultivation of microalgae or cyanobacteria in suspension, at its most basic, requires making nutrients and light available to the algae, which utilize the nutrients and light to power cellular metabolism, producing metabolic products and algal cell biomass. Numerous systems for suspension phase cultivation have been developed, for the most part falling into three categories: 1) closed photobioreactor systems (**Figure 3-6**) in which the culture is held within a closed physical container; and 2) open ponds (**Figure 3-5**), in which the culture is contained in a pond but exposed to the environment and 3) attached growth systems (biofilm production).<sup>99-105</sup> The choice between the different options for cultivation is often based on the ultimate application for the algal biomass and derived products, and it is unlikely that, in the various schemes for producing fuels or high value bio-products, that one cultivation system will fit all approaches. In this context, we discuss briefly the existing systems and, where information is available, link this with currently installed production capacities. Generally, it is assumed that about 13,600 tonnes (15,000 tons) of algae biomass, dry basis, is commercially produced worldwide, almost exclusively in open ponds, mostly of the raceway paddle wheel mixed design.<sup>106</sup> The main microalgal species currently produced (>90% of total) are the cyanobacterium *Arthrospira* sp. (*Spirulina*) (*Arthrospira platensis*, about 9,100 dry tonnes (10,000 tons)) and *Chlorella* (*Chlorella vulgaris*, about 3,600 dry tonnes (4,000 tons)), cultivated in several dozen plants, ranging in size from several tens to a few thousand tons (dry basis) annual production capacity.<sup>107</sup> China accounts for approximately two-thirds of total world production, which is sold mainly for human food products, with bulk (plant gate) selling prices for *Arthrospira* sp. (*Spirulina*) and *Chlorella* dried biomass of typically \$10–25/kg and \$20–40/kg, respectively. These prices are examples only; prices will vary depending on the market supply and

demand. Ultimately, it is thought that the target price point for algal biomass has to drop below \$1/kg for fuel applications to be economically feasible and thus any profit margins would have to be adjusted accordingly.<sup>25</sup>

Open pond systems have often been used for (relatively) low cost production of algal biomass.<sup>108-110</sup> Examples include *Arthrospira* sp. (*Spirulina*) and *Dunaliella* production.<sup>111,112</sup> Open pond systems have been scaled to over 40 hectares in a single system.<sup>111,112</sup> Cooling of the culture in sunny environments is accomplished by evaporation of the culture media, which increases water consumption but removes the need for physical cooling of the culture or by pumping deep sea water from adjacent oceans. Exposure to the atmosphere brings a host of environmental challenges, including introduction of dust, dirt, foreign material, weeds, and even animals into the culture. Careful culture maintenance is required to maintain successful growth in the presence of these challenges.<sup>113,114</sup> As with any form of farming, pests, weeds, and abiotic stresses can negatively impact culture health. Rapid detection, diagnosis, and treatment are critical to return a culture back to robust production and prevent pond crashes.



**Figure 3-5:** Outdoor open pond microalgae production systems at scale. (A) Earthrise production facility, Calipatria, CA, and (B-C) Cyanotech facilities in Kailua Kona, HI. Images from Google Maps.

Closed algal growth systems, known as bioreactors, can be classified as photobioreactors (PBR). PBRs are closed (or almost closed) vessels for phototrophic algal cultivation where light is supplied either directly by the sun or via artificial sources such as LEDs. While a typical open pond system is open to the environment on at least its top surface, in closed systems the exchange of liquid, gas, biologics, dust, and solids input and output from the system are carefully controlled. Typically, closed systems also direct the circulation of the algal culture to maximize the evenness of the culture's exposure to natural or artificial light. Since all liquid and gas streams have to be brought in and out of the bioreactor via pumps or bubbling pressure, the energy requirements for this type of culture can be higher. However, a PBR typically produces a denser culture, which requires less energy to extract algal oils from the remaining solids, and environmental contamination is also minimized. Maintaining and optimizing water chemistry is easier in a closed system that is not exposed to rain and evaporation. The geometric configuration of closed PBRs is

often designed for efficient utilization of natural light. Through a variety of methods, light is more evenly distributed through the growth media in PBRs than in open pond systems. Maximum daily volumetric harvest rates on the order of 40% and dry biomass concentrations up to 5 g/L (dry basis) are feasible (for tubular PBRs).<sup>103</sup> High algal biomass concentrations lead to increased harvest yields and faster and more economical down-stream processing.

Closed systems vary widely in size, material, shape, and technical principles of operation (examples are shown in **Figure 3-6**, including the small-scale open research ponds), but they all attempt to prevent introducing contaminating living organisms into otherwise pure algal cultures, while at the same time preventing escape of algal organisms or media that could produce environmental damage.<sup>115-117</sup> Commonly, closed PBRs require induced turbulence of the algal suspension to avoid large gradients in the cultivation medium and to compensate for cell on cell light shading (the same can be said for open ponds). A detailed overview of several PBR types tested in concert with an open pond technique has previously been published.<sup>118</sup> More recently, an integrated hybrid system of large scale PBRs for high-density inoculation of open ponds was described.<sup>119-121</sup> This hybrid PBR-open pond approach aims to integrate the best of both systems and is thought to mitigate contamination risk by increasing the concentration of cells inoculating the large-scale ponds.



**Figure 3-6:** Cultivation systems for algae growth. (A-B) flat panel photobioreactors at AzCATI),<sup>39</sup> (C) Small, 1000 L research open ponds at AzCATI, and (D-E) horizontal and vertical tubular photobioreactors at Algae PARC (between 12m<sup>2</sup> and 24 m<sup>2</sup> each) <sup>118</sup>

Attached growth (or biofilm) systems are ideally suited in wastewater treatment scenarios. These systems could be highly efficient as the biomass does not require dewatering, can be simple and cost-effective. Often these systems are combined with a closed cultivation reactor and thus the water utilization efficiency is increased, due to the reductions in the evaporative losses. A significant amount of work is currently underway to optimize the productivity, CO<sub>2</sub> uptake and

harvesting efficiency in attached growth reactors.<sup>104,105,116,122-124</sup>

### 3.4. NUTRIENT AND CO<sub>2</sub> UTILIZATION

One of the challenges with sustainable cultivation of algae on a commercial commodity-scale is to supply the nutrients and additionally mitigate the enormous amounts of water needed for feedstock cultivation and processing.<sup>9</sup> Effective wastewater recycling is essential to minimize consumption of freshwater and chemical nutrients.<sup>9,10</sup> Water usage requirements for algal biomass and biofuel production will vary depending on growth conditions and ultimately the lipid or biofuel yield from the algal biomass. For example, for a production system growing algae at approximately 1 g/L (dry basis), with about 20% oil content of the biomass for biofuel applications, a total of ~5000L would need to be processed to generate 1 kg of biofuel (green or bio-diesel). The algal biomass typically contains 45-50% carbon (C), 7.6% nitrogen (N) and 1.4% phosphorus (P) (L. Laurens, NREL, unpublished data). It has to be noted that the elemental composition varies dramatically based on growth conditions and species of algae used, but on average, the above approximation can be made and is consistent with the Redfield ratio based on the atomic ratio (106:16:1 C:N:P) and on a weight basis (40:7:1 C:N:P). Thus, the nutrient requirements to support the same 1 kg of biofuel would be in the range of 0.38 kg N and 0.07 kg P equivalent (corresponding to 0.214 kg phosphate delivered). This is consistent with estimates in the literature, where reports of 3000 liters of water per kg of microalgae-based biodiesel have been estimated,<sup>12</sup> and associated nutrient requirements are reported as 0.33 kg nitrogen if freshwater without any recycling is used for open pond cultivation.<sup>13</sup> (Note: This same report incorrectly states that 0.71 kg Phosphate (which equates to 0.326 kg P) would be needed to produce the same 1 kg biodiesel.<sup>13</sup> According to our calculations and other published reports, this is excessive and inconsistent with the average elemental composition of algae detailed above (~7.6% N and ~1.4% P) and published in the literature.<sup>14,21</sup>) There is a distinct relationship between nutrient requirements and productivity and proximate algal biomass composition, which partly explains the ranges observed in the literature, e.g., our estimate of 20% oil content in harvested algae is relatively conservative, while Pate et al. assumed algae with a 50% oil content, which reduced the nutrient requirements to produce 1 kg bio-diesel to 0.18 kg N and 0.025 kg P.<sup>14</sup> While closed photo-bioreactors can be used to reduce water losses due to evaporation,<sup>9</sup> this imposes additional costs in installed capital equipment (CAPEX). Higher efficiency water use and wastewater recycle may further reduce water consumption, and the direct use of wastewater may provide an inexpensive and effective source of nutrients that also reduces freshwater use.<sup>15</sup> The use of wastewater, however, may introduce other complications in an open system such as complexity of diverse microbial populations (see **Section 3.5**). It is likely that for large scale deployment, a combination of technologies will be required, for example a 2-5% of the volume of open ponds will likely need to be installed as PBR for inoculation. Such 'hybrid' approaches may mitigate some of the contamination risks.

In general, any source of CO<sub>2</sub> can be used for cultivating algae, however some options are more advantageous than others. Using pure CO<sub>2</sub> is very expensive, using air does not require transport, however air does not contain a large amount of CO<sub>2</sub> (~0.04 w%) and more must be supplied to facilitate optimal algae growth.<sup>125,126</sup> Since procuring CO<sub>2</sub> and pumping it to the algae is one of the more costly and energy tasking aspects of algae cultivation, using gas derived from industrial productions can be used to mitigate these challenges.<sup>126</sup> Flue gas usually contains a large amount of CO<sub>2</sub>, though the exact concentration depends on the process and the origin. For example, flue gas from natural gas-fired power plants can be much higher in CO<sub>2</sub> concentration compared with flue gas from coal-fired power plants).<sup>127</sup> In general, flue gas from a coal power plant will have 10-20% CO<sub>2</sub>.<sup>128</sup> A natural gas power plant and a fermentation-based ethanol plant will have around 5% and 99% CO<sub>2</sub> respectively.<sup>129</sup> The ability to utilize such industrial gases, however, is limited by certain constraints, most notably, the difficulties in transporting the CO<sub>2</sub> to the algae ponds. The algae production facility would have to be located near a suitable gas source, such as a power plant, potentially making it more difficult to obtain other critical resources, such as water,

nutrients and land.<sup>130</sup> In addition, flue gases can contain high amounts of NO<sub>x</sub> and SO<sub>x</sub>, which can change the pH of the algae cultivation medium solution to inhibit algae growth. In some countries, legal barriers prevent flue gas from being used as a feedstock in the production of pharmaceuticals, food or feed, as it is classified as waste.<sup>131</sup> In addition, the potential for accumulation of heavy metals in the biomass, which in addition to the waste designation could have negative impacts on the integration potential of CO<sub>2</sub> emissions mitigation strategies.<sup>132</sup> Alternative sources can be made available for algae cultivation, for example the exhaust released after combustion of biogas from anaerobic digestion plants, can provide CO<sub>2</sub> in a more decentralized manner.

In general, it takes an approximate mass ratio 2.0 CO<sub>2</sub> to produce a quantity of algae biomass, e.g., around 2.0 g of CO<sub>2</sub> to produce 1 g of ash-free dry algae.<sup>133</sup> The actual mass ratio is dependent on algal species and its composition and the uptake (assimilation) kinetics follow photosynthetic activity and thus exhibit a day-night cycle. The amount of flue gas needed per acre of algae in an open pond greatly depends on the flow rate of the CO<sub>2</sub> source, the concentration of CO<sub>2</sub> in the source, as different concentrations of CO<sub>2</sub> will have different levels of absorbance by the algae, and the dimensional parameters of an open pond or closed system.<sup>125</sup> Since CO<sub>2</sub> uptake is directly related to efficiency of photosynthesis and light availability, the intricacies of the underlying algae physiology provide flexibility in the interpretation of this range and the potential CO<sub>2</sub> assimilation potential of algae cultivation.

To gain some perspective, we include here a number of calculations that can be used to estimate the amount of emissions that could be captured using algae. In one scenario, a coal plant, which processes 2 million tons (MT) of coal per year, produces 800 MWe, and creates an estimated 5,000,000 tons of CO<sub>2</sub> per year. Once transported to the pond, the system has 67% transfer efficiency (most losses are attributed to outgassing, which is a phenomenon dependent on pH, culture media, cell density, culture health, and turbulence.). The algae processed have on average 46.5% carbon in ash-free dry weight (AFDW). Multiplying this number by the weight ratio of CO<sub>2</sub> to carbon and dividing by the transfer efficiency (67%) yields the amount of CO<sub>2</sub> consumed to produce 1 metric ton (MT) of algal biomass.

$$1 \text{ MT } afdw \text{ algae} \times \frac{0.465 \frac{MT \text{ C}}{MT \text{ afdw algae}} \times \frac{44}{12}}{0.67} = 2.5 \text{ MT } CO_2$$

Using a productivity of 54 MT ha<sup>-1</sup> y<sup>-1</sup> harvest, or 15g m<sup>-2</sup> d<sup>-1</sup> AFDW biomass, the amount of CO<sub>2</sub> required per ha was calculated, and subsequently the amount of land needed to sequester all of the CO<sub>2</sub> produced by the power plant in one year was estimated to be roughly 36,000 ha.<sup>107</sup>

$$\frac{5,000,000 \frac{MT \text{ CO2}}{y}}{54 \frac{MT \text{ algae}}{ha \cdot y} \times \frac{2.5 \text{ MT CO2}}{MT \text{ algae}}} = 36,000 \text{ ha}$$

This calculation assumes that the algae are continuously fixing CO<sub>2</sub>, however energy generation and associated CO<sub>2</sub> production by the coal plant vary depending on the time of year and time of day, and the algae are unable to perform photosynthesis without sufficient light. For the estimates we present here, there is a need to account for a lack of CO<sub>2</sub> consumption during the night by the algae, but also reduced production by the power plant, which causes a highly complex picture of CO<sub>2</sub> assimilation potential. To account for the availability of CO<sub>2</sub> at certain times of the year, and peak CO<sub>2</sub> production during the day, the amount of CO<sub>2</sub> the algae would be able to process was estimated at 30% of emissions.<sup>129</sup> This lowers the yield to around 1,500,000 MT y<sup>-1</sup>, which requires approximately 10,800 ha of land, still much larger than an envisioned algae production site of between 400 – 2000 ha.

Though theoretically algae cultivated in 10,800 ha of ponds could process around 1,500,000 MT  $y^{-1}$  of CO<sub>2</sub> emissions, this figure does not allow for slowed production caused by routine maintenance or contaminants found in the algae. Using a 10% reduction in algae productivity to 49.5 MT ha<sup>-1</sup> y<sup>-1</sup> lowers the amount of CO<sub>2</sub> captured in the algal biomass to 500,000 MT, or about 18% of annual power plant emissions. This figure is based on an annualized average productivity, carbon content of algae, and the type of cultivation equipment.

The reliability of the numbers used in these calculations are highly dependent on the accuracy of the underlying assumptions about areal productivity and algal carbon content, which can vary dramatically with location and season of cultivation. Similarly, the 30% efficiency of CO<sub>2</sub> uptake potential is likely an overestimation of the CO<sub>2</sub> actually assimilated by algae. According to some accounts, this figure is more like 16%, after taking into account the losses at the transfer station and at the ponds (photosynthesis 50% of the day and summer versus winter productivity at a 2:1 ratio).<sup>107</sup> Integrating CO<sub>2</sub> transfer efficiency with the variable production of CO<sub>2</sub> at the stack of the power plants, peak production (day time) accounts for only 67% of the overall emissions. The in-depth, granular carbon assimilation potential for algae follows a daily supply/demand curve and is not easily calculated, but undeniably algae provide an opportunity for capture and sequestration of otherwise wasted CO<sub>2</sub>. Integration of algae cultivation with ethanol fermentation plants could provide an opportunity to supply higher concentration and higher purity CO<sub>2</sub>. Research is needed in the feasibility of CO<sub>2</sub> delivery and assimilation potential at the large scale.

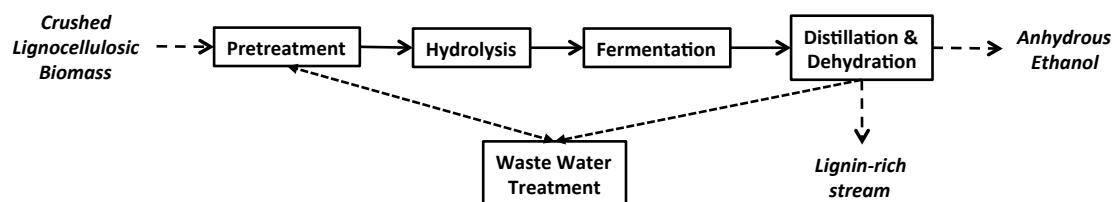
### 3.5. INTEGRATION WITH WASTEWATER TREATMENT

By integrating algal production and wastewater treatment (WWT), both processes might be accomplished with improved economic and environmental sustainability. The integration with wastewater is thought to be one of the only economically feasible pathway for the large-scale production of fuels from algae. The two main areas of intersection for algal cultivation for biofuels and wastewater are in: 1) WWT with discharge or offsite reuse of the treated effluent (the wastewater is only used once for algal production); and 2) use of treated or untreated wastewater as a culture medium that is recycled repeatedly for production of algal biofuel feedstock. In the WWT application, the main products would be reclaimed water, algae-based fertilizer, and algal biofuels (both gaseous – from anaerobic digestion – and liquid fuels). However, biofuels and fertilizers would not be major economic drivers at current prices.<sup>16–19</sup> Instead WWT fees and reclaimed water sales would provide most of the revenue. The dedicated biofuels application has thus far only been carried-out experimentally or at a small pre-pilot plant scale. Algae have been grown on a wide variety of wastewaters, most prominently municipal, but also agricultural (animal barn flush water and field drainage) and industrial (food processing, aquaculture, etc.) wastewaters. For municipal wastewaters, the limiting nutrients for algal growth are typically (in sequence of limitation) inorganic carbon, nitrogen, possibly some trace metals, and phosphorus.<sup>15,20</sup> Nevertheless, the application of municipal wastewater for algae production holds promise to be economically feasible even in the short term.<sup>15,134</sup>

Some wastewaters contain inhibitors for algal growth, for example, high ammonia concentrations in animal waste and toxic compounds in industrial wastewaters. Such wastes are often also highly turbid, reducing light availability to the algae. When algal growth media is recycled, inhibitory organic compounds, including allelopathic agents excreted by algae themselves, can accumulate in the media and potentially inhibit growth of competing algae.<sup>51</sup> Typically, the biomass produced from a WWT facility is restricted in the applications and types of final products that it can be used for. The most common bioenergy product from WWT algae cultivation is methane (from biogas rich in methane) generated through anaerobic digestion (AD), a process which is understood to be mainly agnostic to feedstock, with yields mainly driven by the C:N content of the feedstock. However, recent work has shown that WWT cultivated algae is amenable for hydrothermal liquefaction (HTL) conversion of the algal biomass (see **Chapter 5**),<sup>135</sup> where the process of conversion is more feedstock-quality agnostic and thus the contribution of carbon from the

microbial population grown on wastewater is converted to liquid fuel intermediates and thus provides an avenue for liquid as well as the gaseous bioenergy from WWT.<sup>136</sup>

Among many reported process possibilities, an alternative process option would be to integrate algae production with WWT from ethanol fermentation plants, such as a lignocellulosic or a sugarcane plants.<sup>137</sup> The lignocellulosic ethanol chain (biochemical route) is the industrial process that converts lignocellulosic materials into ethanol through various steps. A schematic adopted in industrial scale demonstration/pre-commercial plants typically comprises the following unit operations: crushing or size reduction of the biomass, biological, chemical or physical pretreatment, enzymatic hydrolysis and fermentation followed by distillation and dehydration of the final fuel-grade ethanol product (**Figure 3-7**). However, for each of the wastewater installations, the regional legislative landscape needs to be taken into account.



**Figure 3-7:** Illustration of a sequence of typical process operations in a lignocellulosic ethanol production plant. The waste water treatment step is able to support algae production operations.

A significant amount of water is required by this type of process, which has to be recycled and reused to make the system environmentally sustainable. Thus, a waste water treatment unit must be designed and installed at the biorefinery to deal with the high organic and inorganic content of process water. Efficient fresh water use represents a growing environmental concern. In order to ensure the sustainable production of biofuels, an efficient water management system must be adopted that allows recycling as much of the water as possible to the process. Lignocellulosic ethanol generates wastewater streams containing high concentrations of organic compounds, measured as chemical oxygen demand (COD) and biochemical oxygen demand (BOD), as well as high concentrations of ammonia, which all need to be reduced to allow for water recycling. Moreover, high solids loadings, contaminants such as hydrogen sulfide (depending on pretreatment process), and potentially problematic metals and salts including silica, calcium, magnesium, manganese, sodium, potassium also can be present at different levels.<sup>138</sup> A 80-100 kT/year lignocellulosic ethanol plant will generate an approximate wasterwater flow of 8,140 m<sup>3</sup>/day. Investigating the integration of algae into the biorefinery scheme could shift the issue of large volumes of wastewater to be treated from a problem to an opportunity. A typical wastewater sample provided from a lignocellulosic plant has a very high total chemical oxygen demand (TCOD) of approximately 125,000 mg/L.<sup>138</sup> Provided the wastewater is amenable to anaerobic treatment, there is potential to generate large quantities of methane-rich biogas. The high influent organic load will also result in large quantities of biological sludge being produced, which must also be handled, dewatered, and disposed.

Biorefinery wastewaters will contain different types and concentrations of contaminants depending on the type of pretreatment process, the efficiencies of the different sections of the biorefinery, the design choice and constraints, etc. Algae growth could benefit from some of the components contained in the wastewater stream, and this represents a potentially effective means and ideal solution for concentrating/separating and valorizing the wastewater stream. The use of high organic carbon wastewater could provide a good medium for algae production, in a heterotrophic or mixotrophic setting, likely with consortia of different species of algae. Some of the advantages of such a proposed integration are to exploit the highly pure and easily recoverable CO<sub>2</sub> available on site at fermentation-based ethanol plants as well as the plant's utility infrastructure and logistics already in place.

Even if most of the research and demonstration effort has so far concentrated on phototrophic species, from an industrial point of view the heterotrophic (or mixotrophic) option can be well adapted to a lignocellulosic ethanol biorefinery context, since the equipment, the skills, and the area needed would fit with the existing installation. In addition, while a phototrophic cultivation can be developed only where climatic conditions are appropriate for the microalgal strain, heterotrophic cultivation can be carried out co-located with a lignocellulosic ethanol plant and operated year round without experiencing seasonal variations in productivity, thus facilitating a more optimal use of plant capital equipment. Furthermore, from a broader perspective, the use of algae to treat terrestrial feedstock biorefinery wastewater could generate a positive return also for the biofuel itself, since the GHG emission reduction performance will most likely be improved when substituting algal cultivation for conventional WWT.

Finally, biofuel feedstock supply chains are normally traced and certified, so that the origin and the characteristics of the feedstock that generates the wastewater used to cultivate the strains is also well known, which opens opportunities for marketing the algae products as certified bio-products as well. The higher quality and more consistent nature of the cellulosic biorefinery wastewater (relative to municipal wastewater) could support the co-production of some of these higher value products in an approach that extends beyond thermochemical treatment of the algal biomass residue. However, until now, this approach has not been demonstrated in the published literature or commercial domain and thus no information on the feasibility is available. Based on the combination of the characteristics of the wastewater and the chosen microorganism (algae), the growth conditions could be met and different products obtained. Again, depending on the feasibility of separation techniques (i.e. obtainable purity of extracted algae), various markets could be addressed, from those for small-scale high added value products to large-scale lower value biofuels for the energy sector (discussed in **Section 6**).

The efficient combination of two different biomass chains, while certainly adding further complexity to a biorefinery scheme, could help to improve wastewater issues while increasing overall sustainability, which could be beneficial for the biofuel economics (by reducing the overall cost of required nutrients for the plant as well as providing a CO<sub>2</sub> source that would otherwise be a cost factor in the biofuel production process). Possible algae plant configurations based on such a co-location approach could be more cost-effective than stand-alone schemes, bringing the economic feasibility of algae-based bioenergy production closer to economic feasibility. Alternative scenarios where there is a price placed on carbon, would help with providing incentives beyond just meeting the RFS threshold for qualifying as an advanced biofuel (reducing carbon emissions >60%).

### 3.6. ALTERNATIVE ALGAE PRODUCTION SCENARIOS

One alternative form of algal production is to metabolically engineer algae to produce volatile compounds that can be directly harvested from the culture headspace as products for direct sale or for further conversion into more highly valued products or biopolymer feedstocks, e.g. **ethanol** and **ethylene**.<sup>139-141</sup> This relatively recent development in algal production has the potential to contribute significantly to the photosynthetic generation of gaseous and liquid biofuels. The commercial development of Algenol in producing ethanol from closed cultivation of cyanobacteria on a demonstration scale could be commercially viable as well as sustainable in the context of providing significant additional reductions in greenhouse gas (GHG) emissions of the overall production system.<sup>141,142</sup> In Algenol's process, ethanol is collected from closed photobioreactors, where it is photosynthetically produced and secreted.<sup>141,142</sup> Unlike in other biofuel pathways, due to the continuous production of ethanol from the cultures, there is little waste biomass available to provide process heat and electricity to offset those energy requirements. In the US, the EPA recently certified Algenol's *Direct-to-Ethanol* fuel as an advanced biofuel with a 69% reduction in life cycle GHG emission compared to gasoline.<sup>143</sup> Energy consumption and GHG emissions can be further reduced by using higher efficiency heat exchangers in ethanol purification and/or by use of

solar thermal energy to supply some of the process heat.<sup>141</sup> It has to be noted here that most recently, Algenol has transitioned to producing biomass from their cyanobacterial production platform, that is slated to be treated in a HTL process for liquid fuel production.<sup>144</sup>

Algal biomass feedstocks can also be generated from a **heterotrophic fermentation** approach, where organic carbon, often in the form of sugars derived from terrestrial feedstocks, such as corn or sugar cane, is supplied to algae as their carbon source for aerobic growth and intracellular lipid production. The primary targets of the heterotrophic cultivation development are for the high-value food and feed applications and are typically not produced as fuel feedstocks. The advantages of heterotrophic cultivation systems are often related to their higher productivity, as there is a higher level of control over the cultivation process. Heterotrophic cultivation can be performed to achieve high lipid contents and high algae cell concentrations, and offers the possibility of working with genetically modified organisms. For example, heterotrophic fermentor systems with algae can produce extremely high biomass concentrations in the cultivation medium, on the order of 25 g/L and sometimes over 100 g/L.<sup>145,146</sup>

Companies employing heterotrophic growth of algae include Solazyme, Roquette and DSM (including now the company formerly known as Martek), even though all of these companies are focused on producing higher value products from a select number of algal strains, with targeted products including nutraceutical omega-3 and omega-6 fatty acids. Combined phototrophic and heterotrophic systems have been demonstrated in the literature and allow for a metabolic 'boost' of the lipid content of phototrophic cultures by providing a source of organic carbon (mixotrophic growth)<sup>147,148</sup> which, in theory, combines the best of both growth options. However, challenges remain to control contamination accompanying scale up of mixotrophic growth technology. The discussion that follows will focus on phototrophic cultivation, as this is seen as a much more cost effective approach to generating biomass at the scales needed to produce commodity fuels.

## **4. Biochemical Processes for Algal-Biomass-Derived Fuels**

Conversion pathways for fuel production refer to the cultivation and processing of algae and include harvesting and some form of cell pretreatment to prepare the algal biomass for extraction of the intracellular lipids, and ultimate upgrading of lipids oil to finished product(s), in combination with the recovery and purification of other products. While aquatic cultivation is often compared to large-scale agriculture, the conversion processes are more analogous to chemical engineering processes, in particular petrochemical refineries and biobased refineries, such as those at ADM and Cargill, to produce fuels and products. There are a wide range of different processes and fuel products reported in the literature, mostly focused around the extraction and upgrading of algal lipids, and referred to as 'Algal Lipid Extraction and Upgrading' (ALU) pathways, producing renewable fuels such as fatty acid methyl ester (FAME) biodiesel or hydrocarbon-based renewable diesel blendstock.<sup>5,149,150</sup> In the context of this report, the background information for describing a consistent basis of the processes and the products generated is included here. The pathways we focus on are considered 'biochemical processes', allowing for the utilization of the non-lipid portion of the cell mass (e.g., for the development of additional bio-products).<sup>25,151,152</sup> There are numerous permutations of a simple base-case process scenario, through either biochemical or thermochemical processing scenarios (**Figure 4-1**). A large number of reports are published in the literature on a variety of processing approaches and some of these include techno-economic analyses. This section aims to clarify the different approaches and provide a critical assessment of the merits of each.<sup>151,153–159</sup>

### **4.1. OVERVIEW OF CONVERSION PATHWAY STRUCTURE**

The algae conversion pathways typically consist of a series of steps: harvested biomass from the cultures is concentrated (and sometimes dried), subjected to a lipid extraction protocol, after which the options are numerous on converting the residual (oil extracted) biomass to additional bio-products which include converting the spent algal biomass to biogas using anaerobic digestion (AD). One major technological challenge for fuel-scale operations of algae is cost-effective separation or harvesting of produced algae from cultivation media.<sup>160</sup> Cultivation at large scale typically only achieves an algal cell concentration of 0.5-2 g/L (dry mass basis). The cells must be concentrated (dewatered) over about 2 orders of magnitude to form a paste or slurry that can be efficiently processed. The dewatering steps typically consist of settling (auto-flocculation) followed by dissolved air flotation (DAF) assisted by adding a flocculant (e.g., chitosan), and centrifugation.<sup>25</sup> Even though the primary settling step is the simplest of the three dewatering operations, due to the high volumes to be processed, this step also represents one of the most costly within the overall harvesting process.<sup>25</sup> We want to highlight two important caveats here. First, this type of settling is assumed in a number of techno-economical models and would need to be tested and optimized for different species of algae. Second, not all algal species will exhibit suitable self-associating behaviors to enable auto-flocculation (e.g., *Scenedesmus* will auto-flocculate but *Nannochloropsis* will not) and thus the processing options have to be customized for the particular species. After primary settling, the biomass achieves a concentration of approximately 10 g/L (1 wt% solids, dry basis), and this can concentration can be increased up to 60 g/L (6 wt%, dry basis) after flocculant-assisted DAF,<sup>160</sup> and then to approximately 200 g/L (20 wt%, dry basis) after centrifugation. Sometimes primary dewatering is achieved by induced settling instead of DAF and then use centrifugation for the remaining slurry. While literature supports the use and efficacy of these operations for harvesting cultivated algal biomass, it must be emphasized that processing specifics are highly species-dependent and good detailed comparative studies are not yet available.<sup>160,161</sup> Most literature reports are based on the somewhat antiquated techniques for wastewater processing, and may leave room for more cost-effective dewatering methods with the use of alternative technologies currently being investigated for algal processing. Similarly, the impact of algal cell mass composition and cell wall structure as well as of excreted organic carbon in cultivation media on harvesting effectiveness is only recently starting

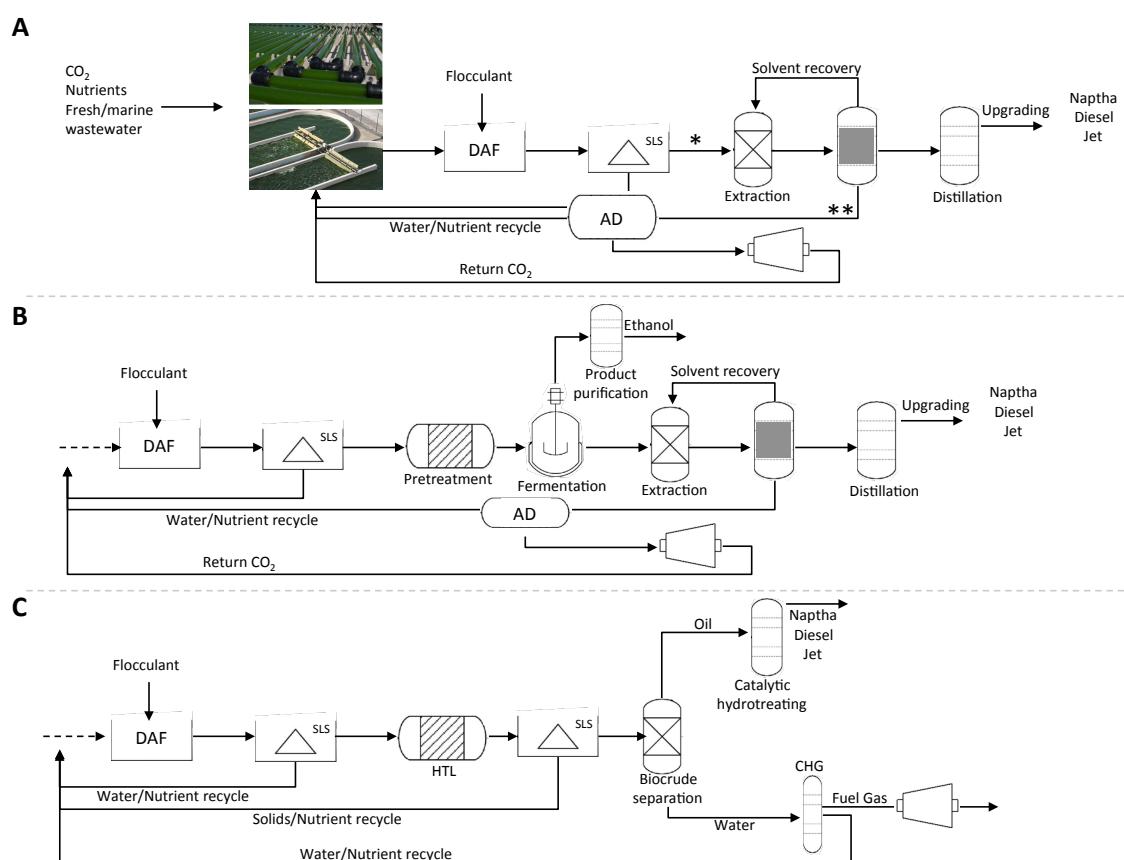
to be investigated. Such remaining unknowns and complexities complicate the implementation of harvesting at the large scales needed for fuel production.<sup>162</sup>

A visual overview of the conversion pathway process-flow for a base-case scenario, in comparison to a biochemical multiple-component biorefinery and a hydrothermal liquefaction (HTL) whole biomass conversion approach is shown in **Figure 4-1.A**. In brief, the process-flow that is typically modeled includes a harvesting and concentration step to get the algal biomass to the desired solids concentration for the extraction step. The following steps are highly variable and depend on the specific process. In the base-case design pathway (shown in **Figure 4-1.A**), a cell rupture step precedes lipid extraction, which uses a hydrophobic solvent (e.g., hexane) that can be recycled after distillation, leaving an enriched lipid (algal oil) product for subsequent upgrading. The recovered oil is either hydrotreated to produce a renewable diesel blendstock (RDB) (or gasoline or jet-range hydrocarbon blendstocks) or transesterified to produce fatty acid methyl ester (FAME) biodiesel.<sup>116,127,163,164</sup> After the (liquid-liquid) extraction of the lipid fraction, the remaining biomass is either used to produce biogas via AD<sup>165</sup> or converted to additional liquid fuels such as ethanol through hydrolysis and fermentation.<sup>166</sup> Any generated biogas (methane) can subsequently be used as fuel to supply process heat and power, and using an AD process to convert the spent biomass to biogas provides a means to recycle CO<sub>2</sub>, nitrogen, and phosphorous to algal cultivation to lower the requirement for fresh nutrients. The AD residual digestate solids fraction can be sold as a fertilizer bio-product. The AD digestate liquor can be returned to the cultivation ponds to recycle essential nitrogen (N) and phosphorus (P) nutrients.<sup>23,167,168</sup>

The different permutations of the base-case lipid extraction scenario described in the literature can be categorized as follows: 1) those that rely on different pretreatment methods to increase solvent accessibility to the intracellular lipids, e.g., mechanical cell rupture to improve mass transfer of the solvent to the constituent lipids; and 2) those that employ a form of whole algal cell mass treatment prior to extraction. Depending on the conversion technology envisaged, microalgae can be processed dried or wet, but typically employ organic solvent in either case. A pretreatment can consist of microwaving or sonication, which both increase the extractable lipid yield from algae.<sup>151,169-171</sup> Beyond these techniques, supercritical CO<sub>2</sub> extraction is gaining popularity as a 'green chemistry' approach for lipid extraction.<sup>157,172-176</sup> Supercritical CO<sub>2</sub> extraction is performed on dried algal biomass, after which the residual cell mass and CO<sub>2</sub> (and in many cases a co-solvent such as ethanol) are brought up to pressure (~200-250 bar). After extraction, the oil and CO<sub>2</sub> are recovered.<sup>158,175,177</sup> Typically, pure CO<sub>2</sub> at supercritical conditions can achieve a polarity (depending on the co-solvents used), and thus lipid selectively, similar to hexane (i.e., a highly non-polar solvent), and this can be modified based on the co-solvents used (e.g. methanol, propanol, etc.), and thus lipid recovery can be optimized. The major sustainability advantages of supercritical extractions are that they eliminate the use of toxic extraction solvents and permit complete and efficient recovery of the CO<sub>2</sub>. However, these benefits come at a cost of expensive capital equipment and substantially increased compression requirements, as well as the need to dry algal biomass prior to processing.

It is worth noting that gravimetric extractable lipid yields do not necessarily represent achievable fuel yields because solvent-based lipid recoveries are purely based on polarity matching of biochemical constituents with the extraction solvent and not exclusively selective for lipids, often "over-extracting" components (extracting other compounds in addition to neutral lipids), which can result in overestimating the true lipid content of the algal biomass.<sup>65,151</sup> A number of publications report laboratory-scale obtained gravimetric yields and extrapolate these yields to envisioned commercial processing scenarios involving large-scale implementation of an extraction system. However, in addition to risks associated with extrapolating potentially inflated gravimetric yields, as well as a lack of data on the quality of the extracted oil, the extraction performance observed at lab-scale may not directly scale to larger systems due to practical limitations in mass transfer of solvent to lipids at larger scales,. In addition, comprehensive comparisons of the impact of cell wall characteristics, lipid molecular composition, etc. is only rarely provided in

literature reports; however all these factors likely influence extraction recovery yields and ultimately the fuel yields. Recently, there has been a transition in the literature towards using algal fatty acid content as a basis for standardizing discussion and reporting of data and biofuel potential. In particular, extraction efficiencies are increasingly being related and correlated with the original lipid content of the algal cell mass.<sup>151,178-180</sup> Beyond the analytical application of *in situ* transesterification, there have been reports investigating its application to large-scale direct FAME biodiesel production from algal biomass.<sup>181,182</sup> While this approach typically generates close to the maximum fuel potential from algal biomass oil, the process economics are challenging and do not support large-scale implementation, mostly due to high methanol consumption and especially the need to dry the biomass, which is generally considered cost-prohibitive. Challenges with biodiesel production also lie in the logistics of delivery, uncertainties on product properties, quality and blending properties. Going forward, it is important for any of the processes and unit operations being demonstrated at the laboratory-scale to define and report their yields at each step in sufficient detail that comparisons of literature reports across the field are possible, which is not currently the case. For the field to be able to advance more rapidly, it is necessary to meaningfully compare literature reports from different groups developing various novel algae-based processes to be enable reliable and comparable economic projections about scalability (towards biofuel production scales) and commercial viability. At present there seems to be a lack of well-established, cost-effective oil extraction technologies available and demonstrated for algae.



**Figure 4-1 :** Illustration of major algae conversion pathways under development: (A) base-case algal lipid extraction and upgrading (ALU) approach; algae are grown in open ponds, or photobioreactors, or hybrid systems after which the algal cell mass is harvested by either decantation, flocculation, centrifugation or filtration. It is then either dried or processed wet through extraction of lipids, which are further upgraded via hydrotreating to renewable diesel, jet or via transesterification to FAME biodiesel. After which, the residual cell mass is anaerobically digested, with the produced biogas being used for power generation for the entire plant;<sup>165</sup> \*core aspect of conversion process acting on either wet or dry algal biomass; can include mechanical or

physical cell rupture, direct (or *in situ*) transesterification, biocrude conversion, preceded by any type of cell rupture, pretreatment, wet or dry extraction, supercritical, etc.; (B) Current base-case of combined algal processing (CAP) pathway, where bioenergy-products are derived from both the carbohydrate (after dilute acid pretreatment and fermentation) and lipid fractions of the biomass; (C) Hydrothermal liquefaction process as described and modeled. DAF = dissolved air flotation, SLS = solid liquid separation, AD = anaerobic digestion, HTL = hydrothermal liquefaction, CHG = catalytic hydrothermal gasification.

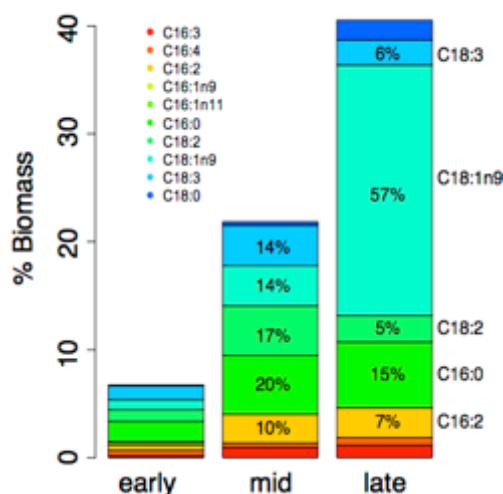
One lipid extraction approach that is demonstrated in the laboratory and described and modeled to be applicable to a 5000 acre farm-based biofuels production pathway is based on a biochemical processing strategy, the Combined Algal Processing (CAP) pathway, to selectively recover and convert specific algal biomass components to specific fuels, e.g., carbohydrates to ethanol and lipids to a renewable diesel blendstock (RDB) product (**Figure 4-1.B**).<sup>5,155</sup> In brief, this type of process can be described as follows: whole algal biomass, grown phototrophically in open pond systems, is dewatered to an algae paste concentration of 20% (dry basis) and fed directly into a dilute sulfuric acid catalysed pretreatment process, followed by either solid/liquid separation of the residue and hexane solvent extraction to separate the neutral lipid-rich oil from the residual cell mass, with fermentation to ethanol of sugars liberated after pretreatment.<sup>155</sup> Alternatively, the pretreated slurry is first fermented to ethanol, after which the cell mass residue is extracted.<sup>156</sup> Both processes improve the overall pathway economics relative to the base-case scenario using lipid extraction alone by providing an avenue for biofuel production from both carbohydrate and lipid fractions of algal biomass. The lipid-extracted residual cell mass is sent to AD and follows the same route to nutrient recycling and powering the overall plant as described above for the base-case process. An alternative processing approach is to also route the protein fraction towards fuel production. This process is being investigated on whole algal biomass and takes advantage of a novel *E. coli* that was developed for the fermentation of amino acids to mixed alcohols.<sup>183-186</sup> The approach is especially well suited to biochemical upgrading schemes because it can take advantage of the substrate specificity of biocatalysts. Through it, lipid-rich algal slurry can be fermented to ethanol with no loss of fatty acids. This biological specificity can reduce process complexity and the need for specific recovery steps to provide clean feedstock streams.

## 4.2. FEEDSTOCK EFFECTS ON BIOCHEMICAL PROCESS EFFECTIVENESS

For any of the published lipid-based process pathways there is naturally a strong dependence on the lipid content of algal biomass. Ideally, to increase value and thus revenue for an alga-process, a biorefinery approach for full utilization of all biomass components is an attractive solution. Each of the process options discussed above is highly dependent on the lipid content and composition of the biomass, with mass transfer and solvent polarity compatibility with the composition determining the yields, quality and efficiency of extraction.<sup>153,187,188</sup> Furthermore, the effect of the cell composition may influence the susceptibility of the cells to pretreatment, e.g. cell rupture using mechanical or chemical means. For example, the dynamic compositional changes in the biomass, the lipid composition (**Figure 3-1.G&H** and **Figure 4-2**) and the cell walls,<sup>65,189</sup> where the cell wall may become impenetrable to solvents as lipid content increases, and thus increase the complexity of a conversion pathway reliant on lipid extraction.<sup>190</sup>

The composition (fatty acid chain length and fermentable sugar concentration) plays an even larger role in defining the suitability of an algal biomass feedstock to enable the respective process yields in a Combined Algal Processing pathway (discussed above), where both the lipid and the carbohydrates become feedstocks for developing fuels.<sup>5,155,156</sup> One advantage of a lipid-extraction biochemical conversion pathway is the relatively non-destructive nature of this fractionation approach (as compared to thermochemical treatments, such as HTL) and its ability to generate relatively clean product streams. This approach not only increases the overall fuel fraction obtained from the algal biomass, but also allows for the implementation of a modular approach to the valorization of each of the fractions. Increasing the recovery of high-quality and potentially

high value products replaces a lipid-extraction approach. The initial demonstration and theoretical calculations include fermentative routes to fuels, including renewable diesel and ethanol. However, there is no reason to discount the option of diverting a fraction of each of these streams (slipstreams) to alternative higher value products. In the biorefinery for bio-products section (**section 6**), we explore options that are compatible with slipstreams implemented as the next stage of fractionation supporting maximal algal biomass utilization.



**Figure 4-2 :** Total lipid content (as FAME) shown on a biomass basis (% DW) in early, mid or late-harvested *Scenedesmus acutus* biomass, illustrating the individual relative contributions of the different fatty acids to the total lipids and the dynamic changes in lipid content (<10% to >40%) and composition over the course of harvest timing

This fractionation pathway is however highly dependent on the composition of the algal biomass, with the highest value attributed to the algal biomass with the highest lipid content. A range of different scenarios were recently compared with respect to their theoretical yields, with results indicating that the calculations underpinning the respective lipid- and carbohydrate-based yields also deeply influence process economics.<sup>5</sup> In addition, different species of algae will exhibit distinct patterns of pretreatment and component release,<sup>190</sup> presumably due to the variations in biochemical composition and susceptibility of different algal species cell walls to cell disruption.

### 4.3. PROCESS OPTIONS FOR FUEL PRODUCTION

The lipids (or oils) extracted using either of the scenarios described above need considerable processing before they can become a fuel-blendstock. In the case of fatty acid-rich algal lipids, the processing options are either transesterification to produce FAME biodiesel or hydrotreating the oils to generate a renewable diesel blendstock (RDB), which will be a mixture of long chain alkanes similar in fuel properties to traditional diesel. Both processes have their respective advantages, with FAME biodiesel production based on well-established transesterification technology that has been applied to vegetable oils for decades, and RDB being completely compatible (fungible) with the existing fuel infrastructure. Comparisons of RDB and FAME biodiesel production have been published that highlight some of the challenges.<sup>191-194</sup>

After separation from the hexane solvent, the extracted raw algal oil is sent to a central hydrotreating unit where catalytic deoxygenation and denitrification is carried out, often followed by isomerization over heterogeneous catalysts to reduce the chainlength and thus improve the cold-flow properties of the resulting fuel.<sup>194-199</sup> Because of the relatively high cost of hydrogen and LCA costs due to production of hydrogen largely from natural gas, other process configurations remove a large fraction of the oxygen as CO<sub>2</sub> by decarboxylation, reducing carbon conversion

efficiency of the process. Using a C16/C18 algal oil feedstock (most common fatty acids in algal feedstocks), the products from these reactions are C15 to C18 normal alkanes, which are likely to be solid at room temperature. Therefore, an isomerization catalyst is also needed to introduce branching, which can produce a dramatic lowering of cloud point and a moderate reduction in cetane number.<sup>197</sup> The resulting RDB fuels have the advantage of being very similar to petroleum-derived fuels and containing few impurities that might cause operational issues. The downside is the significantly higher capital, operating, and energy cost.<sup>200</sup> Hydrotreating and hydrocracking, technologies originally developed for oil refineries, have been found to be particularly useful for processing vegetable oil, greases, and pyrolysis oils. The level of hydrogen required for hydrotreating can be controlled by processing conditions and choice of catalyst because the same materials that catalyze hydrodeoxygenation also catalyze decarboxylation. Optimization of this technology for algae-based biorefineries is necessary before commercial deployment of renewable fuel production from algae. Often, the assumptions utilized for the hydrotreating step and specific algal oil characteristics are currently less detailed because of a significant lack of relevant literature. Available technology models often assume that the stated "lipid content" is essentially 100% triglyceride, and thus ignore other co-extracted compounds that may be present such as phospholipids, pigments and nitrogen-containing impurities such as hydrophobic amino acids that could require additional cleanup. Additionally, hydrotreating process specifications such as hydrogen demand, pressure, and temperature are based on a compilation of literature studies for hydrotreating vegetable oils,<sup>201</sup> and thus are currently independent of the specific oil characteristics such as degree of saturation or even specific oil composition characteristics. These are parameters that are critically important to estimate the final fuel cost as well as predict the extent to which the hydrotreated oils (RDB) will blend into existing fuel infrastructure.

