Algae Production Platforms for Canada's Northern Climate

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Abstract

Large resources are being invested globally in algae research in the anticipation that these microorganisms will become the "silver bullets" that lead to economic bio-renewable fuels, new food sources, and a host of high value products and simultaneously mitigate rising atmospheric CO₂ levels. A great deal of research has been completed on strains of algae with the potential to produce high lipid yields that make the biomass suitable for biofuel production. Many production systems for algae cultivation continue to be developed for moderate and hot climates (e.g., USA, Europe, and Australia). The largest algae cultivation systems to date use open pond systems. These autotrophic systems, however, have limited applicability in Canada's northern climatic conditions. There is consensus that closed photobioreactor systems are required to control environmental conditions (including temperature), minimize evaporation and contamination, and augment the limited sunlight available during winter to generate consistent biomass yields for economically sustainable crops. Given the high capital and operating costs, however, many are skeptical that meaningful and economically sustainable algae cultivation can take place in Canada. This paper identifies nine scalable algae photobioreactor cultivation technologies that may suit Canadian northern climates. The information provides insights related to the developing ¹ Corresponding author. Tel.: +1-780-492-7797.

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algae industry in Canada as well as highlighting opportunities for further technological development specific to cold climates. Although the review demonstrates that exciting headway has been made, significant technological challenges remain and require that further innovations be developed.

Keywords: Microalgae; photobioreactor; biomass feedstock; bioproducts

1. Introduction

Over the past relatively short period, there has been a renewed interest in growing and cultivating microalgae for commercial purposes. These single cell plants are extraordinary in their capacity to more than double biomass within a single day [1]. Research shows the plant's ability to synthesize a host of highly valued compounds, including bio-oils for energy [2-10], hydrogen and isoprene production [11], food, livestock and fish feed [12, 13], and coveted health and nutrition ingredients [14-18] while simultaneously improving water [19-21] and air quality [22-26]. It is for this reason that algae is seen to hold enormous potential for meeting a number of our world's pressing challenges.

There are more than 40,000 species of algae [26, 27], each with a unique composition and grown in micro-environments suitable for their existence. Considerable research has been conducted to isolate strains of algae with high growth yields and high lipid content [28]. Both qualities are important to building a business case for a sustainable renewable energy industry.

For commercial purposes, natural environmental growth conditions are weighed against artificial environments that can be tightly controlled and generally lead to much higher yields [26]. When considering artificial environments, emphasis shifts from working only with indigenous algae

strains found in particular geographic locations to cultivating strains that offer the greatest yield potential for desired products and potential bi-products.

In Canada's challenging northern climate, unless there is a specific environmental burden that can be improved through the cultivation of algae in situ, i.e., oil sand tailing ponds, control of algae blooms (or blue-green algae, also known as cyanobacteria, which can negatively affect habitats), it is generally necessary to create artificial environments to achieve meaningful commercial yields of algae biomass.

Each strain will have a unique composition and makeup and likewise require a tightly controlled growing environment to optimize yield. Photobioreactors are constructed to tightly monitor and control all aspects of the growth conditions including lighting, nutrients, temperature, pH, media composition, etc., and ensure optimal growth of a specific algae strain. The challenge in photobioreactor construction is to minimize capital and operating costs to the point where the cost of the biomass produced for industry is less than other competing renewable inputs to biofuel production [26] and / or other valued production endpoints.

An overarching goal of a series of studies by this research group is to develop a model that allows researchers to benchmark technology, performance evaluate and compare different technologies and processes to identify those technologies and processes that will support an economically viable and sustainable algae biomass industry.

2. Microalgae Review

Generally, when considering microalgae for economic and commercial uses, it is important to first identify algae strains naturally growing in a region of interest, document the composition of

the media and environmental conditions in which they naturally grow, characterize the composition makeup of the algae, and select those species that already demonstrate a natural capacity to synthesize compounds of interest.