Fatty acid Methyl Ester (FAME) biodiesel production relies heavily on existing infrastructure and thus applications to algal oils are expected to be relatively straightforward, but true assessments will only be possible when sufficient quantities of algae-derived FAME biodiesel are produced. FAME biodiesel is commercially produced from triacylglycerides (TAG) by (most often) base-catalyzed transesterification or from free fatty acids (FFA) by acid catalyst esterification. The alcohol used is most commonly methanol such that the product consists predominantly of fatty acid methyl esters (FAMEs), though a number of impurities are typically present. The structure of the fatty acid chains present in the feedstock has a determining effect on many of the critical quality parameters for FAME biodiesel. The two most important properties for fuel quality are i) the chain length, followed by ii) the degree of fatty acid unsaturation. The degree of unsaturation can be quantified as the iodine value (IV), which refers to the moles of double bonds per mass of sample. Typical terrestrial crop oils and animal fats consist almost exclusively of C16 and C18 fatty acid chains. For this narrow range of materials, IV can be correlated with many important properties such as cetane number, viscosity, density, and molar H/C ratio.<sup>196,202</sup> A significant fraction of mono and polyunsaturated FAMEs are desirable in FAME biodiesel, which make algal oils a desirable feedstock (**Figure 4-2**). Polyunsaturated FAMEs have much lower cetane numbers than mono-unsaturated FAMEs but also much lower melting points (and much greater solubility at cold temperatures). There have been concerns that polyunsaturated FAMEs are not adequately stable to oxidation, however this problem can be mitigated by the use of antioxidant additives.<sup>203,204</sup> As noted above, the effect of FAME makeup on biodiesel properties and performance is well understood. A much more challenging area is the impact of impurities. For FAME biodiesel made from conventional terrestrial crop oils and animal fats these impurities are mono- and diglycerides, plant sterols and steryl glucosides, free fatty acids, and residual metals. Monoglycerides and other impurities are known to have a dramatic effect on cold temperature operability, which led to the introduction of a cold soak filterability test and a limit on total monoglycerides.<sup>205</sup> Additionally, there is concern that residual metals such as Na or K from transesterification catalysts, Mg from adsorbents, or Ca from hard water could poison emission control catalysts and filters. There are potential challenges to the commercial deployment of FAME biodiesel technology from algae, and these are mostly related to variability in oil composition, not only on fuel properties relating to physiological and phylogenetic variability in the fatty acid

profile,<sup>191</sup> but also on conversion effectiveness and catalyst performance on oils that are not primarily triglycerides; in some cases, as much as 80% of the oils were found to be free fatty acids.<sup>155,156</sup> Because of the lack of sufficient lipid-rich algal biomass for oil extraction and conversion, actual fuel property measurements are scarce in the literature.

#### 4.4. MICROALGAE FOR BIOGAS AND BIOMETHANE

Alternative biochemical conversion pathways for microalgae involve generating biogas through AD of the intact algal biomass, without prior lipid extraction. These approaches have been reviewed in a recent report published by IEA Bioenergy Task 37.<sup>206</sup> Thanks to their high energy content, microalgae are considered an advantageous substrate for AD, but biogas yields from AD will be highly dependent on the particular algal strain and its cell mass composition. Many algae have low ash content (<10%), which is advantageous for AD, however their often low C:N ratio may make the process challenging. The choice of optimal algal species can lead to faster conversion of algal biomass to biogas. Some species possess no cell walls; some have protein-based cell walls without cellulose or hemicellulose. These attributes make them easier to degrade.<sup>207</sup> Besides easy degradability, other features, like productivity or sensitivity to contamination, have to be considered as well for microalgae production. If the species of choice possesses rigid cell walls and is natively resistant to AD, the application of a suitable pretreatment prior to AD is necessary.<sup>208,209</sup> Similarly, the digestibility of the residual biomass after e.g. lipid extraction has to be considered, since this process impacts the C:N ratio of the material and thus not all AD microbial consortia are equally adjusted to such feedstocks.<sup>210</sup>

As mentioned above, a large focus of biofuels from microalgae research and development (R&D) is on maximizing lipid production. Lipids also yield high levels of biogas but microalgae with excess levels of lipids are not amenable to stable anaerobic digestion.<sup>211</sup> The big advantages of anaerobically digesting microalgae is that neither a pure culture is needed nor does a specific compound (e.g., lipids for biodiesel) need to be produced. Both these advantages can significantly reduce the cost of producing microalgae biomass and also enable microalgae production to be part of another process like wastewater treatment. The microalgae may be digested to produce biogas, which releases CO<sub>2</sub> when combusted to generate power for the plant. Therefore, carbon accounting can become complex. The overall return captured carbon needs to be considered in the context of a life cycle analysis (see Section 8). Similarly to other conversion processes discussed in this section, the energy input in mixing, harvesting and conversion of microalgae to biogas is very significant and may be of a scale that more energy is used in the process than is contained in the produced biogas. A microalgae-based biogas industry is far from commercialization, although significant steps are being taken in the wastewater treatment sector to demonstrate facilities of a significant scale. Innovation is required in optimizing microalgae systems. Currently, the microalgae industry is focused on producing products of higher value than can be afforded by bioenergy applications in order to offset high production costs and thus AD for conversion of whole algae may not be economical. In principle, a more economic approach to producing biogas from microalgae is cascade usage in a biorefinery concept wherein higher value products yield the most revenue, leaving residual cell mass that can be transformed into lower value biogas.

Biogas is typically expressed in units of L biogas per kg volatile solids (VS), with VS corresponding to the AFDW of the algal biomass. In the literature, measured specific biogas yields of microalgae vary between 287 and 611 L/kg VS and specific methane yields between 100 to 450 L/kg VS (**Table 4-1**).<sup>207,212,213</sup> The reason for these broad ranges is that AD performance is highly species-specific, reflecting the significant differences in cell composition as well as cell wall characteristics that exist between algal species. In addition to variability of effective AD, it usually takes a long time for the methanogenic community to adapt to the feedstocks, which may contribute to the reported variability, along with different methodologies employed by different research groups in the cited literature. After AD, intact cells of *Scenedesmus* sp. were detected in dark AD fermenter.<sup>207,214</sup> This can be explained by the fact that *Scenedesmus* sp. is able to utilize a variety

of sugars and organic acids to support its heterotrophic growth.<sup>148</sup> The variation in biomethane yields may also be explained by the use of differing test systems to measure biomethane potential (BMP). Some practical recommendations can be found for digesting microalgae. The thermophilic digestion of microalgae shows higher biogas yields than mesophilic digestion.<sup>212,214</sup> Thermophilic digestion of *Scenedesmus obliquus* resulted in a biogas production rate 30% higher than for mesophilic digestion.<sup>168</sup> Drying of microalgae reduces biogas yields and is therefore not recommended. A biogas yield decrease of between 16 and 20% was reported.<sup>207</sup>

**Table 4-1:** Methane and biogas production yields from different microalgal species measured by BMP tests)<sup>207,212,213</sup> NS = not specified

Species	Temp. [°C]	Biogas prod. [L/kg VS]	CH <sub>4</sub> prod. [L/kg VS]	CH <sub>4</sub> content [%]	Ref.
<i>Arthrospira platensis</i>	-	481 ± 14	293	61	207
<i>Chlamydomonas reinhardtii</i>	-	587 ± 9	387	66	207
<i>Chlorella kessleri</i>	-	335 ± 8	218	65	207
<i>Chlorella vulgaris</i>	28-31	-	310-350	68-75	215
<i>Dunaliella salina</i>	-	505 ± 25	323	64	207
<i>Dunaliela</i>	35	-	420	-	216
<i>Euglena gracilis</i>		485 ± 3	325	67	207
<i>Nannochloropsis</i> sp.	38	388	312	80.5	217
<i>Scenedesmus obliquus</i>	-	287 ± 10	178	62	207
<i>Anthrospira</i> sp.	35	-	320-310	-	216
	38	556	424	76.3	217
<i>Anthrospira maxima</i>	35	-	190-340	-	218
Mixed algae sludge ( <i>Chlorella</i> - <i>Scenedesmus</i> )	35-50	-	170-320	62-64	214
	50	500	NS	-	214
	35	405	NS	-	219
	45	611	NS	-	214
	35	-	100-140	-	220
	38	420	310	73.9	217

Some microalgae possess extremely thick cell walls, which can make AD quite challenging. For example, the thickness of the relatively stable cell wall of *Chlorella pyrenoidosa* is 0.1 – 0.3 µm.<sup>221</sup> Treatment methods commonly used to break the cell walls include: thermal hydrolysis (> 100°C); mechanical treatment, e.g., ultrasound, lysis, centrifuge and liquid shear as occurs in a high-pressure homogenizer; chemical treatment such as oxidation or alkali treatment; and enzymatic pretreatment with cell wall-degrading enzymes or even known predators. Thermal pre-treatment of *Nannochloropsis salina*, prior to AD, significantly increased the methane yield.<sup>208</sup> In activated and primary sludge treatment, different technologies have been successfully applied to pretreat biomass prior to AD to increase methane yield. Alzate et al. tested the AD potential of three microalgae mixtures.<sup>222</sup> Pretreatments investigated included thermal, ultrasound, and biological (enzymatic) methods. Biological pretreatments showed negligible enhancement of CH<sub>4</sub> productivity, with the highest CH<sub>4</sub> productivity increase (46–62%) achieved using thermal hydrolysis; the optimum temperature for this pre-treatment depended on the microalgal species.<sup>222</sup> Ultrasound pre-treatment at 10,000 kJ/kg total solids (TS) increased CH<sub>4</sub> productivity

up to 24%; no further increase in productivity was noted at higher energy input.<sup>222</sup> The influence of low temperature thermal (50-57°C) and freeze-thaw pretreatments on algae prior to AD showed that compared to AD of untreated microalgal biomass both pretreatments promoted protein hydrolysis and increased methane yields by 32-50% when digested at 20°C.<sup>223</sup> The application of high pressure treatment by a French press or enzymatic treatment to *Chlorella vulgaris* also increased methane yields compared to untreated cell mass.<sup>212</sup> Finally, we note that there are potentially additional challenges to implementation of AD for algae, mainly related to compounds potentially present in the algal biomass that can inhibit effective functioning of digesters, e.g., including ammonia, sulfide, light metal ions, heavy metals, and various organics. Due to differences in AD inocula, waste compositions, and experimental methods and conditions, literature results on inhibition caused by specific toxicants vary widely.<sup>224</sup>

One challenge with the implementation of microalgal biogas installations is the availability of suitable land and sufficient algal cultivation capacity to supply AD. If this amount of algal biomass was converted by AD it could theoretically produce biogas containing 35% of the primary energy in the coal being combusted, however this could still be less than the energy required to pump the microalgae culture, which could result in a negative energy return on investment for the overall process' sustainability. For this technology and any subsequently discussed algae-based technology, future research should focus on overall process sustainability, putting emphasis on the process' life cycle analysis projecting a favourable energy return on investment.

Other implementation issues with microalgae include the variable length of the growing season depending upon location and the lack of light (and growth) at night. Optimal temperatures for cultivation of microalgal biomass are on the order of 27°C. This will not be attainable in temperate oceanic climates and may limit technology deployment to tropical or Mediterranean climates. Contamination of cultivated microalgal species by higher trophic life forms and other species of microalgae also may be a challenge to achieving and sustaining commercial scale microalgae R&D and FAME biodiesel production, however it is not a problem for AD-based conversion. It is likely that innovative integrated production and conversion processes will be required to optimize algal biogas production. Such integrated systems may include coupling bioenergy production with microalgae production to scrub CO<sub>2</sub> from combustion emissions in power plants. They may involve the use of microalgae to upgrade biogas and co-digesting the produced microalgae with slurries of agricultural production and food processing wastes. Numerous aspects need to be evaluated to design optimal algal biofuel systems including the particular species of algae, cultivation and harvesting techniques, pretreatments for produced algae, configuration of the AD system, composition and concentration of produced biogas (biohydrogen or biomethane), choice of co-substrates and finally integration of the technology with upstream cultivation and downstream recycling of nutrients. There are still significant gaps in understanding and commercializing biogas production of biogas from microalgae, e.g., the energy and carbon balances as well as the minimum viable cost for the produced biogas are not yet known. It may well be that multi-product biorefineries that include the production of higher value bio-products will be required to allow financially sustainable biofuel production systems that incorporate AD of whole or residual algal cell biomass. Recently, the European Commission funded project All-Gas is specifically investigating biogas production from consortia of algae and bacteria in support of a wastewater treatment facility. The project is based around the concept of using a mixture of algae and bacteria to clean waste water and produce fuel. The project is led by Aqualia, the third-largest private water and wastewater company in the world. All-Gas has built a 1 hectare pilot facility in Chiclana de la Frontera, Spain, and will soon expand to 3.5 hectare system.

By co-processing the residual biomass in a lipid-extraction or biochemical conversion process, through AD, the recycling of a large fraction of the nutrients is possible, as has been demonstrated in the recent literature.<sup>23</sup> An additional advantage of AD is the generation of energy in the form of biogas to power the production plant. Some of the challenges that are associated with an efficient AD platform after lipid extraction lies in the bioavailability of the carbon left over for AD, the

relatively high N:C ratio of the cell mass residue after AD, and the bioavailability of N and P in the AD effluent.

#### **4.5. CONCLUSIONS**

Biochemical processing or fractionation of algal biomass presents opportunities to take advantage of multiple feedstock streams from the biomass (e.g., lipid, carbohydrate and protein-based fuels). This opens up the possibility to develop a biorefinery approach based on these fuel feedstocks, i.e., to develop specific fuels from each of the major biochemical constituents in the algal biomass. This approach extends the yields from algae beyond just the use of the lipid fraction. Most of the process options described in the literature rely on AD for some of the final fuel recovery, with conversion of residual cell mass carbon to methane (biogas) used to power the plant. This aspect is critical to the sustainability of the conversion process since it is the main route for recycling nutrients to cultivation. The overall yields and process challenges are intimately related to specific species and their respective cultivation conditions used to generate the algal biomass and thus care has to be taken in interpreting yields reported in the literature without a demonstration of the integrated process from cultivation to processing. In conclusion, the options are diverse for algae conversion and extraction. If a whole algal biomass biorefinery approach is taken, then the yields of biofuels from algal biomass may, even with conservative assumptions, exceed the biomass yields typically achieved using terrestrial feedstocks.

## **5. Processes for Thermochemical Conversion of Algae**

Thermochemical processing of algae involves a high-temperature conversion process of whole algae towards a renewable fuel feedstock in the form of a bio-oil. These thermochemical processes can be hydrothermal liquefaction or pyrolysis, the differences being the moisture content of the feedstock and the properties of the resulting 'bio-oil' feedstock. In this section, progress in high-temperature conversion of algal biomass is reviewed and placed in context as an alternative to a lipid-based extraction process. Much of this discussion is directly from or adapted from a recently published review by one of this report's co-authors.<sup>135</sup>

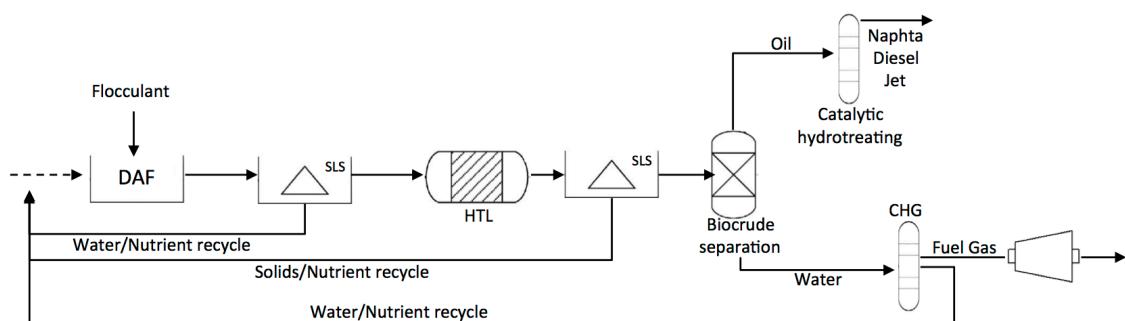
In the last five years, a tremendous expansion of research and development was focused on the thermochemical processing of whole algae for the production of fuels.<sup>24,135,225</sup> There are several key elements to this expanded interest in thermochemical processing: 1) Processing is applied to whole algae, not just lipid extracts, resulting in higher product yields; 2) Feedstock composition is less critical to the process, allowing a wider range of algae growth scenarios to be considered; and 3) Envisioned products are hydrocarbon fuels compatible with current infrastructure. Because of these three attributes, products produced through this pathway have more flexible future growth options as well as direct market applications.<sup>6</sup>

The vast majority of the research and development (R&D) in thermochemical conversion of algae to fuels is based on hydrothermal processing, and, specifically, hydrothermal liquefaction (HTL) to produce a biocrude product. Thermochemical conversion of algae can be divided into the direct pyrolysis of dry algae and the high-pressure processing of algae in water slurries. Wet (hydrothermal) processing is better suited for applying to algae because algae are grown in extremely dilute aqueous systems. The partial dewatering of algae-containing solutions to the level of 10-20% dry solids, usually accomplished by mechanical means for HTL, is less energy intensive than the thermal drying to >90% dry solids required for pyrolysis. The required moisture content for the two processing options differs because the value of liquid water is different for both. In HTL, the pressurized system serves as a heat transfer medium and moderator. Pyrolysis requires boiling off water in the reactor, which results in a large heat sink. This slows the heating process and interferes with fast pyrolysis reaction mechanisms.

### **5.1. PROCESS OPTIONS**

As algae are grown in dilute water media, the recovery of the algae for subsequent processing is a critical step. The amount of energy required to concentrate the algae to a form in which it can be effectively processed is a major consideration. The two processing options for thermochemical processing, pyrolysis (temperatures in excess of 500°C, in the absence of air, for short residence of about a second) and hydrothermal (typically 350°C, 200 bar pressure for some minutes of residence time), both require removal of the bulk of the water and recovery of algae biomass at dry solids concentration much above the 0.1 wt% concentration at which algae is typically grown. However, the water removal requirement is an order of magnitude higher for pyrolysis (the feedstock is typically processed at <10 wt% moisture) and typically requires energy and cost intensive thermal drying of the algal biomass. Because of this requirement for dry biomass prior to pyrolysis, the overall techno-economics make this process prohibitively expensive. The dewatering for hydrothermal processing is more typically accomplished by less energy intensive physical means, as it is meant to concentrate the algae only to a slurry with typically 15-25 wt% dry solids (75-85 wt% moisture). As a result, relatively little process research and development has focused on pyrolysis of algal biomass compared to the use of hydrothermal conditions.<sup>226,227</sup> There have been some more fundamental studies of pyrolysis of algae performed at very small scale, however, such as by thermal gravimetric analysis (TGA).<sup>228</sup> Small laboratory reactor studies of slow pyrolysis can also be found.<sup>229-231</sup> The examples of commercial progress on thermochemical conversion of whole algae utilize hydrothermal processing.

The slurry of algae, typically at a concentration of 15-25 wt% dry solids in water, is pumped through the hot reaction zone, such that the typical residence time is 10 to 30 min. Pretreatment of the algae by microwave heating prior to HTL may offer some improvement in algal biocrude oil yield and quality, but the effect was found in only one of three species tested.<sup>232</sup> Following HTL, the biocrude and aqueous phases can be separated. This phase separation is facilitated by removal of any solids phase, whose composition will vary with algal species, reaction conditions and residence time (**Figure 5-1**).<sup>24</sup> Pressure letdown and heat recovery play into the energy efficiency of the process, and will likely be key elements of design in any commercial process. One method of biocrude recovery often used, particularly with small batch reactor tests, requires the use of additional chemical treatment and solvents to facilitate the phase separation.



**Figure 5-1:** Process flow diagram for hydrothermal processing of whole algae, DAF = dissolved air flotation, SLS = solid liquid separation, HTL = hydrothermal liquefaction, CHG = catalytic hydrothermal gasification. Adapted from<sup>24</sup>

Reviews of hydrothermal processing of algae have been published recently by groups active in the research field.<sup>232,233</sup> According to one group, the engineering challenges are straightforward with the main hurdle being sufficient and economical production of the algal biomass. A second group identified research and development issues such as HTL heat-up rate and biocrude product recovery; this review was written before there were continuous-flow results in the literature, and they specify that such information is needed to build on all the batch data that has been published. Fortunately, continuous-flow process results are now appearing in the open literature.<sup>234</sup>

Several attempts have been made recently to catalyze the otherwise thermally driven reactions of HTL. The effect of pH on the chemical conversion mechanisms for HTL of biomass has long been recognized.<sup>235</sup> One group made comparative tests with added base and acids to determine the effect on HTL of *Arthrospira* sp. (*Spirulina*).<sup>236</sup> They determined that the addition of organic acid resulted in higher yield of biocrude exhibiting a lower boiling point and improved flow properties, while the addition of base resulted in more extensive deoxygenation. Subsequent tests suggest that addition of sodium carbonate results in higher biocrude yield relative to uncatalyzed conditions, while calcium phosphate addition or nickel oxide addition reduces the biocrude yield while increasing the gas yield.<sup>237</sup> The use of heterogeneous catalysts in HTL has also been investigated to a very limited extent by short tests in batch reactor systems. The Leeds group reports that heterogeneous catalysts cause an increase in de-oxygenation, with CoMo and Pt affecting carbohydrate and protein fractions, while Ni deoxygenates lipids and promotes gasification.<sup>238</sup> Savage's group has studied catalytic HTL more extensively and has even devised a special reactor configuration to separate the liquefaction from the catalytic step.<sup>239,240</sup> In both of these test programs all the catalysts were added to the reactor without any indication of either reduction or sulfiding, however no analysis was performed on the used catalysts and the potential for sulfur poisoning or coke formation on the catalysts was not addressed. The minimal effects reported are probably not important in the long run as catalyst stability in an HTL environment with a sulfur containing feedstock is highly suspect without results showing to the contrary.

## 5.2. FEEDSTOCK EFFECTS

A key element of thermochemical conversion of whole algae is the wide applicability of thermochemical processes. All forms of biopolymers partially break down under thermochemical processing conditions.<sup>241</sup> Therefore, whole algae are processed, not just lipid extracts. The chemistry involves a complex set of reactions from hydrolysis to dehydration, depolymerization to condensation, as well as various forms of heteroatom (nitrogen (N), sulphur (S), phosphorous (P), potassium (K)) removal. The former reduces the heteroatom "contaminants" and other trace elements; it concentrates the energy in the algae biopolymers into more hydrocarbon-like structures. A comparative feedstock study of whole algae biomass and other lignocellulosic biomasses (pine wood and grape residue) showed algae achieved a higher biocrude yield.<sup>242</sup> As a result, an algal species does not need to be grown under strictly controlled conditions to specifically maximize the lipid content because carbohydrate and protein structures, as well as lipids, can be converted directly into fuels by thermochemical processes.<sup>243</sup> While lipids produce the highest yield of biocrude, >90 wt%, proteins and carbohydrates also produce significant yields of biocrude, particularly at higher temperature, 350°C. Further, it was reported that these two components produced higher biocrude yields when mixed than when processed individually, achieving higher biocrude production from whole biomass.<sup>241</sup> Mixed algae culture grown in open wastewater treatment systems has shown even better HTL biocrude yield and quality than that from laboratory grown monocultures.<sup>244</sup> These advantages for whole algae processing contrast with other methods being developed for direct recovery of the lipids as a FAME biodiesel product through transesterification in supercritical conditions with and without hydrothermal carbonization pretreatment.<sup>245,246</sup>

In order to better understand the effects of feedstock composition variation, tests have been performed with model compounds and biochemical components. Maillard reactions, condensations of sugars (carbohydrates or carbohydrate fragments) and amino acids (proteins or protein fragments), have been found to occur under the conditions of hydrothermal biomass processing thus suggesting a method for biocrude production from non-lipid biopolymers.<sup>247</sup> Lipid transformation at hydrothermal conditions showed that triglyceride hydrolysis proceeded at sufficient velocity that fatty acid yields could be maximized at short residence time (<30 min) at up to 350°C.<sup>248</sup> Model development was undertaken to develop the relationship between biocrude yield and the biochemical makeup of the algae. Broad agreement was reached between predicted and actual yields for microalgae and this also showed that the biocrude yield was 5-25% higher than just the lipid content depending on the biochemical makeup of the algal cell mass.<sup>249</sup>

HTL of macroalgae has also been studied in the laboratory and are included here for comparison. University of Leeds performed batch reactor studies with macroalgal biomass slurries at 21 wt% dry solids and concluded that the highest yields of biocrude (17-18%) were derived from *L. digitata* and *A. esculenta*, while biochar yields were highest for *L. saccharina* and *A. esculenta* (18-19%). By claiming both biocrude and biochar as energy products, the authors concluded that these species produced energy yields equivalent to anaerobic digestion but greater than fermentation.<sup>250</sup> PNNL reported HTL of macroalgae in a continuous-flow reactor, processing *L. saccharina* over a range of slurry concentrations up to 22% and achieving biocrude yields as high as 28% (58% on carbon basis), and without using a solvent extraction recovery step.<sup>251</sup> Using the PNNL system, the biochar yield was reported as part of the mineral precipitate, which was very low (up to 4%). In addition, the PNNL system incorporated catalytic hydrothermal gasification (CHG) for byproduct aqueous processing wherein another 34% of the carbon in the feed was recovered as a fuel gas product, which had a composition similar to anaerobic digester gas.

## 5.3. BIOCRAVE PRODUCT DESCRIPTION

The bio-oil (or biocrude) produced and isolated after HTL is a black tarry substance that is compared with petroleum crude, with the exception that this biocrude is oxygenated, acidic, and contains various elements from the original biomass, such as nitrogen and sulfur. Thus, HTL

biocrude from algae needs additional processing before it can be used. The elemental analysis of algal biomass-derived biocrudes shows a range of compositions dependent upon processing severity and feedstock identity. Biller and Ross report carbon contents in algal biocrude ranging from 68 to 73 wt% across four algal species, with hydrogen contents around 9 wt%.<sup>249</sup> The oxygen content (by difference) ranged from 10 to 19 wt% and the nitrogen content from 4 to 7 wt%. These two elements are key to determining the extent of hydrotreating required to produce useful liquid hydrocarbon fuels. Analysis by GC/MS indicates that the biocrude contains aromatic hydrocarbons, nitrogen heterocycles and long-chain fatty acids and alcohols. The higher heating values (HHV) are variously reported in the literature in the range of 30 to 38 MJ kg<sup>-1</sup>.<sup>169</sup> Ultra-high resolution mass spectrometry (Fourier Transform Ion Cyclotron Resonance, FT-ICR MS) has also been applied to the HTL biocrude directly recovered (without solvent extraction) from a continuous-flow reactor system to provide data on the heteroatom content of higher molecular weight material.<sup>252</sup> The biocrudes are about 70 wt% distillable under vacuum and the distillate contains only a minor fraction of the oxygen and essentially none of the trace elements.<sup>253</sup> Further processing of the heavier fraction has yet to be demonstrated.

#### 5.4. UPGRADING OF BIOCRADE TO LIQUID FUELS

The biocrude derived from HTL of algae may be suitable for use as heavy fuel oil, but significant upgrading is required before it can be used as a transportation fuel. Different methods have been evaluated in the laboratory including catalytic hydrotreatment and catalytic cracking.<sup>24,254</sup> Hydrotreating of HTL oils, which is done in a way similar to hydrotreating of fossil crude oil, results in a nearly hydrocarbon product, like a sweet crude oil, when done in a fixed-bed continuous-flow reactor.<sup>24</sup> Biocrude hydrotreatment processing was accomplished with a catalyst similar to fossil oil hydrotreating catalysts (Co promoted Mo sulfide), together with pressure (130 bar) and temperature (400 °C) similar to other hydrotreaters. Although these results are from relatively short term tests, catalytic hydrotreatment of the biocrude significantly reduced oxygen content to a range of 0.8 to 1.8 wt% in these tests. HTL resulted in desulfurization and denitrogenation down to nearly immeasurably low levels. Total Acid Number (TAN) was reduced to below the level of detection due to oxygen removal, but the effect may also result from ammonium (formed by hydrodenitrogenation, HDN) neutralization of the remaining acids. The viscosity and density both correlate with the high hydrogen to carbon atomic ratio 1.85 to 1.98. With such low remaining oxygen content, the solubility of the oil in water was quite low and the carbon content in the aqueous byproduct was very low (0.7-1.4 wt%). The nitrogen content of the aqueous was relatively high, suggesting a substantial amount of ammonium is generated during the upgrading HDN process. The gas products were mostly hydrocarbon, with a tentative identification of ammonia, with little carbon oxides recovered. The result is upgraded oil fairly similar to sweet fossil crude oil. After such upgrading, this biocrude could be inserted into a traditional refinery for final conversion to traditional gasoline, jet or diesel fuels. Comparable results have more recently been reported when using a batch reactor system wherein the authors conclude that the yield of hydrocarbon product by HTL and hydrotreating exceeds the amount of lipid originally in the algae (*Chlorella sp.*).<sup>255</sup>

An alternative hydrotreating concept is based on processing in a supercritical water environment.<sup>256</sup> A range of catalysts, mostly metals, have been tested, in short time, batch reactor tests with a hydrogen-limited environment. The level of deoxygenation, denitrogenation, and saturation of hydrocarbons was less than in the continuous-reactor results.<sup>251</sup> A key conclusion was that the biocrude was essentially desulfurized by the treatment. A key omission is the analysis of the catalyst following the tests. Sulfur removal by formation of metal sulfides on the catalyst, as would be expected in this type of test, is not a basis for a sustainable process, as sulfiding is a well-known catalyst poisoning process. A separate study using HZSM-5 (a catalytic cracking catalyst) in the presence of hydrogen but without water was also reported, but again only in a sealed batch reactor.<sup>254</sup> The authors report that a high yield of aliphatic hydrocarbon product was achieved at 400°C while a more aromatic product was produced at lower yield at 500°C. No

analysis of the used catalyst was reported, such as to quantify coking, which is a typical result with this catalyst.

## 5.5. BYPRODUCT WATER DESCRIPTION AND UTILIZATION

During HTL processing of whole algae cell mass, 25-40% of the carbon in the feedstock remains as dissolved organics in the byproduct aqueous phase. In addition, the aqueous phase also contains about 50% of the nitrogen and other soluble minerals and dissolved CO<sub>2</sub>. Recycle of this water and potential nutrient stream is another key element in the HTL scheme for algae utilization, and critical for the overall process sustainability. Analysis of the dissolved organics has been performed in a few studies, to evaluate the presence and identity of high-molecular weight species and identify nitrogen and oxygen containing compounds, using GC with nitrogen and phosphorus detection (NPD) analysis to more specifically identify nitrogen containing compounds in the aqueous phase.<sup>233,257</sup> The toxicity of these compounds was quantified and whole aqueous sample tests verified the potent cell cytotoxic activity with Chinese hamster ovary cell assay with LC<sub>50</sub> at 7.5% of aqueous byproduct in DI water. Another study concluded that microbial growth on HTL aqueous phase from *Nannochloropsis oculata* at concentrations up to 10-40% was possible using *Escherichia coli*, *Pseudomonas putida* and *Saccharomyces cerevisiae*, although the *S. cerevisiae* needed glucose supplementation.<sup>258</sup> Recycle of the aqueous phase back to the HTL reactor has also been tested, and one research team concluded that there was an increase in biocrude yield and that the same level of reaction could be accomplished at lower temperature. Apparently, the soluble organic compounds were further reacted to higher molecular weight compounds which could then be recovered in the biocrude fraction.<sup>259</sup> Other studies have evaluated the recycle of the aqueous byproduct directly to the algae growth pond. An initial study used aqueous byproduct from *Arthrospira* sp. (*Spirulina*) HTL as a growth medium supplement for *Chlorella minutissima*. The supplement contained significant N (as ammonium) and P (as phosphate), which, at concentrations of 1% or less of the aqueous, was utilized by the cells at lower rates than when using the control medium alone. At higher concentration (10%), all algae growth was inhibited, indicating that the aqueous phase can not be recycled directly to the algae cultivation system without significant clean up.<sup>260</sup> Tests of *Desmodesmus* sp. HTL and regrowth on diluted aqueous byproduct demonstrated that up to 50% N nutrient replacement could be achieved without reduction in growth. The authors concluded that the lack of macro-/micro nutrients, other than N or P, such as Mg, in the aqueous phase is the main cause of growth reduction rather than toxicity due to insufficient dilution of organic components, such as phenols.<sup>261</sup> A more detailed study should be carried out to investigate the exact composition of the aqueous phase and then test the respective toxicity of each of its major components. The survey study of *Scenedesmus dimorphus*, *Chlorogloeopsis fritschii*, *Arthrospira* sp. (*Spirulina*) and *Chlorella vulgaris* in HTL and algae growth on the aqueous byproduct provides important guidance for future process developers.<sup>262</sup> The aqueous byproduct from the HTL process using several algal species was orders of magnitude higher in nutrients compared to standard growth media. These growth trials showed that heavy dilution of these aqueous streams is necessary to avoid growth inhibition by phenols, fatty acids and nickel. Several algal species were all able to grow on the aqueous byproduct but different levels of dilution were required. Mixotrophic growth was evident such that *Chlorogloeopsis fritschii* (at 400X dilution) and *Chlorella vulgaris* (at 200X dilution) achieved higher algal biomass yields than in their respective growth media. A more recent survey of *Scenedesmus almeriensis*, *Nannochloropsis gaditana*, *Phaeodactylum tricornutum* and *Chlorella vulgaris* in HTL and algae growth on the aqueous byproduct provides additional information.<sup>263</sup> These authors report that regrowth is strain specific as *Scenedesmus almeriensis* and *Phaeodactylum tricornutum* were not able to grow satisfactorily. Up to 75% of the nutrients could be replaced by recovered aqueous byproduct for *Nannochloropsis gaditana* and *Chlorella vulgaris*.

Catalytic Hydrothermal Gasification (CHG) is a novel process that employs a catalytic upgrading pathway to recover energy and nutrients from the rich aqueous phase solution. CHG is carried out at subcritical water conditions and produces methane and CO<sub>2</sub> gases from the dissolved organic

residues because of the catalyst.<sup>24</sup> An important fuel gas byproduct is formed that can be used to provide power and energy to support the overall plant. The product gas can be burned to produce combined heat and power (CHP) for the hydrothermal processing system (**Figure 5-1**). The combination of HTL with CHG as a second stage provides a liquid fuel product, while the CHG step cleans up the aqueous byproduct to facilitate its reuse or disposal. In this configuration, the effluent water from HTL is passed to CHG for processing of the remaining organics. This remainder contains significant energy, and would also be difficult and costly to dispose of or treat in another way, so CHG presents an ideal second stage. The CHG stage recovers more than 99% of the remaining organic material, leaving only methane and CO<sub>2</sub> gas and clean sterile water as product outputs. CHG can also be used alone to process algal biomass directly to gas products, and this has been demonstrated in a batch reactor with *Nannochloropsis* sp. and in a continuous-flow reactor with *Arthrosphaera* sp. (*Spirulina*).<sup>231,264</sup> This technology enables >99% of the chemical oxygen demand (COD) of the algae slurry to be converted to a fuel gas and CO<sub>2</sub> product. The direct processing of the algae with the preferred metal catalyst is complicated by poisoning of the catalyst by sulfur in the algae. However, use of HTL with mineral separation or appropriate aqueous processing before CHG may allow the complete cycle to be realized.

## 5.6. RECYCLING OF NUTRIENTS

A significant advantage of hydrothermal processing, both HTL and CHG, is that it recovers a high percentage of the nutrients in the original algae (including CO<sub>2</sub>), which can be recycled to support new algae growth (**Figure 5-1**). Nutrient recycle is achieved in several ways. In the continuous HTL,<sup>6</sup> one of the process steps is a precipitation/solids separation step which precipitates minerals, principally phosphate.<sup>24</sup> The phosphorus precipitate is very dense and small in volume, so can easily be recovered and processed into phosphorus fertilizer. From one third to one half of the nitrogen in the feedstock will appear in the biocrude, but the remainder is present in the effluent water and is recovered later after CHG, along with other plant nutrients and CO<sub>2</sub>. The gas mixture from the catalytic reactor is a 60/40 mix of methane and CO<sub>2</sub>, and the water is saturated with CO<sub>2</sub>. Since the water may be returned to the algae growth ponds to provide plant nutrients, it will also carry with it the dissolved CO<sub>2</sub>. In addition, the CO<sub>2</sub> in the gas product should be able to be separated from methane by conventional means, such as membranes, and returned to the algae growth pond. However, this has not yet been demonstrated. If the product gas is burned to produce combined heat and power (CHP), it can also be scrubbed to recover CO<sub>2</sub>. With these steps, nutrient recycle is nearly complete, almost as a closed loop.

## 5.7. CONCLUSIONS

Within the realm of thermochemical conversion technologies used for fuel production from algae, HTL is one of several promising avenues for near-term commercialization. The performance differences seen across the range of species tested and reported in the literature suggests species type has only minimal impact on the HTL process with differences in yield indistinguishable from the experimental variation due to the different batch reactor methods used, however detailed compositional characterization of the product needs to be carried out to assess the quality differences. Tests in continuous-flow systems should provide more definitive details of product variation with species. The composition of the algal biomass will impact the composition and yield of the biocrude. The cost of the feedstock is a critical barrier to commercial viability. Utilization of the biocrude product has had only limited study, but the direct hydroprocessing to liquid hydrocarbon fuels appears to be fairly straightforward based on initial laboratory tests. Recycling nutrients has been recognized as a key to sustainable operation and hydrothermal processing and provides the means to accomplish the recovery of the elements of primary concern, nitrogen and phosphorus. Expanded development and demonstration of conversion technology is needed in process-representative continuous-flow reactor systems. Scale-up for biocrude production is needed to allow testing of the liquid product to validate its application as a fungible biofuel.

## **6. Biorefineries and Bioproducts from Algae**

One priority for algal biofuels research is reducing the cost of the resulting fuel. One way to achieve a cost-reduction is through identifying high value primary and bio-products and ultimately by increasing the inherent value of the algal biomass for different conversion or upgrading pathways. This section considers issues at the interface between production and conversion processes, including discovery of novel compounds in algal biomass as well as establishing a link with scaled conversion process characteristics, primarily tailored around established lipid extraction or biochemical processing or fractionation pathway.<sup>5,149,150,265–267</sup> Bioproducts recovered in an algal bio-refinery approach are by definition highly dependent on the composition of the algal biomass, which, as discussed in earlier sections, is not static as often erroneously assumed, but rather highly dynamic and dependent on both the strain and the physiological environment the cells are grown in. A biorefinery is defined as a facility in which algal biomass can be sustainably processed into a spectrum of bio-based products (food, animal feed, chemicals, and materials) and bioenergy products (biofuels, power and/or heat).

### **6.1. MICROALGAE BIOREFINERY**

There are various biorefinery approaches for utilizing algae biomass or algae derived components in combination with energy production. The major objectives of research towards successful biorefineries are focused on identifying critical factors for economic development and deployment of algal biofuels that can achieve targeted levels of algal biomass productivity and composition and conversion efficiencies. This area of research is highly relevant to reducing costs for future algal biofuels commercialization, and integrating the dynamic algal biomass composition with downstream process characteristics by providing options for the development of fuel-relevant products derived from either lipid, carbohydrate or protein fractions. The rationale described here allows for the transition to intrinsic algal biomass value, providing a better link with algal biomass production costs. This approach could eliminate the potential conflict between maximizing biofuel yields and maximizing potential revenue, which provides a better sense of the path to commercialization. As new fuel-scale components are discovered, a higher value can be assigned to the algal biomass, and by doing so, there is subsequently less pressure on further increasing algal biomass productivity to reach cost targets. This implies that the cost of biofuel production could be reduced and new pathways or bio-product technologies can be identified for future strain and process development, to further aid with process economics.

A large number of potential products can be identified, as shown by the preliminary list in **Table 6-1**, which breaks out bio-products by their approximate concentration in algal biomass and their projected market size. This list serves as an example and is not comprehensive; several additional potential bio-products can be found in different species. The products found can be separated into groups relating to their applications. Bio-products with applications in food and feed markets (including nutraceuticals) are shown to have relatively small market sizes (25,000 tonnes (T)) but can command a high unit price (\$30,000 - \$100,000/T). The second large market segment is displacing products from petrochemical markets (e.g. polyurethane replacements, bioplastics), which each have a large potential market (11,000,000 – 40,000,000 T). These types of products reduce constraints around the purity of the cultivation environment and serve as a basis for product development in a manner that is scalable with a fuels production biorefinery scenario. For example, an economical projection of algal productivity for fuels is based on a 5,000 acre (2024 ha) open pond farm cultivation.<sup>5</sup> Assuming a  $25 \text{ g m}^{-2} \text{ day}^{-1}$  productivity, a total of 184,717 T algal biomass would be produced by a single farm each year. Even at 3% of the total algal biomass produced, an isolated nutraceutical product based on a single farm's output would be likely to saturate the market (5,542 T) or at least limit the biofuel production from that same farm. This same projection is true for several other higher value, but smaller market products. Thus, in order to stay relevant to a large-scale biorefinery approach in context of a fuel production scenario, prospective bio-product calculations should be carried out relative to fuel-scale production.

**Table 6-1:** Bioderived products from algae biochemical components. Biomass composition shown as wt% of dry biomass ranges, based on observed, literature-reported or measured. ¶market size based on IHS report on sorbitol. Where market sizes are missing, they are either not available, or highly dependent on the specific application (NREL, *unpublished data*)<sup>268</sup>

Feedstock	Wt %	Product	Market size (T)
Fatty acids	10-45%	Hydrocarbon fuel products	5,000,000
Omega-3-fatty acids	3-6%	Polyols	11,000,000
	3-6%	Polyurethane	11,000,000
	3-6%	Nutraceuticals	22,000
Hydroxy fatty acids	~1%	Surfactants, fuel additives	3,500,000
Branched chain fatty acids	~1%	Surfactants, fuel additives	3,500,000
Fatty alcohols	~1%	Surfactants, fuel additives	3,500,000
Sterols	2-4%	Surfactants/emulsifiers	2,000,000
	2-4%	Hydrocarbon fuel products	5,000,000
	2-4%	Phytosterol nutra/pharmaceuticals	25,000
Phytol	3-4%	Raw material for vitamin E, fragrance	1
	3-4%	Surfactants, fuel additives	3,500,000
Polar lipids	10-35%	Ethanolamine	600,000
	10-35%	Phosphatidylcholine, phosphoinositol and phosphatidyl ethanolamine (lecithin) <sup>†</sup>	20,000-30,000
Glycerol	2-6%	Di-acids for nylon production	2,500,000
	2-6%	Feed, pharmaceuticals	25,000
Fermentable sugars (glucose, mannose)	10-45%	Polylactic acid (PLA) polymers	300,000
	10-45%	Di-acids (e.g. adipic acid)	2,500,000
	10-45%	Ethanol	68,000,000
Mannitol	3-6%	Polyether polyols	2,300,000
Alginate	~3-5%	Alginate additives	12,000
Starch	5-40%	Polysaccharide-derived bioplastics <sup>°</sup>	2,000,000
Protein	19-40%	Thermoplastics	5,000,000
Amino acids/peptides	19-20%	Polyurethane	11,000,000
Amino acids/peptides	19-20%	Biobutanol, mixed alcohol fuels	40,000,000

<sup>†</sup> Nutraceutical, dietary supplement, at this point, without doing much more research, an unknown market size, though likely in the range of 20,000-30,000 T/yr.

The feed industry offers a large opportunity for the commercialization of microalgae. The global production of feed has increased every year for the past five years; the current levels of feed production is summarized in **Table 6-2**.<sup>269</sup> Aquaculture feed production has seen a 1.8% increase in feed demand.<sup>269</sup> This corresponds with a rise in demand for aquaculture itself, as natural sources of marine resources are exhausted and more people need the nutrition provided by

Omega-3 fatty acids. In order to maintain their nutritional value, fish need feed that support fatty acid production.<sup>270</sup> Microalgae are currently used as feed for the larva of fish and crustaceans, and have potential as a feed source for adult species due to their nutritional properties.<sup>270,271</sup> Demand for livestock feed has also increased.<sup>269</sup> Microalgae could offer a supplement to existing produced for livestock consumption and comprise anywhere from 7-20% of feed composition depending on the species.<sup>272</sup> Though, technological barriers exist for greater algae production, 30% of algae production contributed to the animal feed industry in 2004.<sup>270,272</sup> Increasing this contribution could reduce use of crops used for human consumption and increase the cost-effectiveness of biofuel production.

**Table 6-2:** Summary of feed production for different markets

	Total	All Livestock	Poultry	Pig	Ruminant	Aquaculture
<b>Production (10<sup>6</sup> Tonnes)</b>	980	939	439	256	196	41
<b>Percentage</b>	100%	96%	45%	27%	20%	4%
<b>China (10<sup>6</sup> Tonnes)</b>	183	158.2	65	85	8.2	18
<b>USA (10<sup>6</sup> Tonnes)</b>	173	146	82	24	40	11

One important consideration that relates to the discussion around a fully integrated approach is the quality of the biomass and products when the algae are cultivated in wastewater. The biomass (and residue after processing) may be contaminated and can only be used for energy purposes (anaerobic digestion, and/or co-generation for electricity and heat production) or for fertilizer. Potentially, additional uses as petrochemical replacements may be possible, but the quality limits and requirements have not been reported. When microalgae are cultivated in a synthetic medium, besides the uses listed for microalgae in wastewater medium, the biomass residue can be used for many other purposes, such as fish feed in aquaculture, animal feed (substituting meals), human food, food supplements, pharmaceutical products, and cosmetic products.

## 6.2. MICROALGAE-BASED FEEDSTOCKS FOR COMMODITY BIO-PRODUCTS

Microalgal biomass derived from cultivation can be refined to produce a wide range of biobased products for different applications (e.g., paint, bioplastics, chemical building blocks, food and feed ingredients, and biofuels). The technology for production is still immature, but if developed, it is expected that more market combinations of commodities could be within reach. Collaboration with industry is essential because up to now it has been primarily a technology push in this field, and for further implementation of algae biomass as a feedstock for biobased products, a market pull is required. In order to establish algal biomass as a sustainable feedstock for biofuels and other commodities, market competitiveness needs to be improved and development and implementation need to be facilitated. The long-term challenge is to produce commodities at competing prices. The market demand for products from microalgae is reflected by the current industrial stakeholders involved in microalgae research and development (R&D), mostly global players in the fuel, chemical and food sectors. For a further decrease in production cost, it is necessary to enter markets for commodities that are ready to accept and distribute algae-based products. For this, a series of improvements need to be carried out in different steps along the production chain: strain development, new reactor concepts and new process strategies, use of residual streams and optimization of the integrated biorefinery approach to whole algal biomass fractionation. The commodities should meet market demand in terms of steady supply with sufficient quality, safety and reliability. Research on full production chains at larger scale will enable the evaluation of economics and sustainability. There are opportunities to produce and market whole algal biomass

in the shorter term (2-5 years).

The following issues need to be addressed to facilitate development and implementation of sustainable biobased production strategies for microalgae. First, the economic and sustainability assessment of prospective production chains needs to be established, by harmonizing techno-economic and LCA models and using empirical data coming from pilot/demonstration plant facilities. These models will be used to indicate and prioritize the focus areas for future experimental work in order to make the industrial process cost competitive. Second, new products need to be introduced into the market place, and at least for some novel, biobased products a market pull needs to be established. Third, the necessary stakeholders throughout the value chain need to be connected, by bringing together the necessary academic/ industrial networks that cover the entire chain(algal biomass producers, technology developers and bi-product application end users).

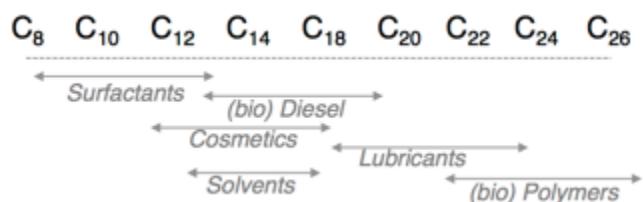
As mentioned already, the biochemical composition of an algal biomass will define and direct the bio-based product options for biorefinery development. As described earlier, algal biomass composition is made up of protein, lipids, carbohydrates, ash, and a range of minor constituents, such as nucleic acids, pigments, etc. Each of these potential bio-products' concentration varies significantly based on the algal strain and physiological inputs to the cultivation system.<sup>65,66,273</sup> The dynamic algal biomass composition indicates distinct accumulation profiles and rates of protein, lipids and carbohydrate biosynthesis during nitrate starvation. Even though compositional shifts are typically associated with longer cultivation times and thus higher production costs, the potential to obtain extra value from different components of the algal biomass needs to be weighed against any extra cultivation or recovery expenses.

An example of a subset of bio-products that can be derived from each of the three major component fractions (lipids, proteins and carbohydrates) is bioplastics. Biobased or biodegradable plastics are a small, but growing, segment of the enormous plastics market. With a large global demand for plastics and a relatively small market share for the bioplastics industry today, at approximately 0.36%, there is clearly room for growth as the switch to renewable sources begins to happen. Renewable carbon sources, such as fermentable sugars, starch, cellulose, lignin, chitosan and protein, can all be used to produce various bio-based plastics.<sup>274,275</sup> Common bioplastics currently being produced or researched today include polylactic acid (PLA), polyhydroxyalkanoates (PHA), cellulose esters, and starch- and protein-based plastics (often plant or animal proteins).<sup>274,275</sup> Several researchers have described blending whole algae as filler material for different types of plastics. For example, whole algae has been mixed in various proportions as filler material for polypropylene,<sup>276</sup> polyvinyl chloride,<sup>277</sup> polyethylene,<sup>278,279</sup> and blends of algae and starch<sup>280</sup> and other polymers have also been studied.<sup>281</sup> Poly-β-hydroxybutyrate (PHB), a form of PHA, is another storage polymer that can be used to produce high-quality biodegradable plastics.<sup>282</sup> PHBs can be produced by cyanobacteria,<sup>283</sup> though examples exist where algae such as *Phaeodactylum tricornutum*<sup>284</sup> and *Chlamydomonas reinhardtii*<sup>285</sup> have also been transformed to produce PHB. Alternatively, plasticizers derived from oleic acid (C18:1), after cracking to form pelargonic (C9) and azelaic acids are feasible and widely commercialized as non-toxic and low migration properties.<sup>286</sup> This group of plasticizers also include epoxidized triglyceride vegetable oils from soybean, linseed, castor and sunflower oils, and algal oils can be immediately added to the suite of bio-based plastics from oils (**Table 6-2**).<sup>286</sup>

### 6.3. MICROALGAE-BASED OLEOCHEMICALS

Another highly valuable class of products ideally suited for using algal lipids is oleochemicals, chemicals derived from oils and fats that are similar to and could potentially replace selected petrochemicals. These oleochemical products include triglycerides, fatty acids, fatty acid methyl esters, fatty alcohols and fatty amines, as well as glycerol, typically derived from high-triglyceride content plant-derived feedstocks, with the level of unsaturation and chain length of the fatty acids

defining the ultimate product properties. For example, cosmetics, pharmaceuticals, nutraceuticals, paints, lubricants, surfactants and polymer additives are all common products that can be derived from products typically present in microalgal oils. The triglyceride fraction, similar to vegetable oils, is the most abundant lipid class in nutrient deprived algae.<sup>31</sup> As with the dynamic algal cell mass composition shifts as described above, the lipid composition can vary dramatically with cultivation conditions. In particular, the chain length distribution of the fatty acids that make up the lipids will help define a suitable oleochemical application, with the approximate acyl-chain ranges for different product classes shown in **Figure 6-1**.



**Figure 6-1:** Illustration of relationship between fatty acid profile (C<sub>8</sub> = 8-carbon fatty acyl chain) and application to different oleochemical industrial chemical

One subcategory of oleochemicals with a large market opportunity is surfactants or surface active agents, broadly defined as organic compounds that can enhance cleaning efficiency, emulsifying, wetting, dispersing, solvency, foaming or defoaming and lubricity of water-based compositions.<sup>287</sup> Typically, surfactant molecules are amphiphilic, i.e., they contain a polar, hydrophilic headgroup and a non-polar, hydrophobic tail, which allows them to form water-soluble micelles. The annual surfactant demand in the United States is estimated to be 1,500,000 tonnes. The largest end use market for surfactants is in household cleaning detergents.<sup>287</sup>

Oil-based epoxies and polyols are important starting materials for making polyurethanes and epoxy resins that exhibit similar characteristics to petrochemical polyurethanes, and such materials have been produced from crude algal oils.<sup>288</sup> Vegetable oils are widely used as plasticizers in the form of epoxidized oils because of their high numbers of carbon-carbon double bonds, which make them a good target for manipulation into useful products.<sup>289,290</sup> Epoxidized oils are natural, nontoxic, non-corrosive and biodegradable substitutes for phthalates and other plasticizers derived by petroleum. Epoxidized oils are also compatible with polyvinylchloride, and as stabilizers for resins to improve flexibility, elasticity and stability of polymers towards heat and UV radiation. Epoxides can also be used as high-temperature lubricants, and the products obtained from ring opening to polyols can be employed as low temperature lubricants.<sup>291,292</sup> The efficiency of these epoxides is directly related to the amount of epoxy groups per molecule, expressed as an oxirane number. Epoxides with higher oxirane values and lower iodine values are considered high-quality plasticizers.<sup>291</sup> Even though epoxidation of algal oils has been demonstrated, the purification of a highly unsaturated feedstock, such as that produced by, e.g., *Nannochloropsis* sp., by selecting specific lipid molecular components or manipulating the feedstock's chemical composition, e.g., level of unsaturation, could allow the influence of composition on the polymer properties or epoxidation effectiveness to be more rigorously tested.

The synthesis of algal lipid-based epoxies and polyols would require precise control of the overall oxirane and hydroxyl functionalities given the high concentration of highly unsaturated double bonds in algal oil. The fatty acid distribution of algal oil from *Chlorella*, *Scenedesmus* and *Nannochloropsis*, relative to more traditional vegetable oil feedstocks for epoxidation, is listed in **Table 6-3**. The double bonds in the higher concentration and more highly unsaturated C20:5 fatty acids in *Nannochloropsis* oils have a higher probability of reacting than the double bonds in the lower concentration and less unsaturated C16:1, C18:1, C18:2, and C18:3 fatty acids.

**Table 6-3:** Fatty acid profile of three algal species compared to typical linseed and soybean oil. Algae fatty acid profiles are obtained from early harvest, high protein algal biomass (NREL, *unpublished data*)<sup>270</sup>

	<i>Scenedesmus</i>	<i>Chlorella</i>	<i>Nannochloropsis</i>	Linseed	Soybean	Fish Oil
<b>C14</b>	1.3	1.1	5.4	0	0.5	7.5
<b>C16</b>	18.4	11.5	15.6	5.4	8.5	18.0
<b>C16:1n9</b>	3.6	0.7	19.4	0	0	0
<b>C18</b>	1.3	1.1	0.3	3.5	4.0	3.6
<b>C18:1n9</b>	5.9	3.5	5.2	19.0	28.2	7.7
<b>C18:2</b>	14.1	11.4	4.1	24.0	49.2	1.2
<b>C18:3</b>	31.5	34.9	0	47.0	7.4	0.3
<b>C20</b>	1.0	0	0	0	0	0.2
<b>C20:4</b>	0	0	6.1	0	0	1.0
<b>C20:5</b>	0	0	38.7	0	0	0.4
<b>C22</b>	1.9	0	0	0	0	0
<b>C22:1n9</b>	1.2	0.8	0	0	0	0.1
<b>C24</b>	1.6	1.1	0	0	0	0

#### 6.4. MICROALGAL CARBOHYDRATE-BIO-PRODUCTS

Carbohydrates and protein form ideal feedstocks for additional bio-product generation. In particular, microalgal carbohydrates present an opportunity for the production of an inexpensive sugar stream for potential upgrading to a variety of fuels, biobased chemicals, or as stand-alone value-added bio-products. Although fuel production from sugars has dominated most of the bio-product literature, the need for a more integrated, economical, and holistic approaches to the use of sustainable energy resources has researchers and industry looking more closely at non-fuel uses for renewable feedstock streams. The US Department of Energy (DOE) publication "Top Value Added Chemicals from Biomass"<sup>293</sup> highlighted the most promising candidates for valorization – primarily from sugars or their derivatives, which makes the sugar-to-bio-product area well-covered in the literature. We focus here on some of the possibilities that exist for upgrading and utilizing microalgal sugars for the production of value-added, viable bio-products.

The carbohydrate fraction of algal feedstock can end up as soluble monomeric components in the aqueous phase after an acid pretreatment process such as in the CAP pathway (see **section 4.1** for a more detailed description) and if so lends itself well to biological fermentation-based upgrading, or it can remain in a polymeric form if an extraction-based approach for lipid-recovery is used.<sup>155,294</sup> Of the monosaccharides, glucose and mannose are the most common and typically dominate the carbohydrate fraction in microalgae.<sup>295</sup> The best-established routes to valorize glucose are through bacterial or fungal fermentation to compounds such as 1,4 diacids, 3-hydroxypropionic acid, itaconic acid, glutamic acid, adipic and muconic acid or sorbitol. Each of these products can in turn become feedstock for subsequent upgrading to final product(s) such as solvents, polyesters, nylon and equivalents, fabrics, inks, paints, carpet fibers, plastics, adhesives, superabsorbent polymers, personal care products (contact lenses), rubber (tires), flavor augmenters, sweeteners, de-icers, and abrasion resistant coatings.<sup>293,296</sup> In brief, beyond the biological upgrading pathways, there are a range of chemical upgrading routes that can be applied to glucose, e.g., chemical dehydration to form 2,5 furandicarboxylic acid (FDCA) and levulinic acid, which can be used in the production of plastic polymers, fabrics, nylon, carpet fibers, fuel ingredients, solvents, polyesters, and herbicides. Similarly, chemical oxidation of glucose to

glucaric acid is feasible, and glucaric acid can be used to produce solvents, nylon equivalents, polyesters, fabrics, plastics, and detergents.<sup>297,298</sup>

A more unusual sugar compound found in some algal species is mannitol, a natural polyol product that can constitute between 2-3% of the cell mass in *Nannochloropsis sp.*,<sup>66,295</sup> the majority of which would end up in the soluble liquor fraction during the conversion process,<sup>5</sup> and thus recovering mannitol as a slipstream might have economical benefits. Similarly, sorbitol can be produced from glucose through chemical or biological hydrogenation and, together with mannitol, enter as a feedstock for bio-product(s) in a range of different applications. Sorbitol differs from mannitol principally by having a different optical rotation.<sup>293,299</sup> It is probable that other pathways exist for using microalgal sugars to produce value added bio-products, however, we have focused mainly on those applications that have been recognized to have the most potential.<sup>293</sup> With the advancement of technology and the intensification of research in this area, an increasing number of upgrading routes are likely to become feasible for the use of glucose and other, more unique, microalgal-derived carbohydrates.

## 6.5. MICROALGAL PROTEIN PRODUCTS

One significant natural use for lipid-extracted algal biomass is as a source of human or animal nutrients and protein. Several reviews have covered algae as sources of protein for human nutrition,<sup>300-306</sup> mostly because protein from algae shows good nutritional characteristics.<sup>301,307,308</sup> A typical amino acid composition of several leading algal species has been reported in the recent literature and supports the finding of good nutritional value for algal biomass protein.<sup>267,309,310</sup> That algae can be grown to contain good protein nutritional value is not the only hurdle to overcome for food and feed uses of algal protein, however. Microalgae are often subjected to nutrient (most often nitrogen) deprivation to induce high lipid production, which can also cause catabolism of native proteins and thus change the amino acid profile and perhaps the nutritional value of the algae. The inverse relationship between algal biomass and lipid and protein productivity needs to be carefully balanced to maximize the efficiency of the entire biorefinery.<sup>311,312</sup> Related work on producing protein products for human nutrition from terrestrial biomass-based biorefineries may also be applicable to algal biorefineries.<sup>267,309,310</sup> The quality of algal protein for human or animal consumption depends on its amino acid composition, the limiting amino acid(s), the palatability of the material, the digestibility of the proteins, the amount of non-protein nitrogen, and the potential for the presence of other anti-nutritional components and the severity of the conditions required to remove them.

Integrating food or feed uses of extracted algae will need to be tested to ensure these processed residues remain a good nutritional source at large scale. The presence of heavy metals from flue gasses, flocculating agents used for dewatering, solvents used to extract algal oil, or acid residuals from algal biomass pretreatment may interfere with protein nutrition. Realistic biorefinery algae samples therefore need to be tested, in addition to laboratory grown samples.<sup>310</sup> The costs of drying or otherwise stabilizing protein needs to be reduced to economical levels to enable feed bio-product transport to support large scale feeding operations. One attempt to value post-extracted algal residue uses comparisons to soybean meal as a basis for a pricing model.<sup>313</sup> Feeding trials of lipid-extracted algal residues have occurred for ruminant cows<sup>314</sup> and for aquaculture.<sup>67,315-318</sup>

An approach to deaminate amino acids before converting the remaining carbon backbones to fuels and chemicals also has been proposed in the literature,<sup>183,186</sup> which allows ammonia to be recycled and used as fertilizer. When applied to algal biomass or algal protein-enriched residues, this also allows for the harvest of fast-growing, protein-rich algae without the need for stress conditions to induce lipid production, along with slower growth. Lan and Liao reviewed some of the engineering strategies that can be used to channel microbial products to higher alcohols, such as n-butanol and isobutanol.<sup>184</sup> The transformation of *E. coli* was carried out with the aim of being able to

convert proteins into higher alcohols.<sup>186</sup> Recently this approach has been applied to algal biomass-derived proteins, with the successful production of a mixed-alcohol stream, at over 75% efficiency, with composition consistent with the originating amino acid composition.<sup>319</sup>

## 6.6. RESEARCH PROGRAM FRAMEWORK

It is important in the development of research programs and commercialization targets to view an algal biorefinery as an integrated platform.<sup>3</sup> The production of bio-products and biofuels are intimately connected and the processing scenarios will need to be customized for each pathway pursued. Three features are essential in future research activities to commercialize the concept of an algal biorefinery: **1) Whole chain approach:** Integrating the full production chain. This comprehensive research comprises the study of cellular processes, strain improvement, cultivation optimization, scale-up, biorefinery, product development, chain analysis and design analysis. It allows a continuous feedback at all levels, as well as to evaluate the economics and sustainability of the whole process. For example, a biorefinery with different product streams will be highly strain and cultivation system dependent (e.g. producing high-quality omega-3-fatty acids from a *Nannochloropsis* sp.) and thus integrating and customizing the cultivation, harvesting, production and recovery of different bio-products is necessary to provide a commercial route to bio-products. **2) Multidisciplinary approach:** Different expertise areas need to be integrated. Both biological and engineering aspects of cultivation and biorefinery of algae must be included. A multidisciplinary team combines specialist knowledge with practical insight, which unifies laboratory and pilot scale research. **3) Bridge from fundamental research to applications:** Collaborations between research institutions, industry and governments will connect R&D to marketable products and business opportunities. Technologies need to be developed both on a lab and pilot scale and move from initial idea to the production processes that deliver competitive and innovative products for industrial partners.

## 6.7. CONCLUSIONS

Algal biomass-based bio-products can provide the critically needed revenue to aid with the economics of an algae-based biorefinery installation. Some of the bio-products discussed here are reviewed as bio-products to a biofuel production pathway in a biorefinery setting based on their applications and their concentration in the algal biomass. As such, a biorefinery approach appears essential to realize the full value of algal biomass and is able to generate different product streams from the original biomass with respective commensurate market sizes. Each biorefinery scenario must be evaluated in the context of a defined conversion pathway (based on the recently demonstrated pretreatment fractionation approach that leaves lipids extractable, solubilized carbohydrates and protein fractions accessible for respective bio-product recovery and/or upgrading). Alternative scenarios based on different fractionation schemes can be similarly approached and evaluated. The highly complex and specific nature of product separations and the multiple hypothetical bio-product options that exist need to be prioritized as research topics to provide the maximum value for ongoing work. For each of the fractions we have highlighted, there are a subset of products and pathways to experimentally demonstrate the valorization approaches discussed in this report. Perhaps the technical area closest to being further developed and experimentally demonstrated is the production of oleochemical products from algal oils. As with any bio-product, the selling price of each component has to be assumed according to the market application. The overall value of the algal biomass can be calculated, allocating different components to several end uses. For each scenario, the most profitable application of each component of the algal biomass should be selected in order to maximize the overall economic potential of the biorefinery.

## **7. Techno-economic Analysis of Current Pathways to Biofuels and Bioproducts**

Techno-economic analysis (TEA) is an essential aspect of any bioenergy producing process or biorefinery operation. In particular, TEAs are a key tool for assessing economic feasibility and commercial viability based on defined process conditions. Such analyses, as simplistically represented by **Equation 7-1**, allow technical progress or absolute performance to be quantified as cost per unit product, in most cases the ultimate fuel produced, or in terms of installed capital and operating costs (including variable and fixed operating costs). At the same time the analyses also enable the valuations of potential bio-products to be taken into account. The equation represents a break-even cost of production. If profit needs to be included, there would be two additional items, implicitly tied to the cost of capital; return on capital investment and income taxes. A similar calculation and cost quantification can be carried out for any potential process or biorefinery permutation, however, since most of the literature reports on TEA are focused on a biofuel production scenario using microalgae, cultivated in a photobioreactor or an open pond, this will also be the focus here. In particular, a number of TEA have been developed for both biological and thermochemical pathways for converting algal biomass to fuels and chemicals (including highly valuable nutraceutical fatty acids), to assist in realizing the goals of increasing bioenergy production from algae.<sup>2,5,6,17,165,193,320-329</sup> However, for algae-derived processes, the immaturity of technologies has dramatically impacted the fidelity of process modeling which serves as the backbone for economic assessment and reduces the accuracy of the calculated costs of the ultimate fuel produced.

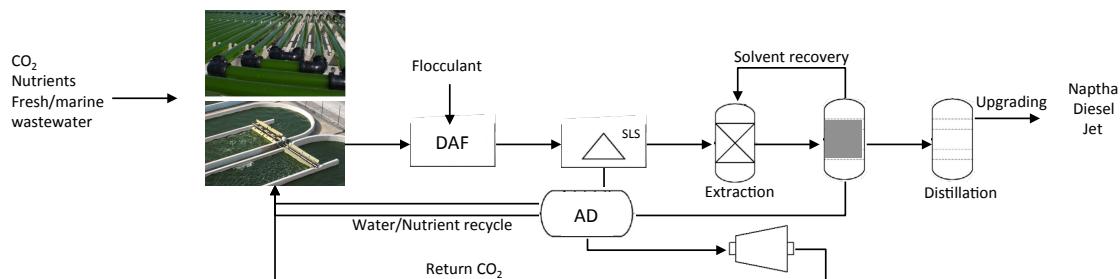
$$C_{production} = \sum_i C_{capital,i} + \left( \sum_j C_{operating,j} - \sum_k V_{bio-products,k} \right)$$

**Equation 7-1:** General and simplistic principle of cost calculations for TEA, where  $C_{production}$  = Total cost of production per unit product,  $C_{capital,i}$  = Capital cost (installed), including direct and indirect capital costs, of sub-category i.  $C_{operating,j}$  = Operating cost of sub-category j.  $V_{bio-products,k}$  = Value of k<sup>th</sup> co-product, capital and operating costs are time dependent and assumed to be over the lifetime of the facility ( $C_{capital}$ ) or, for  $C_{operating}$  and  $V_{bio-products}$  on an annual basis and dependent on the yield of the products

These conceptual TEAs of target or example processes provide a detailed basis for understanding the potential of various conversion technologies and help identify specific technical barriers where research and development progress could potentially lead to significant cost improvements. Because the overall final cost estimations are highly dependent on i) biological parameters underpinning cultivation performance and biomass composition, which in turn are the summative result of meteorological and geographical influences as well as chemical properties and conversion reactor engineering, and ii) local economic conditions, including engineering, construction and labor costs, as well as policy and financial incentives, the absolute numbers provided by a TEA may not translate towards a global accounting of algal biofuels. What we describe here is the potential to produce a highly modular TEA that allows for a case-by-case comparison of inputs and their performance and cost impacts. Consistent assumptions for items such as plant lifetimes, rates of return, and other factors need to be used in all cases if the various conversion pathways are to be assessed on a comparative basis. While the clearest and simplest question to ask at this time is "What is the cost of algal biofuels?" the answer is highly complex and requires considerable background information and many assumptions. The above discussions around algae cultivation, algal biomass composition and the integration of processing pathway(s) with the recovery of fuels and bio-products are necessary to understand the complexity of this process. Techno-economic assessments and the ultimate estimated cost of the fuel product are inherently linked to each of these parameters.

## 7.1. REVIEW OF ASSUMPTIONS AND SENSITIVITIES AROUND TEA

TEA tends to be complex, not only because of the many inputs, outputs, and inter-relationships that are involved, but also because algal product manufacturing processes vary widely and continue to be developed and improved. In the following discussion around TEA of algae-based fuels, it is necessary to keep the different possible fuel pathways in consideration, i.e., for example, both biochemical and thermochemical routes have been described and used as the basis of detailed TEA reports supported by the US Department of Energy (DOE).<sup>2,5,330</sup> To generate input data for TEA, a highly flexible framework in the form of robust process engineering models is needed that anticipates both existing and future pathways for algae-based production of food/feed, fuel, and higher value chemicals.<sup>320</sup> This framework should be established by describing in a comprehensive manner the many inputs and outputs that occur, or can occur, in algae production and upgrading engineering operations, in addition to identifying the methodologies required to accurately measure these data. While the algal industry continues to grow, greater harmonization between life cycle analysis (LCA) and TEA methods is necessary, as current evaluations of industry processes often require extrapolating laboratory data and are affected by differences between prospective production pathways and inconsistencies in how system boundaries are defined. Similarly, the legislative landscape in different countries can impact the rate of adoption of renewable fuels (or chemicals), and any applicable credits may be accounted for (though often credits or policy incentives are not included in the baseline TEA methods). For example, the EU requires certain sustainability criteria to be met and liquid fuels to have a sustainability certification prior to their implementation into existing infrastructure. Similarly, as part of the US Renewable Fuel Standard, there are defined sustainability and GHG emission criteria that need to be met by any of the proposed new renewable fuels produced. There is currently no consistent or standardized reporting on TEA approaches.<sup>25,320,323</sup> A generic process is shown in **Figure 7-1**, which illustrates some of the main aspects (with a number of permutations from this scenario discussed in previous sections) that are present in a conversion/extraction process and that should be captured in any technoeconomic process simulation model. A comprehensive list of parameters of critical importance in the establishment of a robust TEA for an algae process is shown in **Appendix A**. The impact of different process technology sequences is described in great detail in a recently published report, which provides detailed energy balances and cost breakdowns for 10 different case-study scenarios, ranging from lipid extraction through HTL for whole algal biomass conversion, all based on open pond algal production carried out at the 100 ha scale.<sup>324</sup> The authors conclude that there is a positive energy return on most of the processes studied, however the total estimated production costs still exceed current commodity prices for commercial fuels now being distributed. The inclusion of high value bio-products in these scenarios (with the exception of HTL conversion, which due to its destructive nature eliminates most bio-product streams) is envisioned to reduce the overall calculated cost of fuel production and thereby render a subset of the processes economically feasible.



**Figure 7-1:** Overview of a generic algae production and conversion/extraction process; algae are grown in open ponds, or photobioreactors, or hybrid systems after which the cell biomass is harvested by for example flocculation and dissolved air flotation (DAF), followed by centrifugation and processed through to the extraction of lipids, which can be further upgraded via hydrotreating to renewable diesel, jet fuel, after which the residue is transferred to anaerobic digestion (AD)

where the biogas produced is used for additional power generation for the entire plant<sup>165</sup>

A study published in 2011 established a framework for TEA and provided initial notions about the need to harmonize process assumptions, concluding that a consistent set of definitions were needed to be able to effectively compare cost information (**Appendix A**).<sup>323</sup> This analysis also identified areas for further improvement, such in consistency of units of measure and cost categorization. For example, units of measure used in each source investigated were disparate enough to require great care to harmonize volumetric or mass basis results to be able to accurately compare cost information for different scenarios. While some analyses indicate that the estimated cost of algal biomass production is attractive, converting results to a cost based on lipid production may provide a different outlook. Hence, the extractable oil content still represents one of the most important (though certainly not the sole) metric in studying economic feasibility of algal biofuel production. At the same time, the measurement of algal lipid contents remains a key challenge in research laboratories. Many of the methods used by different research groups (especially those based on extraction and gravimetric analysis) give rise to product streams of uncertain purity and therefore even experimentally generated productivity numbers must be considered with caution.

In response to the challenges and differences reported throughout the literature, an initiative was launched by the DOE to align the simulation models being used for feasibility assessment of algal biofuel production. This initiative brought together modeling partners from the National Renewable Energy Laboratory (NREL), Argonne National Laboratory (ANL), and Pacific Northwest National Laboratory (PNNL) to harmonize their conceptual algal biorefinery models around TEA, life-cycle assessment (LCA), and resource assessment (RA), respectively, such that the results from each model carry the same implications as they are all based on consistent inputs and assumptions. This effort began validating and improving upon core modeling assumptions included a workshop, which served a vetting process for the respective collaborators' models, i.e., modeling approaches and assumptions were critiqued when they presented the details of their respective models to an expert stakeholders group comprised of industry, academia, and other national laboratory participants. This ultimately resulted in the publication of a algal harmonization report<sup>25</sup> documenting model details and the resulting near-term cost, sustainability, and resource implications for producing 5 billion gallons per year (BGY) of renewable diesel at the U.S. national scale spread across a large consortium of individual unit farms.<sup>23</sup> While this harmonization effort represented an important step forward in better understanding plausible processing details and costs (**Table 7-1**), including cultivation and harvesting steps, the study was limited by: 1) being based on a single set of design and cost inputs, largely obtained from available public domain literature, which at the time remained scarce; and 2) focusing on what economics would look like "today" (in 2012) based on modeled estimates for benchmark productivity performance for current productivity performance ( $\sim 13 \text{ g m}^{-2} \text{ day}^{-1}$ ) and dewatering technologies (leveraging from industrial practices for wastewater processing), but largely avoiding projections for future performance and cost targets.

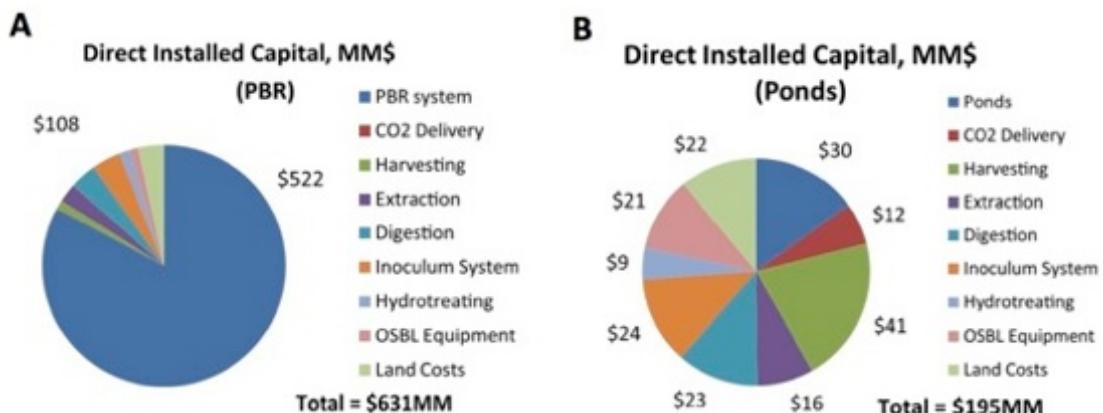
**Table 7-1:** Final harmonization assumptions for base-case scenario process inputs (from 2012).<sup>25</sup>

Metric	Before Harmonization		After Harmonization	
	LCA	TEA	LCA	TEA
Productivity, g m <sup>-2</sup> day <sup>-1</sup>	25	25	per site from RA	
Water demand, cm/d	0.6	0.3	per site from RA	
Lipid fraction, wt%	25%	25%	25%	25%
Net harvesting efficiency <sup>[1]</sup>	85.50%	99%	95%	95%
Net harvesting efficiency <sup>[2]</sup>	85.50%	85.50%	85.50%	85.50%
RD yield from raw oil, wt% <sup>[3]</sup>	85%	78%	85%	78%
Nitrogen (N) recovery to culture, net	0.76	0.75	0.76	0.76
Phosphorus (P) recover to culture, net	0.5	0.5	0.5	0.5
Net N demand, mg/g-algae	14	32	19.5	20.0
Net P demand, mg/g-algae	6.3	6.4	4.1	4.1
Pond mixing, KWh/ha/d	48	48	48	48
Recycle pump, KWh/L	4.80E-05	1.95E-05	2.50E-05	2.50E-05
Water pump from off-site, KWh/L	4.80E-05	3.00E-04	1.23E-05	1.23E-05
DAF, output solids content	10 wt%	11 wt%	12 wt%	13 wt%
Centrifuge power, KWh/g-out	5.77E-05	1.01E-05	1.93E-05	1.94E-05
Solvent extraction heat, KWh/kg-oil	1.39	4.48	3.09	3.15
Solvent extraction electricity, KWh/kg-oil	0.54	0.05	0.069	0.069
AD heat demand, KWh/kg-TS	0.54	NA	0.22	0.22
AD electricity demand, KWh/kg-TS	0.136	0.027	0.085	0.085
AD yield, L-CH <sub>4</sub> /g-TS	0.3	0.333	0.3	0.3
Gross Electricity demand (including all CO <sub>2</sub> ), KWh/kg-oil <sup>[4]</sup>	5.7	3.7	5.1	4.9
Net electriciy imported, KWh/kg-oil <sup>[5]</sup>	1.4	-1.8	1.32	1.16

<sup>[1]</sup> Algae that are not retained during dewatering, but are ultimately returned to the pond with the supernatant are not counted as loss. <sup>[2]</sup> Product of disruption efficiency (90%) and lipid recovery efficiency (95%). <sup>[3]</sup> Not harmonized to facilitate comparison with previous LCA and TEA studies of other biofuels, as explained above. <sup>[4]</sup> Gross facility power demand, including off-site and recycle CO<sub>2</sub> considerations, for 25 g m<sup>-2</sup> day<sup>-1</sup>, 25 wt% lipids. CHP power generation is excluded. <sup>[5]</sup> Net facility power balance, including CHP power generation. Positive value denotes net power import; negative value denotes net power export.

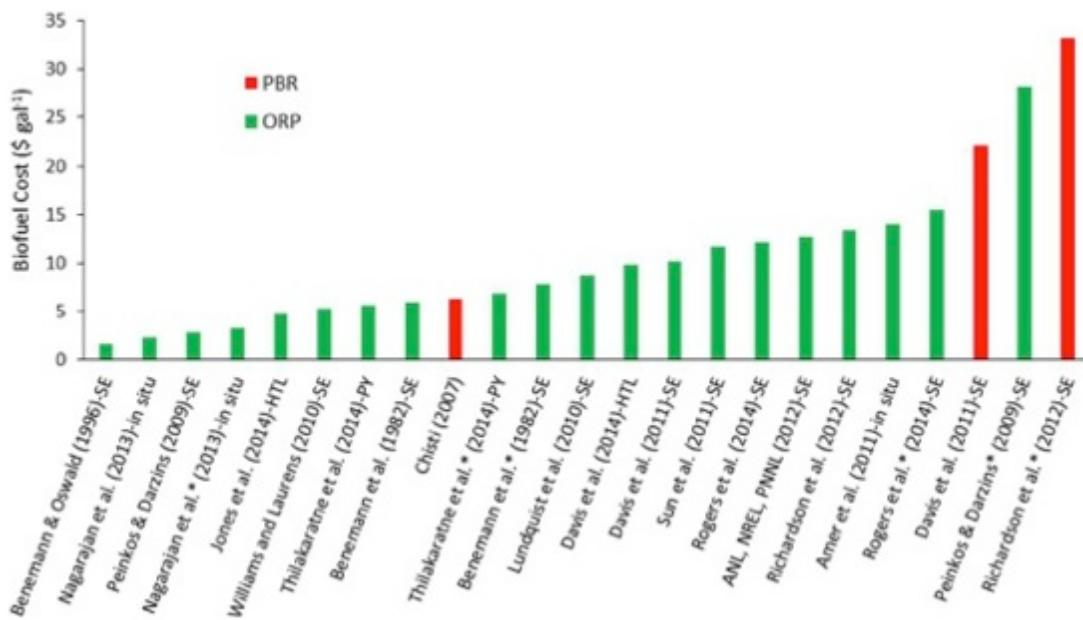
The cited literature shows that since the publication of the previous IEA Bioenergy Task 39 algae report in 2010, significant progress has been made in increasing the rigor and detail that is included in TEA of algae systems. Typically, a TEA is supporting a particular processing pathway, which is simulated using software programs like Aspen Plus. Because the basis of TEA is both technical and financial, an in depth understanding of the process is required, but also access to experimental data to aid in understanding the accuracy of the simulated models. Early on, little experimental data at scale was available, and a lot of scale up information was gained from early reports such as those by Benemann and Oswald.<sup>101</sup> One especially highly cited report used the available data and established a thorough TEA framework, thereby providing a highly transparent overview of inputs into an algal production oriented TEA.<sup>165</sup> While the results of a prospective TEA should generally not be interpreted to reflect absolute cost estimates (this report is outdated both with respect to the technology modeled as well as with the underlying cost assumptions), the type of

analysis and the overview it provides of what parameters are most impactful on the processes techno-economic performance can serve as the basis for future developments. For example, an investigation of cost sensitivity was carried out to identify the highest impact factors over the entire process, which showed algal strain-specific biological parameters, specifically lipid content and growth rate, were the model inputs with the largest cost sensitivity.<sup>165</sup> However, several other parameters more related to operational/engineering aspects were also identified that could be improved through adjustments to the process. Of the additional sensitivity parameters examined, those with the strongest cost impact were operating days per year and degree of nutrient recycle.<sup>165</sup> It was concluded then that there is room for substantial improvement in algal oil production economics for both the open pond and PBR systems, assuming a strain can be identified or engineered to sustain a high growth rate while also maintaining a high lipid content. The majority of the cost improvement accrued from reductions in capital equipment (**Figure 7-2**). In particular, the high PBR installed capital cost estimate, which overall is threefold higher than the installed cost of an open pond, with >80% of the higher PBR cost driven by the actual tubes that make up the PBR systems. Some of this extra cost could be offset through increased productivity as well as reduced algal culture contamination-induced losses anticipated in PBR systems. However, the overall cost for fuel production based on PBRs is still anticipated to be higher than for open pond systems.<sup>165,321,331</sup> A detailed dataset provided by Davis et al. was used as the basis for a number of scenario analyses, including an assessment of the financial risk to commercial deployment of open ponds or closed PBR systems for cultivating photosynthetic algae.



**Figure 7-2:** Direct installed capital cost allocation for PBR (A) and open pond (B) cultivation. Data based on sizing the facility of 4800 acres (1942 ha) to producing 10 MM gal yr<sup>-1</sup> (38 MM L yr<sup>-1</sup>) based on 2011 technical and economic assumptions.<sup>165</sup>

A comprehensive survey of available TEA reports for algal biofuels was recently published and can serve as a framework for assessing the uncertainty associated with algal process TEAs (**Figure 7-3**).<sup>320</sup> Current TEA results for algae-based production reported in the literature range widely, projecting that algal production routes can be threefold cheaper to an order of magnitude more expensive than conventional diesel (**Figure 7-3**).<sup>101,322</sup> A major contributor to these large differences is the different system boundary or processing pathway assumed in the calculations. For example, the contribution of CO<sub>2</sub> to the operation costs can be as high as 50% in some TEA models.<sup>332,333</sup> Another factor is the level of maturity assumed, with some modeling considering current systems and others examining future potential. Finally, there is also uncertainty about the large-scale algal biomass productivity that can be realized for microalgae, and different assumptions about this represent the functional unit for such assessments and production pathway assumptions. Further differences in model financial assumptions as well as in the level of technical and engineering rigor contribute to the variability in end results obtained using different models.



**Figure 7-3:** Techno-economic assessment results color-coded by growth platform and conversion technology. Studies span reported approaches, including SE-solvent extraction, HTL-hydrothermal liquefaction, PY-pyrolysis. \*Denotes high value reported in the study. Some studies report current costs while others estimate future costs based on advancements in sub-processing technologies. Costs are reported in 2014 dollars based on an inflation rate of 2.4%.<sup>320</sup>

## 7.2. TEA OF ALGAL BIOMASS PRODUCTION

Typically, algal biomass production and conversion TEAs are integrated into an overall process TEA model, which supports an overall integrated process and allows for financial, bio-product and nutrient recycle credits to be applied across the value chain. The algal biomass production portion, in the NREL TEA studies accounts for between 65% and 85% of the overall total cost of production of the final fuel.<sup>5</sup> In light of the complexity and wide range of different approaches to process integration reported in the literature, in recent years a case was made to separate the 'upstream' algal biomass production cost estimates from the 'downstream' conversion process costs. We want to note here that the costs used in the highlighted reports are to be used as examples only.

For the models supported by the DOE Bioenergy Technologies Office, a similar protocol is used to estimate the overall cost of production. The details presented here serve as an example for a rigorous TEA study. The material and energy balance data from the computational simulation quantify total stream flows are used to assist in determining the number and size of required capital equipment items. As process conditions and flowrates change, baseline equipment costs are automatically adjusted using a scaling factor. These baseline costs usually come from vendor quotes but sometimes they are obtained from existing established cost databases. Once equipment costs are determined, direct and indirect overhead cost factors (e.g., installation costs and project contingency) are applied to determine the total capital investment (TCI). The TCI and the plant's operating expenses (also developed using modeled process flowrates) are used in a discounted cash flow rate of return analysis to determine a plant-gate price for refined renewable diesel blendstock (RDB) for a given discount rate. This plant-gate price is also referred to as the minimum fuel selling price (MFSP, in \$/gallon). The product of the analysis described here is a TEA model that reasonably estimates a product price for a pre-commercial process. The resultant MFSP is unique for the set of process conditions simulated, and it should be emphasized that a certain amount of uncertainty always exists around the chosen conditions, as well as around the assumptions made about capital equipment and raw material costs. Without a detailed understanding of the basis behind it, the absolute computed MFSP carries a risk of being taken out of context. While an MFSP value can be used to assess the marketplace competitiveness of a given

process, it is best suited for comparing technological variations against one another or for performing sensitivity analyses to identify where economic or process performance improvements are possible or needed.<sup>2,165,320</sup>

The conclusions from the most recent modeling effort to estimate the cost of algal biomass production in open ponds, in particular in the context of a fuel-producing algae production-based biorefinery, are a set of numbers for algal biomass selling price that are inherently linked to the scale of production and the growth rate of the particular strain under investigation (*Scenedesmus acutus*, assumed growth rate of 25 g m<sup>-2</sup> day<sup>-1</sup> and 27% FAME lipid content). In summary, an average cost of \$0.54/kg (\$491/ton ash-free dry weight biomass) was estimated (all data based on 2011\$).<sup>2</sup> The modeled facility consists of approximately 2,020 ha (5,000 acres) of production pond cultivation area, with a total facility footprint of 3,075 ha (7,600 acres). This facility is assumed to achieve an annual algal biomass product yield (dry basis) of 14 T ha<sup>-1</sup> y<sup>-1</sup> (38 U.S. ton/ acre/year, which is consistent with a cultivation productivity target of 25 g m<sup>-2</sup> day<sup>-1</sup> as an annual average across varying seasonal rates). Based on the design assumptions, project costs, and financing, the minimum algal biomass selling price (MBSP) follows a trend inversely proportional to individual pond size, varying from \$0.63/kg-\$0.72/kg (\$576-\$649/ton) algae feedstock (ash-free dry weight (AFDW) basis) (average \$0.67/kg (\$612/ton) in 2011 dollars) of dewatered algal biomass for production in "small" 0.8 ha (2-acre) pond designs, to \$0.50/kg-\$0.60/kg (\$452-\$545/ton) (average \$0.55/kg (\$491/ton)) for production in "medium" 4 ha (10-acre) pond designs. This result suggests that although algal cultivation ponds larger than approximately 0.8-1.2 ha (2-3 acres) in size do not currently exist commercially today (with the exception of wastewater treatment ponds that approach 4 ha (10 acre) in size), moving toward larger pond sizes on the order of 4 ha (10 acres) is key to reduce biomass costs towards viable levels, as required for subsequent conversion to produce a commodity fuel product at a market competitive price.<sup>2</sup>

This most recent analysis reinforces the importance of the finding that algal production economics are influenced strongly by achievable cultivation productivity, with particularly dramatic penalties on minimum (algal) biomass selling price (MBSP) if productivity is lower than the target 25 g m<sup>-2</sup> day<sup>-1</sup> annual average. However, this trade-off must be balanced carefully against associated nutrient requirements, given known linkages between high-nutrient (particularly nitrogen) feeding strategies and increased algal biomass productivity, where a sensitivity analysis found that if nutrient inputs and resultant algal biomass composition were to be adjusted from the nutrient-limited mid-lipid algal biomass baseline to a nutrient-replete low-lipid (high-protein) algal biomass assumption, the resulting nutrient costs increase to such a level that it would require more than a 40% improvement in productivity to ultimately lower the MBSP. Recycling nutrients stored in the algal biomass back to the production ponds is critical for both controlling costs and minimizing the greenhouse gas footprint of an algal production process. However, to ensure applicability of this effort's outputs to any downstream conversion-processing pathway, no credit is assumed for such recycles (instead, any such credit may be applied on the downstream conversion process to reduce final fuel/product costs). If recycle credits were accounted for here instead, the cost impacts attributed to compositional variations may be less pronounced. Additionally, this work also showed that it is critical to avoid the use of fully lined ponds to keep pond costs reasonable, focusing on situating pond facilities in locations with soils having high native clay contents such that relatively expensive plastic liners are only required to cover small targeted areas of the ponds for erosion control (in the base case scenarios, liners only covered 2-25% of total pond area depending on specific pond design). If instead ponds were fully lined across the full 2024 ha (5,000 acres) of cultivation area, MBSP costs would increase on average more than \$0.14/kg (\$125/ton) for the 4 ha (10 acre) pond design scenarios relative to the costs indicated above. Finally, this report<sup>2</sup> also includes a high-level discussion on cost tradeoffs and logistical issues between sourcing CO<sub>2</sub> via carbon capture from power plant flue gases versus direct use of bulk flue gases, with concentrated CO<sub>2</sub> costs adding significantly to MBSP; however, use of flue gases is challenged by substantial logistical and practicality constraints for a facility of this size.

### **7.3. TEA CASE STUDY FOR OPEN POND CULTIVATION AND BIOCHEMICAL AND THERMOCHEMICAL CONVERSION TO FUELS**

To highlight one example of a conversion pathway TEA, an algal lipid extraction route is chosen here to provide background information and run through the process of describing the technical and economical features of the analysis of a biofuels production system.<sup>5,156</sup> This pathway is one of two main pathways being pursued by the DOE for producing liquid biofuels from algae (the other pathway is the HTL route based on whole algal biomass conversion). These two pathways have been adopted by the DOE as baseline cases for technology and process optimization towards future design cases that improve the cost basis for production of algae-based fuels, as shown through full TEA and life cycle analysis (LCA) and for which comparative sustainability assessment reports are available in the peer-reviewed literature.<sup>334,335</sup>

The overarching process design described earlier for an lipid extraction and upgrading case study,<sup>5</sup> converts algal biomass, delivered from upstream cultivation and dewatering, to ethanol, RDB, and minor bio-products, using dilute-acid pretreatment, fermentation, lipid extraction, and hydrotreatment. Additional areas, e.g., anaerobic digestion of spent algal residues, combined heat and power generation, and utilities are also included in the design, and so are detailed material and energy balances and capital and operating costs for this baseline process. This case study techno-economic model provides a production cost for the fuel products that can be used to gauge the techno-economic potential and to quantify critical cost drivers. This study estimated an overall RDB production cost of \$4.35/gallon gasoline equivalent (gge) (\$1.15/L)<sup>5</sup> and also identified a number of remaining technology gaps and uncertainties (all 2011\$). Further research and development to reduce costs will be needed for this pathway to be able to achieve fuel production costs that meet the US target of \$3/gge. Overall algal biomass feedstock cost was identified as one major determinant of final fuel production cost. At the time this study was published algal feedstock cost was assumed to be \$0.47/kg (\$430/ton). However, since then, a new report on the economics of algal biomass production pathway was published<sup>2</sup> and the revised cost estimates are higher (\$0.54/kg or \$491/ton).

Even though thermochemical conversion technologies for processing algal biomass are at an early stage of development, attempts have been made to quantify their economic potential. Based on limited data now available and a high level of interest in algae-based fuels production, a number of studies including a TEA analysis and several LCA analyses have been undertaken that evaluate the techno-economics and sustainability attributes of a hydrothermal processing option.<sup>6,119,320</sup> As stated in the TEA study, the cost of the algal biomass feedstock is significant<sup>5,6</sup> and as such is the major determinant of the ultimate fuel cost. While values being used at this point are speculative and should be considered simply as a placeholder, the algal biomass cost of production needs to be better determined to inform future process TEAs. A comparative analysis of the HTL option versus conventional algae oil extraction and biodiesel production based on early literature data suggests that HTL uses biomass more efficiently but has comparatively higher emissions and nutrient requirements, which is discussed in more detail in previously published reports.<sup>335,336</sup>

### **7.4. EXAMPLE STUDIES OF BIOREFINERY ECONOMIC ASSESSMENTS**

Previously, reports have appeared in the literature on the generation and exploitation of bio-products from algae- and terrestrial-biomass-based biorefineries.<sup>149,150,185,266,337-339</sup> These reports are often broad and not tied into a particular conversion pathway, rather describing a generic process that is agnostic to a particular conversion approach that is based on hypothetical assumptions about biomass composition and assumes each of the major feedstock fractions can be separately recovered at high yield.<sup>149</sup> In order to assess the impact of potential bio-product development as part of any bioenergy pathway, it is important to keep the discussion relevant to a fractionation approach that has proven to be economically superior to a harmonized baseline biofuels production process based on lipid extraction and anaerobic digestion of the residual extracted biomass.<sup>5,155</sup> Any increases in the value of the biomass, by the summation of the value

and compositional fractions and their resultant potential derivative products of the individual components, as discussed in the next sections, will aid with the overall biorefinery economics.

Considering the technoeconomic analysis assumptions for photobioreactors, different scenarios<sup>118,340</sup> were simulated in order to have estimations on the current status of phototropic microalgae production. The forecast of expected improvements over the coming years, to achieve a better performance of the system, allows assessment of different projections about what can plausibly be achieved in the near future. The same approach as carried out for cultivation system comparisons should be implemented to compare prospective biorefinery applications. Even though microalgaebased biorefineries are still in an early stage of development compared with microalgal cultivation per se, as a starting point we can make use of existing process modeling programs with incorporated databases now being used in the food, biotechnology and chemical industries. The challenge here is to have sufficiently clear knowledge about the viability of using each of the above described technologies for microalgae, as well as in incorporating novel technologies for which information is scant.