By way of example, a recent study on the cultivation of *Nannochloropsis sp. F&M-M24* under altered media nitrogen availability demonstrates how cultivation conditions may alter the elemental composition of produced microalgae biomass [29] (see Table 1).

Table 1

Mostafa [30] reviewed metabolites as well as phytochemical and biologically active compounds including fatty acids, sterols, carotenoid pigments, antioxidants, anti-cancer, anti-microbial, anti-viral, nematicidal activity and molluscicidal activity. Microalgae can also be used for feed, fertilizer, CO₂ sequestration, wastewater treatment, biofuel production, and phytoremediation for heavy metals.

There is also an ongoing investigative task to determine ways to augment the growth media and environment to optimize both algae growth and the expression of the compounds of interest. Sustainable commercial viability is determined by the balance of capital and input costs versus revenues gained from saleable output products and avoided operating costs (i.e., GHG penalties). There are more exciting possibilities with the investigation of enhanced genetically modified organisms (GMOs) [31]. Acién et al., Norsker et al., and Chen et al. emphasize the importance of achieving biomass production yields that will support economic and commercial viability [32-34]. Abdelaziz et al. and Slade et al. point to the importance of reaching a net positive energy balance for all processes leading to the production of commercial end products [35, 36].

Industry is looking for economically sustainable and scalable algae production platforms that will deliver algae biomass at costs that are lower than existing competing inputs. Recent literature provides a useful background on this topic [37-40]. It is the purpose of this research paper to determine the current ability of the algae industry to deliver algae biomass in Canada's northern climate by:

- reviewing cultivation technologies involving artificial environments that are currently being developed and are deployable in Canada;
- identifying a range of cultivation technologies with potential for Canada;
- identifying gaps in knowledge on algae cultivation in Canada.

3. Algae Cultivation Techniques

The algae cultivation method significantly influences growth characteristics, yield, and composition [41]. Algae growth generally depends on sufficient light and a carbon source for photosynthesis, but depending on the environmental conditions algae may assume a different metabolism approach [42]. The various cultivation methods include phototrophic, mixotrophic, heterotrophic, and photoheterotrophic [43, 44]. Chen et al. summarized biomass productivity, lipid content, and productivity for different algae species under various cultivation methods [34]. Their review shows that the phototrophic approach is the most common, although biomass and lipid productivities were relatively low compared with the heterotrophic method when the same algae species were considered. The work by Liang et al. [45] showed that phototrophic cultivation provided higher cellular lipid content (38% for *Chlorella vulgaris*) but much lower lipid productivity compared with algae growth under heterotrophic conditions. Different algae strains can grow using different cultivation techniques. For example, while *Chlorella vulgaris*,

Arthrospira (Spirulina) platensis, and *Haematococcus pluvialis* grow under phototrophic, heterotrophic, and mixotrophic conditions, strains such as *Selenastrum capricornutum* and *Scenedesmus acutus* grow favourably under phototrophic, heterotrophic, and photoheterotrophic conditions.

Bacterial contamination and infestation by predatory microorganisms, i.e., rotiphers, is a major concern in algae cultivation and addressing it may become even more challenging with high levels of organic substrates that support predator rapid growth. An infestation can rapidly destroy the culture. Great care is generally taken to ensure that the culture is anexic and is maintained in that way [46]. Given the challenges associated with maintaining anexic cultures, there has been a shift, especially over the past five years, toward exploring the benefits of maintaining a biodiverse polyculture [47]. Different cultivation methods are discussed briefly.

3.1 Phototrophic Cultivation Method

The phototrophic algae cultivation method involves the consumption of light and CO_2 as a source of energy and inorganic carbon [43]. The phototrophic, also known as photoautotrophic, culture method converts light into chemical energy via photosynthetic reactions [34, 41, 42]. This method of culturing algae is the most commonly used cultivation condition for algae growth; it generally results in media with low cell density but at relatively low cost. The method provides scalability with relative ease, although the low cell density leads to higher costs to concentrate the media [34]. The benefit of phototrophic cultivation is the potential use of CO_2 from flue gases emitted from power plants and heavy industries for its biological fixation [34, 43].