The overall turnover coming from the exploitation of the different fractions of the algal biomass depends on the end use(s) of the product(s). The market analysis can be conducted looking at five different market scenarios according to the biomass value pyramid: biofuel(s), chemical/technical product(s), food/feed product(s), and specialty products for food, cosmetics and pharmaceutical industries. An overview of the potential markets and products that can be derived from microalgal biomass is discussed in the literature,<sup>149,150,265</sup> with many more potential products being discovered in ongoing research projects.

## **7.5. TEA CASE STUDY OF PHOTOBIOREACTOR CULTIVATION FOR BIOREFINERY APPLICATIONS**

With the discussion of economical viability of algae-derived products increasingly focusing in on higher value bio-products and biorefinery applications, the value of bringing in PBRs for demonstration and deployment of technologies becomes sensible. Projections on algal biomass production costs in PBR systems have been carried out.<sup>321</sup> These techno-economic models need to be continuously revisited and supported by new experimental results being obtained and verified at pilot and preferably also at demonstration scales. In addition, costs for the balance of the biorefinery need to be included. Up to now there has been a lack of information available regarding biorefinery costs, and more work needs to be done in this direction. Biorefinery and production costs need to be combined and compared for different business scenarios. Market values result from different combinations of end products from microalgae: biofuel, bulk chemicals, food/feed as commodities, food specialties, skin care products/cosmetics and pharmaceutical products. Estimations of algal biomass production costs can be refined for algae cultivation using currently installed reactor systems. Projections of different scenarios allow the effects of key variables to be compared, such as location of the facility or type of cultivation system. The modification of other specific parameters for a certain scenario, like performance, flow and aeration in the system, can be used to perform sensitivity analyses. As result, such modeling tools can be used to show the most suitable location and system for algae production, as well as to highlight the main cost drivers. In addition, these techno-economic models also provide the basis for deriving further information about energy consumption.

As part of an evaluation of technology development at AlgaePARC in the Netherlands, six different locations around the world were compared as potential locations for an algal biomass production facility: The Netherlands, Saudi Arabia, Canary Islands, Turkish Riviera, Southern Spain and Curaçao. The effect of location results in different weather conditions (light, temperature and humidity), electricity and labor costs, taxes or number of operational days per year. The projection included four state-of-the-art cultivation systems now operating at AlgaePARC: open raceway ponds, horizontal tubular PBR reactors, vertically stacked tubular PBR reactors and flat panel PBR

reactors (**Figure 7-4**).<sup>103,118</sup> Empirical data obtained from these outdoor systems at AlgaePARC locations has been used for this analysis, and the effect of the different performances of these different cultivation systems (**Table 7-2**) is reflected in the results. Productivity data achieved in the south of Spain are approximately 27 T ha<sup>-1</sup> y<sup>-1</sup> which corresponds to 7.4 g m<sup>-2</sup> day<sup>-1</sup> for open raceway ponds, whereas closed photobioreactors achieved between 34 and 61 T ha<sup>-1</sup> y<sup>-1</sup>, which corresponds to between 9.3 and 17 g m<sup>-2</sup> day<sup>-1</sup>.<sup>3</sup> These data underpin the results presented in **Figure 7.6** and can explain some, not all, of the discrepancies between the reported technoeconomical considerations.



**Figure 7-4:** The four types of algal cultivation reactor systems being investigated at AlgaePARC installation at Wageningen UR, The Netherlands: (left to right) open raceway pond, horizontal tubular PBR reactor, vertical stacked tubular PBR reactor and flat panel PBR reactor).

**Table 7-2:** Experimental data used in the study; obtained outdoors at AlgaePARC in pilot plant production systems.<sup>103</sup>

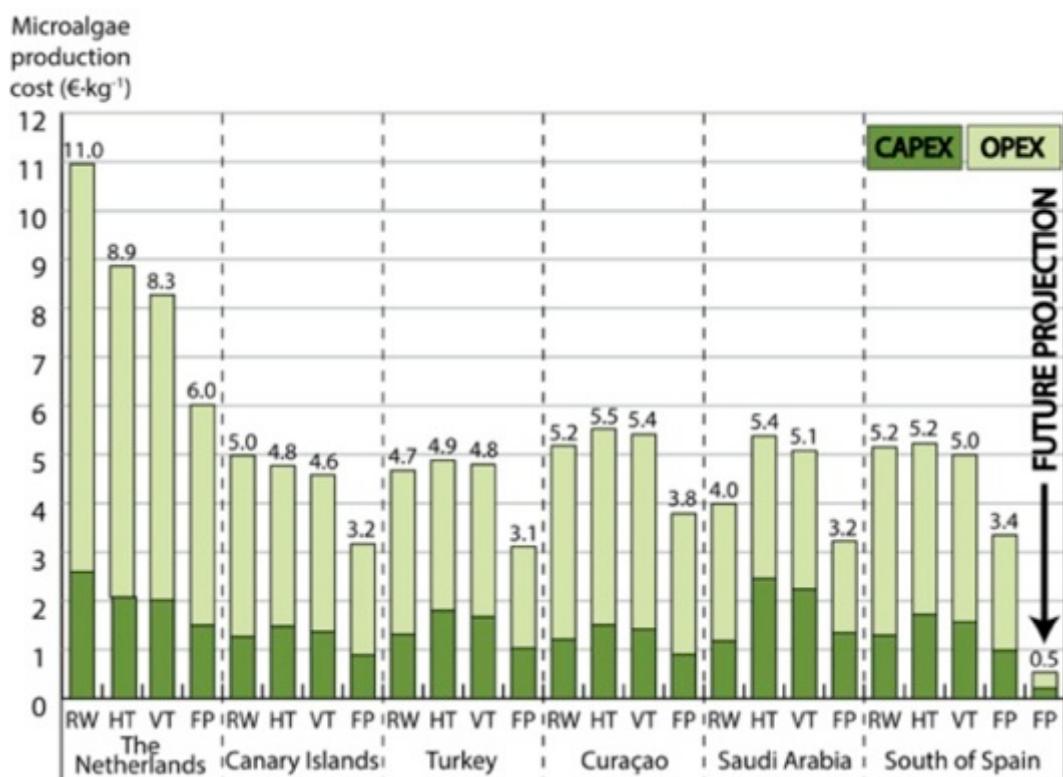
Reactor	Raceway pond	Horizontal tubular	Vertical stacked tubular	Flat panels
<b>Photosynthetic efficiency (% sunlight)</b>	1.2	1.5	2.4	2.7
<b>Daily dilution (%)</b>	16	25	27	27
<b>Days</b>	24	36	36	36
<b>Flow of culture (m·s<sup>-1</sup>)</b>	0.25	0.45	0.45	-
<b>Aeration (vvm)</b>	-	-	-	0.3-0.6

The inputs and outputs of TEA models are similar to the inputs into the models described above: location, including light intensity, electricity costs, taxes and labor, cultivation system, empirical growth data, and specific parameters, such as culture temperature, daily dilution rate, mixing and operational days per year. The ultimate outputs of these models are the cost of algal biomass production, broken out into capital and operating costs, as well as the Net Energy Ratio (NER) or energy return on fossil energy invested (EROI). NER is a ratio of energy returned to energy invested. For algae production, it was calculated as the ratio of the chemical energy produced as algal biomass and the grid electricity needed for its production. NER includes all raw energy and should not be limited to electricity and should include natural gas as well. All these data are then used to perform a detailed sensitivity analysis to identify and rank order high priority areas for subsequent research.

**Figure 7-6** shows the results of a simulation performed based on the different cultivation systems being evaluated in The Netherlands. It includes projections for how these systems would perform in different locations. The data shown exhibit a range between €3.2 and €11/kg for biomass, depending on the type and location of the installation (in a current state of technology scenario). The authors included an outyear projection to €0.5/kg, assuming a decade of research and development (R&D) to bring improvements into the process that could change the overall

economics, including improvements in biomass productivity.<sup>3</sup> The selection of a certain location for the analysis directly affects the algal biomass production (related to solar irradiance and other meteorological parameters) as well as taxes and energy and labor costs. The study incorporated monthly averages of climatologic data and assumed different downtimes for different locations (due to differing winter seasons and the need for maintenance operations). According to the levels defined by the Association for Advancement of Cost Engineering, deviation of the results from the real cost should be lower than 30%. Capital investment was approximated using Lang factors, by multiplying the major equipment cost by appropriate factors to obtain the different items in the cost. Major equipment (ME) cost has been obtained directly from suppliers when possible (vendor quotes), otherwise prices have been obtained using standard engineering estimates, such as Prijzenboekje (29<sup>e</sup> Editie. DACE-Dutch Association of Cost Engineers) or literature. In case the ME cost for certain equipment was not from the current year, the price was updated to 2014 using the consumer price index (CPI). The study considered rented land, but the price was not location-specific. In the sensitivity analysis, land was owned, and in this case it would be free.

Mass balances were used to calculate parameters, such as daily volumes of medium, nutrients and CO<sub>2</sub> to be supplied. Energy cost was estimated as the product of the total power consumption and the industrial price paid for the energy in the studied location. Total power consumption was calculated from the number of units of major equipment, the power consumption and the time of operation of each unit. The calculated energy to overcome the head losses for the systems was also added to the power consumption; concretely the energy dissipated due to major losses from friction and minor losses from bends in the tubular photobioreactors, as well as head losses in bends, curves and friction in the raceway pond. The study did not consider embodied energy in materials (PBRs, fertilizers, etc.).



**Figure 7-5:** Projected biomass production costs (cultivation and harvesting) in the studied locations for current scenarios and the future projection for south of Spain. Costs as the sum of CAPEX and OPEX. RW: raceway pond; HT: horizontal tubular photobioreactor; VT: vertically stacked horizontal tubular photobioreactor; FP: flat panels photobioreactor.<sup>3</sup>

Net energy ratio (NER) is a ratio of energy returned to energy invested. For algae production, it

was calculated as the ratio of the chemical energy produced as algal biomass and the grid electricity needed for its production; values above unity imply more energy produced than invested. Conventional fossil-fuel-based electricity is the source of electrical energy in the study. Nevertheless, a simulation of the use of photovoltaic energy as source of electricity during the day was also examined. In this scenario, conventional electricity was still used to supply electricity during the night. Harvesting of the biomass was based on the combination of microfiltration followed by centrifugation, a combination that has been shown to be more cost effective than centrifugation alone.<sup>341</sup> An ideal heat exchanger performed on demand temperature control in the culture by pumping water at a constant temperature of 25°C. The analysis included the required pumps and energy. For the temperature control, the energy balance in the PBRs was calculated. In an open system analysis, irradiance, radiation and convection, as well as evaporation and condensation are the factors considered most influential to the heat flow. The heat flows due to algae growth, conduction from the ground and evaporation and condensation in closed systems, were not included due to their smaller influence on the overall heat balance.

Business viability for different scenarios can be accessed by deducting algal biomass production and biorefinery cost from the corresponding market value. Net costs above market value mean financial losses. On the other hand, scenarios showing market values higher than costs indicate economically feasible production chains. An initial analysis done at AlgaePARC for different scenarios shows that pathways aiming to produce medium to high value products or specialty chemicals are within reach to yield a significant financial return. Opposite results pertain if production is rather focused on bulk commodities such as commodity chemicals and biofuels. The low market values for such products do not cover their production and processing costs. Therefore, further reduction in costs is required to achieve such large volume market combinations. Market price of algal biomass for bulk chemicals and food commodities is below 2 €/kg, which makes it imperative to drop costs below this level to be competitive in the market. AlgaePARC's projections show the possibility of achieving this in the mid-term. For biofuels, reducing processing costs combined with an increased governmental and societal pressure on sustainability, coupled with increasing fossil oil prices, may make it possible in the medium-long term.

## 7.6. CONCLUSIONS

TEA represents a powerful tool that can be used to better understand the current commercial viability of algae-based biofuel and bioenergy systems. The relatively high cost of producing algal biomass remains the most critical barrier to commercial viability of algae-based production. For example published projected future costs range from between \$541/tonne (\$0.54/kg) for open pond production in Arizona, USA<sup>2</sup>) and \$10,177 (€9,000)/tonne (\$10.2/kg) for photobioreactor cultivation in The Netherlands. The largest impact on the cost was the productivity of the algae and cultivation engineering system, ranging from <10 g m<sup>-2</sup> day<sup>-1</sup> to 25 g m<sup>-2</sup> day<sup>-1</sup> for the outyear projections of the design cases. A detailed algae-farm design report supporting the current baseline assumptions for biomass production was integrated with state of technology biomass productivity data from an open testbed for algae cultivation, if algae cultivation were to be scaled up to a 2023 ha (5000 acre) farm.<sup>7</sup> The projected target for 2022 cost of biomass production is \$0.54/kg (\$491/ton), which supports moving towards cost-effective production of biofuels, although further cost reductions to achieve viability will likely require coproduction of value-added products.<sup>2</sup>

Two potential processing pathways to fuel are described because of the availability of a highly detailed description of the processing assumptions as projected large-scale biorefinery installations. In the U.S., the National Renewable Energy Laboratory (NREL) and the Pacific Northwest National Laboratory (PNNL) published conceptual design reports in 2014 projecting algal biofuel minimum fuel selling price (MFSP) targets achievable by year 2022 for the conversion of algal biomass to biofuels either via algal lipid extraction and upgrading (ALU) or via

hydrothermal liquefaction (HTL), respectively.<sup>5,6</sup> Both reports documented a set of targets for yields and processing conditions that would support a modeled MFSP of roughly \$4–4.5/ gasoline gallon equivalent - GGE (\$1.1 - \$1.19/L) for the respective conversion technology pathways, dependent on an assumed algal biomass feedstock cost of \$0.47/kg (\$430/ton) algal biomass AFDW following upstream dewatering to 20 wt% solids, and extrapolated to 2022. Reflecting the primacy of the cost of algal biomass production to biofuel production economics, both conversion pathways exhibit strong sensitivities to the cost of the algal biomass; MFSP is reduced by nearly \$1/GGE if algal production cost is reduced \$0.14/kg (\$130/ton) from the base case (i.e., to \$0.36/kg or \$300/ton), and reciprocally increases by slightly less than \$1/GGE if the algal production cost increases by \$0.13/kg (i.e., to \$0.61/kg or \$550/ton), which is more in line with the updated biomass cost targets of \$0.54/kg (\$491/ton) in the most recent 2016 biomass production design case.<sup>2,5,6</sup>

There are **two challenges** and steps remaining regarding TEA of cultivation of microalgae: i) **harmonization and standardization of the models, assumptions and methodologies**; and ii) **accessibility to pilot and demonstration experimental data from different locations to permit model validation**. In addition, there is a need for improving the fidelity of modelling, incorporating temporal and geographic resolution, as well as stochastic modelling of commodity prices. TEA model validation requires access to reliable data sources, both technical and socio-economic. In particular, the development of scalable and cost-effective technologies need to focus on generating and collecting large-scale data to feed the TEA and LCA computational models that are being developed. Furthermore, multi-year continuous production data needs to be made available to the community and could help validate the numerous productivity models around photosynthetic production potential for algae that are available.

## **8. Sustainability and Life-Cycle Assessment of Algal Bioenergy**

Life Cycle Assessment (LCA) is a decision-support tool used to comprehensively quantify the environmental impacts (including those on climate change) of a product or service, by adopting a product system perspective (from cradle to grave, i.e., from cultivation for feedstock production, to processing to extract intermediates and products, to final waste management, including transport where applicable). The ISO standards 14040 and 14044 (2006) provide the basic requirements on conducting LCA, while ISO/TS 14067 (2013) gives specific guidance on quantifying the carbon footprint of a product, and ISO 13065 (2015) supplements these with details on quantifying GHG emissions from bioenergy systems. An LCA study should clearly define the scope of analysis, including what processes are within its boundary, and quantify the inputs and outputs of each step. In terms of algal bioenergy, the processes with greatest environmental impacts typically include fossil energy input to algae production, as well as conversion to fuels and other products. LCAs of algal biofuels often are based on a biorefinery concept or installation and also consider delivering the biorefinery products to various markets; this enables the displacement of equivalent products, such as fossil fuels or other bio-product substitutes. The outcomes of LCA studies are highly dependent on their system boundaries and assumptions, which often differ between studies.

### **8.1. MICROALGAE SUSTAINABILITY CONSIDERATIONS**

LCA commonly assesses a range of impact categories, such as climate change (due to CO<sub>2</sub> and other GHG emissions), fossil energy use, associated with producing a unit of product such as 1MJ of fuel. It could also consider particulate air emissions, eutrophication and water consumption. Eutrophication is the detrimental effect of oxygen depletion in waterways due to algae blooms' decaying biomass, causing fish kills. The distribution and combustion of the resulting algal biofuel is sometimes excluded from the LCA to avoid confounding the assessment of the algae process with the transportation network for fuels.<sup>342</sup> The consideration of bio-products of biofuels adds complexity to the assessment. There are different methods available for handling bio-products (as defined in ISO 14040/44, ISO, 2006) , which adds further variation to the results between different studies.<sup>342</sup> Even though progress has been made to encourage the consistent modeling of LCA of algae processes, the ISO LCA standards still leave the selection of system boundary and functional units to be chosen by the particular researcher.<sup>342</sup> The more recent ISO standard on sustainability criteria for bioenergy (ISO 13065:2015) provides more specific advice on the functional unit and handling bio-products. Handling bio-products is difficult unless the product system can be divided into separate processes for each bio-product. LCA may be used in determining eligibility for government incentive programs, e.g. eligibility of fuels under the purview of the Renewable Fuel Standard in the US, and the European Renewable Energies Directive is based on LCA and carbon accounting standards. One challenge in the interpretation of a multitude of technical reports on LCA in the algae literature is inconsistency in system boundary and assumptions. This has led to a number of meta-analyses, i.e. reviews of the existing literature on LCA and attempts to interpret the reports on a consistent basis.<sup>115,320,342-346</sup>

LCA involves compiling an inventory of inputs and emissions associated with each stage across the life cycle.<sup>200,347-349</sup> LCA enables the comparison of alternative systems on the basis of the same functional unit (e.g. 1 MJ fuel-energy produced). Bioenergy focused product pathways are being investigated with a conscious effort on unifying an approach taken to assess the environmental impact of the algal growth and processing operations. This emphasizes that LCA (like TEA) cannot be considered in a vacuum, but is inherently linked with the entire pathway.<sup>342</sup> LCA can be used to compare between the environmental and human health impacts of renewable and conventional products. Recently, a set of indicators has been proposed in the assessment of algal fuels, which ranges from soil quality, water quality and quantity used, nutrient utilization, greenhouse gas emissions, biodiversity, air quality and overall system productivity (**Table 8-1**).<sup>342,350</sup>

**Table 8-1:** Proposed generic environmental indicators for assessing sustainability of algal biofuels, compiled from tables and information in published literature<sup>342,350,351</sup>

Category	Indicator	Unit	Potential environmental effects	Ref.
Soil quality	Bulk density	g cm <sup>-3</sup>	Water holding capacity, infiltration, crop nutrient availability	350
	Terrestrial acidification	kg SO <sub>2</sub> equivalent to air		342
	Terrestrial eco-toxicity	kg 1,4 dichlorobenzene to industrial soil		342
Water quantity	Peak storm flow	L s <sup>-1</sup>	Erosion, sediment loading, infiltration	351
	Minimum base flow	L s <sup>-1</sup>	Habitat degradation, lack of dissolved oxygen	351
	Consumptive water use (incorporates base flow)	feedstock production: m <sup>3</sup> ha <sup>-1</sup> day <sup>-1</sup> ; biorefinery: m <sup>3</sup> day <sup>-1</sup>	Availability of water for other uses	350
Water quality	Nitrate concentration in streams (and export)	concentration (mg L <sup>-1</sup> , ppm); export kg <sup>-1</sup> ha <sup>-1</sup> yr <sup>-1</sup>	Eutrophication, hypoxia, potability	350
	Total phosphorus (P) concentration in streams (and export)	concentration (mg L <sup>-1</sup> , ppm); export kg ha <sup>-1</sup> yr <sup>-1</sup>	Eutrophication, hypoxia	350
	Salinity	Conductivity (no unit)	water composition change	350
	Fresh/Marine water eutrofication	kg P and N equivalent		342
Energy	Fossil energy consumption	MJ kg ha <sup>-1</sup> yr <sup>-1</sup>	Fossil resource depletion	this report
Greenhouse gases	CO <sub>2</sub> equivalent emissions (CO <sub>2</sub> and N <sub>2</sub> O)	kg C eq GJ <sup>-1</sup>	Climate change, plant growth	350
Land use	Agricultural/Urban land occupation	m <sup>2</sup> x year of land		342
	Natural land transformation	m <sup>2</sup> x year of natural land		342
Resource depletion	Mineral resource depletion	kg Fe equivalent		342
	Fossil resource depletion	kg oil eq		342
Biodiversity	Presence of taxa of special concern	presence	increased or decreased biodiversity	350
	Habitat of taxa of special concern	ha	increased or decreased biodiversity	350
	Abundance of released algae	Number L <sup>-1</sup>	increased or decreased biodiversity	350
Air quality	Tropospheric ozone	ppb	human and plant health	350

	carbon monoxide	ppm	human health	350
	Total particulate matter less than 2.5 um diameter (PM2.5)	$\mu\text{g m}^{-3}$	visibility and human health	350
	Total particulate matter less than 10 um diameter (PM10)	$\mu\text{g m}^{-3}$	visability and human health	342,350
	Ozone depletion	kg CFC-11 equivalent		342
	Human air toxicity	kg 1,4 dichlorobenzene to urban air		342
	Photochemical oxidant formation	kg NMVOC compound equivalent to air		342
Productivity	Primary productivity or yield	$\text{gC L}^{-1} \text{ year}^{-1}$ or based on chlorophyll a	Climate change, soil fertility, cycling of carbon and other nutrients	350

Of the sustainability indicators introduced above, water is one of the more important resources to be considered by LCA. However, the consistent assessment of water usage remains a complex challenge and sits at the core of the current energy-food-water nexus.<sup>337,352</sup> Water itself is a renewable resource, but the ways in which it is used for different energy strategies are not directly comparable. For instance, underground injection of water for hydraulic fracturing and emulsification with fracking fluids has a much different environmental implication than the use of water to produce electricity via hydroelectric power or cooling water for thermoelectric generation. Furthermore, algal cultivation may be able to treat agricultural wastewater thereby potentially lowering water pollution associated with chemical fertilizers and allowing for recycling of water for other purposes, which may lower demand for freshwater (e.g., if used for power plant cooling) or ground water pumping. Other potential contributions to the food sector by algal cultivation include providing bio-products that can be used for human nutrition, animal feed, and aquaculture and fertilizer. These applications are all very different from the transpiration of water by organisms during biomass production or its recycling during biomass processing.<sup>352</sup>

The impact of water use also varies dramatically by region, and therefore, a universal or even national framework is rarely appropriate. Water is lost through evaporation during cultivation and by drying of harvested microalgae.<sup>353,354</sup> The evaporation rate depends on local environmental and geographical conditions such as temperature, humidity and wind velocity.<sup>12</sup> It should be noted that most of the countries that have favorable climate conditions to achieve high lipid productivity can encounter serious water stress situations.<sup>12</sup> The reported values for water consumption of microalgae-based biodiesel vary from a few liters to a few thousand of liters per liter of microalgae biodiesel.<sup>12</sup> In addition to precipitation and evaporation rate, sudden changes in weather patterns and the occurrence of extreme events such as floods, drought, monsoons and hurricane will have a significant impact on water management for algal biofuel.<sup>12</sup> The use of water consumption can be reduced by approximately 80% if harvest water is fully recycled, and up to 90% if seawater or wastewater is used for culture.<sup>13</sup> As losses due to evaporation during biomass production and drying are almost unavoidable, the freshwater requirement still remains significant.<sup>13</sup> Fully recycling the harvest water and the use of wastewater for culture can reduce nutrient input requirements, decreasing nitrogen usage by 94% and eliminating the need for addition of potassium, magnesium and sulfur.<sup>13</sup> However, the suitability of wastewater to subsequent cultivation is not straightforward. The reuse of water may be limited due to the extracellular metabolites released and gradual accumulation of toxic compounds by microalgae in water.<sup>355</sup> Wastewater is susceptible to bacteria and virus contamination, which may in the worst case devastate the whole colony of microalgae.<sup>9</sup> This effect can be avoided by frequent cleaning of the

raceway pond, two or four times a year,<sup>12</sup> which results in a discharge of substantial amount of water.<sup>164,356,357</sup> Currently, there is no compliance threshold or inclusion of water in LCAs required by the EISA.

Following EISA enactment, the EPA and the **National Academies of Sciences with the National Research Council (NRC)** have completed qualitative assessment of the sustainability impact of renewable fuels complying with the RFS. The goal of that work was to assess and avoid potential negative environmental impacts of the RFS.<sup>358</sup> Again, as commercial algal facilities are being developed and research funded and prioritized, some of the major concerns identified by the NRC may need follow-up. The major sustainability concerns identified by the NRC report relating to resource and environmental effects of large-scale development of algal biofuels, and that would have to be addressed prior to deployment, can be summarized as follows:

- *The quantity of water (whether fresh water or saline water) required for algae cultivation and the quantity of freshwater addition and water purge to maintain the appropriate water chemistry.* Maintenance of water level and quality in open-pond systems or evaporative loss of cooling water if it is used to maintain temperature in photobioreactors could be a concern because of the potential for high net evaporative losses, particularly in arid regions where solar resources are most suitable for cultivation.
- *Supply of the key nutrients for algal growth—nitrogen, phosphorus,<sup>359</sup> and CO<sub>2</sub>.* Nutrient sources can include virgin sources and waste streams such as flue gas. Preparation and transport of these waste streams for reuse, nutrient recycling, production of bio-products, and fossil inputs required to produce necessary nutrients all affect the energy return and GHG emissions.
- *Appropriate land area with suitable climate and slope, near water and nutrient sources* (for example, a stationary source of CO<sub>2</sub> such as a coal-fired power plant or a wastewater source such as municipality, industry, or agriculture).
- *Energy return on investment (EROI).* Algal biofuel production would have to produce sufficiently more energy than is required in cultivation and fuel conversion to be sustainable.
- *GHG emissions over the life cycle of algal biofuels.* Algal biofuel production would have to produce a GHG benefit relative to other fuel options such as fossil fuels. Yet, estimates of life-cycle GHG emissions of algal biofuels span a wide range, and depend on many factors including the source of CO<sub>2</sub> and the disposition of bio-products.

## 8.2. LIFE CYCLE ASSESSMENT OF MICROALGAE BIOENERGY SYSTEMS

A review of LCA studies on climate effects of microalgae biofuel production is provided here, including an analysis of modelling choices and assumptions. A total of 29 algal production-oriented LCA studies published between 2009 and 2015 were identified for review. Eleven other studies were excluded due to a lack of transparency or because they provided insufficient quantitative information or limited their assessment a single life-cycle stage of the algal biofuel production chain. The regions covered by the 29 studies selected were (number of studies included in parentheses): Australia (1), Canada (1), China (2), Colombia (1), Europe (2), Finland (1), India (1), Israel (1), Singapore (1), UK (3) and USA (15). System boundaries varied from cradle-to-gate (10), cradle-to-pump (aka well-to-tank or well-to-pump, 3), cradle-to-grave (aka well-to-wheels or well-to-wake, 15) and hybrid input-output LCA (1). The difference between these boundaries is that cradle-to-gate includes emissions up to the conversion process, whereas cradle-to-pump adds the distribution of the biofuel and cradle-to-grave adds the combustion of the biofuel. The reviewed studies employed functional units based on energy or mass. A total of 23 studies adopted energy-based functional units. Other studies considered a mass-based functional

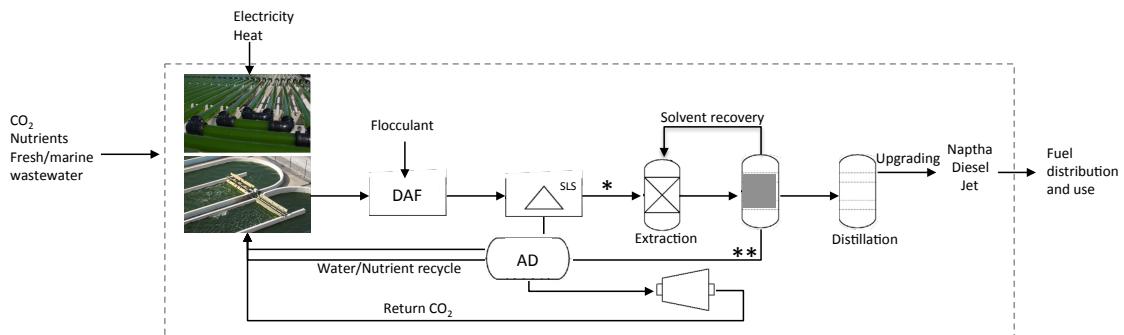
unit and one study used a distance-travelled approach. To enable comparison between the studies we converted the results of each study to 1MJ as the functional unit.

It has been argued that biofuels from microalgae present a sustainable option for producing biofuel, as this approach avoids competition for land use and, hence, circumvents several of the criticisms made about terrestrial feedstock-based biofuels, especially direct and indirect land-use change.<sup>360,21,110,361,362</sup> Nonetheless, the climate advantages of using biodiesel from algae relative to fossil diesel can only be ascertained through the use of Life Cycle Assessment (LCA). In the most recent EU financed projects (FP7), the inclusion of LCA is mandatory to allow for comparative assessment of newly developed technologies.

As the technology for microalgae biofuel production is still in its infancy, the optimal production techniques are not yet demonstrated ubiquitously at large scale. There are few commercial-scale installations, so most of the data published in the literature are largely derived from bench or pilot scale studies.<sup>108</sup> **Figure 8-1** shows the basic premise of cultivation, harvesting, conversion and fuel production from microalgae, as identified in the literature, with the assumed boundary for LCA shown as a dashed line box. Even though the steps in algal biofuel production are reviewed in previous chapters, we briefly reiterate the most pertinent aspects here.

For harvesting, several technologies can be employed (example options among many other responsibilities are listed here): Microalgae can be decanted and/or flocculated followed by a centrifugation or filtration step to recover wet algal biomass. Depending on the conversion technology, microalgae can also be dried. In the extraction step, oil can be extracted from the microalgae biomass with a solvent (through dry or wet extraction), including with supercritical CO<sub>2</sub> (dry extraction). Conversion routes to biofuels are highly diverse, and, in particular, production of FAME biodiesel using methanol can be accomplished using the traditional transesterification reaction, which is the most common technology assumed in the reviewed studies, or it can be produced using more recently developed technologies like ultrasonication with direct transesterification and supercritical methanol.<sup>116,127,163,164</sup> Beyond FAME biodiesel, a renewable diesel hydrocarbon product can be produced by deoxygenation and isomerization over heterogenous catalysts of extractable lipids from microalgae.<sup>198,199</sup> Alternatively, hydrothermal liquefaction (HTL) (a more recent technology compatible with wet extraction) can be used to convert algae biomass to biocrude, which can then be upgraded to liquid fuels and other chemicals.<sup>6,135</sup> Finally, the resulting biofuel is distributed and ultimately combusted.

An LCA of HTL conversion of whole algal biomass incorporates information on utilization of the aqueous byproduct stream and reports that the resulting main fuel produced has considerably lower green house gas (GHG) emissions than petroleum fuels and even less than corn ethanol.<sup>363</sup> The EROI remains lower than for petroleum fuels, but with significant potential for process efficiency improvements.<sup>364</sup> A separate LCA confirmed the value of using CHG to improve GHG emissions as well as the benefits to minimizing product cost by using the aqueous byproduct stream for additional fuel production while also allowing recycling of nutrients.<sup>365</sup> Another LCA suggests that consideration be given to the source of any CO<sub>2</sub> added to the algae growth environment as well as the perceived difference in effect between ground transport use of the fuel versus atmospheric combustion as jet fuel.<sup>366</sup>



**Figure 8-1:** Illustration of boundary conditions (dashed line box) for Life Cycle Analysis (LCA) of microalgal biofuels. Base-case algal lipid extraction and upgrading approach; algae are grown in open ponds, or photobioreactors, or hybrid systems after which the cell biomass is harvested and processed wet through extraction of lipids, which are further upgraded via hydrotreating to renewable diesel, jet fuel, after which the residue is transferred to anaerobic digestion (AD) where the biogas produced is used for power generation for the entire plant<sup>165</sup> [additional details discussed in section 7]

The systems studied vary considerably in terms of cultivation, harvesting, extraction and conversion processes, and assumed growth rates and lipid content. The productivities in the reviewed studies varied from 2 g DW m<sup>-2</sup> day<sup>-1</sup><sup>121</sup> to 42 g DW m<sup>-2</sup> day<sup>-1</sup>,<sup>116</sup> while the lipid content varied between 7% and 50% (DW basis).

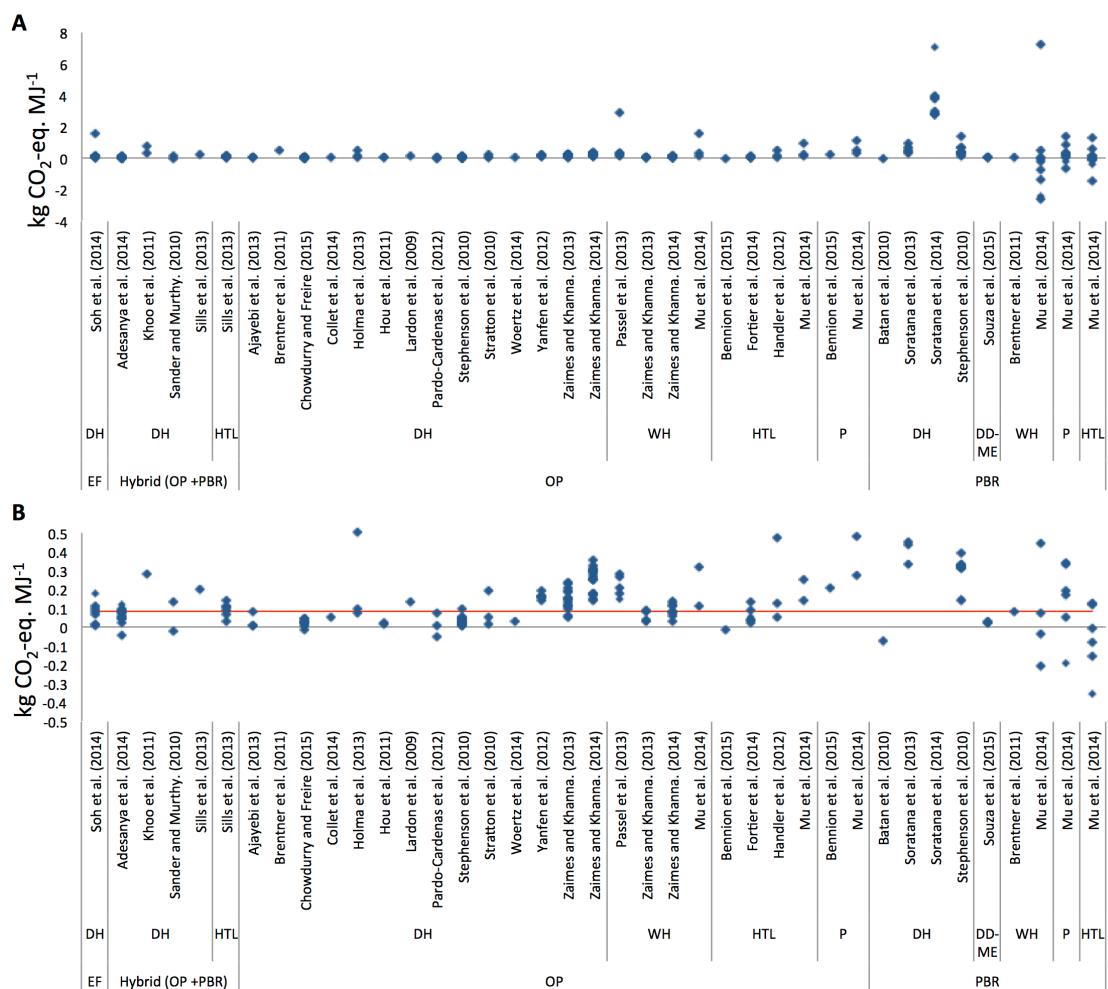
The reviewed studies handle bio-products differently. A total of 13 out of 29 studies expanded the system boundaries of microalgae biodiesel to include alternative functions of the bio-products. The remainder of the reviewed studies considered different allocation methods: mass, energy or economic (market prices) allocation. Four studies employed both system expansion and allocation, and 1 study performed a sensitivity analysis to different allocation methods. Finally, 1 study attributed all impacts to the main product.

An extremely large variation in estimated net GHG emissions is reported: between -2.6 and 7.3 kg CO<sub>2</sub>eq MJ<sup>-1</sup> biofuel produced; however, more than 85% of the reported emissions fell between the narrower range of -0.35 and 0.5 kg CO<sub>2</sub>eq MJ<sup>-1</sup>). These findings are consistent with earlier published reviews of the LCA literature.<sup>320,363</sup>

The variations are mainly due to differences in modeling choices (e.g., the approach adopted to deal with co-production), followed by high uncertainty and variation in the reported performance of processes used for microalgae cultivation, harvesting and oil extraction processes. Overall, these studies show that the development of less energy-intensive technologies for the cultivation and harvesting steps is critical for reducing the life cycle GHG emissions of producing microalgal oil-based biodiesel. An uncertainty assessment, e.g., based on Monte-Carlo simulation, should be included in future studies of algae-to-biofuel systems to increase the robustness and transparency of the outcomes and help guide further research towards reducing overall uncertainty. Moreover, a complete meta-analysis will aid in identifying opportunities for harmonizing several of the (different) assumptions in the various studies, which will also help reduce overall uncertainty.

Greenhouse gas (GHG) emissions (expressed as kg CO<sub>2</sub> equivalents released over the entire process) is the standard way to quantify potential climate change impact of a particular product. Non-CO<sub>2</sub> GHG emissions are converted to CO<sub>2</sub> equivalents by multiplying by the global warming potential (GWP) (ISO 14067, 2013), where the GWP is usually calculated over 100 years (GWP100) but it is recommended to also include the 20 year impact (GWP20). The range of GHG emissions across the published reports is shown in Figure 8-2. For comparison purposes, the GHG results of the reviewed studies were normalized to 1 MJ of energy content (Lower Heating Value). Cradle-to-grave GHG emissions of fossil diesel are also shown, 0.083 kg CO<sub>2</sub>-eq. MJ<sup>-1</sup> (European

Commission's Renewable Energies Directive, 2009), as a red line in **Figure 8-2**. The GHG emissions of the reviewed studies varied from -2.6 to 7.3 kg CO<sub>2</sub>eq MJ<sup>-1</sup> (**Figure 8-2.a**); however, more than 85% of the reported emissions lie between -0.35 and 0.5 kg CO<sub>2</sub>-eq. MJ<sup>-1</sup> (**Figure 8-2.b**). The main reasons for these very wide ranges are associated with modeling choices (e.g. approach to handling bio-products), and the substantial differences related to the alternative cultivation, harvesting and conversion processes. Seven studies reported negative GHG emissions due to *i) handling bio-products via substitution*<sup>117,120,367,368</sup> or *ii) exclusion of combustion from system boundary, as accounting for biogenic CO<sub>2</sub> absorbed during microalgae growth was accounted for but not its release through combustion.*<sup>363,369,370</sup> More than 60% of the LCA results reported GHG emissions higher than those for fossil diesel, which is mainly due to the energy-intensive technologies used in microalgae cultivation and harvesting.



**Figure 8-2:** GHG intensity of microalgae biodiesel in the reviewed studies: A) overall results; B) detailed range [-0.5 – 0.5 kg CO<sub>2</sub>-eq. MJ<sup>-1</sup>], relevant for more than 85% of the reviewed studies. The red line shows the GHG intensity of fossil diesel. DH - Dry extraction with hexane; EF - Erlenmeyer flasks; HTL - Hidrotermal liquefaction; WH - Wet extraction with hexane; P - Pyrolysis; DD-ME -Dry extraction di-methyl ether; OP - Open pond; PBR - Photobioreactor

The results of the 29 LCA studies (**Figure 8-2**) show a wide variation in GHG emissions for algal biofuel systems, with many results exceeding the emissions for fossil diesel. Nevertheless, microalgae biodiesel production systems are very recent and technology developments are focused on finding higher production efficiencies. In this context, many studies included several scenarios comparing different technologies, different uses of bio-products and future changes in microalgae biofuel production systems with expected GHG emission reductions, showing a high

variation between scenarios. The development of less energy-intensive technologies for microalgae cultivation and harvesting steps is critical in order to reduce the life cycle GHG emissions of microalgae biofuels.

Meaningful computations of GHG emissions for the production and use of microalgae biofuel must consider a number of contributions. Since many aspects of the microalgae biofuel production pathway are experimental, or even hypothetical, specific numerical values for emissions used in many recent assessments should be viewed with caution. The focus here is on identifying the critical parameters that most significantly affect the results.

The structure of a GHG emission computation and the selection of emissions sources to include depend upon the question being investigated. Here we have assumed the purpose to be a comparison of microalgae-derived biofuel versus petroleum-derived diesel to inform assessment of the potential emissions savings that could be realized by converting from fossil- to algae-based diesel fuel, or to guide and constrain price- and volume-based optimization of a microalgae-based fuel production pathway. Whether the objective is to compare between fuels or to choose amongst options for a microalgae pathway, the computations must be performed on a full life-cycle basis, i.e., they must include the emissions associated with all activities that may differ between options being compared, especially including so-called upstream operations, such as the provision of electricity and the processes that supply any fossil fuels used in the algae process, as well as distribution and use of the produced biofuel. The scope of this ensemble is referred to as the system boundary.

In selecting the system boundary for microalgae biofuel LCAs, the researcher must recognize that, compared to other fuels, microalgae cultivation and processing requires large amounts of electrical power and, because microalgae biomass contains substantial amounts of protein, might require more nutrients than other biomass feedstocks, depending upon the degree of nutrient recycling.

### **8.3. SYSTEM BOUNDARIES**

The system boundary determines which processes and emissions sources are included in the analysis, and should be selected carefully, in accordance with the goal of the study. In selecting the system boundary for microalgae biofuel LCAs it should be noted that, compared to other fuels, microalgae cultivation and processing requires large amounts of electrical power and, because microalgae biomass contains substantial protein, might require more nutrients than other biomass feedstocks, depending upon the degree of nutrient recycling. Also, only a portion of the microalgal biomass yields biofuel and bio-products, and the remaining fraction can contain substantial amounts of organic carbon and nitrogen, corresponding to substantial potential for methane ( $\text{CH}_4$ ) and nitrous oxide emissions ( $\text{N}_2\text{O}$ ), which are GHGs more potent than  $\text{CO}_2$ . Therefore, the system boundary for microalgae LCA must include all nutrient manufacturing and supply, including  $\text{CO}_2$  or other carbon sources, electricity production, natural gas and other fuel provisioning, all operations for cultivation, harvesting, conversion to fuel, fate and valorisation of bio-products, co-generation of heat and power on site, and treatment of any waste. The fate of all nitrogen and carbon in all process fractions must be followed all the way to the end, e.g., if AD is employed, then disposal of the AD digestate (residue left over after AD) and associated emissions must be considered, including possible  $\text{N}_2\text{O}$  emissions if the digestate is used as fertiliser.<sup>371</sup> Nutrient assumptions should be based on commodity chemicals rather than small niche chemicals that may not be available at large scale, yet may have anomalously low manufacturing emissions.<sup>372</sup> Fugitive  $\text{CH}_4$  emissions should be considered, e.g., from AD facilities, or  $\text{CH}_4$  losses from internal combustion engines used in co-generation systems. The system boundary should include distribution of fuels to the end user and ultimate combustion in a vehicle (well to wheel).

The system boundary for microalgae LCA should include emissions associated with the construction of the microalgae cultivation, processing, and conversion facilities.<sup>373</sup> Microalgae

cultivation can require substantial infrastructure compared to other biomass cultivation systems, e.g., raceway ponds require liners to protect their berms from erosion and, in some soils or under some regulatory constraints, may require liners covering the entire pond. Concrete for sumps and paddlewheel footings can be substantial. Since other biomass, e.g., grasses and corn stover, do not require large quantities of materials, microalgae GHG emissions must include these “infrastructure cycle” emissions to enable a fair comparison with other biofuels. Also, since construction can require site levelling, which involves large-scale disturbance of soil, microalgae LCA should include the emissions associated with fuel use for these operations, and also direct land use change (dLUC) emissions, that is from loss of biomass and soil carbon. Furthermore, emissions from indirect land use change (iLUC) should also be considered, if use of land for microalgae production displaces some other productive land use. Neither dLUC or iLUC emissions due to microalgae production have received much attention to date but may significantly impact the GHG emissions from different geographical locations,<sup>374</sup> in part because of expected high productivities and expected use of marginal land with low carbon stocks in biomass and soil. However, demonstrated productivities have remained lower than expected and water-use considerations may force microalgae cultivation towards land with non-negligible soil organic carbon.<sup>25,375</sup> If algae are grown in low rainfall land that is marginal for agriculture production, the evaporation rate will be high, and there may be limited access to fresh water. Thus, it is more likely that algae production will occur on land closer to fresh water supplies, which is often more productive, and has higher carbon stocks.

Two unique aspects of microalgae biofuel production are the nearly continuous use of electricity during cultivation for culture circulation and mixing, both in open raceway ponds and in photobioreactors, and, second, the rapid harvest cycle. The cultivation mixing energy demand, expressed as  $\text{kWh ha}^{-1} \text{ day}^{-1}$  can be divided by the productivity, expressed as  $\text{g m}^{-2} \text{ day}^{-1}$ , giving a value of energy demand for mixing per gram of biomass. Thus, the GHG emissions associated with culture mixing are inversely proportional to the productivity. Since the harvest cycle is so rapid for microalgae, occurring many times in any given season, the GHG emissions must be considered on a season-by-season basis and the analysis must consider carefully how to combine the results into a representative, annual-average, value. It is quite possible to have substantial GHG emissions reduction during the summer while having emissions in excess of fossil diesel during the winter when growth rates are slower.<sup>376</sup>

The non-linear dependence of GHG emissions on productivity has two consequences for GHG emission estimation. First, because of the non-linearity, it is generally inappropriate to perform analysis based upon an annual-average productivity. Second, because of site-to-site variation in productivity, GHG emissions corresponding to fuel production at regional and national scales should consider a geographical ensemble of sites with site-level productivity estimated in some way. This is where the interaction with a resource assessment model would be highly advantageous. Failure to consider these variations can cause errors in both the mean value of the GHG emissions and under-estimation of associated uncertainties.

The approach to quantifying  $\text{CO}_2$  fluxes must be considered carefully. Microalgae cultivation systems are often supplemented with  $\text{CO}_2$ . The  $\text{CO}_2$  can be obtained from the combustion of fossil fuels, e.g., from flue gases from electrical power generation or industrial processes, from vented gas from fermentation, or from underground  $\text{CO}_2$  sources. Although the latter would never be considered a carbon-neutral source, it is common practice to treat  $\text{CO}_2$  from fossil fuel combustion during power generation and industrial processes as carbon neutral. The argument is that the industrial process is a sunk cost that will occur whether microalgae are produced or not so that utilizing the  $\text{CO}_2$  for microalgae biofuel implies avoidance of the carbon emissions from the fossil diesel that are displaced. These assumptions require careful evaluation in each case. For example, if a small on-site power plant is constructed for the sole purpose of supplying flue gas and electricity for microalgae cultivation, then the associated  $\text{CO}_2$  may no longer be considered neutral. In some scenarios involving co-generation of power on-site from the microalgae residues,

it is sometimes necessary to generate heat or power beyond that which can be produced from the residues. In these cases, natural gas (fossil methane) is sometimes used to supplement the co-generation plant. If so, the emissions associated with the supplemental natural gas would be taken as a burden for the biofuel because the emissions would not have occurred without the production of microalgae. It is also important to track the fate of the carbon. For example, if carbon in CO<sub>2</sub> from flue gas is taken up in the microalgae biomass, and if that carbon is later converted to CH<sub>4</sub> and lost to the atmosphere, the additional climate effect (measured as GWP) of the CH<sub>4</sub> compared to CO<sub>2</sub> should be included in the LCA models. Though since both CO<sub>2</sub> and CH<sub>4</sub> are of biogenic origin, the calculated impact on net emissions should not be impacted.

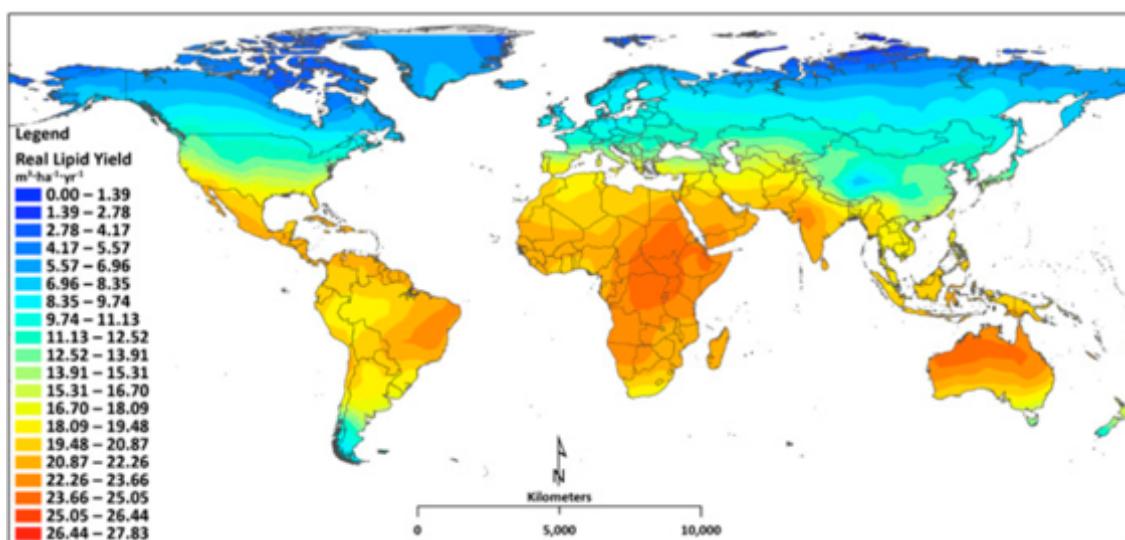
The discussion here has focused on emissions that occur within the well-to-wheel system boundary described above. Accounting of indirect effects, as is common in consequential LCA, goes further and considers changes in emissions that occur as a result of changes elsewhere in the economic network that are affected by the production and sale of the microalgae biofuel and associated bio-products. While consequential LCA studies give a more comprehensive picture of the consequences of one economic choice versus another, e.g., utilising a parcel of land for microalgae cultivation compared to utilising it for an alternative purpose, the computations also introduce additional challenges related to unknowns in the economic modeling. Attributional LCAs – those that exclude these complexities by cutting off part of the system with allocation – have been used for guiding the optimization of microalgae pathways towards affordable products, but some questions can only be addressed with consequential-style analyses. For example, growing microalgae from power plant flue gas adds utility to the power plant and therefore may extend its service lifetime beyond a scenario without microalgae. If this were to delay the replacement of a fossil-fuel based power plant with an alternative power source with lower emissions, then it is possible that investing in microalgae production will lead to total emissions higher than an alternative scenario without microalgae. Thus, while attributional style analyses may be adequate for optimisation of pathways in some cases and for comparing between fuel alternatives, questions regarding the total efficacy of microalgae biofuel production, such as for informing policy development, require further analysis performed with consequential LCA methods.

This review of published values for GHG intensity of algae-based biofuels did not find any clear difference between the different algae cultivation methods, or between methods for obtaining biofuels from algae (**Figure 8-2**). A comprehensive meta-analysis, which would account for the impacts of variation in method of assessment discussed above, may detect consistent differences that are not apparent from the absolute values presented here.

#### 8.4. RESOURCE ASSESSMENT FOR ALGAE OPERATIONS

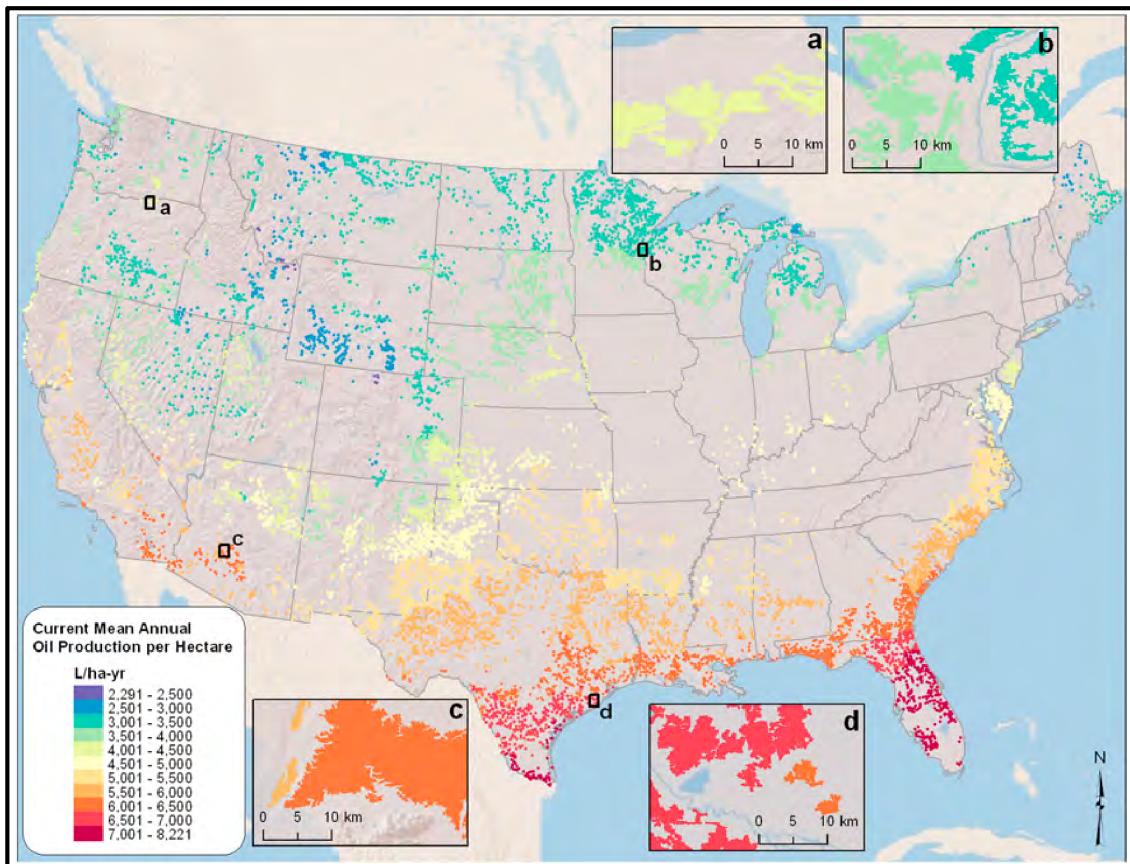
An important consideration that underpins the overall sustainability discussion is land use and integrating a cultivation facility within a food-energy-water network.<sup>352,357</sup> A resource assessment (RA) accompanying an LCA may be used to evaluate the impact of farm operations on (direct and indirect) land use changes and to calculate the total amount of fuel or other product able to be manufactured using a specific process given the amount of input resources available within a specific area. Specifically, algae resource assessments heavily depend on available data on solar radiation (MJ m<sup>-2</sup> yr<sup>-1</sup>) in combination with the number of days with full sunlight per year. Additionally, RA informs the TEA and LCA of the need to bring resources to the cultivation facility from remote locations.<sup>374,375,377–379</sup> In all cases, the importance of uniform approaches to these analyses is increasing as the algae industry seeks to rapidly develop, finance, and build out its operations. Land and water in suitable climates for large-scale algal biofuels production exist, but the economics of production, and the embodied energy and GHG mitigation of the biofuel will be influenced by the proximity of these resources. It is less likely that the CO<sub>2</sub> is available in regions most suited to year round algal growth. Optimal siting of large-scale algal biofuels production facilities will require that the resources exist in close proximity, or that there are drivers to ensure the provision of the missing resource (most likely CO<sub>2</sub>).

A recent development on a global scale for the evaluation of biofuel potential of algae concluded that, solely based on historical meteorological data from 4,388 global locations, a biomass productivity potential could be estimated.<sup>380</sup> The overall summary (**Figure 8-3**) of this work was that microalgae can have a positive impact on the transportation energy portfolio of various countries, assuming water, nutrients and CO<sub>2</sub> are not limiting. This assessment was based on one growth model for one species and does not do the wide span of algal biological diversity and physiological response to nutrient, heat and light availability justice, but it can be interpreted as a general map of algae biofuel contribution potential.



**Figure 8-3:** Overview of global current near term lipid productivity of microalgae based on a validated biological growth model of *Nannochloropsis* cultivated in a photobioreactor, based on meteorological data from 4,388 geographical locations.<sup>380</sup>

An assessment of the land, water and resource availability specifically in the US concluded that approximately 430,000 km<sup>2</sup> (166,000 miles<sup>2</sup>) is available and suitable for algal cultivation in open ponds, which was estimated to be able to produce  $220 \times 10^9$  L ( $57 \times 10^9$  gallons) of oil per year, which, in 2011, was equivalent to 48% of the US petroleum imports for transportation.<sup>375</sup> To achieve these levels of production, it was estimated that the land requirement amounted to 5.5% of the contiguous US land area and the water consumption exceeded the current agricultural water needs by 3-fold. When the resource assessment analysis was carried out for the contiguous US, different regions exhibited up to 3-fold ranges in projected oil productivity, with the highest productivity expected around the Gulf regions (Florida, southern Texas) (**Figure 8-4**). A different, more recent, study included a direct land use change factor in the assessment of sites for algae cultivation.<sup>374</sup> The main conclusions are that the previously published studies overestimate the GHG benefits if forested lands are included as part of the predicted attractive sites.



**Figure 8-4:** Overview of GIS siting information correlated with biomass and oil predicted productivity across the contiguous US, shown as the mean annual algal oil production ( $\text{L ha}^{-1} \text{ yr}^{-1}$ ) at technology and assumptions<sup>375</sup>

The results from a similar European study illustrate the areas within Europe that are suitable for the high efficiency production of microalgae in either open ponds (A) or photobioreactors (B).<sup>379</sup> Three macro-areas for algae cultivation were identified (suitable areas, non-suitable areas and buffer zones), using indicators similar to the above US studies; solar irradiation and annual mean air temperature. Within each of these sites, photobioreactor (PBR) and open ponds were simulated as cultivation conditions, within a fresh, waste- or seawater environment. The European locations identified indicate that the following areas can be considered a priority towards deployment of open pond algae cultivation; southern Portugal and south-west Spain, Sardinia, Sicily and Apulia in Italy, Greece and Cyprus. For the photobioreactor cultivation, the same areas were selected except that the available areas are wider due to the more flexible siting consideration of closed reactors. However, it has to be noted that a detailed research and land use change study is currently lacking for the European continent and should be included in any future projections.

## 8.5. CONCLUSIONS

Even though algae-derived biofuels and bioenergy applications, as discussed throughout this report, present a promising technology route towards future energy security and energy independence, the scalability considerations suffer from general extrapolation from laboratory data. The lack of a consistent reference framework and pathway infrastructure makes side-by-side comparison of sustainability metrics very difficult. Overall, LCA is a powerful tool to compare different processes, but differences in assumptions, bio-product credits and system boundaries make comparisons difficult and limit the conclusions that can be drawn.

Most analyses of GHG balance reported in recent years have fallen short of the high expectations

placed on biofuel from microalgae relative to its fossil counterpart. The review of recently published LCA studies of microalgae biofuel production shows a very wide range of GHG emissions: between -2.6 and 7.3 kg CO<sub>2</sub>eq MJ<sup>-1</sup>; however, more than 85% of the reported results lie between -0.35 and 0.5 kg CO<sub>2</sub>eq MJ<sup>-1</sup>. The main causes for this variation are related to contrasting modeling choices (e.g. the approach adopted to deal with co-production), the high uncertainty in microalgae cultivation, (harvesting and oil extraction processes) and lack of harmonization of LCA approaches by different research groups. This review of published values for GHG intensity of algae-based biofuels did not find any clear difference between the different algae cultivation methods, or between methods for obtaining biofuels from algae, however, the variations in methods for assessment may have masked differences. A comprehensive meta-analysis, which would account for the impacts of variation in method of assessment discussed above, may detect consistent differences that are not apparent from the absolute values presented here.

The high parameter uncertainty in microalgae cultivation, harvesting and oil extraction reduces the overall confidence and conclusions that can be drawn from a LCA. An uncertainty assessment (e.g. based on Monte-Carlo simulations) should be conducted to increase the robustness and transparency of the outcomes and guide further research towards reducing the overall uncertainty. Moreover, a meta-analysis will reduce the range of variability by harmonizing several of the (different) assumptions applied in the various studies. Despite the uncertainties in the results, it is clear that the development of less energy-intensive technologies for microalgae cultivation and harvesting steps is critical in order to reduce the life cycle GHG emissions of microalgae biofuel. Microalgae biofuel production systems are very recent and the development of improved production technologies is still taking place. Many of the studies were based on data from pilot and lab-scale facilities. It is likely that larger scale operations would yield more favourable results. As a final recommendation, future LCA studies should also be performed for commercial systems to better support the selection of the best production pathways and to confirm the results from lab and pilot scale assessment.

## **9. Biogas from Macroalgae**

### **9.1. INTRODUCTION**

The scientific literature on liquid biofuel production from algae is relatively extensive compared to the literature on biogas production from algae. Prior to 2010, very few academic papers dealt with biogas from macroalgae (seaweed). However, since 2010, a significant number of papers have been published on this topic. The potential for research on biogas production from seaweed is extensive due to: the myriad of species available; the seasonal variation in the composition of these seaweeds; the logistics of harvesting the seaweeds; the differences in beach cast seaweeds, natural stocks of seaweeds and cultivated seaweeds; the potential growth rates per unit of area; the integration with aquaculture; the potential for co-digestion with other biomass feedstocks. The list of variables, many of which at present remain unknown, is long.

There are numerous species of seaweed that may be segregated or distinguished in a number of ways. For example they may be distinguished by colour; seaweeds may be green, red or brown. The genetic difference between the green seaweed *Ulva lactuca* (**Figure 9-1.f**) and the brown seaweed *Fucus serratus* (**Figure 9-1.c**) is larger than that between *U. lactuca* and an Oak tree.<sup>206</sup> *U. lactuca* contains a lot of sulphur leading to the production of hydrogen sulphide ( $H_2S$ ) in digesters (or on beaches).<sup>381</sup> The ideal ratio of carbon to nitrogen (C:N) for stable anaerobic digestion is between 20 and 30.<sup>382</sup> *U. lactuca* has a C:N ratio of less than 10,<sup>383,384</sup> making mono-digestion extremely difficult due to increased levels of ammonia in the digestate. Brown seaweeds such as *Laminaria* species (**Figure 9-1.b**) have very different composition as compared to *U. lactuca*; typically the C:N ratio is well over 20 at the end of the summer period and sulfur levels are minimal. The carbohydrate content of *L. digitata* increases and the ash content decreases from winter to summer.<sup>385</sup> This leads to a situation whereby both the C:N ratio and the specific biomethane yield rise and peak in late summer.<sup>385</sup>

Seaweed may be collected as a residue (such as the algae bloom associated with the green seaweed *U. lactuca*); may be cast on beaches (such as *F. serratus* and *A. nodosum* (**Figure 9-1.e**), may be harvested naturally from shallow waters (*H. elongata* (**Figure 9-1.a**) and *L. digitata*) or may be cultivated in aquaculture systems. Integrated multi-trophic aquaculture (IMTA) involves growing seaweed in association with fish farms, typically seaweeds such as *L. digitata* or *S. latissima* (**Figure 9-1**).<sup>386</sup>

A sustainable significant biofuel industry would probably require the scale associated with aquaculture. The economics of a seaweed biofuel industry are dubious as certain seaweeds are used for food and have high economic value. Technology development for seaweed biomethane is still at an early stage, with most work being evaluated at lab scale and very few seaweed digesters operating at pilot or commercial scales.<sup>206</sup> The authors believe that biogas from cast seaweed will have applications in the short term, however the quantities of seaweed required to match a significant portion of renewable energy are very large and it is as yet unknown as to how this can be achieved in a sustainable manner. In Denmark, a biogas facility digests cast seaweeds and the residues of seaweed processing industries.<sup>387</sup> In this section, we aim to provide a perspective on the potential for biogas production from seaweed.



**Figure 9-1:** Seaweeds collected from West Cork (a) *Himanthalia elongate* (b) *Laminaria digitata* (c) *Fucus serratus* (d) *Saccorhiza latissima* (e) *Ascophyllum nodosum* (f) *Ulva lactuca* (photos from Eoin Allen and Muhammad Rizwan Tabassum, Environmental Research Institute, University College Cork, Ireland)

## 9.2. CHARACTERISTICS OF SEAWEEDS

Macroalgae, more commonly known as seaweeds, are highly efficient aquatic organisms, capable of rapidly growing biomass utilizing sunlight, CO<sub>2</sub> and nutrients extracted from the sea.<sup>383</sup>

Seaweeds are in general characterised as having cell walls containing no lignin and only low amounts of cellulose and lipids.<sup>383,388</sup> Particular brown seaweeds (such as *A. nodosum*) can be rich in polyphenols which are difficult to degrade under anaerobic conditions and can inhibit anaerobic digestion.<sup>382,389</sup> Brown seaweeds are used to produce alginates, which find uses as thickeners, gelling agents and stabilizers for frozen food and cosmetics.<sup>388</sup> Red seaweeds are used for anti-fouling, antibiotic and anti-malarial applications.<sup>390</sup>

Seaweeds are also excellent indicators of pollution.<sup>390</sup> Algae blooms of *U. lactuca* are an indicator of eutrophication by excess nitrogen in estuarine waterways associated with non-point source pollution (run off from fields) and point source pollution (sewage outfalls).<sup>384</sup> However, growing and harvesting seaweed can remove nutrients from water and therefore can be used as a means to reduce eutrophication.<sup>381,389</sup>

Optimum levels of the C:N ratio for a substrate for anaerobic digestion (AD) are in the range of 20:1 to 30:1.<sup>391</sup> Digestion of nitrogenous substrates (C:N ratio less than 15) can lead to problems caused by excess levels of ammonia in the digester.<sup>391</sup> Protein (primary source of nitrogen) concentrations are low in brown seaweeds, whilst high in red and green seaweeds.<sup>388</sup> This can lead to situations whereby *U. lactuca* may have a C:N ratio of less than 10 while species like *S. latissima* can have a C:N ratio well above 20.<sup>206,384,388</sup>

The protein content of seaweed also can vary with season.<sup>206,382,385,388</sup> For example, *S. latissima* had a maximum value of protein in May (150 g/kg Total Solids (TS)) and a minimum (at half the protein content) in summer (73 g/kg TS).<sup>388</sup> Higher protein means increased N and lower C:N

ratios. Thus, as the year progresses from spring through summer the C:N ratio rises. This in turn can lead to higher biomethane potential assay results. Values of 204 L CH<sub>4</sub>/kg Volatile Solids (VS) were recorded in May (spring in the northern hemisphere) digesting *S. latissima*, rising to 256 L CH<sub>4</sub>/kg VS in August.<sup>388</sup>

When cultivated in ponds, the C:N ratio of *U. lactuca* was found to vary from 7.9 to 24.4.<sup>392</sup> Incoming irradiance was suggested as the factor controlling the C:N ratio, as with more light seaweed accumulates more carbon (and carbohydrates), which leads to an increase in the C:N ratio. Nitrogen starved *U. lactuca* produced more biomethane than nitrogen replete *U. lactuca*. A critical value of N of 2.17% of TS has been reported by Pedersen and Borum (1996) for maximum macroalgal growth, versus a subsistence value of 0.71% of TS as N.<sup>392,393</sup>

### 9.3. COMPOSITION OF SEAWEED

#### 9.3.1. Proximate Analysis

Proximate analysis gives data on the Total Solids (TS), Volatile Solids (VS) and ash content. The biodegradable element of the seaweed is the Volatile Solids. Salt is a major constituent of ash in seaweed. The TS content of brown seaweeds ranges with species and with season. Allen and co-workers gave a range of TS varying from 12.65% for *H. elongata* to 23.2% for *A. nodosum* (**Table 9-1**).<sup>394</sup> Tabassum and co-workers in assessing the seasonal variation of *A. nodosum*, showed that the TS content of *A. nodosum* varied from 19.2% in May to 34.5% in September.<sup>382</sup> In assessing the variation of *L. digitata*, it was shown that the TS varied from 8.4% (December) to 19.7% (August).<sup>385</sup>

More pertinent to bioenergy recovery is the volatile solid content. In seaweeds, VS content tends to be lower than other biogas substrates due to salt content. In assessing a wide range of different seaweeds collected in Ireland, a range of VS/TS of 60.3% (*U. lactuca*) to 86% (*S. polyschides*) was found (**Table 9-1**).<sup>394</sup>

The ash content not only reduces the biodegradability of the seaweed but the associated salt content can accumulate in the digestion process and suppress biogas production. Tabassum and co-workers found that the ash content in *A. nodosum* varied from a high of 30.4% in March to a low of 18.3% in November.<sup>382</sup> For *L. digitata* the ash content ranged from a high of 38.8% in December to a low of 19.5% in December.<sup>385</sup>

#### 9.3.2. Ultimate Analysis

Ultimate analysis of the substrate assesses the portion of carbon, hydrogen and nitrogen in a dry solid sample. This allows an elemental formula to be developed to describe the total solids content of the substrate. For example, *Ulva* sp. generated the elemental formula C<sub>9</sub>H<sub>16</sub>O<sub>7</sub>N.<sup>384</sup> The Buswell Equation can then be used to estimate the maximum theoretical potential for production of biogas from the substrate. Using the elemental formula for *Ulva* sp. a theoretical maximum methane production potential of 431 L CH<sub>4</sub>/kg VS at 51.5% methane content is predicted.<sup>384</sup> *U. lactuca* collected from West Cork, Ireland had a biomethane potential (BMP) of 183 L CH<sub>4</sub>/kg VS.<sup>384</sup> Thus, only 42% of the potential was converted to methane, indicating poor conversion efficiency by AD. Brown seaweeds collected from the coast of West Cork in 2013 had C:N ratios in excess of 15, with many in excess of 20 (**Table 9-1**).<sup>394</sup> The green seaweed *U. lactuca* had the lowest C:N ratio of 8.5.

#### 9.3.3. Biomethane Potential from Monodigestion of Seaweed

The BioMethane Potential (BMP) results from the literature are summarised in **Table 9-2**. The results are varied and reflect the fact that the seaweed was collected from different countries, at different times of year, with differing day length and light radiation, with different levels of nitrogen in the water, etc. The methodologies for assessing BMP may also differ; employing different inoculum, different inoculum to substrate ratio, different reactor volumes. However, it

can be stated that brown seaweeds (excluding *F. serratus*) tend to generate between 150 and 350 L CH<sub>4</sub>/kgVS.

It should be borne in mind that the yield per ton of substrate (expressed in m<sup>3</sup> CH<sub>4</sub>/t wet weight) is a function of both the BMP (expressed in L CH<sub>4</sub>/kgVS) and the portion of VS per unit wet weight (wwt). Thus, for example, if *A. nodosum* has a VS content of 19.4% (**Table 9-1**) and a BMP of 166 L CH<sub>4</sub>/kgVS (**Table 9-2**), then a value of 32m<sup>3</sup> CH<sub>4</sub>/t wwt may be achieved. This may be compared to *S. latissima*, which has a far higher BMP of 342 L CH<sub>4</sub>/kgVS (**Table 9-2**) but a lower VS content of 10.09% (**Table 9-1**) resulting in a yield value of 34.5m<sup>3</sup> CH<sub>4</sub>/t wwt.

**Table 9-1:** Characteristics of raw seaweeds collected in Cork in 2013 (TS = total solids; VS = volatile solids)<sup>394</sup>

Substrate	TS % wwt	VS % wwt	Ash % TS	C % TS	H % TS	N % TS	O % TS	C:N ratio
<i>A. nodosum</i>	23.2	19.4	16.1	40.4	5.3	1.6	36.6	26.0
<i>H. elongate</i>	12.65	8.10	36.0	30.8	4.1	1.4	27.7	21.4
<i>L. digitata</i>	14.20	10.34	27.2	34.2	4.8	1.5	32.3	22.3
<i>F. spiralis</i>	19.72	13.92	29.4	36.1	4.7	2.1	27.7	17.3
<i>F. serratus</i>	20.07	14.74	26.6	37.1	4.8	2.4	29.1	15.5
<i>F. vesiculosus</i>	21.18	16.11	24.0	26.8	3.2	1.5	44.5	17.6
<i>S. polyschides</i>	15.25	13.11	14.0	36.1	5.0	1.6	44.3	23.2
<i>S. latissima</i>	15.49	10.09	34.9	29.1	3.8	1.2	31.0	24.0
<i>A. esculenta</i>	18.72	11.91	36.4	29.3	4.2	1.9	28.2	15.5
<i>U. lactuca</i>	18.03	10.88	39.7	30.0	4.4	3.5	22.4	8.5

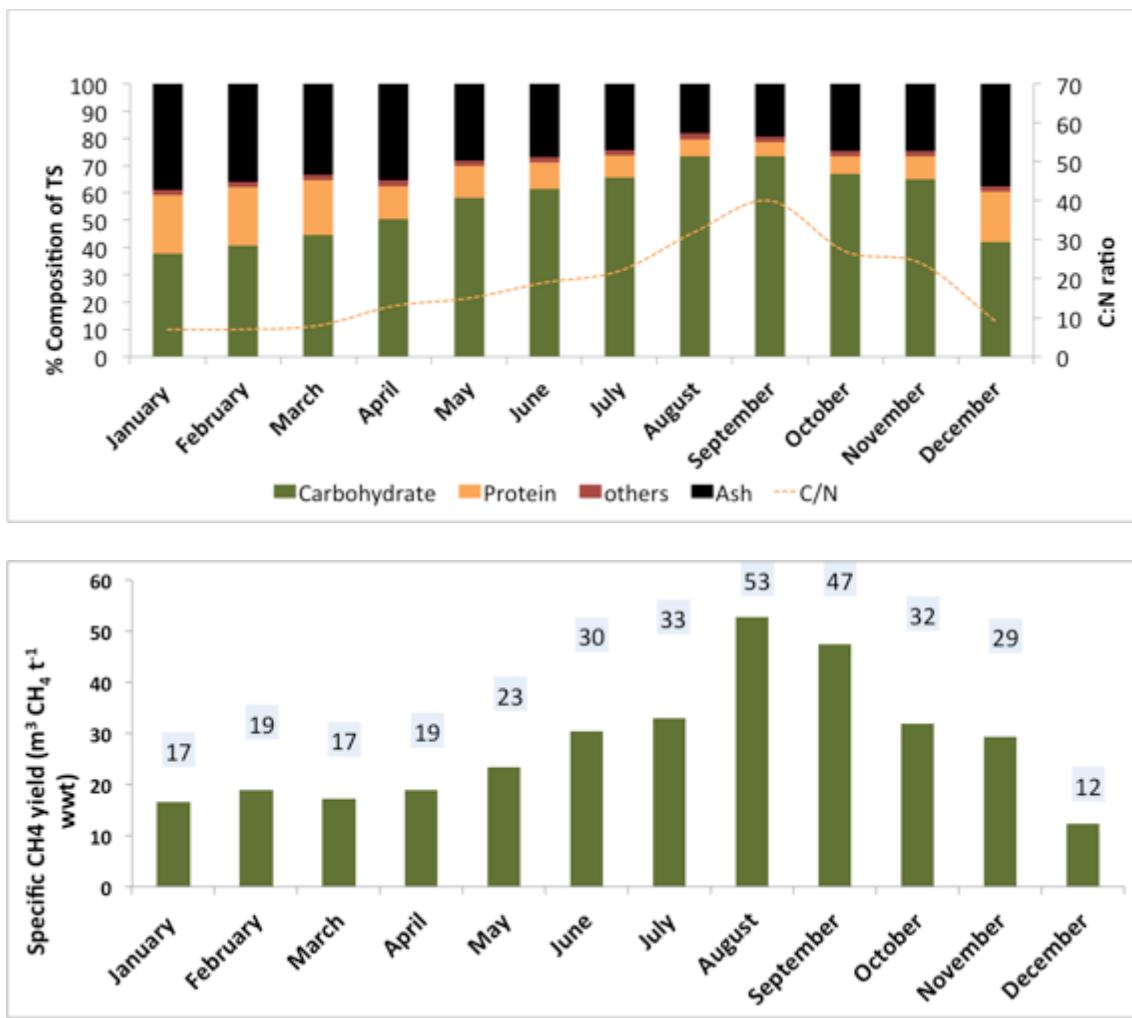
### 9.3.4. Annual Variation in Composition and Biomethane Potential in Brown Seaweed

Tabassum and co-workers assessed *L. digitata* collected from the shores of West Cork in the 12 months of the year (**Figure 9-2**).<sup>385</sup> *L. digitata* collected in January is not a good substrate for anaerobic digestion (AD) as the C:N ratio is less than 10, the carbohydrate content is less than 40% and the Specific Methane Yield (SMY) is 17m<sup>3</sup> CH<sub>4</sub>/t wwt. The carbohydrate content increased (with a corresponding decrease in protein and ash content) from January till August/September, when the carbohydrate content peaked (as did the C:N ratio). The highest SMY was recorded in August, 53m<sup>3</sup> CH<sub>4</sub>/t wwt with a C:N ratio close to the optimal range for AD. Adams and co-workers found this trend mirrored by *L. digitata* sourced in Wales.<sup>395</sup> The salt content (reflected in the ash content) is also the lowest obtained during the year. Thus, in northern latitudes *L. digitata* should be harvested in August for optimal substrate composition for AD.

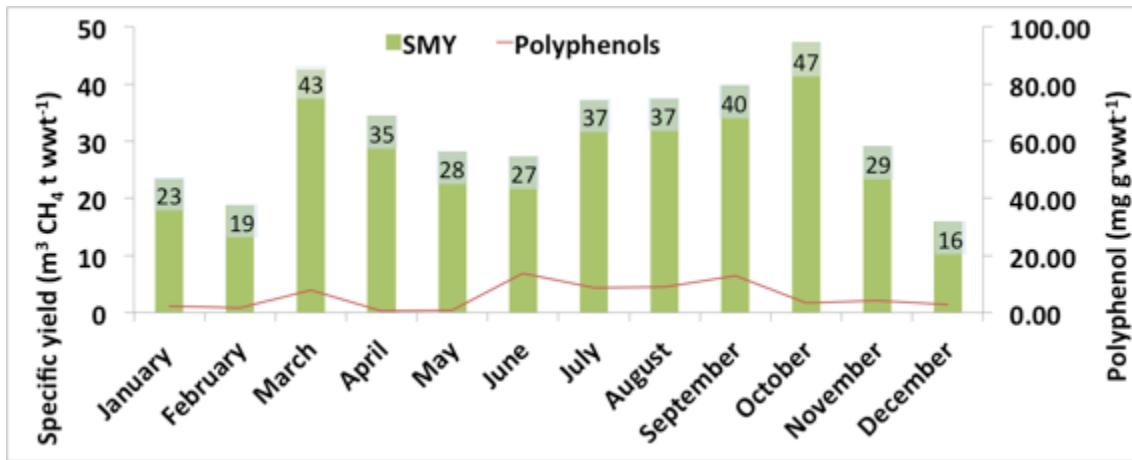
**Table 9-2:** Overview of Biomethane Potential (BMP) for different types of macroalgae

Seaweed	BMP Yield L CH <sub>4</sub> /kg VS	Region, Country	Reference
<b>Brown Seaweeds</b>			
<i>H. elongata</i>	261	West Cork, Ireland	394
	202	Brittany, France	388
<i>L. digitata</i>	218	West Cork, Ireland	394
	246	Sligo, Ireland	396
<i>F. serratus</i>	96	West Cork, Ireland	394
<i>S. latissima</i>	342	West Cork, Ireland	394
	335	Sligo, Ireland	396
	223	Trondheim, Norway	397
	220	Norway	398
	209	Brittany, France	388
<i>A. nodosum</i>	166	West Cork, Ireland	394
<i>U. pinnatifida</i>	242	Brittany, France	388
<i>S. polyschides</i>	255	Sligo, Ireland	396
	216	Brittany, France	388
<i>S. muticum</i>	130	Brittany, France	388
<b>Red Seaweeds</b>			
<i>P. palmata</i>	279	Brittany, France	388
<i>G. verrucosa</i>	144	Brittany, France	388
<b>Green Seaweeds</b>			
<i>U. lactuca</i>	183	West Cork, Ireland	384

Tabassum and co-workers assessed *A. nodosum* in a similar manner. A significant differentiation in these two seaweed species, *L. digitata* and *A. nodosum*, is in their polyphenol content.<sup>382</sup> The portion of polyphenol increases during the summer months inhibiting the production of biomethane. From **Figure 9-3** it may be noted that for *A. nodosum* there are two times during the year when SMY peaks, one in March (yielding 43m<sup>3</sup> CH<sub>4</sub> /t wwt) and a second in October (yielding 47m<sup>3</sup> CH<sub>4</sub> /t wwt).



**Figure 9-2:** (top) Annual variation in composition of *L. digitata* in Ireland and (bottom) associated biomethane potential.<sup>385</sup>



**Figure 9-3:** Annual variation in polyphenol and biomethane potential of *A. nodosum*.<sup>382</sup>

#### 9.4. ENSILING OF SEAWEEDS

If the suitability of seaweeds for anaerobic digestion peaks once a year, it is necessary to harvest at that time and store the seaweed for year round availability to feed the biogas system. An alternative approach could be to harvest seaweed throughout the year from a smart mix of

species.

Herrmann and co-workers investigated natural silage fermentation of five seaweed species from West Cork, one green seaweed species, *U. lactuca*, and four brown seaweed species, *A. nodosum*, *L. digitata*, *S. polyschides* and *S. latissima*. All seaweeds were collected at expected stages of optimal biomethane potential, from end of August to start of October.<sup>399</sup>

Ensiling of the seaweeds was assessed over 90 days. Between 10 and 28% of the original mass of seaweed was released as an effluent. The cost of silage would have to be considered against the benefits of increased yields. This effluent was rich in volatile fatty acids, which was very amenable to biomethane production. For four of the five seaweeds (excluding *S. polyschides*), ensiling increased methane yields (based on BMP of original fresh seaweed) by up to 28% provided that silage effluent is collected and utilised. Thus, the optimal logistics of a seaweed biogas industry should include provisions for ensiling of seaweed.

## 9.5. CONTINUOUS DIGESTION OF SEAWEED

### Difficulties in Long-Term Digestion of Seaweed

Biogas production from seaweed using AD is innovative, challenging and does not have a lot of empirical data from which to learn. High concentrations of sulphur, sodium chloride and heavy metals in certain seaweeds can lead to potential inhibition.<sup>400</sup> Sodium chloride is an AD process inhibitor at high concentrations but is still required in small amounts.<sup>401</sup> Sodium ions are required at levels between 100 mg/L and 350 mg/L for healthy AD microbial community metabolism. However, at higher sodium ion levels of 3,500 mg/L to 5,500 mg/L a medium inhibitory effect to methane-producing microorganisms is caused, while a strong inhibitory effect occurs above 8,000 mg/L. Acclimatisation of inoculum to higher sodium concentrations over a long period, such as 12 months to 24 months, can significantly increase the tolerance and reduce the lag phase time during digestion. Alternatively, direct use of inoculum sourced from marine environments may be a cost-effective approach to minimise sodium inhibition.<sup>224</sup> As discussed previously, inhibition of the digestion process can also occur when the C:N ratio is lower than 15, as this can lead to increased levels of ammonia in the reactor, which can eventually lead to failure.<sup>391</sup> For stable digestion, the ratio of alkalinity to acidity in the biogas digester (the FOS:TAC ratio) should be maintained at 0.3 or less.

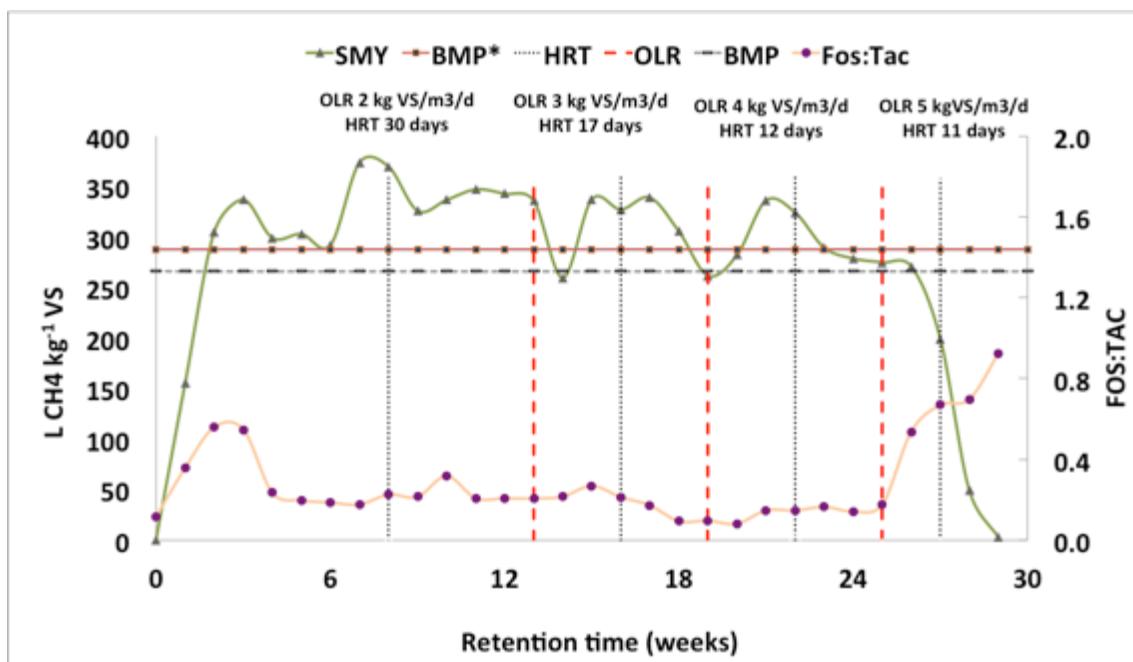
### Co-Digestion of Green Seaweed with Slurry

*U. lactuca* is a problematic seaweed because it reduces the amenity of the shore where it is collected and has a particularly low C:N ratio and a high sulphur content that is difficult for AD. Co-digestion with cattle manure can overcome some of these problems.<sup>402</sup> Allen et al. (2014) co-digested both fresh and dried *U. lactuca* with cattle manure slurry in long term continuous AD in laboratory 5-L scale reactors.<sup>381</sup> Three reactors co-digested *U. lactuca* with manure slurry comprising 25%, 50% and 75% of the VS in the feedstock, respectively. The optimum mix was determined to be 25% fresh *U. lactuca* and 75% manure slurry, with this mixture achieving 93% (170 L CH<sub>4</sub>/kg VS) of the biomethane potential at an organic loading rate (OLR) of 2.5 kg VS/m<sup>3</sup>/d with a FOS:TAC ratio of 0.3 (stable) and total ammonical nitrogen levels (TAN) of 3000 mg/l. The worst performing mixture was 75% fresh *U. lactuca* and 25% manure slurry which was only able to operate at an OLR of 1 kg VS/m<sup>3</sup>/d with a FOS:TAC of 0.45 (unstable).

### Mono-Digestion of Brown Seaweed

Tabassum and co-workers assessed mono-digestion of *L. digitata* over 30 weeks.<sup>403</sup> As would be expected from differences in composition, the performance was greatly different than *U. lactuca*. Of interest was the fact that the original BMP value recorded (266 L CH<sub>4</sub>/kg VS) was exceeded by the SMY achieved during continuous digestion (**Figure 9-4**). The BMP assay was repeated (BMP\* in **Figure 9-4**) with inoculum from the digester and the value increased to 288 L CH<sub>4</sub>/kg VS indicating the acclimatization potential of the microbial community to seaweed. The loading rate of

the reactor was increased in stepwise fashion up to 4 kg VS/m<sup>3</sup>/d with stable performance (defined as FOS:TAC less than 0.3 and the SMY having a value similar to the BMP value). The chloride content rose to 13 g/L, without great disruption to the process. The reactor failed when a loading rate of 5 kg VS/m<sup>3</sup>/d at a retention time of 11 days was imposed on the system.



**Figure 9-4:** Evaluation of 30 weeks of mono-digestion of *L. digitata* with increasing organic loading rate.<sup>403</sup> SMY, Specific Methane Yield; BMP, Biomethane Potential; HRT, hydraulic retention time; OLR, Organic Loading Rate; Fos:Tac, the ratio of alkalinity to acidity in the biogas digester

## 9.6. GROSS ENERGY YIELDS OF SEAWEED BIOMETHANE PRODUCTION

### Yields per Hectare of Seaweed Biomethane Systems

There is little agreement or definite knowledge on the yields of seaweed per hectare per annum. This obviously varies by species, by geographical location, by nutrient levels, by method of cultivation, and on whether the seaweed is cast or cultivated. One estimate suggests that a one hectare farm could yield 130 wet tonnes of kelp per annum, however another postulates 15 t TS/ha/yr for brown algae in temperate water.<sup>404</sup>

The sugar kelp, *S. latissima*, is one of the fastest-growing European kelp species and has the highest carbohydrate content. *S. latissima* produce large amounts of aquatic biomass and can be cultivated without the use of fresh water, farmlands, fertilizers and pesticides needed for land-based cultivation. These large, brown seaweed prefer cold-temperate zones and arctic growth conditions, which, in Europe, stretch from northern Portugal to northern Norway. This makes them attractive as future biomass producers for diverse industrial applications. This species resembles the Japanese kelp *S. japonica*, of which 4 million tons wet weight are cultivated annually in China, Korea, and Japan for use as food (kombu) and production of chemicals. Cultivation experiments with *S. latissima* in the North Atlantic coastal areas predict algal biomass production potentials of up to 340 t wwt ha<sup>-1</sup>,<sup>405</sup> however more conservative numbers range from 170-220 tons.<sup>406-408</sup> Indeed, there are still large variations in algal biomass production levels observed in cultivation trials and precautions should be taken in extrapolating small-scale trial results to industrial scale.

In natural environments, *S. latissima* can grow to 30 m depth and resist wave heights

corresponding to storm conditions. Cultivation should, however, preferably be done only in the more photosynthetically productive upper 10 m. Strong water current means higher nutrient supply per unit time and corresponding potential for higher growth productivity. Recent work has demonstrated that *S. latissima* has greater biomass production per individual when cultivated in strong water currents compared to more sheltered sites.<sup>408</sup>

The winged kelp *Alaria esculenta* is also among the most productive macroalgal biomass producers and has been cultivated in Ireland for the last 10 years.<sup>409</sup> It is reported to produce from 5-14 kg up to 45 kg wwt m<sup>-1</sup> rope (on which it is cultivated), the latter amount being equivalent to up to 90 tons ha<sup>-1</sup>. *A. esculenta* can grow naturally to at least 8 m depth in moderately to highly exposed areas.<sup>410</sup> The dry biomass weight of harvested *S. latissima* and *A. esculenta* are reported to vary between 8% and 20%, with the content of their storage carbohydrates mannitol and laminaran varying between 8-19% and 2-34% of their dry matter, respectively.<sup>411,412</sup>

It may be simplified to state that from a cultivation area of 1 hectare in the sea an amount of algal seaweed biomass of up to 100 - 200 tons wet weight can be harvested, containing 15-30 ton dry matter and 9-18 tons carbohydrates that can be converted to 6,000-12,000 m<sup>3</sup> methane or 6-12 tons ethanol. In cultivation, when used as feedstock for bioenergy production, the short growth phase of 6 to 9 months before harvesting leads to an advantageously short carbon cycle.

### Comparison of Energy Yields per Hectare with Land Based Systems

These yields can be compared with grass silage yields of 10 to 15 t TS ha<sup>-1</sup> yr<sup>-1</sup>.<sup>413</sup> **Table 9-3** provides estimations of the gross energy yields per hectare for a number of seaweeds and energy crops. The yields of seaweed vary greatly depending on variety and method of cultivation. Existing methods of growing seaweed on ropes with separation to allow boat travel between lines for harvest leads to relatively low potential yields. This is apparent in the first entry in **Table 9-3** where the yield of *L. digitata* is only 5 t TS ha<sup>-1</sup> yr<sup>-1</sup>. Higher yields are possible with innovation in cultivation methods. For example, the European Commission funded research project AT~SEA investigated advanced textiles for cultivating seaweed. These textiles were seeded in-house and taken to sites at sea for further growth and seaweed biomass production. Several test facilities were used, such as in Galway Bay, Ireland, Oban in Scotland and Solund in Norway. Yields of 16 kg m<sup>-2</sup> substrate were achieved. This equates to 160 tons wet weight per hectare per year or approximately 24 t TS ha<sup>-1</sup> yr<sup>-1</sup> (assuming 15% TS; see **Table 9-1**). This number will reduce, if for example, only 60% of the sea area is covered by membranes, to allow light to penetrate to the seabed.

**Table 9-3:** Potential gross energy production per hectare per annum based on a variety of species of seaweed<sup>357,394,413</sup> (wwt = wet weight)

Substrate	Yield (harvest)		Biomethane yield	Biomethane yield	Gross Energy	Source
	t TS/ha*yr (*t VS/ha/yr)	t wwt ha/yr	m <sup>3</sup> CH <sub>4</sub> / t wwt	m <sup>3</sup> /ha/yr	GJ/ha/yr	
<b>Seaweeds/Macroalgae</b>						
<i>L. digitata</i>	5.0	35.2	22.5	792	28	414
<i>S. polyschides</i>	22.5	147.5	34.5	5,090	181	415
<i>S. latissima</i>	30.0*	297.3	34.5	10,260	365	407
<i>A. esculenta</i>	36.0*	302.2	26.9	8130	289	416
<i>U. lactuca</i>	45.0	249.6	20.9	5216	186	392
<i>L. hyperboreana</i>	30.0 – 90.0			6,630 – 19,890	239 – 716	417
<i>L. japonica</i>	31.0* – 80.0*			8,060 – 20,800	290 – 749	407,418
<i>M. pyrifera</i>	34.0* – 50.0*			13,260 – 19,500	477 – 702	416,419
<b>Terrestrial crops</b>						
Fodder beet	16			6,624	250	420
Maize	19.5			5,748	217	420
Grass	12.5			4,303	163	413,420
Rye	2.1			732	28	420

For the purpose of comparison, maize is the dominant terrestrial crop used for biomethane production.<sup>420</sup> The biomass yield per hectare is high (**Table 9-3**), particularly in warm continental summers. Fodder beet also has a high biomass yield though its use is less common than maize. Grass would be an optimal crop for biomethane production in oceanic temperate climates, such as Ireland.<sup>413</sup> There is a wide range of data on potential yields of biomethane from seaweeds, but taking conservative values the energy yield per hectare from seaweeds could be similar to that from grass feedstocks.

The net energy per hectare of seaweed biomethane is unknown. It depends on the parasitic energy demand of harvesting or cultivation and of the process to convert the seaweed to usable bioenergy. To assess net energy seaweed can be categorised into three cases:

1. Seaweeds which are detrimental to the amenity of a bay, such as *U. lactuca*, which may be cast or found in long shallow estuaries and may require removal to ensure the amenity of a bay.
2. Cast or naturally occurring seaweed collected from the shore or harvested from shallow waters
3. Aquaculture: harvesting of seaweed from cultivated stock

The energy necessary for aquatic biomass feedstock production increases from case 1 to case 3. If *U. lactuca* needs to be removed from a bay, the energy involved in transport for collection may be neglected as the *U. lactuca* must be removed, whether it is digested or not. This is comparable to digestion of food waste, whereby food waste is collected from houses whether or not it is digested. Cast or naturally occurring seaweed is not intentionally cultivated but simply gathered. Thus, the

only energy involved in its production is in harvesting and transport to a processing facility. Aquaculture will require the highest energy for macroalgae biomass production, as it also requires energy for establishing growth in addition to harvesting and transport. It is unlikely, however, that it will require the same level of energy inputs as production of terrestrial crops. Fertiliser, herbicides and lime should not be required for its cultivation, for example. Typically the seaweed will draw nitrogen from polluted waters (such as those in close proximity to salmon farms) and in this manner can beneficially act to enhance environmental protection in such regions.

#### **9.6.1. Potential of Seaweed Resource**

Although seaweed is not available for digestion in continental areas remote from the sea, it has large potential as a biogas crop in coastal areas with temperate oceanic climates where it could be co-digested with grasses, slurries or food wastes. The exact length of coastline depends on the grid scale used to make the measurement, however according to Wikipedia, the UK has a coastline length of 19,700 km, South Korea 12,500km, France 7,300 km and Ireland 6,400 km. The amount of harvestable natural and cast seaweed resources associated with these long coastlines is as yet undefined but there is obviously a significant quantity available. Of issue, however is the ecological impact of harvesting natural resources and the legal authorisation to do so.

The view of the authors is that for a significant industry, seaweed should be cultivated at sea to minimise environmental impact and maximise resource potential. Optimal solutions would involve the circular economy whereby for example the seaweed farms can reduce eutrophication in waters associated with fish farms. According to Jacob and co-workers worldwide aquaculture contributed 66.6 million tonnes of fish in 2012.<sup>386</sup> It requires 12.9 t of *S. lattissima* to sequester the nitrogen excreted per t of Atlantic salmon.<sup>386</sup> Thus the potential resource of seaweed in integrated multi-trophic aquaculture is of the order of 850Mt, which greatly exceeds the ca. 26Mt of seaweed cultivated in 2013.<sup>386</sup>

### **9.7. CONCLUSIONS AND RECOMMENDATIONS**

On the 24<sup>th</sup> February 2015, the Environment Committee of the European Parliament (European Parliament News, 2015) stated that "Advanced biofuels sourced from seaweeds or certain kinds of wastes should account for at least 1.25 % of energy consumption in transport by 2020". This statement would suggest that seaweed biofuel technology is sufficiently developed and proven to begin to be deployed at scale. However, there are now very few seaweed digesters operating at commercial scale. There are also a myriad of seaweed species and numerous potential pathways to produce energy from seaweed. Long-term, anaerobic digestion may be problematic due to sand accumulation and due to salinity. It is unlikely that cast seaweed can be harvested at a scale to provide significant quantities of liquid transport fuel, but biomethane production and upgrading for injection into a pre-existing natural gas (methane) grid to support transport or heat and power production are possible. The more likely scenario is new cultivation in large sea farms, either in combination with fish farming (such as IMTA) or in areas dedicated to seaweed production. It is not yet known which particular species of seaweed would be best suited but the fast growing kelps are robust and probably the best candidates for large biomass production in areas where sea temperatures do not exceed 18-20°C. Numerous parameters (such as the method of cultivation, species of seaweed, seaweed yield per hectare, time of harvest, method of harvesting, suitability of seaweed to ensiling, gross and net energy yields in biogas, carbon balance, cost of the harvested seaweed, cost of the produced biofuel) have not been assessed. Much additional research is still required. An optimum pathway needs to be agreed for seaweed biofuels. The authors' view is that seaweed to biogas technology is understood at the lab scale but requires much more piloting and demonstration to be proven for commercial deployment. The economics of macroalgal biogas systems would benefit with the co-production of several products in a bio-refinery context, in which biogas is produced from residual macroalgal biomass fractions after extracting more highly valued compounds. However, considerable further research in this area is required to identify economically viable macroalgae biorefining scenarios.

## **10. Macroalgae for Higher Value Products and Liquid Fuels**

### **10.1. MACROALGAE POTENTIAL**

With the world's oceans covering over 70% of the planet's surface and the need to develop more sustainable routes to fuels and chemicals increasing, it is anticipated that over the medium- to long-term ocean-grown biomass, especially macroalgae or seaweeds exhibiting areal growth productivities exceeding terrestrial crops (see **Chapter 9**), will become an ever larger contributor of renewable feedstock for the bio-based products industry. In 2014, 27.3 million tons wet weight of seaweed were produced globally<sup>421</sup> for use mainly in production of hydrocolloids, food and feed.<sup>422</sup> Most of the macroalgae production in the world is from farms in China (80-90%), Phillipines, Indonesia and Japan. The European macroalgae recovery is typically from the environment, but a significant area of research is underway to support active cultivation. There is limited use of seaweeds for energy production; whole seaweed is generally not considered viable for producing energy alone due to its high feedstock price, but using seaweed processing side streams or by-products to produce energy or fuel bio-products in seaweed-based biorefineries may become viable in the future. Co-production of bioenergy products is seen as an interesting option for obtaining value from side-streams that don't have higher value uses. However, there are only a few stakeholders considering bioenergy products beyond biogas at this point. The main reason is the relatively high price of seaweed and the need to produce higher value products than energy from the seaweed biomass for production to be economically viable.

For liquid biofuels in particular, macroalgae is a biomass feedstock with great production potential but also considerable obstacles to being used, the main obstacle being its relatively high price for cultivation and conversion.<sup>423</sup> Currently the biorefinery concept, where smaller amounts of multiple higher value products are produced together with a few larger volume lower value bulk products like liquid biofuels, is seen as the way forward.<sup>424-427</sup> Depending on the streams available at a future biorefinery there are mainly two routes for energy production: biological conversion and hydrothermal processing. Some of the technologies used for macroalgal biomass conversion are similar to the process pathways discussed for microalgae in **Chapters 4 and 5**, and the respective differences are discussed there in more detail. Thermochemical conversion by pyrolysis or gasification of macroalgae into biofuels is not energy efficient because of the high water content of the algal biomass.<sup>428</sup> At the time of report preparation, energy and chemicals via sugar routes remain the most researched and understood, although these routes are still not straightforward. However, hydrothermal processing, an area of active research that shows promise for algae feedstocks (see **Chapter 5**), may ultimately prove to be a better fit for macroalgae feedstocks than biological conversion. During biological processing, either the carbohydrate macromolecules of a high-carbohydrate containing seaweed species (such as *Saccharina latissima*) are broken down to sugar monomers and fermented to ethanol, butanol or other sugar-fermentation products similarly to land-based biomass processing, or the seaweed is digested anaerobically to produce biogas that can be used as is or upgraded to pipeline quality methane (see **Chapter 9**).

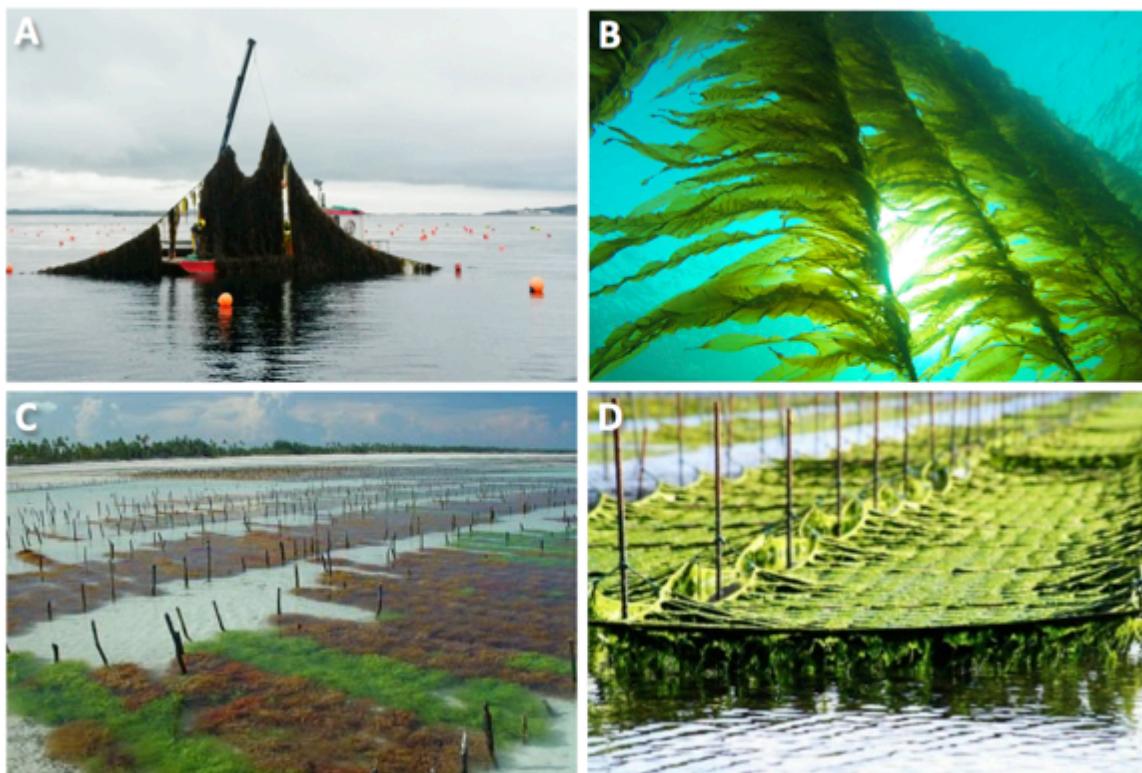
The many identified macroalgae projects described below are mostly focusing on improving cultivation efficiency and economics, not specifically on production of bioenergy, e.g., liquid or gaseous biofuels. Biofuel production processes from macroalgae are more or less similar to the routes used for terrestrial biomass feedstocks. The main concerns for the viability of the value chain are the feedstock production scale and price.

In addition to the current uses of whole seaweeds as foods and feeds and of macroalgal polysaccharides as hydrocolloids, macroalgae also contain a variety of compounds possessing a wide range of bioactive properties, such as anti-tumor, antiviral, anticoagulant, mucus protecting, LDL cholesterol reducing, prebiotic, anti-inflammatory and anti-hypertension effects.<sup>429</sup> One example is the sulphated polysaccharide fucoidan in brown seaweed, which has been extensively

studied with respect to its potential pharmacological properties.<sup>429</sup> Industry based on marketing of extracted bioactives or other high market value compounds represents a new bio-economy opportunity. However, while bioactive compounds can command a high market price, they represent a relatively small percentage of seaweeds dry weight. In such cases, residues from seaweed processing will constitute the major part of the seaweed biomass and are expected to be available for production of additional products, potentially including bioenergy products like biofuels. An on-going challenge to achieve such bio-production of higher value speciality products and lower value bioenergy products remains the disparity in scales of markets and production volumes between speciality chemicals/bio-actives and commodity biofuels.

The majority of published literature, studies and projects on macroalgal cultivation and conversion available to the authors is coming out of Europe, especially from the countries in northern Europe and Scandinavia. Due to the great interest in the potential for algal biofuels and the increasing importance of cultivated macroalgae in certain regions of Europe, especially in northern Europe, there have been numerous projects financed at both European and national levels. As such, the balance of this chapter focuses on recent developments in Northern Europe, summarizing major projects and companies actively researching or commercializing seaweed biomass. The majority of projects are not focused on applications but rather simply on lowering the cost and improving macroalgae cultivation efficiency, rate, yield and biomass quality.

Due to the fact that seaweed cultivation is labor intensive, with mechanized and automated cultivation technologies still at the development phase, most of the seaweed currently being used in Europe is wild harvest.<sup>422</sup> The number of sites with dedicated seaweed cultivation is growing rapidly, although the total amount being produced is still quite small, at maximum a few hundred tons wet weight per year. Even though seaweed cultivation is becoming a large-scale business, in general the feedstock remains too pricey to be used solely for energy production. Examples of natural and cultivated seaweed farming are shown in **Figure 10-1**.



**Figure 10-1:** Illustration of commercial seaweed farming (A-B) Cultivated seaweed harvesting by Seaweed Energy Solutions (SES), Norway (Photo: Judit Sandquist (A) and SES<sup>430</sup> (C) Seaweed

farming in Zanzibar<sup>431</sup> (D) Nori farming in Japan<sup>432</sup>

New cultivation technologies for seaweed feedstocks are being developed in research and development (R&D)-projects in Scotland, Ireland, Norway, Denmark, Sweden, Faroe Island, the Netherlands, Spain and Portugal, aiming to improve macroalgal growth productivity and biomass quality, enhance the predictability and increase the degree of mechanization and automation, thereby lowering cultivation cost. Large sea areas are available for aquatic biomass production without the conflicts that characterize corresponding terrestrial biomass production (e.g., arable land, fresh water, fertilizers, pesticides, GMO, etc.). One driver of increasing importance in the Nordic region is Integrated Multitrophic Aquaculture (IMTA), in which seaweeds are used to alleviate the dissolved effluents from fish farms.<sup>406</sup> This development is expected to increase the availability of seaweed and conversely lower its price.

## 10.2. MAJOR EUROPEAN PROJECTS

The **AT~SEA** project, advanced textiles for open sea biomass cultivation, was an EU 7th framework research project started in 2012 and ended in July 2015.<sup>433</sup> This project targeted the development of advanced, 2D seaweed cultivation substrates in order to demonstrate the technical and economic feasibility of seaweed cultivation in Europe. The project homepage states that the project identified suitable textiles for open sea seaweed biomass cultivation. Furthermore, project members founded a start up company (AT~SEA Technologies) to help commercialize the project's developed technologies. Seaweed cultivation is the focus in this project. Applications for seaweed biomass are not addressed.

**MERMAID** was an EU 7th framework project started in 2012 and ended in 2015.<sup>434</sup> This project targeted the integration of seaweed cultivation sites with offshore energy production, such as wind and wave energy production. Seaweed application was not targeted specifically, but co-produced seaweed biomass was assumed to be a marketable product in the business cases.

**EnAlgae** (Energetic Algae, <http://www.enalgae.eu/>) was a collaboration project within the INTERREG IVB North West Europe (NWE) Programme carried out March 2011 to June 2015 focused mostly on the following:

- micro- and macroalgae production in European pilot facilities, demonstration of strain management and common data management.
- Identification of opportunities within political, economic, and technology sectors to promote the adoption of algal biomass for the European energy market
- development of new tools to support decision- and policy makers as well as investors.<sup>435</sup>

Its overall objective was to develop algae-based technologies to reduce net CO<sub>2</sub> emissions and dependency on unsustainable energy sources in North West Europe. Sustainable technologies for algal biomass production, bioenergy production and greenhouse gas (GHG) mitigation were developed in the project and taken from pilot facilities through to market-place products and services.

**MacroFuels** (<http://www.macrofuels.eu/>) is a newly started project in the EU Horizon2020 framework that aims to produce advanced biofuels from macroalgae. The targeted liquid and gaseous biofuels are ethanol, butanol, furanics and biogas.<sup>436</sup> The conversion routes applied will be enzymatic hydrolysis with subsequent fermentation to ethanol, anaerobic digestion to biogas and thermochemical conversion to furanics. The project participants have started to grow seaweed but no results are available yet.

## 10.3. NATIONAL COMPANIES AND PROJECTS

### Denmark

**MacroAlgaeBiorefinery - MAB3** was a four-year project focused on assessing the potential for

macroalgal biorefineries to produce food, feed and fuel products.<sup>437</sup> The project vision was to ferment the carbohydrates to ethanol and use the protein rich residues as feed.

The subsequent **MAB4** project also focuses on macroalgae-based biorefineries. This project includes activities on seaweed cultivation and chemical extraction of products from seaweed. The chemicals of interest are food, feed and cosmetics ingredients.<sup>438</sup>

DTU and Steeper Energy have been working on HTL conversion of algae, both microalgae and macroalgae, and have found this conversion method promising for both alga types.<sup>439</sup>

### **The Netherlands**

**Hortimare** is a Netherlands-based company that operates in Norway and the Netherlands. They offer "Seaweed Genetics and Hatchery" where seaweed juveniles, bred for high contents and yields of marine proteins, mannitol, alginate and bio-active ingredients, are developed and sold to seaweed farmers. Hortimare also offers an "Integrated Aquaculture Service" supplying services to seaweed cultivation in the direct proximity of salmon farms. These seaweeds absorb significant amounts of the valuable nutrients released from aquaculture farms and are typically rich in proteins, mannitol and other ingredients, and according to Hortimare this type of integrated aquaculture also helps salmon farmers in maintaining and restoring marine ecosystems by improving bio-diversity and combatting sea lice.

In Hortimare's "Seaweed Bio-Refinery Plant," cultivated macroalgae is processed and refined into high quality protein for feed and food applications, feed for salmon being one of them. Other products are higher priced compounds for the global chemical-, pharma- and nutraceutical markets. There are probably side-streams from seaweed processing that can be utilized for fuel or energy production, although the issue of disparity in scales between higher value and commodity bioenergy products still needs to be overcome.

The Dutch Seaweed Biorefinery Program was a four-year project that ran between September 2009 and August 2013.<sup>440</sup> This project aimed to assess the concept of large-scale biorefining of seaweeds to produce CO<sub>2</sub> neutral chemicals, third generation biofuels and bio-energy. The project investigated several seaweed types as well as conversion and application strategies in a cascading biorefinery concept. The authors concluded that technical feasibility was demonstrated, however, several challenges remain before such seaweed-based biorefineries will be economically viable.<sup>441</sup>

North-sea-weed-chain: This one-year project assessed two business cases with two seaweed species, *Saccharina latissima* for winter cultivation and *Ulva sp.* for summer cultivation. Among the products, sugars from the sugar kelp were identified as potential biofuel intermediates, but the project concluded that since seaweed will be an expensive feedstock, the highest possible value needs to be obtained from the products extracted from the marine feedstock.<sup>442</sup>

### **Norway**

**Seaweed Energy Solutions AS (SES)** focuses on large-scale cultivation of seaweed primarily for feed and food purposes, but energy production from fractions and residuals is also part of the scope. SES operates Europe's probably largest seaweed farm in Mid-Norway with access to 70 hectare for cultivation of different seaweed species like the large biomass producing kelps sugar kelp *Saccharina latissima* and winged kelp *Alaria esculenta*. From their 300x300 m large pilot, SES produced 100 tons sugar kelp in 2015.<sup>443</sup> SES participates in various research projects focussed on finding innovative uses for cultivated seaweed and seaweed processing residues. Previously, in 2011-13, SES ran several projects with financial support from the Research Council of Norway (SeaBreed, SeaweedTech) and Eurostars (SeaweedStar), all focusing on macroalgae cultivation and conversion of macroalgal biomass to bioethanol.

There are several smaller Norwegian companies that produce seaweed-based food and feed

products, e.g., Austevoll Seaweed Farm, Seaweed AS and Algea.<sup>443</sup> More recently established companies like Ocean Forest, Folla Alger, Frøya Tare and Alginor also aim to cultivate or process seaweed.<sup>443</sup> All of these companies have so far no waste streams that can be used for energy production but their knowledge can contribute to developing and improving commercial seaweed cultivation and processing, and some of them will probably be important participants in the rapidly growing seaweed industry in Europe.

**SINTEF** Fisheries and Aquaculture also conducts research to develop industrial scale macroalgae cultivation technology, as well as on integrated multi-trophic aquaculture (IMTA).<sup>444</sup> Of note, SINTEF also has a 4 year Priority Project to develop technology for the production of biofuels and chemicals from seaweed.<sup>445</sup>

Of on-going seaweed projects, the following three, all financed by The Research Council of Norway, are the largest ones: 1) PROMAC aims to develop energy efficient processing of cultivated macroalgae for use in food and feed-products;<sup>446</sup> 2) MACROSEA seeks to establish a knowledge platform for industrial macroalgae cultivation, focusing on understanding and overcoming biological, ecological and technological challenges;<sup>447</sup> and 3) MARPOL is to apply enzyme technology to develop innovative biomaterials by modify and upgrading of macroalgae polysaccharides.<sup>448</sup>

### **Others**

FMC Health and Nutrition, a producer of functional ingredients for foods and dry-tablet medications, harvests wild seaweed for alginate and other polysaccharides production. In their processing operations there are waste streams not being productively used today, which could potentially be used for additional bio-products or bioenergy production.

**France's** Center of Studies and Valorization of Algae (CEVA) is well known for their competence on cultivation and processing of algal biomass into high value products. Also in France, **Cargill** is very active in the harvesting and conversion of seaweed, in particular to extract hydrocolloids and other products.

**Ireland's** MaREI Centre at University College Cork performs significant research on biogas and biohydrogen production from seaweed.

There are several other universities and research institutions in Europe, which have research groups actively researching seaweed-based production. These include: Energy Research Centre of the Netherlands (ECN); Scottish Association for Marine Sciences (SAMS); National University of Ireland, Galway; Irish Seaweed Center, Harper-Adams University; Teagasc (Agricultural Technological Institute in Ireland); Queen's University, Belfast; Aarhus University; Danish Technological Institute; Technical University of Denmark (DTU); Chalmers University; Gøteborg Universitet; Linné Universitet; Scandinavian Biogas; and the University of Linköping.

Several of these institutions were partners in the recently completed EnAlgae project (INTERREG 2011-2015), which brought together 19 partners and 14 observers from across seven EU member states described previously.<sup>435</sup>

Another large on-going research project on bioenergy production from cultivated macroalgae is Sweden's SEAFARM, which is focused on developing techniques for cultivating seaweeds to be used as raw materials for future seaweed-based biorefineries producing food, feed, bio-based materials and bioenergy products.<sup>449</sup>

## **11. Conclusions and Recommendations**

This report provides an overview of the state of technology of algae, both micro- and macroalgae, as feedstocks for bioenergy applications. Their photosynthetic efficiency far outpaces terrestrial feedstocks and it is generally accepted that there is a tremendous opportunity to exploit algae for bioenergy applications because of their high yielding biomass potential and favorable process energetics. However, there remain substantial technical, economical and sustainability barriers in place that slow down the successful commercial deployment of algae-based technologies for bioenergy applications. These barriers, generally applicable as barriers to algae commercialization and not specifically for bioenergy applications, are discussed throughout the report and can be categorized as follows: 1) Biomass productivity needs to be optimized with respect to energy, water and nutrient balance, to ensure a sustainable overall value chain; 2) Ecological, genetic and biochemical development of algal species is needed to improve productivity and robustness of algal species against perturbations such as temperature, seasonality, predation, and competition; 3) Physical, chemical, biological, and post-harvest physiological variations of produced algal biomass as a function of cultivation and production practices needs to be understood and integrated with the algae process operations; 4) Co-located inoculation, cultivation, primary harvest, concentration, and preprocessing systems need to be developed to aid economical viability; 5) Technologies for efficient on-site processing or fractionation of algal biomass into lipids, carbohydrates, and/or proteins needs to be developed at scales compatible with large-scale cultivation and farming; 6) Development and implementation of methods to maximize recycle of nitrogen and phosphorus compounds and other essential nutrients from residual materials need to be promoted to minimize fresh fertilizer and other nutritional input requirements.

Since the 2010 report was published, the economic and policy challenges have become more pronounced despite tremendous advances in understanding and manipulating algae biology, larger scale cultivation demonstration, and valorizing algal feedstocks for a variety of higher value product applications. In essence, it is understood that *high uncertainty still exists in how soon algae-based routes can become cost competitive for bioenergy, and how big algae for bioenergy ultimately can be*. This uncertainty stems from and extended period of low fossil fuel prices (in particular in comparison to 2010-2014), coupled with an on-going lack of clarity regarding future policy on carbon pricing. The cost targets for competitiveness in the market have become significantly more difficult to reach, despite the substantial improvements being achieved in the underlying core algal cultivation and upgrading technologies. As a consequence, companies that were leading commercial development of algae-based biofuels are increasingly redirecting their commercial focus towards production of higher value food, feed and specialty products. This report's comprehensive review of international commercial and research algae installations illustrates this shift in market focus.

Beside the economic challenges, there are additional concerns around the sustainability of large, commodity-scale algae cultivation. For example, there could be unsustainable demands on nutrients if algae were grown at a level sufficient to replace even a small fraction of transportation fuels. The nutrients available in wastewater (e.g., municipal or cellulosic biorefinery-derived) provide an opportunity to mitigate the cost of meeting the nutrient demand for algal growth while still allowing for the production of high quality algal biomass. Alternatively, the different bioenergy conversion options, e.g. lipid extraction, fractionation or biogas production processes, allow for different levels of nutrient recycling that will partially reduce an overall cultivation facility's net nutrient demand. The wide ranges of reported economic cost projections and algae process life cycle assessments illustrate the high level of complexity and uncertainty still facing the nascent algae production and refining industry.

This report provides a comprehensive overview of the recent progress in the fields of biotechnology for strain improvement of microalgae. In particular, the ability to manipulate the cell's biochemistry independent of the growth mechanism has been and remains one of the major

challenges in algal (and other) strain improvement. Increasing the algal cell lipid content typically negatively affects growth rate and biomass productivity. With the advent of genomic information becoming readily availability and substantial advances in metabolic engineering over the past 5 years, tremendous improvements have been made in reconfiguring metabolic networks without impacting growth rates. Manipulation of the cell's metabolism upstream of lipid synthesis, e.g., by increasing the availability of pyruvate for production of acetyl-CoA as a substrate for the initial steps in lipid biosynthesis, has increased cellular lipid content without impacting growth rate. Similarly, improvements in photosynthetic efficiency to achieve actual efficiencies closer to the theoretically possible 8-12% have been carried out in model organisms. An increased rate of photosynthesis was observed after reducing the size of the light-harvesting complex, with a simultaneous reduction in respiration. This is an area that should continue to be investigated in future research. Translating learnings and advances demonstrated in model organisms to large-scale-relevant species should also become a future research priority. There is a highly dynamic relationship between algal oil content and algal biomass growth productivity, which depends on the integration of species and the physiological conditions it is exposed to. There are opportunities to improve the productivity of algae through minimizing losses occurring during photosynthesis while avoiding impairing algal cells' robustness for outdoor deployment. This overall issue represents both one of the greatest technical opportunities and challenges to advancing microalgae-for-bioenergy deployment, and should be a major emphasis area for future research.

Numerous new promising conversion approaches have been developed, at least to a preliminary level, since the 2010 report was published. Of these approaches, two have gained traction as distinct pathways to pursue for the production of algae-derived fuel and products. These pathways can be categorized in broad terms as: 1) pretreatment of algal biomass in the presence or absence of acid to fractionate whole algae cell biomass into lipid, carbohydrate and protein fractions; and 2) processing whole cell algae under high temperature and pressure conditions to an upgradable biocrude liquid using hydrothermal liquefaction (HTL). Both pathways include a route to fuel while at the same time allowing for nutrient recycling and thus gain ground in the area of achieving more sustainable operations. While the core technologies are very different, both pathways are being pursued in parallel as a means to increase biofuel yield from algae. The first, the fractionation pathway, thanks to its less destructive nature (compared with HTL), can be integrated with multiple routes to bio-products to maximize the valorization of the algal biomass. As long as the on-going challenge of achieving cost-competitive production of bioenergy products in the current low energy market price environment persists, greater industrial research emphasis is likely to be placed on identifying and developing new higher value bio-products.

In terms of macroalgae (seaweeds), conversion to biogas using anaerobic digestion (AD) technologies is among the most promising approaches, with many research studies on use of macroalgae as a renewable feedstock reported since the 2010 report was published. The promise of a macroalgal biomass to biogas approach for algal bioenergy production is that lower cost cast seaweed could be used and AD-derived biogas could be used directly or upgraded to pipeline quality methane for injection into the existing gas grid to bolster gaseous fuel supply. Such conversion and bioenergy generation is not necessarily dependent on a continuous supply of macroalgae feedstock, as at least in some locations it will also be possible to feed (or co-feed) terrestrially-sourced biomass to supplement intermittent supplies of cast seaweed. A mixed feedstock approach like this could also improve economic viability. Feedstock flexibility coupled with the ability to integrate with existing gaseous fueling infrastructure makes an AD-based bioenergy route attractive for further study and development. However, AD-based approaches for macroalgae are not yet fully proven and may be problematic in the longer term due to issues such as high salinity and accumulation of sand in the reactors. It is also unlikely that cast seaweed can be harvested at a scale sufficient to provide significant quantities of transport fuel or on a consistent enough basis to meet the continuous supply needs for a biofuel-focused biorefinery. The more likely scenarios are co-feeding of land-based biomass as well as new large scale cultivation of seaweeds being established, more than likely associated with aquaculture. Seaweed-

based production for bioenergy products (as opposed to higher value food, nutritional and chemical products, which is already commercialized to a significant extent) is at an early stage of development. It is not yet known which species would be best suited for such a bioenergy application. Numerous parameters, including species, method of cultivation, harvest method, suitability of various feedstock storage methods, cost of the harvested seaweed, cost of the produced biofuel, etc., have not yet been adequately assessed and much additional research is required.

At least for the foreseeable future, primary strategies for liquid biofuels production from algae will need to rely on producing products from algae that will command a higher market value than liquid fuels. Alternatively, approaches that can valorize integration of algal production with wastewater treatment or carbon capture from high CO<sub>2</sub> emitters such as power plants or cement plants may aid the economical viability of algal biofuels production. In all cases, the production of algae for biomass and bioenergy applications will need to be integrated with existing markets and demand trends for products and fuels and will be guided by the quality and cost of the algal biomass.

For the algae bioenergy field to move forward and commercial operations to be able to begin to deploy at scale cost-competitive technologies for fuel-production from algae in the future, both improved policy support and well coordinated long-term and preferably highly international research and development (R&D) programs are needed. Despite wide-spread criticisms about the considerable demands that large scale cultivation of algae for bioenergy will place on nutrient, energy and water availability, these issues can be overcome with a long-term commitment to R&D and a focus on overcoming the major barriers that are limiting the realization of algae-based systems. In addition to meeting the economic targets mentioned above, it is imperative that algae-based processes meet sustainability goals, including having an overall positive return on expended energy, accompanied ultimately by a reduction in greenhouse gas emissions for the production of fuels or products. Furthermore, to support process and operation sustainability, there is a need to maximize the recycle of nitrogen, phosphorus, carbon and other nutrients from residual materials remaining after preprocessing and/or residual processing to minimize fresh fertilizer input requirements in upstream cultivation and reduce the demand on ever more constrained global nutrient resources.

As a final note, there have been challenges during the writing of this report in comparing the technical, economic and sustainability metrics across different technologies, as well as results being reported on similar systems by different laboratories, both nationally and internationally. This situation reflects the current lack of a transparent framework for describing and reporting on algal research and algae processing operations. In light of this, we want to close by emphasizing that there is **a clear and urgent need for more open data sharing and harmonization of analytical approaches, spanning the full range of issues being investigated, from cultivation and processing of algae, to product isolation and marketing, to TEA and LCA modeling methodologies.** A harmonization of methodologies in the international algal bioenergy community is imperative to increase the efficiency and pace of progress in the high priority areas of research needed to advance development and deployment of more sustainable algae-based bioenergy production.

## **Appendix A: Overview of Input Metrics for Describing Algae Bioenergy Operations**

**Table A-11-1:** Overview of suggested harmonized inputs in measurements used for reporting on algae operations, compiled from tables in ABO's Industrial Algae Measurements document (IAM 7.0, <http://algaebiomass.org/resource-center/technical-standards/IAM7.pdf>) and Batan et al<sup>117</sup>

Metric	Unit	Notes
<b>1. Cultivation: Continuous data - weather</b>		
Precipitation	cm day <sup>-1</sup>	Precipitation data (as available from weather events)
Air temperature	°C	Minimum hourly basis
Dew point temperature	°C	Hourly basis
Solar radiation/insolation	W m <sup>-2</sup>	Hourly basis
Wind speed	m s <sup>-1</sup>	Hourly basis
Air pressure	mm Hg	Hourly basis
<b>2. Cultivation: Continuous data - culture</b>		
Water salinity	mg L <sup>-1</sup>	
Water pH	pH	
Water temperature	°C	
Dissolved oxygen	mg L <sup>-1</sup>	
Oxidation reductive potential	mV	
Photosynthetically active radiation (PAR)	µmol m <sup>-2</sup> sec <sup>-1</sup>	Hourly basis
<b>3. Cultivation: Installation/logistics</b>		
Land use/cost		Upon installation
Polyethylene consumption	m <sup>3</sup> ha <sup>-1</sup>	Pond liner
Scale of production (pond/cultivation size)	ha	
Days of operation		Steady state/dynamic/culture crash ratio
Diesel Fuel Consumption	L ha <sup>-1</sup>	<sup>117</sup>
Polyethylene consumption	m <sup>3</sup> ha <sup>-1</sup>	
Natural Gas Consumption	MJ ha <sup>-1</sup>	
Electricity Consumption	kWa ha <sup>-1</sup>	
Photosynthetic Area per Facility Area	ha ha <sup>-1</sup>	
Transportation Costs	L kg <sup>-1</sup> biofuel	
<b>4. Cultivation: Discrete data – culture</b>		
Pond depth	cm day <sup>-1</sup>	Daily basis
Make-up water (evaporation)	L day <sup>-1</sup>	Volume of make-up water added to the pond (if applicable)
Make-up water (after harvest)	L day <sup>-1</sup>	Volume of water added back after harvest (if applicable)
Nutrients – nitrogen	mg N L <sup>-1</sup>	Daily basis, measured as ppm N
Nutrients – phosphorus	mg P L <sup>-1</sup>	Daily basis, measured as ppm P
Optical density (OD)	absorbance	
CO <sub>2</sub> source (flue gas/purified CO <sub>2</sub> )	Wt %	
Water supply		Fresh/saline/brackish water, stating source

Biomass concentration (AFDW)	g L <sup>-1</sup>	Measured according to standard procedure of total suspended solids
Contamination count	count (type) mL <sup>-1</sup>	
Salt consumption	g kg <sup>-1</sup> algae	

#### 5. Cultivation/productivity and other calculated metrics

Total productivity (AFDW)	g	$\frac{AFDW_{t\ final}(g) - AFDW_{g\ initial}(g)}{\text{pond volume (L)}}$ represents total biomass produced during an experiment or batch
Average biomass areal productivity	g m <sup>-2</sup> day <sup>-1</sup>	$\frac{AFDW_{total}(g)}{\text{pond area (m}^2\text{)} \times \text{days}}$
Daily Biomass areal or volumetric productivity	g m <sup>-2</sup> day <sup>-1</sup> or g L <sup>-1</sup> day <sup>-1</sup>	$\frac{AFDW_{t+n}(g) - AFDW_t(g)}{V \times n}$ where n = number of days between measurements, allowing for n > 1, typical sampling plans are AFDW every other day and calculated on a m <sup>2</sup> or L basis
Average biomass volumetric productivity	g L <sup>-1</sup> day <sup>-1</sup>	$\frac{AFDW_{total}(g)}{\text{pond volume (L)} \times \text{days}}$
Nitrogen depletion rate	mg L <sup>-1</sup> day <sup>-1</sup>	$\frac{\text{nutrients } N_t(\text{mg}) - \text{nutrients } N_{t+1}(\text{mg})}{n}$ where n = number of days between measurements and nutrient N > 0
Phosphorus depletion rate	mg L <sup>-1</sup> day <sup>-1</sup>	$\frac{\text{nutrients } P_t(\text{mg}) - \text{nutrients } P_{t+1}(\text{mg})}{n}$ where n = number of days between measurements and nutrient P > 0

#### 6. Cultivation/strain specific parameters for productivity

Light absorption coefficient		Needed for physics-based modeling of strain productivity
Light extinction coefficient		Needed for physics-based modeling of strain productivity

#### 7. Cultivation/other LCA/TEA metrics

Water evaporation rate	cm day <sup>-1</sup>	$\frac{\text{pond depth}_{t+n}(\text{cm}) - \text{pond depth}_t(\text{cm})}{n}$ where n = number of days between measurements
Pond downtime (unplanned)	% of month	% downtime due to unplanned events, crashes, contamination, emergency maintenance
Pond mixing energy	KWh day <sup>-1</sup> m <sup>-3</sup>	

	volume	
<b>8. Cultivation: Biomass component analysis</b>		
Moisture/Ash	% DW	Based on harvested, centrifuged material
Total lipids	% DW	Based on harvested, centrifuged material
Total protein	% DW	Based on harvested, centrifuged material
Total carbohydrates	% DW	Based on harvested, centrifuged material
C:N:P molar ratio		Based on harvested, centrifuged material
Biomass elemental composition (C, H, N, S, O, P)	Wt %	Based on harvested, centrifuged material
<b>9. Harvesting and conversion</b>		
Dewatered algal biomass concentration	g L <sup>-1</sup>	
Harvesting efficiency	%	Specify at each stage of harvesting process
Processing	As applicable	As much detailed information on conversion process, heat supply and efficiency of conversion or extraction as possible
Natural gas consumption	MJ ha <sup>-1</sup>	
Methanol Consumption	g kg <sup>-1</sup> biofuel	
Sodium hydroxide Consumption	g kg <sup>-1</sup> biofuel	
Sodium methoxide Consumption	g kg <sup>-1</sup> biofuel	
Hydrochloric Acid Consumption	g kg <sup>-1</sup> biofuel	
Spent biomass usage	As applicable	As much detailed information on processing of residual biomass as possible, including recycling nutrient and energy credits

## **Appendix B: Company and Research Group Overview**

An overview of global installed commercial facilities with capacity and target products is included here. We first highlight a couple of commercial installations here, with no particular preference other than that these represent installed operations across the value chain; from biomass production, volatile fuel production (Algenol) to biochemical pretreatment and extract and heterotrophic fermentation of microalgae. Commercial seaweed operations are presented as well, to highlight the

### **11.1. EXAMPLES OF COMMERCIAL PHOTOTROPHIC ALGAE CULTIVATION OPERATIONS**

There are many commercial algae cultivation companies currently in operation around the world. We will not summarize all companies here, but refer to the summarizing table of commercial operations, which is included as **Appendix B**. We selected a subset of the commercial operations here to highlight the different approaches that are currently undertaken as a viable approach to algae commercial deployment.

**Sapphire** has been developing the algae liquefaction technology since 2007 and has now moved to a pilot plant scale of operation. Sapphire has three facilities across California and New Mexico. Its headquarters and primary lab are in San Diego, California, there is a Research and Development Facility in Las Cruces, New Mexico. In 2010, the company began construction of the world's first commercial demonstration algae-to-energy farm in Columbus, New Mexico. Construction of Phase 1, constituting of the first 40 ha (100 acres) of ponds was completed in 2012. The company has the full technology pathway from algae growth to harvesting to conversion and fuels marketing. The algae growth system is an open pond design using non-potable water based on non-arable land. With the planned 120 ha (300 acres) of cultivation, the annual product yield is estimated to be around 3,780,000 L (1 million gallons) of transportation fuels.

**Algenol** uses a proprietary strain of cyanobacteria to produce an ethanol product, which is directly recovered from their photobioreactors.<sup>450</sup> The algae biomass is periodically harvested and processed by HTL to produce a biocrude. Algenol has an integrated biorefinery pilot plant in Fort Myers, Florida, with a capacity of 37,800 L (10,000 gal) per year of ethanol. In 2015, Algenol plans to announce their first commercial facility, to be located in the United States.

### **11.2. EXAMPLES OF INSTALLED OPERATIONS OF HYDROTHERMAL LIQUEFACTION OF ALGAE**

Hydrothermal processing of algae to fuels is still primarily a subject of laboratory R&D. The bulk of the research is still performed in batch reactor systems and cannot even be considered actual process development.<sup>234</sup> However, there are a few examples of the technology coming out of the laboratory into the marketplace.

As part of their patent portfolio (over 300 patents and patent applications) **Sapphire** has a patented process for liquefaction which includes a hydrothermal step with biocrude treatment and recovery including acidification and solvent extraction.<sup>451</sup> They also have a patent application describing the upgrading of the biocrude product.<sup>452</sup> The Sapphire biocrude ("Green Crude Oil") has been tested in partnership with petroleum refiners, such as Tesoro, in coprocessing with petroleum streams in a range of applications including hydrotreating, catalytic cracking, and delayed coker.

Algal biomass collected following ethanol production at the **Algenol** plant provides the feedstock for the biomass-to-hydrocarbon fuels process. The biomass is dewatered before being fed into the HTL unit, which Algenol has developed in collaboration with PNNL. The HTL biocrude oil is upgraded in a hydrotreater unit to a hydrocarbon product that essentially contains a mixture of liquid hydrocarbons in the range of diesel, jet and gasoline fuels. The upgraded product contains none of the oxygen, nitrogen or sulfur present in the biocrude from HTL and can be distilled into diesel, jet fuel and gasoline fractions. On one wet acre of algal cultivation Algenol can produce around 30,200 L (8,000 gallons) of liquid fuels per year, mainly ethanol, with 1,890 L (500 gallons) of jet ultra-low sulfur diesel, 1,440 L (380 gallons) of gasoline and 1,190 L (315 gallons) of jet fuel. This makes Algenol's technology compare favorably to corn at 3,900 L/ha (420 gallons per acre) per year.

**Genifuel Corporation and Reliance Industries, Ltd.** were partners with Pacific Northwest National Laboratory (PNNL) and others in the National Association for Advanced Biofuels and Bioproducts (NAABB), which coordinated research on the fuels pathway of algae strain development, growth, harvesting, and conversion. Reliance has now contracted with Genifuel to fabricate a 1 ton per day pilot plant for hydrothermal processing of algae biomass to liquid and gaseous fuels. Construction of the pilot plant is complete and start-up is underway, with delivery to India planned for later in 2015. The hydrothermal processing technology is licensed by Genifuel from PNNL.<sup>453</sup>

**Muradel** has a HTL demonstration plant at Whyalla, Australia, which can produce 30,000 liters per year of biocrude. A planned commercial plant of 1000 hectare would produce 500,000 barrels of biocrude per year. Muradel uses marine algae grown in seawater on marginal land for their feedstock. They earlier decommissioned their 2-year old pilot plant near Karratha, NWA. The projected cost for biocrude were \$9.90/L using the pilot plant data, but costs are expected at about 1\$/L in the new plant.

A continuous-flow HTL pilot plant was designed and built at the University of Sydney in Australia. Although there is no commercial interest involved in this work, it is a significant element in the process development effort for HTL of algae. The design flow rate of the pilot plant is 15-90 L of algae slurry at 10 wt% dry solids per hour. The process design does not include a biocrude separation technology, but biocrude extraction by dichloromethane is handled batchwise off-line. Processing results for *Chlorella* and *Arthrospira* sp. (*Spirulina*) have been published.<sup>454</sup>

### **11.3. EXAMPLES OF COMMERCIAL HETEROTROPHIC ALGAE OPERATIONS**

**Solazyme**, recently rebranded as **TerraVia**, is a San Francisco based corporation which cultivates *Chlorella*, a type of microalgae.<sup>455</sup> The microalgae are grown in fermentation tanks, and use the sugar derived from a variety of crop plants. Though previously a prominent producer of fuel derived from microalgae, they currently market food and nutrition products.<sup>456,457</sup>

**DSM** is a Dutch company that produces a variety of commodities pertaining to health and nutrition. It utilizes algae to produce some of its nutritional lipid products, primarily those which incorporate Omega-3.<sup>458</sup> In 2010, DSM acquired Martek, a company which produced DHA using *Schizochytrium*.<sup>459,460</sup> DSM also collaborates with other companies, such as Evonik Nutrition and Care GmbH and Sanofi to produce other algae related products.<sup>461</sup>

**ADM, the Archer Daniels Midland Company**, is a health and nutrition company. In 2014, ADM and Synthetic Genomics, Inc entered into a joint venture, which explored the use of microalgae to produce omega-3 fatty acids.<sup>462</sup> Synthetic Genomics works with a number of algal species, including *Chlorella*, to create their products.<sup>463</sup>

**Bunge** is a global agribusiness, which produces food and fertilizer. The company partners with

Solazyme/TerraVia, and has a line of algae related products called algawise, which contain Omega-9 fatty acids.<sup>464,465</sup> It also operates several plants which produce ethanol from crops.<sup>466</sup>

**Roquette** is a French company which processes plant based raw materials, and their feedstocks include maize, wheat, potatoes, peas, and microalgae. They cultivate Chlorella, and have a microalgae brand called algohub, which relates to pharmaceuticals, cosmetics, animal nutrition, infant nutrition, and nutraceuticals.<sup>467,468</sup>

## **11.4. OVERVIEW OF GLOBAL COMMERCIAL AND RESEARCH OPERATIONS**

**Table A-11-2:** Summary of commercial and research operations working towards commodity algae-based (both micro- and macroalgae) products globally, separated by region and by commercial installation. N/A = No information available, Fermentation includes predominantly heterotrophic cultivation companies, Suppliers include cultivation systems, measurement and general equipment manufacturers, Research includes large government supported academic and public private partnerships projects and consortia. All weblinks were accessed between September 2016 and January 2017

Company or Institution	URL	Latest Web Update Year	Cultivation (only commercial) open pond (raceway)/PBR (PBR)/Other	Focus	Algae species used	Country
<b>Commercial</b>						
<b>Europe</b>						
<b>Production Method - PBR</b>						
Agro tech	<a href="http://www.agrotech.dk/uk/facilities/microalgae-lab">www.agrotech.dk/uk/facilities/microalgae-lab</a>	2016	PBR	The production of high value bio-products, for example, fish farming, food or ingredients for medicine and industry.	microalgae	Denmark
Algae pangea	<a href="http://www.alga-pangea.de/">www.alga-pangea.de/</a>	2016	PBR	grow algae to be used in the Pharma industry, cosmetics industry, Food industry/Feed industry	microalgae	Austria
AlgaeLink	<a href="http://www.algaelink.nl/joomla/">www.algaelink.nl/joomla/</a>	2012	PBR	producing algal biomass for animal and human nutrition		The Netherlands
AlgaEnergy	<a href="http://www.algaenergy.es/">www.algaenergy.es/</a>	2016	PBR at laboratory, pilot and Industrial scale	developing cultivation systems	microalgae	Spain
Algalif	<a href="http://www.algalif.com/">www.algalif.com/</a>	2015	PBR	Astaxanthin for nutraceuticals, pharmaceuticals, food and cosmetics	Haematococcus	Iceland
Algainenergy	<a href="http://www.algainenenergy.com/ita/index.php">www.algainenenergy.com/ita/index.php</a>	2015	PBR	use algae as fertilizor		Italy
Algasol	<a href="http://www.algasol.info/">www.algasol.info/</a>	2015	industrial scale PBR (flexible, modular PBR, floating on water that can be deployed on land, pond, or the ocean)	develops industrial algae cultivation systems and performs research for production of nutraceuticals, water treatment, animal feed and biofuels.	microalgae	Spain
Algaspri	<a href="http://www.algaspri.com/">www.algaspri.com/</a>	2014	1.3 hectare PBR system			Almere, The Netherlands
Algenuity	<a href="http://www.algenuity.com/">www.algenuity.com/</a>	2016	PBR	produces equipment for algae growth		UK
Archimedea Ricerca	<a href="http://www.archimedericicerca.com/en.html">www.archimedericicerca.com/en.html</a>	2011	industrial scale PBR (Green Wall Panel)	Microalgal biomass production for natural cosmetics, oils, pigments, aquaculture and food supplement applications	microalgae	Italy
AstaReal, AB, Owned by Fuji Chemical Astax	<a href="http://www.astareal.com">www.astareal.com</a>	2016	PBR	CO2 technology	microalgae	Sweden
BiotechMarine(SECMA Biotechnologies Marines now Rouiller Group)	<a href="http://www.biotechmarine.com">www.biotechmarine.com</a>	2015	PBR	microalgae biotechnology company	microalgae	Germany, Milz
Blue Biotech GmbH	<a href="http://www.bluebiotech.de">www.bluebiotech.de</a>	2012	PBR	Focused on ethanol, bio-gas, bio-jet fuel, and biodiesel commercially. First phase of commercial production begins in mid-2015. Annual per-acre production of 8,000 gallons of fuel.	microalgae	France
Boots/PML	<a href="http://www.photobioreactor.co.uk">www.photobioreactor.co.uk</a>	2015	PBR	Provides a green solution for variety of industrial waste-greenhouse and toxic gases, wastewater and solid waste based on microalgae and bacteria. Biotransformation of waste products into valuable products such as biomass, 3rd generation biofuels – bioethanol, biodiesel, ecobriquettes, valuable and heavy metals, glycerine, etc		UK
Buggy Power Chlorella Trebon	<a href="http://www.buggypower.eu/">www.buggypower.eu/</a> <a href="http://ftp.alga.cz/cs/kvrobky-2-ras.html">ftp.alga.cz/cs/kvrobky-2-ras.html</a>	2015 2014	PBR (1100m3) PBR	Producing skin treatments for cosmetic purposes dedicated to biengineering projects for microalgae, production of Chlorella	Chlorella	Spain, Portugal Czech Republic
Ecoduna	<a href="http://www.ecoduna.com/">www.ecoduna.com/</a>	2016	PBR	produces equipment for algae growth		Austria
EcoFuel Lab, Ltd	<a href="http://www.ecofuel.cz">www.ecofuel.cz</a>	2015	PBR	microalgae consulting agency	microalgae	Czech Republic, Prague
Greenovation	<a href="http://www.greenovation.com">www.greenovation.com</a>	2016	PBR	Providing algae related technology	microalgae and macroalgae	Germany
Igem MIAL	<a href="http://www.igem.nl">www.igem.nl</a> <a href="http://www.mial.eu/index.php/en/">www.mial.eu/index.php/en/</a>	2012 2016	PBR PBR	Produces algae for skin treatment	microalgae	The Netherlands Germany
Microphyt	<a href="http://www.microphyt.eu/">www.microphyt.eu/</a>	2015	pilot, laboratory and Industrial Scale PBR	Uses technique to reduce pretreatment enzymes Sells food products	microalgae	France
Necton	<a href="http://phytobloom.com/">phytobloom.com/</a>	2014	flat and tubular PBR, air-lift-bags	Operates a pilot facility to cultivate algae, and produce biodiesel. Currently produces about 2,000 gallons of fuel per one acre.	microalgae	Portugal
Neocarbons	<a href="http://www.neocarbons.com">www.neocarbons.com</a>	2012	PBR	In research and development phase of an algae cultivation system. Approximately \$120,000 in yearly revenue from jetphtha		
Phycom (owns Nutress and algae orange)	<a href="http://www.phycom.eu/">www.phycom.eu/</a>	2016	PBR	Partnered with Diversified energy to develop an algae production system that to be incorporated into XL's bio refinery. Estimated to provide 100-200 dry tons of algae per acre	microalgae	Switzerland, Gockhausen
Phycosource	<a href="http://www.phycosource.com">www.phycosource.com</a>	2013	PBR	Services related to the processing and refinement of algae biomass, processing services: cell disruption, drying, extraction, purification, encapsulation for polysaccharide, carotenoids, pigments, oleoresins	Nannochloropsis, Tetraselmis, Haematococcus Pluvialis, Isochrysis	France, Cergy-Pontoise
Phyto-Aqua	<a href="http://www.phytoaqua.com">www.phytoaqua.com</a>	2016	PBR	Building photobioreactor and algae cultivation for vitamins and microbiodives - this sector was taken over by bbi-BioTech (IGV withdrew from PBR sector in 2014), bioextracts from algae (such as vitamins and microbiodives)	misc. microalgae	UK
Phytolutions	<a href="http://www.phytolutions.com/">www.phytololutions.com/</a>	2014	PBR- Phytobag	assists in the development of technology for microalgae cultivation, biorefinery engineering, and integrated system design	microalgae	Germany
Proviron	<a href="http://www.proviron.com/">www.proviron.com/</a>	2015	PBR	produce algae as biofertilizer from waste to mitigate carbon footprint		Belgium, Hemiksem
Subitec	<a href="http://subitec.com/de">subitec.com/de</a>	2016	construction and development of PBR (Flat panel airlift Reactors)	gets rid of algae blooms, and helps ecosystems recover		Germany
Varicon Aqua Solutions Ltd.	<a href="http://www.variconaqua.com/">www.variconaqua.com/</a>	2014	PBR	explores microalgae fuel applications	microalgae	UK, Worcestershire
<b>Production Method - Raceway</b>						
Algazur	<a href="http://www.algazur.fr/">www.algazur.fr/</a>	2015	indoor open pond	food products	spirulina	France
Algosud	<a href="http://www.algosud.com">www.algosud.com</a>	2015	open pond	developing and offering PBRs for cultivation, fully computerized algae production systems, cultivation of microalgae for food, feed, fuel, co2 absorption	Nannochloropsis, Tetraselmis, Isochrysis, France, Montpellier Pavlova pinguis	
ASN Leader	<a href="http://www.asn-espirulina.com">www.asn-espirulina.com</a>	2016	open pond	Aquaculture, cosmetics, aquariology, human nutrition	marine microalgae	Spain, Murcia
BASF (owns Cognis)	<a href="http://www.basf.com/us/en/html">www.basf.com/us/en/html</a>	2016	open pond	production of algae for food		Germany
Couleur Spiruline	<a href="http://www.couleur-spiruline.com/">www.couleur-spiruline.com/</a>	2015	indoor pools	grows spirulina for dietary supplements	spirulina	France
Horus Spiruline	<a href="http://www.horus-spiruline.fr/">www.horus-spiruline.fr/</a>	2016	indoor pools	food from algae	spirulina	France
Le Chant De L'eau	<a href="http://www.lechantdel'eau.fr/">www.lechantdel'eau.fr/</a>	2015	indoor pools	Nutritional supplements from algae	spirulina	France
La spirule D'olt	<a href="http://www.spirulinedelacotelebie.fr/">www.spirulinedelacotelebie.fr/</a>	2016	indoor pools	algae for food	spirulina	France
Monzon Biotech	<a href="http://mznbiotech.com/en">mznbiotech.com/en</a>	2010	open pond	Development stage of a biotechnology company focusing on the research, development, and sale of algae nutritional, food additives, and pharmaceutical products		Spain
Spir'Alpines	<a href="http://www.spiralpines.com/">www.spiralpines.com/</a> <a href="http://www.spiriform.fr/#/s-production/">www.spiriform.fr/#/s-production/</a>	2015	indoor pools	produces algae nutrients	spirulina	France
Spir'lib	<a href="http://www.spirulib.com/">www.spirulib.com/</a>	2016	indoor pools	produces algae nutrients	spirulina	France
Spirulina Algae	<a href="http://www.spirulina.gr/">www.spirulina.gr/</a>	2015	open pond	produces algae nutrients	microalgae	Greece
Spirulina La Capitelle	<a href="http://www.spirulinalacapitelle.com/">www.spirulinalacapitelle.com/</a>	2015	indoor pools	producing Astaxanthin applied research (cultivation systems, harvesting, drying, extracting methods, novel applications for food, pharmaceutical, cosmetic, bio-fuels industries)	Haematococcus pluvialis, misc. microalgae	France, Villecun
Spiruline D'aquí	<a href="http://www.spirulinadaguia.com/">www.spirulinadaguia.com/</a>	2016	indoor pools	food from algae	spirulina	France
Spiruline de beaune	<a href="http://http://www.spirulinedebeaune.com/">http://www.spirulinedebeaune.com/</a>	2015	indoor pools	food from algae	spirulina	France
Spiruline de Bretagne	<a href="http://www.spiruline-de-bretagne.com/">www.spiruline-de-bretagne.com/</a>	2015	indoor pools	food from algae	spirulina	France
Spiruline d'aquitaine	<a href="http://www.spiruline-aquitaine.com/">www.spiruline-aquitaine.com/</a>	2009	open pond	Packaged Spirulina	microalgae	France
Spiruline de haute-provence	<a href="http://www.spirulinedehauteprovence.fr/">www.spirulinedehauteprovence.fr/</a>	2016	indoor pools	Produce food products using algae	Spirulina	France
Spiruline des landes	<a href="http://www.spirulinedeslandes.com/">www.spirulinedeslandes.com/</a>	2015	indoor pools	Produce food products using algae	spirulina	France
Spiruline de la côte bleue	<a href="http://www.spirulinedelacotebleue.fr/">www.spirulinedelacotebleue.fr/</a>	2016	indoor pools	food products	spirulina	France
Spiruline d'Ollioules	<a href="http://https://www.spirulinedeollioules.com/">https://www.spirulinedeollioules.com/</a>	2016	indoor pools	food products	spirulina	France
Spiruline du cap des ailles	<a href="http://https://spirulineducapdesailles.com/">https://spirulineducapdesailles.com/</a>	2016	indoor pools	spirulina flakes	spirulina	France
Spiruline du dauphine	<a href="http://www.spirulinedu-dauphine.fr/">www.spirulinedu-dauphine.fr/</a>	2016	indoor pools	food products	spirulina	France
Spiruline du Garlaban	<a href="http://www.spirulinedugarlaban.com/">www.spirulinedugarlaban.com/</a>	2010	indoor pools	food products	spirulina	France
Spiruline du Moulin	<a href="http://www.spirulinedumoulin.com/">www.spirulinedumoulin.com/</a>	2015	indoor pools	Food products	spirulina	France
Spiruline du val de Dagne	<a href="http://www.spirulinedeveddagene.fr/">www.spirulinedeveddagene.fr/</a>	2016	indoor pools	Food products	spirulina	France
Spiruline du val de l'Eyre	<a href="http://www.spiruline-leyre.fr/">www.spiruline-leyre.fr/</a>	2016	indoor pools	Food products	spirulina	France
Spiruline Emoi	<a href="http://https://spirulinememoi.wordpress.com/origine/">https://spirulinememoi.wordpress.com/origine/</a>	2015	indoor pools	Algae as a food product	Spirulina	France
Spiruline les deux maines	<a href="http://www.spiruline-12m.fr/qui-sommes-nous">www.spiruline-12m.fr/qui-sommes-nous</a>	2015	indoor pools	Algae as a food product	spirulina	France
Spiruline Solaire	<a href="http://www.spirulinesolaire.com/">www.spirulinesolaire.com/</a>	2016	indoor pools	Algae as a food product	spirulina	France
Tomalgae	<a href="http://www.tomalgae.com/">www.tomalgae.com/</a>	2015	indoor open pond	Algae as a food product	spirulina	Belgium

Company or Institution	URL	Latest Web Update Year	Cultivation (only commercial) open pond (raceway)/PBR (PBR)/Other	Focus	Algae species used	Country
Vendee algues	<a href="http://www.spiruline-vendee-algues.com/actualites.html">www.spiruline-vendee-algues.com/actualites.html</a>	2016	indoor pools	Algae as food products	spirulina	France
<b>Production Method - Raceway and PBR</b>						
A4F-AlgaFuel, S.A.	<a href="http://www.algafuel.pt">www.algafuel.pt</a>	2016	PBR, open pond	Develops systems to cultivate algae, still in research and development phase, hopes to go commercial	microalgae	Portugal, Lisboa
Algae Food and Fuel	<a href="http://www.algaefoodfuel.com/english/home/">www.algaefoodfuel.com/english/home/</a>	2016	PBR and open pond	consultants specialized in design processes involving photosynthetic microbes		The Netherlands
Algosome Technologies (owns Alpha Biotech)	<a href="http://www.algosome.com">www.algosome.com</a>	2016	PBR, open open pond	developing PBRs and large scale biomass production (via Algasol Bangladesh Ltd and Algae Biomass Bangladesh Ltd) (mostly for fish feed)		France, Saint-nazaire
Roquette/Bioprodukte Prof. Steinberg GmbH	<a href="http://www.algeomed.de/">www.algeomed.de/</a>	2011	PBR	leading French producer of spirulina addition of selenium to microalgae, adapting microorganism with selective pressure to produce enhanced non-GMO organisms for aquaculture and animal nutrition	microalgae	Germany
Supreme Biotech	<a href="http://www.supremebiotech.com">www.supremebiotech.com</a>	2015	PBR			UK/New Zealand
<b>Production Method - Fermentation</b>						
DSM	<a href="http://www.lifesdha.com/">www.lifesdha.com/</a>	2015	fermentation	Algae as fish food and nutritional supplements	microalgae	Netherlands
Fermentalgal	<a href="http://www.fermentalgal.com/en/">www.fermentalgal.com/en/</a>	2015	fermentation, microalgae bred in a predominantly heterotrophic and mixotrophic environment	Using water from food companies as the feedstock for growing	microalgae	France, Libourne
Lonza	<a href="http://www.lonza.com/">www.lonza.com/</a>	2016	fermentation	supplying the pharmaceutical and biotechnology industries with biopharmaceuticals	ulkenia	Switzerland, basel
Roquette	<a href="http://www.roquette.com/2014-1/">www.roquette.com/2014-1/</a>	2016	fermentation	bioreactor technology (Flat Panel Airlift Reactor), process engineering and plant construction, microalgae cultivation	<i>Chlorella vulgaris</i> , <i>Haematococcus pluvialis</i> , <i>Phaeodactylum tricornutum</i> , <i>Nannochloropsis oculata</i> , <i>Tetraselmis suecica</i> , <i>Chlorella sorokiniana</i> , <i>Isochrysis spec.</i> , <i>Dunaliella tertiolecta</i>	France
<b>Production Method - Unknown or Other</b>						
Activ'alg	<a href="http://www.usinenouvelle.com/article/activ-alg_N44121">www.usinenouvelle.com/article/activ-alg_N44121</a>	2005	-	producing of <i>Nannochloropsis gaditana</i> for aquaculture feed companies	<i>Nannochloropsis gaditana</i>	France, Tourlaville
AlgaeBiotech (Joint Venture from FreyCon)	<a href="http://www.algaebiotech.es/">www.algaebiotech.es/</a>		-	Uses geothermal process to convert biomass to oil. A new clean tech company focused on carbon sequestration and waste remediation	microalgae	The Netherlands, France
AlgaeCytex	<a href="http://algaecytex.com">algaecytex.com</a>	2013	-	Produces microalgae on a commercial scale.	microalgae	UK, Canterbury, Kent
Algae health	<a href="http://www.algaehealth.ie/">www.algaehealth.ie/</a>	2013	-	nutritional compounds from algae producing high value molecules from algae (eg Omega3 fatty acids), developing technology for large scale production of 3rd generation biofuels (in future)		Ireland
Algafluid	<a href="http://www.algafluid.com">www.algafluid.com</a>	2009	-		microalgae	Spain, Lerida
Algallimento	<a href="http://www.algallimento.com/">www.algallimento.com/</a>	2016	-	food product	Tetraselmis, <i>Spirulina</i> and <i>Dunaliella</i> microalgae	Spain
Algamundi	<a href="http://www.algamundi.com/">www.algamundi.com/</a>	2016	-	microalgae for food and feed		Italy
Algea	<a href="http://www.algea.com/">www.algea.com/</a>		Cultivation systems			Norway
Algen (algal technology center)	<a href="http://www.algen.si/">www.algen.si/</a>	2016		producing and manufacturing spirulina and spirulina-based nutritional products	spirulina	Slovenia
Algotech Produkter AS > IGV	<a href="http://www.aquaflor.no">www.aquaflor.no</a>	2015	-	develops algae based products and sells them in bulk to manufacturers and distributors in the health foods and nutritional supplements markets	microalgae	Norway, Oslo
Aligel	<a href="http://www.aligel.pt/#/home">www.aligel.pt/#/home</a>		-	Astaxanthin for nutraceuticals, pharmaceuticals, food and cosmetics	microalgae	Portugal
Algo-spiruline	<a href="http://www.algo-spiruline.fr/les-bassins-2/">www.algo-spiruline.fr/les-bassins-2/</a>	2016	-	food supplements	spirulina	France
Algorigin	<a href="http://www.algorigin.ch/">www.algorigin.ch/</a>	2016	-	food supplements	spirulina	Switzerland
Alice Group	<a href="http://alicegroups.com/">alicegroups.com/</a>		-	Produce algae for superfood, cosmetics and fuel		Iceland
Aqualia	<a href="http://www.aqualia.es/aqualia/conoce-aqualia/index.html">www.aqualia.es/aqualia/conoce-aqualia/index.html</a>	2016	-	Providing water to consumers		Spain
Aragreen	<a href="http://www.aragreen.com/">www.aragreen.com/</a>	2016	-	uses micro algae as a key building block for two distinct industrial processes (i) enhanced waste water treatment and (ii) the production of a range of algae containing anti-oxidants, pigments and proteins for human and animal consumption.	microalgae	UK
Azur Naturel	<a href="http://azur-naturel.fr/">azur-naturel.fr/</a>	2016	-	production of algae for food	spirulina	France
bbi-Biotech	<a href="http://bbi-biotech.com/">bbi-biotech.com/</a>	2015	-	biopharmaceutical company using microalgae based technology to create recombinant therapeutics	microalgae	Germany
BDI	<a href="http://www.bdi-bioenergy.com/">www.bdi-bioenergy.com/</a>	2015	-	The mass culture of cyanobacteria using fermentation for the production of reagents interesting various markets	blue-green algae	Austria
Bio Fuel Systems	<a href="http://www.biopetroleo.com/">www.biopetroleo.com/</a>	2014	-	team up with Synthetic Genomics to commercialize DHA from algae (plant that is owned by Solazyme, ADM, and ANP has a projected capacity of 20,000 MT/yr in 2015)	seaweed	Spain
BioGasol	<a href="http://www.biogasol.com/">www.biogasol.com/</a>	2015	-	technology for the production of microalgae		Denmark
Biopharma	<a href="http://www.biopharma.no/">www.biopharma.no/</a>	2016	-	Equipment technology company for processes involving the harvesting and drying of algae	microalgae	Norway
Biotech Industri, AB	<a href="http://www.allgrow.net">www.allgrow.net</a>	2016	-	Develop technology for large scale processes		Sweden, Askim
Bluemater	<a href="http://bluemater.com/">bluemater.com/</a>	2010	-	manufactures technology for biopharmaceuticals. Develops 5L stirred tanks to 600L bioreactors	microalgae	Spain
CDU- Microalgae Research and Business	<a href="http://www.emekgubre.com">www.emekgubre.com</a>	2014	Pilot Prototype design and production			Turkey
Cellulac (includes Aer-bio merged)	<a href="http://cellulac.co.uk/en/">cellulac.co.uk/en/</a>	2016				UK
CEVA	<a href="http://www.ceva.fr/">www.ceva.fr/</a>	2016	-	It organizes a research applied on algae (macro & micro), seagrass and marine biotechnologies. In particular, it ensures the transfer of scientific knowledge from the academic world to the industry field.		France
Clos Sainte Aurore	<a href="http://www.spirulinaces.com/fr/">www.spirulinaces.com/fr/</a>	2013	-	Selling nutritional products	spirulina	France
Domaine Algal	<a href="http://www.spirulinealferme.com/">www.spirulinealferme.com/</a>		-	grows spirulina for dietary supplements	spirulina	France
Domaine Traverse	<a href="http://domaine-traverse.com/">domaine-traverse.com/</a>	2016	-	grows spirulina for dietary supplements	spirulina	France
Ellinor	<a href="http://www.ellin.gr/en/">www.ellin.gr/en/</a>	2014	-	does not deal with algae	no algae	Greece
EniTecnologie, S.p.A	<a href="http://www.enitecnologie.it">www.enitecnologie.it</a>	2016	-	Attempted to commercial algae based products--no longer appears active	microalgae	Italy
Ennesys	<a href="http://www.ennesys.com/">www.ennesys.com/</a>	2015	-	mnesys develops and commercializes energetically self-sufficient water and organic waste recycling equipment, based on micro-algae.	microalgae	France
ENVI, Ltd. Trebon	<a href="http://www.envi.cz">www.envi.cz</a>	2015	-	teaming up with Solazyme to create a factor in Brazil to produce fuel from algae	microalgae	Czech Republic
Eppendorf	<a href="https://www.eppendorf.com/US-en/">https://www.eppendorf.com/US-en/</a>		-	produces spirulina	microalgae	Germany, Juelich
Etoile Verte	<a href="http://etoile-verte.com/">etoile-verte.com/</a>	2016	-	Sells algae and medicinal plants	spirulina	France
Evodos	<a href="http://www.evodos.eu/index.php/applications/harvesting-algae.html">www.evodos.eu/index.php/applications/harvesting-algae.html</a>	2015	-	organic fertilizer from microalgae, takes part in several projects (AlgaDisk, AlgaeMax)		The Netherlands
Ferme la Pimpreline	<a href="http://www.lapimpreline.fr/">www.lapimpreline.fr/</a>	2011	-	produces spirulina	spirulina	France
Fitoplanton Marino	<a href="http://www.fitoplantonmarino.com/">www.fitoplantonmarino.com/</a>	2016	-	power and gas company, EnelGreenPower (EGP) is the groups renewable energy generation company (Solar, Wind, Geothermal, Hydro, Biomass) no current algae projects reported	no algae	Spain
FMC Biopolymer AS	<a href="http://www.fmc.com/www.stortare.no/">www.fmc.com/www.stortare.no/</a>		Harvest	Commercial operation (for >50 years) No commercial or project utilization of algae for energy	Brown seaweed	Norway
Fotosintetica & Microbiologica s.r.l	<a href="http://www.fmcbiopolymer.com">www.fmcbiopolymer.com</a>	2014		markets and sells Allgrow, a natural growth stimulator	microalgae	Italy
FreyeCon (spin-off company from Delft Technical University)	<a href="http://www.feyecon.com/">www.feyecon.com/</a>	2015	-	developer of algae-based products that include: biofuels, aquaculture, animal feeds, and Omega-3 (Kona Demonstration Facility has produced over 11 tons of algae)	marine microalgae	The Netherlands
Gicon	<a href="http://www.gicon.de/en/home.html">www.gicon.de/en/home.html</a>	2016	-	Engineering and consulting firm that works on environmental approval and soil and water management		Germany
Greenaltech	<a href="http://www.greenaltech.com/">www.greenaltech.com/</a>		-	Products for human health, skin care		Scotland
Greenskill	<a href="http://photobioreactor.co.uk/">photobioreactor.co.uk/</a>	2015	-	makes photobioreactors		
GreenTech (bought Greensea)	<a href="http://www.greentech.fr/en/">www.greentech.fr/en/</a>	2016	-	Producing skin treatments for cosmetic purposes	microalgae	France
Greon	<a href="http://www.greon.eu/">www.greon.eu/</a>	2010	-	creates DHA from algae	microalgae	Bulgaria

Company or Institution	URL	Latest Web Update Year	Cultivation (only commercial) open pond (raceway)/PBR (PBI/Other)	Focus	Algae species used	Country	
Hortimare	<a href="http://www.hortimare.com/">www.hortimare.com/</a>	-	grow seaweed using the by-products of salmon farming	-	macroalgae	The Netherlands	
Hortimax	<a href="http://www.hortimax.com/">www.hortimax.com/</a>	2016	-	horticulture products	-	The Netherlands	
IGV Institut für Getreideverarbeitung, GmbH	<a href="http://www.igv-gmbh.de">www.igv-gmbh.de</a>	2015	-	focuses on developing a cost-effective method for farming algae (needs 250 acres to be commercially successful)	salt-water microalgae	Germany, Nuthein	
Innov'alg	<a href="http://www.bluecluster.fr/entreprise/detail/AQF8AA00026N">www.bluecluster.fr/entreprise/detail/AQF8AA00026N</a>	2015	-	culture of macroalgae and microalgae and extracting principles gelling agents used for food or cosmetics	-	France	
JCO Spiruline	<a href="http://www.jco-spiruline.fr/">www.jco-spiruline.fr/</a>	2013	-	Nutritional supplements from algae	spirulina	France	
Les Jardins Coquet	<a href="http://lesjardinscoquet.weebly.com/">lesjardinscoquet.weebly.com/</a>	2015	-	food from algae	spirulina	France	
La spiruline de Cabrafol	<a href="http://spiruline-cabrafol.fr/">spiruline-cabrafol.fr/</a>	2014	-	sells food products	spirulina	France	
La Spiruline de Haute Saintonge	<a href="http://www.spiruline-fr.com/">www.spiruline-fr.com/</a>	2015	-	Packaged Spirulina	spirulina	France	
LaSpirale Verte	<a href="http://www.laspiraleverte.com">www.laspiraleverte.com</a>	-	-	sells food products	spirulina	France	
Linde Group	<a href="http://www.linde.com/en/index.html">www.linde.com/en/index.html</a>	2014	-	-	-	Germany	
Manjolive	<a href="http://www.manjolive.fr/">www.manjolive.fr/</a>	2016	-	Sells food products	-	France	
Marine Farm Madrid	<a href="http://marinefarm.blogspot.com/">marinefarm.blogspot.com/</a>	2008	-	supplying live food options	spirulina	Spain	
Metabolium	<a href="http://www.metabolium.com/">www.metabolium.com/</a>	2014	-	trying to lower the cost of production of algae continuous algae production system, research into biodiesel, bioplastics, etc.	-	France	
Metabolium	<a href="http://en.metabolium.com/">en.metabolium.com/</a>	2014	-	-	-	France, Paris	
mibellebiochemistry	<a href="http://mibellebiochemistry.com/">mibellebiochemistry.com/</a>	2016	-	skin products	-	Switzerland	
Micro Algal Solutions	<a href="http://microa.no/">microa.no/</a>	2016	-	growth and harvesting of various micro-algae strains	Nannochloropsis gaditana	Norway	
MicroA	<a href="http://www.biopharmia.no/">www.biopharmia.no/</a>	2016	-	astaxanthin and polysaccharides from microalgae	-	Norway	
MicrOLife	<a href="http://microlife.bio/">microlife.bio/</a>	2016	-	produces products for cosmetics industry from macro and microalgae	-	Italy	
Mikralgen, SARL	<a href="http://www.mikralgen.com">www.mikralgen.com</a>	-	-	focused on developing technology that captures waste carbon dioxide to produce commercial quantities of algae for use in the food and fuel sectors With eventual goal of commercial plant	-	France, Neuville sur Ain	
NeoAlgae	<a href="http://neoalgae.es/en/">neoalgae.es/en/</a>	2016	-	major integrated energy company dedicated to transforming and marketing oil and gas	no algae	Spain	
New Horizons global	<a href="http://www.newhorizonsglobal.com/">www.newhorizonsglobal.com/</a>	2009	-	Advertised a "carbon negative" energy production process by burning organic matter, and using the carbon dioxide as feedstock for algae, which would in turn produce biodiesel. Built a 30 million gallon demonstration plant in 2008, and then was severely hit by recession	microalgae	UK	
Nordlux	<a href="http://futuresystem-public.sharepoint.com/">futuresystem-public.sharepoint.com/</a>	2015	-	Omega-3 from algae	spirulina	UK	
Nuternel	<a href="http://nuternel.com/">nuternel.com/</a>	2015	-	Nutritional supplements from algae	Haematococcus pluvialis	France	
Nutrinova (formerly Protos Biotech, owned by Celanese)	<a href="http://www.celaneseventures.com, www.nutrinova.com">www.celaneseventures.com, www.nutrinova.com</a>	2015	-	produces astaxanthin products as food supplements	-	Germany, Kelsterbach	
Ocean Harvest	<a href="http://oceanharvest.is/">oceanharvest.is/</a>	2015	-	-	-	Iceland	
Omegaalgae	<a href="http://www.omegalgae.is/">www.omegalgae.is/</a>	2015	-	producing algae	-	Iceland	
Omegagreen	<a href="http://www.omegagreen.nl/">www.omegagreen.nl/</a>	2015	-	algae as feedstock	-	The Netherlands	
Photanol	<a href="http://www.photanol.nl/">www.photanol.nl/</a>	2015	-	technology to convert CO <sub>2</sub> into valuable organic compounds	cyanobacteria	The Netherlands	
Phycolementa	<a href="http://virtualual.es/investigacion/ebt/ebt_seam?ebt=29&amp;lang=es">virtualual.es/investigacion/ebt/ebt_seam?ebt=29&amp;lang=es</a>	2010	-	Dyes used in cosmetic and food industry	-	Spain	
PhycobioTech	<a href="http://phycobio-tech.com/en">phycobio-tech.com/en</a>	2013	-	phycobiliprotein production	microalgae	France	
Phylogenetics	<a href="http://www.phylogenetics.com/">www.phylogenetics.com/</a>	2016	-	genetic engineering of microalgae	microalgae	Spain	
Priiforsk Partners	<a href="http://priiforsk.no/?lang=en">priiforsk.no/?lang=en</a>	2016	-	Nutritional supplements from algae	-	Norway	
Salins du midi	<a href="http://www.salins.com/en/">www.salins.com/en/</a>	2016	-	Produces food products	-	France	
Scottish bioenergy	<a href="http://www.scottishbioenergy.com/">www.scottishbioenergy.com/</a>	2016	-	SUPPLIER OF TRACEABLE PHARMACEUTICAL AND NUTRITIONAL INGREDIENTS	spirulina	UK	
Seamarconi	<a href="http://www.seamarconi.com/">www.seamarconi.com/</a>	2015	-	-	-	Italy, France, Germany	
Seasalter Shellfish, Ltd	<a href="http://www.seasaltershellfish.co.uk, www.seacaps.com, www.oysterhatchery.co.uk/index.shtml">www.seasaltershellfish.co.uk, www.seacaps.com, www.oysterhatchery.co.uk/index.shtml</a>	2008	-	Manufactures algae production systems	microalgae	UK, Reculver, Herne Bay, Kent	
Seaweed Canarias, S.L. (Algacan)	<a href="http://www.algacan.com/">www.algacan.com/</a>	-	-	develops and constructs special machines for the mining of deposits - floating suction dredger	N/A	Spain	
Seaweed Energy Solutions	<a href="http://www.seaweedenergysolutions.com">www.seaweedenergysolutions.com</a>	-	Harvesting and offshore cultivation	commercial wild harvesting and processing, cultivation R&D	Saccharina, Laminaria, Alaria	Norway	
SUN Algae Technology Ltd.	<a href="http://www.sunalgae.com">www.sunalgae.com</a>	2015	-	creates microalgae based products including: food, personal care, industrial products, ochemicals and renewable (plant that is owned by Solazyme, ADM, and ANP has a projected capacity of 20,000 MT/yr in 2015)	microalgae	Austria, Vienna/Timelkam	
Simris	<a href="http://simrisalg.se/">simrisalg.se/</a>	2015	-	Omega-3 from algae	microalgae	Sweden	
Solis Culturae	<a href="http://solis-culturae.com/tr/">solis-culturae.com/tr/</a>	2012	-	food from algae	spirulina	France	
Spirales De lux	<a href="http://www.spiraledelux.fr/">www.spiraledelux.fr/</a>	2016	-	produces algae nutrients	spirulina	France	
Spiruline arc-en-ciel	<a href="http://www.spirulinearcen-ciel.fr/">www.spirulinearcen-ciel.fr/</a>	2016	-	food from algae	spirulina	France	
Spiruline de campagne	<a href="http://www.spirulinedecampagne.fr/">www.spirulinedecampagne.fr/</a>	2015	-	Packaged Spirulina	spirulina	France	
Spiruline de provence	<a href="http://www.spirulinedeprovence.fr/">www.spirulinedeprovence.fr/</a>	2016	-	Produce food products using algae	spirulina	France	
Spiruline des frangines	<a href="http://www.spirulinedesfrangines.com/">www.spirulinedesfrangines.com/</a>	2016	-	Produce food products using algae	spirulina	France	
Spiruline des îles d'or	<a href="http://www.spirulinedes-iles-dor.com/">www.spirulinedes-iles-dor.com/</a>	2014	-	Product for food products using algae	Spirulina	France	
Sun Algae Technology	<a href="http://sunalgae.com/">sunalgae.com/</a>	2016	-	microalgae produced for cosmetics distributes marine strains of quality for	Austria	Austria	
Teramer	<a href="http://www.teramer.eu/complements-alimentaires/">www.teramer.eu/complements-alimentaires/</a>	-	-	farming zooplankton, phytoplankton, live food for aquarium , thephotobioreactors to make them grow and culture media	-	France	
Teregroup	<a href="http://www.teregroup.net/home/english/">www.teregroup.net/home/english/</a>	2014	-	develops cultivation processes using photobioreactors with volumes from 1-10,000 L	microalgae	Italy	
Unilever	<a href="http://www.unilever.com/">www.unilever.com/</a>	2016	-	algae oil for personal care products	-	The Netherlands	
Xanthella	<a href="http://www.xanthella.co.uk/">www.xanthella.co.uk/</a>	2015	-	Grew algae using emissions from fossil fuels, and in turn created biofuel. Had more than \$70 million in investments and then fell victim to the recession in 2008	microalgae	UK	
<b>Suppliers</b>							
Algae Biotech SL / FeyeCon	<a href="http://www.algaebiotech.nl">www.algaebiotech.nl</a>	-	-	Works with producing equipment for a variety of processes	no algae	Spain, Gran Canaria	
Algatek	<a href="http://www.algatek.eu/">www.algatek.eu/</a>	2015	-	Developed a new type of reactor for algae growth	-	Spain	
AquaEcology GmbH & Co. KG	<a href="http://www.aquaecology.de">www.aquaecology.de</a>	2015	-	fermentation and bioreactors	-	Germany, Oldenburg	
Antennae	<a href="http://www.antennae.ch/">www.antennae.ch/</a>	2014	-	durable technologies that are low-cost and simple to use	-	Switzerland	
Aqualgae	<a href="http://aqualgae.com/en/home/">aqualgae.com/en/home/</a>	2016	-	produce raceways phbs and production plants	-	Spain	
Coldep	<a href="http://www.coldep.com/en/">www.coldep.com/en/</a>	2014	-	Creates technology for harvesting algae	-	France	
Enel	<a href="http://www.enel.com/en/G8/">www.enel.com/en/G8/</a>	2016	-	Produces technology for the viable commercialization of algae based products.	microalgae	Italy	
Enlightened designs	<a href="http://enlightened-designs.com/">enlightened-designs.com/</a>	-	-	developer of algae culturing systems.	-	UK	
StatOil ASA	<a href="http://www.statol.com/">www.statol.com/</a>	2016	-	Manufactures algae biofuel equipment	-	Norway	
<b>Shut operations</b>							
Aelio Technologies	<a href="http://coccounter.com/whois/site/aelio-technologies.com.html">coccounter.com/whois/site/aelio-technologies.com.html</a>	-	Shut operations	-	seaweed	France, Paris	
Algaebiotechpro	<a href="http://www.algaebiotechproducts.com/ABP/Home.html">www.algaebiotechproducts.com/ABP/Home.html</a>	-	Shut operations	-	-	UK	
Algae Energy Co Ltd	<a href="http://algae-energy.co.uk">algae-energy.co.uk</a>	-	Shut Operations	-	-	UK	
Algenics	<a href="http://www.algenics.fr">www.algenics.fr</a>	-	Shut Operations	PBR	microalgae and cyanobacteria	France, Nantes	
Alge Oil, GmbH & Co. KG	<a href="http://www.algeoil.com">www.algeoil.com</a>	-	Shut Operations	-	-	Germany	
Alimax	<a href="http://www.alimax.com">www.alimax.com</a>	-	Operations	-	providing services and expertises about algal technologies, development of photobioreactor control system, part of projects in wastewater treatment and biogas (eg. AlgaeBiogas, AlgaDisk), Algal bank,	Germany	
AlgoCyne Ethanol Energy, Inc	<a href="http://www.algodyncene.com">www.algodyncene.com</a>	-	shut operations	-	producing microalgae biomass for cosmetics, aquaculture, food supplements	Germany, Hamburg	
Algues Energy Systems AG	<a href="http://www.algues-energy-systems.com">www.algues-energy-systems.com</a>	-	shut operations	-	-	Italy	
Bio Energy Solutions	<a href="http://www.bionergysolutions.co.uk">www.bionergysolutions.co.uk</a>	-	shut operations	-	compressed and liquified gases, engineering of gas plants, developing the essential process technologies that capture CO <sub>2</sub> emitted by coal-fired power plants, (member of EABA)	no algae	UK, Manchester

Company or Institution	URL	Latest Web Update Year	Cultivation (only commercial) open pond (raceway)/PBR (PBR)/Other	Focus	Algae species used	Country
BisanTech Nuova, GmbH	<a href="http://www.bisanotech.de">www.bisanotech.de</a>	shut operations	-			Germany, Bitterfeld
Clean Algae (Joint Venture from FreyCon)	<a href="http://www.cleanalgaes.es/">www.cleanalgaes.es/</a>	Shut operations	-	owns elinBiofuels, a company that produces biofuel in a biodiesel plant (no algae activities reported)	no algae	Spain
CtoO Energy LTD New	<a href="http://www.c-to-o.com/index.html">www.c-to-o.com/index.html</a>	Operations	Shut			UK, Cranfield
Delta Ruga	<a href="http://www.deltaruga.com/">www.deltaruga.com/</a>	operations	Shut	makes biodiesel		Latvia
Eco-Solids International Limited	<a href="http://www.ecosolids.com">www.ecosolids.com</a>	Operations	Shut			UK, Hampshire
EEM / BFS – Empresa de Electricidade da Madeira / BioFuelSystems		operations	-	Originally aimed at developing technology to remove microalgae from highly diluted state. This was then extended to cleaning waste water and urban waste.	microalgae	Portugal, Porto Santo
Exenia Group, S.r.l	<a href="http://www.exeniacgroup.com/serv02.htm">www.exeniacgroup.com/serv02.htm</a>	shut operations	-			Italy
Grupo Empresarial rafael morales (Algafuel)	<a href="http://www.rafaelmorales.es">www.rafaelmorales.es</a>	Shut operations	-	producing algae separating/harvesting systems	Nannochloropsis, Dunaliella bardawil, Diatoms, Tetraselmis	Spain, Huelva
Heliogreen technologies	<a href="http://www.heliogreen.net">www.heliogreen.net</a>	shut operations	-			Luxembourg
Hezinger Algaetec GmbH	<a href="http://www.hezinger-algaetec.com/">www.hezinger-algaetec.com/</a>	Shut operations	Shut			Germany, Kornwestheim
Ingrepro BV	<a href="http://www.ingrepro.nl">www.ingrepro.nl</a>	Operations	Shut			The Netherlands
Merlin Biodevelopments	<a href="http://www.exalgia.com">www.exalgia.com</a>	Operations	Shut			UK, Wales
Micro-Algues Provence, SARL	<a href="http://provence.spiruline.free.fr">provence.spiruline.free.fr</a>	Operations	Shut			France
MTU Aero Engines	<a href="http://www.mtu.de/">www.mtu.de/</a>	operations	shut			Germany
Oxfordalgae	<a href="http://www.oxfordalgae.com/">www.oxfordalgae.com/</a>	operations	-			UK, Oxford
Preussag, AG GmbH	<a href="http://www.waidler.de/gesundheit/chlorella.htm">www.waidler.de/gesundheit/chlorella.htm</a>	Shut operations	-	Technologies for energy generation from by- and wasteproducts (biodiesel and biogas), (no algae activities reported)	no algae	Germany, Klingenber-Trennfurt
Grupo Aurantia	<a href="http://www.aurantia.es/">www.aurantia.es/</a>	Operations	-			Spain
Uninova	<a href="http://www.uninova.org/ga/default.asp">www.uninova.org/ga/default.asp</a>	Shut operations	-	Coating and food products		Spain
Valorsabio	<a href="http://www.valorsabio.com/index.html">www.valorsabio.com/index.html</a>	Shut operations	-	Private and Independent Engineering and Technology provider company.		Portugal
<b>North America</b>						
<b>Production Method - PBR</b>						
Algae Floating Systems	<a href="http://www.algafloatingsystems.com/">www.algafloatingsystems.com/</a>	2015	PBR	production of microalgae for aquaculture, animal feeds, cosmetics aircraft engines (member of EABA)	Nannochloropsis	United States
Algae Production Systems	<a href="http://www.algaeproductionsystems.com/equipment.html">www.algaeproductionsystems.com/equipment.html</a>	2008	closed photo bioreactor	commercial cultivation of Dunaliella (especially for beta carotene)	N/A	United States
Algae Systems	<a href="http://www.algaesystems.com/">www.algaesystems.com/</a>	2015	floating bioreactors		Dunaliella salina	United States
Algenol (merged with Cyanobiofuels GmbH)	<a href="http://www.algenol.com/">www.algenol.com/</a> <a href="http://www.cyanobiofuels.de/aboutus.html">www.cyanobiofuels.de/aboutus.html</a>	Oct, 2015	vertical PBR	produces high value chemicals from microalgae	microalgae	United States
Algae to Omega	<a href="http://algae2omega.com/">algae2omega.com/</a>	August 2015	PBR	grow algae to feed animals	Seaweed	United States
Aztec Algae	<a href="http://www.aztecalgae.com/About_Us.html">www.aztecalgae.com/About_Us.html</a>	2016	PBR	nutraceutical, pharmaceutical and food industries		United States
BioProcess Algae	<a href="http://www.bioprocessalgae.com/">www.bioprocessalgae.com/</a>	2014	PBR	Synthetics biology company – has researched genetic manipulation of algae. In 2009, started developing a \$600 million deal with Exxon Mobil to make algae fuel commercially, but that deal was downsized after strain didn't hit performance milestones	funded research was with natural strains, not genetically modified	United States
Diversified Energy	<a href="http://www.diversified-energy.com/index.cfm?_s_webAction=singae">www.diversified-energy.com/index.cfm?_s_webAction=singae</a>	2011	closed pond/PBR	designs and produces chemicals for niche markets	isoChrysis, nanno	United States
Garden State BioEnterprises	<a href="http://www.gsbioe.com/">www.gsbioe.com/</a>	2012	PBR	Grows algae to produce astaxanthin	Haematococcus pluvialis	United States
Needful Provision, Inc	<a href="http://www.needfulprovision.org/">www.needfulprovision.org/</a>	1995-2015	PBR	Grow algae for CO2 uptake		United States
Novagreen	<a href="http://www.novagreen.ca/home.html">www.novagreen.ca/home.html</a>	2015	PBR	Food ingredients and some fuel		Canada
photon8	<a href="http://www.photon8.com/">www.photon8.com/</a>	2016	closed system,	Goes algae for liquid protein and omega3s		United States
Phyco2	<a href="http://phyco2.us/coinfo.html">phyco2.us/coinfo.html</a>	2013	PBR	Capture of CO2 using algae system		United States
Solix Biosystems	<a href="http://www.solixbiosystems.com/">www.solixbiosystems.com/</a>	2014	PBR	Runs several testbeds to analyze and access algae's potential as a biofuel.	microalgae	United States
<b>Production Method - Raceway</b>						
Algae to Energy	<a href="http://www.algae-to-energy.com/Aboutus.html">www.algae-to-energy.com/Aboutus.html</a>	2009	open pond	cultivation and commercialisation of microalgae for feeds for aquaculture, cosmetics, food		United States
Alga Labs	<a href="http://www.alga-labs.com/">www.alga-labs.com/</a>	2006-2015	open pond	Until around 2008 produced biomass for oil now produces high value biomass		Canada
Bioalgene	<a href="http://www.bioalgene.com/bio.html">www.bioalgene.com/bio.html</a>	2009	open pond	produces omega-3s from microalgae	microalgae	United States
Cyanotech	<a href="http://www.cyanotech.com/?clid=CP3rwYXnssUCFdSHwodWrbA4A">www.cyanotech.com/?clid=CP3rwYXnssUCFdSHwodWrbA4A</a>	2016	open pond	makes skin care products		United States
Earthrise nutraceuticals	<a href="http://earthrise.com/">earthrise.com/</a>	2015	open pond	Uses light immersion technology with ponds and bioreactors to enhance culture growth	microalgae	United States
Electric Power Research Institute	<a href="http://www.epri.com/search/Pages/results.aspx?k=algae&amp;r=&amp;s=11">www.epri.com/search/Pages/results.aspx?k=algae&amp;r=&amp;s=11</a>	2010	open pond	Get information of feeding algae through flue gas		United States
Kent BioEnergy Corporation	<a href="http://www.kentbioenergy.com/page4/page4.html">www.kentbioenergy.com/page4/page4.html</a>	2012	open pond	make equipment to harvest algae continuously	microalgae	United States
Live Fuels	<a href="http://www.livefuels.com/">www.livefuels.com/</a>	2009	open pond	producing algae for crops	macro and microalgae	United States
PhyCal	<a href="http://www.phycal.com/">www.phycal.com/</a>	2015	open pond	produces algae feedstocks for downstream markets including food, feeds and fuels. Has run a demonstration plant in Iowa since 2009		United States
RAE (Renewable Algal Energy)	<a href="http://www.rae-energy.com/">www.rae-energy.com/</a>	2016	open pond	producing algae for human consumption		United States
ReactWell	<a href="http://www.reactwell.com/">www.reactwell.com/</a>	2016	open pond and geothermal technology	trying to have bacteria convert seaweed into alcohol, and grow large amounts of feedstock	seaweed	United States
Sapphire	<a href="http://www.sapphireenergy.com/">www.sapphireenergy.com/</a>	2015	open pond	creates algae production system for consumers		United States
<b>Production Method - Raceway and PBR</b>						
Algae Aquaculture Technologies	<a href="http://www.algaeaqua.com/">www.algaeaqua.com/</a>	2016	greenhouse, closed pond, PBR	Nutritional supplements and Cosmetics (Spirulina)	Spirulina	United States
Bionavitas	<a href="http://www.bionavitas.com/index.html">www.bionavitas.com/index.html</a>	2009	open pond and PBR	animal nutrition and health	marine algae, seaweeds	United States
Cellana	<a href="http://cellana.com/">cellana.com/</a>	2015	series of PBR coupled with open pond	Process uses waste water and carbon dioxide from industrial sources as feedstocks, and lysis to extract oil for applications in nutritional supplements and combine with biofuel stream	microalgae	United States
Culture Fuels	<a href="http://culturefuels.com/">culturefuels.com/</a>	2015	combined open pond/PBR	explores the use of microalgae in pharmaceutical and biological active substances	microalgae	United States
Petroalgae (now Parabel)	<a href="http://www.parabel.com/">www.parabel.com/</a>	2015	open pond/PBR	Develops photobioreactors	microalgae	United States
Synthetic Genomics	<a href="http://www.syntheticgenomics.com/">www.syntheticgenomics.com/</a>	2015	open pond, PBR	studies about biodiesel from microalgae, pharmaceutical use of microalgae	misc. microalgae	United States
XI renewables	<a href="http://www.xlairiengroup.com">www.xlairiengroup.com</a>	2010	close pond/PBR	Produce freeze dried microalgae for shrimp and fish	microalgae, diatom	United States
<b>Production Method - Fermentation</b>						
Algal Scientific	<a href="http://www.algalscientific.com/">www.algalscientific.com/</a>	2016	fermentation	add microbial culture of wastewater and harvested biomass id dried and sold as natural fertilizer		United States
Alltech	<a href="http://www.alltech.com/">www.alltech.com/</a>	2016	fermentation	Uses algae to grow food and feed		United States
Bunge	<a href="http://www.bunge.com/">www.bunge.com/</a>	2016	fermentation	Used to grow algae, now produces non-algae micro-crops	no algae	United States

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Solazyme/Terravia	<a href="http://solazyme.com/?lang=en">solazyme.com/?lang=en</a>	2016	Uses indirect photosynthesis in dark stainless-steel containers closed system	produces Astaxanthin products grows nongmo chlorella	Haematococcus pluvialis chlorella	United States
Tekmanna	<a href="http://tekmania.com/About_TekManna.html">tekmania.com/About_TekManna.html</a>	2016				United States
<b>Production Method - Unknown or Other</b>						
A2BE Carbon Capture	<a href="http://www.algaeatwork.com/index.html">www.algaeatwork.com/index.html</a>	2013	-	generate sustainable algae industry serving high value markets in food, fuel, agriculture, and nutraceutical production		United States
ADM	<a href="http://origin.adm.com/news/_layouts/PressReleaseDetail.aspx?ID=586">origin.adm.com/news/_layouts/PressReleaseDetail.aspx?ID=586</a>	2014	-	optimizing PBR technology and microalgae growth	microalgae	United States
Advanced Algae	<a href="http://www.advancedalgae.com/">www.advancedalgae.com/</a>	2010	-	Offers design to consume CO2 via Algae		United States
Algaebarn	<a href="https://www.algaebarn.com/">https://www.algaebarn.com/</a>	2016	-	produce marine organism in a sustainable and ecofriendly way, including algae		United States
AFS BioOil	<a href="http://www.afsbiolioil.com/index.html">www.afsbiolioil.com/index.html</a>	2016	-	Builds integrated algae biorefineries near wastewater treatment plants		United States
Algae Bioenergy Solutions LLC	<a href="http://absgreenfuels.com">absgreenfuels.com</a>	2011	-	cultivate and process algae		United States
Algae Collection Technology	<a href="http://28brand.me/mvWeb/kyleale/acl/">28brand.me/mvWeb/kyleale/acl/</a>	2010	harvest	harvests algae that pollutes estuaries, and converts to animal feed, methane, and bio-fuels		United States
Algae Farm International	<a href="https://www.algafarm.us/">https://www.algafarm.us/</a>	2011	-	produce sustainable algae biomass in an indoor climate controlled environment. Solutions to water remediation		United States
AlgaeLab	<a href="http://www.algaelab.org/">www.algaelab.org/</a>	2016	-	Teaches people how to grow their own algae cultures and provides live culture		United States
Algaen Corporation	<a href="http://algaen.com/about/">algaen.com/about/</a>	2016	-	food products from algae		United States
Algae Oil energy LLC	<a href="http://algaeenergyllc.com/">algaeenergyllc.com/</a>	2010-2016	-	Grow, harvest and produce algae based oils		United States
Algaeon	<a href="http://algaeon-inc.com/main">algaeon-inc.com/main</a>	2011	-	beta-1,3-glucan	Euglena gracilis	United States
algawheel	<a href="http://www.algawheel.com/">www.algawheel.com/</a>	2016	-	Grows algae on rotating wheels for water treatment		United States
Algagen	<a href="http://www.algagen.com/">www.algagen.com/</a>	2016	-	Produces algae for animal and human consumption		United States
Algconomics, LLC	<a href="http://www.algoculturing.com/">www.algoculturing.com/</a>	2007-2009	-	produce bio-products, such as biofuels from algae sources		United States
Applied Chemical Technology	<a href="http://appliedchemical.com/services/biomass/">appliedchemical.com/services/biomass/</a>	2016	-	provides biomass and renewable resources		United States
Aquatic Energy	<a href="http://www.aquaticenergy.com/">www.aquaticenergy.com/</a>	2006-2015	-	producing oil and food supplements from algae		United States
Bioenergy Hawaii	<a href="http://www.bioenergyhawaii.com/energy-conversion">www.bioenergyhawaii.com/energy-conversion</a>	2015	-	anaerobic digestion operations to process and treat the organic waste and capture the material's energy value		United States
Biosortia Pharmaceuticals	<a href="http://www.biosortia.com/">www.biosortia.com/</a>	2016	-	microalgae, bacteria and other microorganisms to produce metabolites		United States
Bio-Technical Resources	<a href="http://www.biotechresources.com/">www.biotechresources.com/</a>	2016	-	Algae strain improvement		United States
Bloomfoam	<a href="http://bloomfoam.com/">bloomfoam.com/</a>	2016	open growth (bloom)	Collects algae from open sources and uses them to make foam		United States
BlueOcean NutraSciences	<a href="http://blueoceanutras.ca/">blueoceanutras.ca/</a>	2014	-	Produce Omega-3 fatty acids from Algae		Canada
Buckman Laboratories	<a href="http://https://buckman.com/nr-core-businesses-pchem/performance-chemicals/formulator-water/applications">https://buckman.com/nr-core-businesses-pchem/performance-chemicals/formulator-water/applications</a>	2016	-	algae to clean water		United States
Duke Energy	<a href="http://www.duke-energy.com/environment/carbon-capture-utilization-and-storage.asp">www.duke-energy.com/environment/carbon-capture-utilization-and-storage.asp</a>	2016	-	CO2 capture by algae		United States
eco2	<a href="http://www.eco2capture.com/index.html">www.eco2capture.com/index.html</a>	2016	-	CO2 capture by algae		United States
Eldorado Algaefuels	<a href="http://eldoradofuels.com/">eldoradofuels.com/</a>	2016	-	Treatment of water using algae		United States
Energy Derived	<a href="http://www.energyderived.com/">www.energyderived.com/</a>	2010	-	produces chlorophyll based nutritional supplements	chlorella	United States
Florida Algae	<a href="http://www.floridalinguisina.com/">www.floridalinguisina.com/</a>	2016	-	produces live microalgae for human consumption	Spirulina	United States
General Atomics	<a href="http://www.ga.com/algae-for-aquaculture">www.ga.com/algae-for-aquaculture</a>	2016	-	Produces algae as a nutrient for fish		United States
Genesis Biofuel Inc	<a href="http://www.genesis-biofuel.com/">www.genesis-biofuel.com/</a>	2009-2012	-	uses flue gas from cement plants to grow algae		United States
Global Green Solutions	<a href="http://globalgreensolutionsllc.com/">globalgreensolutionsllc.com/</a>	2007	-	develops chlorophyll products. Capacity: 600,000+ over 20 units started as an environmental cleanup company. Now creates environmentally friendly algae based fuel and food products	microalgae	United States
Green Star Products	<a href="http://www.greenstarusa.com/index.html">www.greenstarusa.com/index.html</a>	1992-2016	-	energy and environment, biomass to energy, no algae related information		United States
Heliae	<a href="http://www.heliae.com/">www.heliae.com/</a>	2015	-	Demonstrated algae growth via CO2		United States
Honeywell UOP	<a href="http://www.uop.com/processing-solutions/renewables/">www.uop.com/processing-solutions/renewables/</a>	2010	-	purifies water through algae and uses resulting algal biomass to create compost or livestock feed		United States
Hydromentia	<a href="http://https://hydromentia.com">https://hydromentia.com</a>	2016	-	biotechnology company. Owns Algenetix which uses algae and yeast for production of fuels		United States
Kapyon Venutures	<a href="http://www.kapyon.com/">www.kapyon.com/</a>	?	-	makes nutritional products		United States
Klamath Algae Products	<a href="http://www.klamathafa.com/">www.klamathafa.com/</a>	1991-2016	-	makes nutritional products	Aphanizomenon flos-aquae	United States
Kuehne Agrosystems	<a href="http://www.kuehneagro.com/">www.kuehneagro.com/</a>	2015	-	development and production of algae-based solutions in and Personal Care, Specialty Chemicals,Wastewater Treatment with CO2 Capture,Aquaculture and Animal Nutrition		United States
Matrix Genetics	<a href="http://matrixgenetics.com/">matrixgenetics.com/</a>	2016	-	engineering to increase algal oil production and resistance to pathogens		United States
Maui Tropical Algae Farm	<a href="http://www.mauitropicalalgaefarm.com/">www.mauitropicalalgaefarm.com/</a>	2016	-	Grows algae for human consumption	Haematococcus pluvialis and spirulina	United States
New Mexico Algae	<a href="http://nmalgae.com/~online77/nmalgae/catalog/nmalic.php">nmalgae.com/~online77/nmalgae/catalog/nmalic.php</a>	2015	-	Nutritional supplements	Haematococcus pluvialis	United States
Nostoca Algae OpenAlgae	<a href="http://www.openalgae.com/">www.openalgae.com/</a>	2008-2016	-	high quality analytical laboratory		United States
PhycoBiologics Inc.	<a href="http://www.phycotransgenics.com/">www.phycotransgenics.com/</a>	2008	-	delivers biologically active proteins to humans using microalgae.		United States
Phytonix Solar Chemicals	<a href="http://phytonix.com/">phytonix.com/</a>	2016	-	Provides vaccines, growth promoters, etc		United States
Plankton Power	<a href="http://www.planktonpower.net/">www.planktonpower.net/</a>	2009	-	transform cyano bacteria to produce chemicals and fuels		United States
POS biosciences	<a href="http://www.pos.ca/opportunities/industries/biofuels/">www.pos.ca/opportunities/industries/biofuels/</a>	2016	-	Create biofuel from algae		Canada
Reed Mariculture Incorporated	<a href="http://reedmariculture.com/index.php">reedmariculture.com/index.php</a>	2016	-	does research and provides cooperations with information on processes		United States
Renew World Energies	<a href="http://www.rwenergies.com/">www.rwenergies.com/</a>	2016	-	producer of marine microalgae concentrates		United States
Scorpio Biofuels	<a href="http://www.scopibiofuels.com/products.html">www.scopibiofuels.com/products.html</a>	2016	-	manufacturing and sale of algae oil		United States
Simplexity Health Sun Chlorella Usa Triton	<a href="http://www.simplexityhealth.com/">www.simplexityhealth.com/</a>	2016	-	supplier of algae oils and algae based biofuels to the country		United States
Sun Chlorella Usa	<a href="http://www.sunchlorellausa.com/">www.sunchlorellausa.com/</a>	2016	-	blue-green algae supplements		United States
Triton	<a href="http://www.tritonhnn.com/">www.tritonhnn.com/</a>	2016	-	health supplement products	Chlorella	United States
VG Energy, Inc	<a href="http://www.vgenergy.net/index.php">www.vgenergy.net/index.php</a>	2016	-	makes health-supporting proteins		United States
WeFeedUs	<a href="http://www.wefeedus.com/">www.wefeedus.com/</a>	2015	-	Uses Metabolic Disruption Technology to provide increased oil production in algae		United States
Whitman Algae Farms	<a href="http://www.wafinc.net/">www.wafinc.net/</a>	2012	-	grows sustainable and ethically raised food. Includes the culture of algae		United States
				process oil for biofuel		United States
<b>Suppliers</b>						
Algae lab systems	<a href="http://algaelabsystems.com/">algaelabsystems.com/</a>	2015	PBR	makes photobioreactors		United States
Algaedyne	<a href="http://algaedyne.com/">algaedyne.com/</a>	2016	-	makes products to grow algae through leds		United States
Algamoil	<a href="http://www.algamoil.com/ www.algamoil.es/">www.algamoil.com/ www.algamoil.es/</a>	?	-	provider of both biodiesel plant consulting and equipment designing service.		USA, Italy, Brazil, Spain, Bulgaria
Alternative Generating Energies and Sustainable Solutions	<a href="http://www.aggessinc.com/home.html">www.aggessinc.com/home.html</a>	2016	-	provides environmental remediation for clients, also has an algae harvesting drone, and designs and installs PBRs		United States
Amec foster wheeler	<a href="http://www.amecfw.com/aboutus/projects/sustainable-and-innovative-solution-renewable-and-alternative-energy-projects">www.amecfw.com/aboutus/projects/sustainable-and-innovative-solution-renewable-and-alternative-energy-projects</a>	2016	-	offer consultancy, engineering, project management, operations and construction services, project delivery and specialised power equipment services to our customers worldwide, advanced biofuels	microalgae	United States
Byrne and Company Ltd	<a href="http://www.byrnefd.com/">www.byrnefd.com/</a>	2010	-	offer consulting to algal, waste to energy, geothermal and other businesses		United States
Commercial Algae Professionals	<a href="http://www.commercialalgae.com/">www.commercialalgae.com/</a>		-	Produce equipment to grow algae	US	
Culturing Solutions Inc.	<a href="http://www.culturingsolutions.com/">www.culturingsolutions.com/</a>	2016	-	produces photobioreactors to grow algae		United States
Global Algae Innovations	<a href="http://www.globalalgaee.com/">www.globalalgaee.com/</a>	2016	-	creates algae related technology		United States
Microbio Engineering	<a href="http://microbioengineering.com/">microbioengineering.com/</a>	2016	-	design and construction of algae ponds for wastewater reclamation, biofuel production		United States
Neste Oil	<a href="http://www.nesteoil.com/">www.nesteoil.com/</a>	2016	-	Photobioreactors and supply		US
Origin Oil (Now Origin Clear)	<a href="http://www.originclar.com/">www.originclar.com/</a>	2015	-	develops infrastructure for the commercial production of algae		United States
Smart Microfarms	<a href="http://www.smartmicrofarms.com/">www.smartmicrofarms.com/</a>	2012- 2016	-	develops and installs microalgae systems		United States
<b>Shut Operations</b>						
Algae Biosciences	No Website	shut operations	salt water aquifers	Nutrition and Cosmetics		United States

Company or Institution	URL	Latest Web Update Year	Cultivation (only commercial) open pond (raceway)/PBR (PBR)/Other	Focus	Algae species used	Country
AlgaeFuel	No Website	shut operations	PBR	Sustainable process to develop biofuel from algae		United States
American Algae LLC	No website	shut operations	PBR	grow algae to sell to other people		United States
Aurora Algae	<a href="http://www.aurorainc.com/">www.aurorainc.com/</a>	Shut Operations	pond	take waste and convert it to fuel	N/A	United States
BioFeedstocks LLC	No Website	Operations	closed loop			United States
Bodega Algae	<a href="http://www.bodegalgae.com/">www.bodegalgae.com/</a>	shut operations	-	producing equipment to produce algae oil, producing small scale, modular PBRs,		United States
Carbon Capture Organization	No Website	shut operations	-	Used to produce oils from algae grown using carbon dioxide captured from stationary sources		United States
Circle Biofuel and Ethanol Corporation	<a href="http://www.circlebio.com/">www.circlebio.com/</a>	Shut operations	PBR	Developing integrated system to produce biofuels from algae. Operates pilot plant in Hawaii. \$27.2 million in federal funds from the DOE as of March 2015		United States
Cosagen Bioscience	No Website	Shut operations	-	Developed products for nutrition and pharmaceutical industry using algae		United States
Dao Energy	<a href="http://www.dao-energy.com/bio-fuels-about-dao.html">www.dao-energy.com/bio-fuels-about-dao.html</a>	shut operations	-	industrial and agricultural systems for production harvesting and downstream processing of algae as food supplement, beta-phycocyanin	marine microalgae	United States
Green Bios Technologies	No Website	shut operations	-	carbon sequestration, wastewater treatment, biofuels, pharmaceuticals		United States
Green Fuel Technologies Corporation	<a href="http://greenfueltechnologies.com/">greenfueltechnologies.com/</a>	shut operations	PBR	production of crude-oil from algae (projects to produce 100 barrels a day)		United States
Kai Bioenergy	<a href="http://www.kaibioenergy.com/">www.kaibioenergy.com/</a>	shut operations	-	made algae biofuels		United States
Lakemastercorp	No Website	shut operations	-	Algae to biofuels		United States
Micro Algae Corporation	No Website	shut operations	-	Unknown		United States
PetroSun	no longer exists	shut operations	open pond	Acquire algae cultivation data for open pond systems	microalgae	United States
Phycosystems	No Website	shut operations	-	algae for feedstock and fuel products		United States
Sunrise Ridge Algae	<a href="http://www.sunrise-ridge.com/old-index.html">www.sunrise-ridge.com/old-index.html</a>	shut operations	-	used wastewater to grow algae		United States
Susquehanna Biotech LLC	No Website	shut operations	-	Grows algae for biofuels		United States
Ternion Bio Industries	No Website	shut operations	PBR	Grew algae for nutraceuticals, cosmetics, biofuels, agriculture		United States
Terrabon	No Website	shut operations	-	bioenergy company		United States
U.S.A. Algae Corporation	No Website	Shut Operations	-			United States

### Middle East

Production Method - PBR						
Algalo	<a href="http://www.algalo.com/">www.algalo.com/</a>	2015	PBR	Growing algae for the production of Astaxanthin	Haematococcus	Israel
Algatechnology	<a href="http://www.algattech.com/">www.algattech.com/</a>	2016	PBR, open pond, semi-closed systems(sleevess, columns, panels)	Has a demonstration facility in CO that has been operating since 2009, and is now poised to produce commercial products in both the fuels and nutrition industry	photosynthetic microalgae	Israel
Frutarom	<a href="http://algguard.frutarom.com/">algguard.frutarom.com/</a>	2016	PBR	Cosmetics	Porphyridium	Isreal
Production Method - Raceway						
NateCo2 (Nature Beta technologies NBT- owned by Nikken Sohonsha)	<a href="http://nikken-miho.com/index_topic.php?did=26&amp;didpath=/26">nikken-miho.com/index_topic.php?did=26&amp;didpath=/26</a>	2015	open pond	Develop microalgae as an economical source for renewable fuels, nutrition, and nutraceutical products	microalgae	Israel
Qualitas Health	<a href="http://www.qualitas-health.com/">www.qualitas-health.com/</a>	2015	open pond	Omega 3 oil	microalgae	Isreal
Production Method - Unknown or Other						
Algaeart	<a href="http://www.algaeart.biz/">www.algaeart.biz/</a>	-	-	feed algae with brackish water of desalination to create feed wastewater treatment	Isreal	Isreal
Aquanos	<a href="http://aquanos.net/">aquanos.net/</a>	2016	-	develops algae based platforms for oral delivery of proteins based drugs and other bio-molecules for the animal healthcare and crop protection markets		Isreal
TransAlgae	<a href="http://www.transalgae.com/">www.transalgae.com/</a>	-	-	constructs biomass and oil biomass farms		Isreal
Univerve Biofuel	<a href="http://www.univerve-biofuel.com/">www.univerve-biofuel.com/</a>	2016	-			Isreal

### Asia

Production Method -PBR						
Yunnan Alphy Biotech Co	<a href="http://www.alphy.net.cn/alphy/index.htm">www.alphy.net.cn/alphy/index.htm</a>	2016	PBR	Astaxanthin	Haematococcus pluvialis	China
Production Method - Raceway						
Denso Corporation	<a href="http://www.globaldenso.com/en/news/2015/20150819_01.html">www.globaldenso.com/en/news/2015/20150819_01.html</a>	2015	open pond	Produces industrial products for the automotive industry	microalgae	Japan
Neoalgae technology	<a href="http://www.neoalgae.com/company-profile/">www.neoalgae.com/company-profile/</a>	2015	open pond	produces algae for human consumption	Spirulina	Thailand
Taiwan Chorella manufacturing company	<a href="http://www.taiwanchorella.com/">www.taiwanchorella.com/</a>	2014	open pond	manufacturers food	Chorella	Taiwan 71, 5F, Sec. 2, Nanking East Road, Taipei 10457
Yaeyama Shokusan Co., Ltd.	<a href="http://www.yaeyamachlorrella.com/">www.yaeyamachlorrella.com/</a>	2011	open pond	Chlorrella as a food supplements	chlorrella	China
Production Method - Fermentation						
Chlorella Industry Co	<a href="http://www.chlorella.co.jp/">www.chlorella.co.jp/</a>	2016	fermentation	Chlorrella as a food supplements	chlorrella	Japan
DaeSang	<a href="http://www.daesangco.com/">www.daesangco.com/</a>	2014	fermentation	Food products, starches, sweetener, health foods	chlorrella	South Korea
Kangcare Bio Industry Co	<a href="http://www.kangcare.com/">www.kangcare.com/</a>	2016	fermentation	Produces algae DHA for vegetarians	chlorrella	China
Xiamen Huisun Biotech Co. Ltd	<a href="http://www.chinahuisun.com/">www.chinahuisun.com/</a>	2016	fermentation	DHA Algal oil	chlorrella	China
Production Method - Unknown or Other						
Alganovo International Co.	<a href="http://www.alganovo.com/">www.alganovo.com/</a>	2010	-	Seaweed products	macroalgae	China
Bangchak Petroleum	<a href="http://www.bangchak.co.th/sunny-bangchak/en/sunny-bangchak.aspx">www.bangchak.co.th/sunny-bangchak/en/sunny-bangchak.aspx</a>	2013	-	Mainly petrochemical business but government initiatives inspire research in the algae area		Thailand
Euglena Co.	<a href="http://www.euglena.jp/en/">www.euglena.jp/en/</a>	2016	-	Making biofuels from a specific algae	microalgae	Japan
Everyone Excellent - algae-bio-tec.co., LTD	<a href="http://www.excellent-algae.com/help.asp?action=top2">www.excellent-algae.com/help.asp?action=top2</a>	2016	-	makes nutrients	microalgae	Taiwan Makung City, Penghu County Western Lane No. 101-16
Gather Great Ocean Algae Industry Group	<a href="http://en.judayang.com/">en.judayang.com/</a>	2016	-	makes food products from marine origins	macroalgae	China
Hubel Ruiren Biotechnology Co., LTD	<a href="http://en.huberuiren.com/">en.huberuiren.com/</a>	2016	-	DHA Algal oil		China
IHI Corporation	<a href="http://www.ihc.co.jp/hi/all_news/2015/press/2015-5-21/index.html">www.ihc.co.jp/hi/all_news/2015/press/2015-5-21/index.html</a>	2015	-	Mainly electrical company, but is experimenting with using algae is involved in a variety of ocean industries but uses Rongcheng Luyuan aquaculture Co. Ltd. To cultivate ocean industry	seaweed	Japan
JingHai Group Co.,	<a href="http://www.jinghaigroup.cc/">www.jinghaigroup.cc/</a>	2015	-	makes nutrients	microalgae	China
Kazuhiro algae Kunshan Co., Ltd. 高島一株有限公司	<a href="http://yihong2.foodmate.net/">yihong2.foodmate.net/</a>	2013	-	Sells seaweed and other ocean products to be used in food	seaweed	China, Suzhou, Jiangsu
Kimyo Sci-Trading Company	<a href="http://www.kimyo.com/">www.kimyo.com/</a>	2016	-	Sells seaweed and other ocean products to be used in food	seaweed	china
Loxley public Company Limited	<a href="http://www.loxley.co.th/news-event-detail.html/106">www.loxley.co.th/news-event-detail.html/106</a>	2011	-	Is mostly an electrical company, but is experimenting with using algae		Thailand
Sun Algae Technology	<a href="http://sunalgae.com/">sunalgae.com/</a>	2016	-	Provides commercial equipment		Hong Kong
Suzhou algae Chen Food Co., Ltd. (苏州藻辰食品有限公司)	<a href="http://msseaweed.cn.gongchang.com/">msseaweed.cn.gongchang.com/</a>	2016	-	Sells seaweed and other ocean products to be used in food	seaweed	China, Suzhou City, Jiangsu Province

Company or Institution	URL	Latest Web Update Year	Cultivation (only commercial) open pond (raceway)/PBR (PBR)/Other	Focus	Algae species used	Country
Shandong First Spirulina Biotech Co	<a href="http://www.35583.tradebig.com/index.php">www.35583.tradebig.com/index.php</a>	2016		grows algae to make power	Chorella, Spirulina Nannochloropsis, Tetraselmis, Haematococcus Pluvialis, Isochrysis	china
WeCareBiofuel Internation Co.	<a href="http://wecareasia.company.weiku.com/">wecareasia.company.weiku.com/</a>	2015	-	end-user retail products		China
Yunnan Green A Biological Project Co, LTD	<a href="http://www.greena.com.cn/">www.greena.com.cn/</a>	2016	havest and PBR	Pharmaceuticals, cosmetics, food	Spirulina	China
Ye Xili Biotechnology Co.	<a href="http://www.yexil.com/">www.yexil.com/</a>	2015		green food, health care products, bio-pharmaceuticals, algae, sand, clean energy	Chorella	China
<b>Oceania</b>						
Algae-Tech LTD Solray Systems	<a href="http://www.algaetec.com.au/index.php">www.algaetec.com.au/index.php</a> <a href="http://www.solrayenergy.co.nz/">www.solrayenergy.co.nz/</a>	2015 2015	Open Air pond	Harvest and manufactures algae for nutritional products Converting Algae into crude oil	microalgae	Netherlands New Zealand
<b>Research and Projects</b>						
<b>Europe</b>						
(European Algae Biomass Association)	<a href="http://www.eaba-association.org/en/about-us/">www.eaba-association.org/en/about-us/</a>	2016	-	Working to make information on algae more available to the public		EU
A4F Algae For Future	<a href="http://www.a4f.pt/">www.a4f.pt/</a>	2016	PBR	research and develop projects for the industrial production of microalgae,	microalgae	Portugal
Aalborg University	<a href="http://www.en.bio.aau.dk/research/biotechnology/">www.en.bio.aau.dk/research/biotechnology/</a>	2015	-	part of D-Factory project		Denmark
ABG AlgaeBioGas	<a href="http://www.algaebiogas.eu/">www.algaebiogas.eu/</a>	2016	-	Algal treatment of biogas digestate and feedstock production		EU
ACCOMPLISH	<a href="http://www.swansea.ac.uk/csr/projects/accomplish/">www.swansea.ac.uk/csr/projects/accomplish/</a>	-				UK
acib (austrian center for industrial biotechnology)	<a href="http://www.acib.at/acib/">www.acib.at/acib/</a>	-		create processes and do research relevant to biotechnological processes		Austria
Agricultural University of Athens (Department of Biotechnology)	<a href="http://www.aau.gr/index.php?item=116">www.aau.gr/index.php?item=116</a>	-		member of EABA		Greece
Algadisk	<a href="http://www.algadisk.eu/">www.algadisk.eu/</a>	2015	-	develop a modular, scaleable and automatic biofilm reactor for algae biomass production		EU
Algae Innovation Center	<a href="http://www.algaenovationcenter.org/eng/">www.algaenovationcenter.org/eng/</a>	2013	-	Research and development of photobioreactor systems		Denmark
Algaerowth	<a href="http://www.algaerowth.de">www.algaerowth.de</a>	-				Munich, Germany
Algaemax	<a href="http://www.algaemax.eu/">www.algaemax.eu/</a>	2015	-	new technology to reduce microalgae harvesting costs		Barcelona, EU
Alganact	<a href="http://alganact.com/">alganact.com/</a>	2015	-	officers integrated and comprehensive R&D services about both microalgae and macroalgae		France
AlgeCenter Denmark (Aarhus University, Danish Technological Institute, Køgegatecenter, Ocean Centre Denmark)	<a href="http://www.algecenterdenmark.dk/">www.algecenterdenmark.dk/</a>	2016	-	research about: Biorefinery, Algae growing, energy production	mostly macroalgae	Denmark
Algoland (Project from Linnaeus University)	<a href="http://lnu.se/en/research/searchresearch/forskningsprojekt/algoland/">lnu.se/en/research/searchresearch/forskningsprojekt/algoland/</a>	2016	-			Sweden
All-Gas	<a href="http://www.all-gas.eu">www.all-gas.eu</a>	2015	-	the project will optimise the production of algae by both heterotrophic and phototrophic routes and will demonstrate integration of these production technologies (Raceway, PhotoBioReactor and Fermentation) to achieve the algae cultivation targets of 90-120 dry tonnes per hectare by annum. establishing the state of the art on research, technological development and demonstration activities regarding the exploitation of various algal and other suitable non-food aquatic biomasses for 2nd generation biofuels production		England, EU
AquaFuels	<a href="http://cordis.europa.eu/result/rcn/53073_en.html">cordis.europa.eu/result/rcn/53073_en.html</a>	2012	-	enabling European SMEs to remediate wastes, reduce Green House Gas emissions and produce biofuels via microalgae cultivation.		EU
BioAlgaeSorb	<a href="http://bioalgae-sorb.com/">bioalgae-sorb.com/</a>	2013	-			EU
Bioalgostral (is owned by cyro)	<a href="http://www.bioalgostral.com/">www.bioalgostral.com/</a>	-		The research program led by Dr. HDR Gabin Tréboux is to sustainably produce microalgae in order to valorize biomass for energy purpose(s). research and development areas regarding small firings, medium and large biomass combustion plants and biomass combined heat and power (CHP) plants		France
Bioenergy 2020+	<a href="http://www.bioenergy2020.eu/">www.bioenergy2020.eu/</a>	2016	-		microalgae	Austria
Biofat	<a href="http://www.biofat-project.eu/">www.biofat-project.eu/</a>	2015	-			EU
Birmingham City University	<a href="http://www.bcu.ac.uk/">www.bcu.ac.uk/</a>	-		memer of EABA		UK
Centre Algatech (Trebon)	<a href="http://www.alga.cz/en/">www.alga.cz/en/</a>	2016	-	research on different properties of algae		Czech Republic
Cyano Biotech	<a href="http://www.cyano-biotech.com/content/home/index.php">www.cyano-biotech.com/content/home/index.php</a>	2016	PBR	applied R&D on cyanobacteria		Germany
Czech Republic Institute of microbiology	<a href="http://ftp.alga.cz/en">ftp.alga.cz/en</a>	2014	PBR	researches photosynthesis, cell cycle of algae and algae technology	microalgae, cyanobacteria	Czech Republic
Danish Technological Institute (Project BioWalk4Biofuels)	<a href="http://www.dti.dk/projects/project-biowaste-and-algae-for-the-production-of-2nd-generation-biofuels/project-stages/28768_2">www.dti.dk/projects/project-biowaste-and-algae-for-the-production-of-2nd-generation-biofuels/project-stages/28768_2</a>	2014	-	Biowaste and algae for the production of 2nd generation biofuels		Denmark
DEMA (Direct Ethanol from MicroAlgae)	<a href="http://cordis.europa.eu/project/rcn/106280_en.html">cordis.europa.eu/project/rcn/106280_en.html</a>	2016	-	develop bioethanol from microalgae with lowcost scalable photobioreactors		EU
D-Factory	<a href="http://www.d-factoryalgae.eu/">www.d-factoryalgae.eu/</a>	2015	-	sustainable CO <sub>2</sub> algae biorefinery	Dunaliella salina	EU
EAWAG	<a href="http://www.eawag.ch">www.eawag.ch</a>	2015	-		N/A	Switzerland
ECN	<a href="http://www.ecn.nl/">www.ecn.nl/</a>		Natural harvest for chemical and bio-chemical fractionation. Due to the variation in seaweed composition, many permutations are possible	Environmentally benign economically viable seaweed fractionation processes, yielding protein, carbohydrate and minerals stream for biochemical conversion to furans based fuels, bioethanol and biobutanol (AEB) and biogas	Laminaria digitata, Saccharina Latissima, Palmaria palmata, Chondrus Crispus, Sargassum, Ulva, etc.	The Netherlands
Enalgae (Energetic Algae)	<a href="http://www.enalgae.eu/">www.enalgae.eu/</a>	2015	-	developing sustainable technologies for algal biomass production		EU
EPFL Ecole Polytechnique federale de Lausanne-Laboratory for environmental biotechnology (LBE)	<a href="http://lbe.epfl.ch/page-34576-en.html">lbe.epfl.ch/page-34576-en.html</a>	2016	-		microalgae	Switzerland
European Biodiesel Board	<a href="http://www.ebb-eu.org/">www.ebb-eu.org/</a>	2016	-		no algae	EU
Frauenhofer IGB	<a href="http://www.igb.fraunhofer.de/">www.igb.fraunhofer.de/</a>	2016	-	memer of EABA, research topics in health, communication, energy, environment, several projects involving algae		Germany
Fuel4me	<a href="http://www.fuel4me.eu/">www.fuel4me.eu/</a>	2016	-	production of high lipid algal biomass,development of a continuous downstream process using all components of the algal biomass (conversion process).	misc. microalgae	EU
GIAVAP	<a href="http://giavap.eu/content/project">giavap.eu/content/project</a>	2013	-	genetic engineering of microalgae to make better suit industrial applications,focusing on carotenoid and PUFA production		Germany, Portugal, France, Italy, UK, Israel
iBet	<a href="http://www.ibet.pt/">www.ibet.pt/</a>	2016	-	brings together, as partners and collaborators, private companies and public institutions, bio-pilot plant, research about microbiology, nutraceuticals, plant genomics and biotechnology, drug discovery, animal cell technology, partnet of D-Factory project		Portugal
IBVF Institute of Plant Biochemistry and Photosynthesis (University of Sevilla)	<a href="http://www.ibvf.csic.es/en">www.ibvf.csic.es/en</a>	2016	-	working group: developmental biology in cyanobacteria		Spain
IBW-Department of Industrial Biological Sciences (University of Ghent)	<a href="http://www.enbichem.ugent.be/">www.enbichem.ugent.be/</a>	2015	pond with microalgal bacterial flocs (MaB-flocs)	wastewater treatment, pilot facility pond with MaB-flocs		Belgium

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IFEU (Institut für Energie- und Umweltforschung Heidelberg)	<a href="http://ifeu.org/">ifeu.org/</a>	2016	-			Germany
IGB Berlin	<a href="http://www.igb-berlin.de">www.igb-berlin.de</a>	2015	-			Germany
InteSusAI	<a href="http://intesus-algae.eu/home/">intesus-algae.eu/home/</a>	2015	PBR, fermentors, open pond	research algae as feedstock for biodiesel		EU
MacroBioCrude	<a href="http://www.swansea.ac.uk/csr/projects/macrobiocrude/">www.swansea.ac.uk/csr/projects/macrobiocrude/</a>	unknown	-		Macroalgae	UK
NTNU	<a href="http://www.ntnu.no">www.ntnu.no</a>		-	process development for production of high value chemicals		Norway
NU Galway (National University of Ireland Galway)-	<a href="http://www.ryaninstitute.ie/">www.ryaninstitute.ie/</a>	2015	-			Ireland
Ryan Institute						
Queens University Belfast	<a href="http://www.qub.ac.uk/">www.qub.ac.uk/</a>	2015	-		no algae	UK
Rothamsted Research	<a href="http://www.rothamsted.ac.uk/">www.rothamsted.ac.uk/</a>	2016	-			UK
SINTEF Energy Research	<a href="http://www.sintef.no">www.sintef.no</a>		-	Thermochemical energy production pathways	Saccharina latissima	Norway
SINTEF Fisheries and Aquaculture	<a href="http://www.sintef.no">www.sintef.no</a>		seaweed cultivation	Seaweed cultivation	Saccharina latissima and Alaria esculenta	Norway
SINTEF Materials and Chemistry	<a href="http://www.sintef.no">www.sintef.no</a>		-	Biochemical energy/molecules production pathways	Saccharina latissima	Norway
SP Technical Research Institute of Sweden	<a href="http://www.sp.se/en/centres/sphbiofuels/sidor/default.aspx">www.sp.se/en/centres/sphbiofuels/sidor/default.aspx</a>	2015	-	part of D-Factory project		Sweden
Suprabio	<a href="http://www.suprabio.eu/">www.suprabio.eu/</a>	2014	-	Sustainable products from economic processing of biomass in highly integrated biorefineries		EU
Swansea University- CSAR (Centre for Sustainable Aquatic Research)	<a href="http://www.swansea.ac.uk/csar/">www.swansea.ac.uk/csar/</a>	2005	-			UK
Università degli studi di Padova	<a href="http://parlab.biologia.unipd.it/">parlab.biologia.unipd.it/</a>	2016	-	continuous cultivation of algae in PBR	microalgae	Italy
University Bielefeld Dr. Olaf Kühn Algae Biotechnology and Bioenergy	<a href="http://www.uni-bielefeld.de/biologie/AlgaeBiotechnology/home.html">www.uni-bielefeld.de/biologie/AlgaeBiotechnology/home.html</a>	2015	-	carbohydrates, lipids, recombinant proteins and other bioactive compounds.		Germany
University Göttingen Algae Culture Collection (SAG)	<a href="http://www.uni-goettingen.de/de/45175.html">www.uni-goettingen.de/de/45175.html</a>	2016	-		microalgae	Germany
University Innsbruck (Research Institute for Limnology (Mondsee))	<a href="http://www.uibk.ac.at/limno/">www.uibk.ac.at/limno/</a>	2016	-			Austria
University La Palma, Gran Canaria (marine biotechnology)	<a href="http://marinebiotechnology.org/en/">marinebiotechnology.org/en/</a>	2013	-	memer of EABA, identifying and cataloging new species of microbiology		Spain
University of Greenwich	<a href="http://www.gre.ac.uk/engsci/research/groups/esrg">www.gre.ac.uk/engsci/research/groups/esrg</a>	2015	-	part of D-Factory project	microalgae	UK
University of Twente (SPT- Sustainable Process Technology)	<a href="http://www.utwente.nl/tnw/spt/">www.utwente.nl/tnw/spt/</a>	2015	-			The Netherlands
University Vienna, Department of Limnology and Bio-Oceanography (Limbo)-Phycology Lab, (Prof Schagerl)	<a href="http://limbo.univie.ac.at/lab_schagerl.php">limbo.univie.ac.at/lab_schagerl.php</a>	2015	-			Austria
Wageningen UR	<a href="http://www.wageningenur.nl/en.htm">www.wageningenur.nl/en.htm</a>	2015	-	part of differente projects (eg EnAlgae, InteSusAI, several smaller studies)		The Netherlands
<b>North America</b>						
Advanced Biofuels Technologies (Advanced biofuels USA)	<a href="http://advancedbiofuelusa.info/">advancedbiofuelusa.info/</a>	2016		Provides education, consulting and advocacy for biofuels including algae		United States
Algae Foundation	<a href="http://thealgaefoundation.org/">thealgaefoundation.org/</a>	2016		promote algae by funding educational outreach, research, development, and other activities		United States
Algae Raft Testbed	<a href="http://raft.arizona.edu/">raft.arizona.edu/</a>	2016	-	turn a profit selling algae based biodiesel by simultaneously making clean water from sewage, using carbon heavy residue as fertilizer, and earning credits for biofuels	microalgae	United States
Arizona State university	<a href="http://larb.asu.edu/">larb.asu.edu/</a>	2011	-			United States
ATP3	<a href="http://atp3.org/">atp3.org/</a>	2016	-	Nutrition, therapeutics, argosciences, health and beauty	both freshwater and marine microalgae	United States
Agricultural Research Center	<a href="http://www.ars.usda.gov/research/projects/projects.htm?ACC_N_NO=42921&amp;N_ID=42921">www.ars.usda.gov/research/projects/projects.htm?ACC_N_NO=42921&amp;N_ID=42921</a>	2016	-	Papayas being digested by algae for biofuels		United States
Cal Poly	<a href="http://www.ee.calpoly.edu/projects/algae-biofuel-interdisciplinary/">www.ee.calpoly.edu/projects/algae-biofuel-interdisciplinary/</a>	2014	-	Use pulsed electric fields to lyse algae cells		United States
Center of Excellence for Hazardous Materials Management – CEHMM	<a href="http://cehmm.org/index.php/programs/algae">cehmm.org/index.php/programs/algae</a>	2016	-	Researches process of commercializing algae to biomass		United States
Colorado School of Mines	<a href="http://chemistry.mines.edu/faculty/mposewitz/mposewitz.htm">chemistry.mines.edu/faculty/mposewitz/mposewitz.htm</a>	2011	-	enzymatic flux of algae and PACE program		United States
Desert Research Institute	<a href="http://www.dri.edu/clean-technologies-and-renewable-energy/clean-technologies-renewable-energy-projects/54-ctrc/3446-doe-algal-based-renewable-energy-for-nevada">www.dri.edu/clean-technologies-and-renewable-energy/clean-technologies-renewable-energy-projects/54-ctrc/3446-doe-algal-based-renewable-energy-for-nevada</a>	2011	-	Interdisciplinary research program will identify promising algal strains from growth in Nevada's geothermal fluids		United States
Eastern Kentuck University Iowa State University	<a href="http://www.biorenew.iastate.edu/research/signature/algae/">www.biorenew.iastate.edu/research/signature/algae/</a>	2016	-	indentify genes which relate to lipid productions		United States
Lawrence Livermore National Laboratory	<a href="http://craft.eku.edu/algae-genetic-research">craft.eku.edu/algae-genetic-research</a>	2016	-	develop bacteria to combat pond infestation in algae ponds		United States
Los Alamos National Labs	<a href="http://lanl.gov/science-innovation/capabilities/bioscience-biosecurity-health/bioenergy/index.php">lanl.gov/science-innovation/capabilities/bioscience-biosecurity-health/bioenergy/index.php</a>	2016	-	used genetic engineering to develop magnetic algae, thus making it much easier to harvest for biofuel production. Harvesting algae accounts for approximately 15–20 percent of the total cost of biofuel production—magnetic algae can reduce such costs by more than 90%.		United States
Louisiana state university	<a href="http://www.algaeadwater.lsu.edu/research.html">www.algaeadwater.lsu.edu/research.html</a>	2012	-	to develop scalable platforms for the manipulation of microalgal and cyanobacterial co-cultures for the production of biofuels and bioproducts.		United States
MAGIC Consortium	<a href="https://www.algaeconsortium.com/magic/">https://www.algaeconsortium.com/magic/</a>	2016	-	produce protein-based human and poultry nutritional products along with hydrated algal oil extract		United States
Michigan Tech	<a href="http://www.mtu.edu/greatlakes/contact/faculty-staff/anderson/">www.mtu.edu/greatlakes/contact/faculty-staff/anderson/</a>	2015	-	classification of algae		United States
Montana State University	<a href="http://biofuels.montana.edu/">biofuels.montana.edu/</a>	2016	-	discovery, growth, and characterization of novel microbial strains		United States
New York University Abu Dhabi	<a href="http://lasb.abudhabi.nyu.edu/index.php">lasb.abudhabi.nyu.edu/index.php</a>	2016	-	evolution, gene expression and metabolism of algae	Chlamydomonas reinhardtii	United States
North Carolina State University	<a href="http://www.ncsu.edu/research/results/vol1n2/08.html">www.ncsu.edu/research/results/vol1n2/08.html</a>	2009	-	cultivation of microbiology from the sea		United States
National Renewable Energy Laboratory	<a href="http://www.nrel.gov/">www.nrel.gov/</a>	2016	-	studies properties of algae and converting algae to biofuels		United States
Sandia National Laboratories	<a href="http://www.sandia.gov/research/research_foundations/bioscience/biofuels.html">www.sandia.gov/research/research_foundations/bioscience/biofuels.html</a>	2016	-			United States
Texas A&M	<a href="http://algaeforfuel.agrilife.org/">algaeforfuel.agrilife.org/</a>	2016	-	algae to fuel		United States
UC Davis	<a href="http://algae.ucsd.edu/about/people/">algae.ucsd.edu/about/people/</a>	2016	-	viable algae-based biotechnology solutions for renewable energy, green chemistry, bio-products, water conservation, and CO2 abatement.		United States
UC San Diego	<a href="http://algae.ucsd.edu/mayfield/index.html">algae.ucsd.edu/mayfield/index.html</a>	2016	-	algae biotechnology research for the production of therapeutic proteins and biofuel molecules.		United States
University of Colorado Boulder	<a href="http://www.colorado.edu/che/StoykovichGroup/index_files/search.htm">www.colorado.edu/che/StoykovichGroup/index_files/search.htm</a>	2015	-	Dewatering microalgae		United States
Colorado State University Fort Collins	<a href="http://www.eci.colostate.edu/research/">www.eci.colostate.edu/research/</a>	2016	-	Characterize emissions from different algae strains		United States
University of Texas at Austin	<a href="http://eureka.utexas.edu/institution/view?institution_id=19">eureka.utexas.edu/institution/view?institution_id=19</a>	2016	-	collects strains of algae to be used in other locations		United States
Washington State University	<a href="http://sites.wsu.edu/sebe/Main/Research/Biofuel.html">sites.wsu.edu/sebe/Main/Research/Biofuel.html</a>	2016	-	studies properties of algae and converting algae to biofuels		United States
<b>Middle East</b>						

Company or Institution	URL	Latest Web Update Year	Cultivation (only commercial open pond (raceway)/PBR (PBR)/Other)	Focus	Algae species used	Country
Ben Gurion University-Jacob Blaustein Institute for Desert Research-Microalgal Biotechnology Laboratory SABIC Corporate Research & Innovation (CRI) Centr Israel Oceanographic and Limnological Research (IOLR)	<a href="http://web2.bgu.ac.il/algal/index.htm">web2.bgu.ac.il/algal/index.htm</a>	2014	-	develop the biotechnology involved in mass production of microalgae for various commercial purposes, utilizing the high temperature, brackish or sea water, and solar irradiance that abound year round in the desert.		Israel
	<a href="http://mrschapter.kaust.edu.sa/Pages/SABIC_Tour.aspx">mrschapter.kaust.edu.sa/Pages/SABIC_Tour.aspx</a>	2016	-	projects on algae fuel		Saudi Arabia
	<a href="http://www.ocean.org.il/mainpageeng.asp">www.ocean.org.il/mainpageeng.asp</a>	2014	-			Israel
<b>Asia</b>						
A*Star	<a href="http://www.a-star.edu.sg/ces/">www.a-star.edu.sg/ces/</a>	2015	-		microalgae	Singapore
Central Salt and Marine Chemicals Research Institute	<a href="http://www.csir.org.in/Pages/Research/Marine_Biotechnology_and_Ecology.php">www.csir.org.in/Pages/Research/Marine_Biotechnology_and_Ecology.php</a>	2015	-	Focusses on large scale cultivation of seaweed for food purposes	seaweed	India
Chinese Academy of Sciences	<a href="http://www.ihb.ac.cn/rsciv/yszl/">www.ihb.ac.cn/rsciv/yszl/</a>	2016	-	algae biology		China
Indian Institute of Technology - Kharagpur	<a href="http://www.iitkgp.ac.in/fac-profiles/showprofile.php?empcode=bVma2">www.iitkgp.ac.in/fac-profiles/showprofile.php?empcode=bVma2</a>	2015	-	converting algae into biofuels	microalgae	Taiwan
Institute of Chemical Technology	<a href="http://www.ictmumbai.edu.in/DisplayPage.aspx?page=g&amp;item=D-12">www.ictmumbai.edu.in/DisplayPage.aspx?page=g&amp;item=D-12</a>	2016	-	Explore algae as a source of biofuel feedstock/biodiesel/ value added products	microalgae	Mumbai
Kyungpook National University		-		the selection of algae strains with desirable traits for biofuel production and development of biodiesel and bioalcohol production systems from marine microalgae and seaweeds, -----		
Marine Bioenergy Research Center	<a href="http://www.mbe.re.kr/">www.mbe.re.kr/</a>	2015	-			South Korea
National Taiwan University	<a href="http://www.ntu.edu.tw/english/">www.ntu.edu.tw/english/</a>	2015	-			Taiwan
Peking University	<a href="http://see.pku.edu.cn/content_view_cn.aspx?pic=edmissions(pic.gif&amp;content_id=816">see.pku.edu.cn/content_view_cn.aspx?pic=edmissions(pic.gif&amp;content_id=816</a>	2012	-		microalgae	China
Qingdao Institute of Bioenergy and Bioprocess Technology	<a href="http://english.qibebt.cas.cn/rh/rs/bc/EnergyAlgae/">english.qibebt.cas.cn/rh/rs/bc/EnergyAlgae/</a>	2013	-	production of lipids for energy	microalgae	China
Sea6 energy	<a href="http://www.sea6energy.com/">www.sea6energy.com/</a>	2015	-	developing enabling technologies to grow and convert seaplants into biofuel, plant growth stimulants, and other bio-renewable products	macro algae	India
University of Tsukuba	<a href="http://plmet.biol.tsukuba.ac.jp/index-en.html">plmet.biol.tsukuba.ac.jp/index-en.html</a>	2015	-	Components which can be produced by algae, and biofuels		Japan
<b>Oceania</b>						
MBD Energy Limited	<a href="https://mbdenergy.com/">https://mbdenergy.com/</a>	2016	-	Develop low cost processes that clean waste cheaply using algae		Australia
Murdoch University	<a href="http://www.murdoch.edu.au/Research-capabilities/Algae-and-O-Centres/">www.murdoch.edu.au/Research-capabilities/Algae-R-and-O-Centres/</a>	2016	-	Developing commercial scale algae culturals	microalgae and seagrass	Australia
National Institute of Water and Atmospheric Research	<a href="http://www.niwa.co.nz/freshwater-and-estuaries/research-projects/bio-oil-from-wastewater-algae">www.niwa.co.nz/freshwater-and-estuaries/research-projects/bio-oil-from-wastewater-algae</a>	2009	-	Determine if High Rate Algal Ponds are feasible		New Zealand
Solar biofuels Consortium	<a href="http://www.solarbiofuels.org/">www.solarbiofuels.org/</a>	2016	-	bio-discovery, structural biology, molecular biology, microbiology, genomics, transcriptomics, proteomics, metabolomics, culture optimisation and bioreactor scale-up within a coordinated research program		Australia
University of Adelaide	<a href="http://chemeng.adelaide.edu.au/research/microalgal/">chemeng.adelaide.edu.au/research/microalgal/</a>	2016	-	the development of commercial-scale microalgal culturing techniques for the production of bioactive compounds, aquaculture feed, fine chemicals, and renewable fuels.		Australia
University of Queensland	<a href="http://www.schenklab.com/algae-energy-farm/">www.schenklab.com/algae-energy-farm/</a>	2016	-	new cost-saving technologies to produce food, feed, nutraceuticals or biodiesel from microalgae.		Australia

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