3.2 Heterotrophic Cultivation Method

The heterotrophic cultivation method uses only organic compounds as sources of carbon and energy and therefore eliminates the requirement for light [41]. This method generally results in higher biomass concentration (cell densities of 50-100 g of dry biomass/L) and lipid productivity than autotrophic cultivation (cell densities of 30 g of dry biomass/L) [46]. Examples of organic carbon sources that can be assimilated by algae for growth include glucose, fructose, sucrose, galactose, acetate, glycerol, and mannose [45]. The cultivation method can be scaled up as a conventional fermenter, but there are issues associated with scaling up, such as contamination and competition with other microorganisms, limited number of microalgae species that may be grown heterotrophically, inhibition from excess organic substrate, the inability to produce light-induced metabolites, and high energy and substrate costs [34, 46].

3.3 Mixotrophic Cultivation Method

This method uses light as the main energy source to perform photosynthesis, although CO₂ and organic compounds are equally essential. In this cultivation method, algae can be cultured phototrophically or heterotrophically depending on the concentration of light intensity and available organic compounds [34, 41, 43]. The carbon sources for this method are both organic and inorganic with medium cell density. The reactor scale-up for the mixotrophic cultivation method is a closed photobioreactor and drawbacks include high equipment cost, high substrate cost, and contamination [34, 46]. Experiments using this approach to cultivate algae have shown maximal growth rates for certain algae species along with higher lipid, starch, and protein productivity than under photoautotrophic regimes, as well as lower production costs [48].

3.4 Photoheterotrophic Cultivation Method

In this cultivation approach, light is required to use the organic compounds as carbon source. Photoheteroterophic cultivation is also known as photoorganitrophy, photoassimilation, and photometabolism [42]. This approach is similar to the mixotrophic cultivation method except for the energy source required for growth and the metabolism reaction [41]. Similar to the mixotrophic method, high equipment and substrate costs and contamination are issues with the reactor scale-up. Medium cell density is also common with this cultivation approach. In any case, it is rarely used for algae growth or biodiesel production [34].

4. Algae Cultivation In Canada

Commercial algae cultivation to date has largely taken place in geographic regions where sunlight energy of is prevalent, temperatures are moderate, and there are ready sources of water and low-cost nutrients. The most prevalent commercial-scale algae cultivation operations use raceway open ponds systems. These are relatively "low tech" and considered the most costeffective, from an initial capital outlay perspective, and thus offer good potential for a viable and economically sustainable operation. However, the system has significant drawbacks and vulnerabilities.

From a geographic climatic perspective, open pond raceway systems are not ideal for a Canadian context. They can only operate for four to six months annually and thus are not economically viable. Although conventional thinking advocating OPR systems persists, there are no known research attempts to experimentally quantify or model these systems in Canada.

To bridge the climatic challenge, several alternative controlled environmental algae growth technologies have been developed. These include photo-bio-reactor (PBR) systems for cultivating algae under phototrophic/autotrophic conditions, flat plate and membrane systems,

plastic / glass tube systems, and fermenters that take advantage of algae's unique capability to grow in heterotrophic conditions in the absence of light and rely on carbon sources other than sunlight for the energy used in growth. Other algae cultivation systems use both autotrophic and heterotrophic conditions (mixotrophic) to achieve growth objectives.

There have been several commercial attempts to cultivate algae at economically sustainable production levels. Most have failed and have thus been withdrawn from active commercial / research and development activities. Companies formerly involved include SFN Biosystems Inc. (Calgary), International Energy Inc. (Vancouver), Centurion BioFuels Corp. (Hamilton) (recently renamed Algaeneers Inc. and looking to convert glycerin to n-butanol), and Algae Fuel Systems (Saskatoon).

National Resources Canada – National Research Council of Canada (NRC) sets a context for algae technology development in Canada. The NRC Institute for Marine Biosciences in Halifax has a history spanning more than 50 years of cultivating algae. Before 2010, the Government of Canada [49] put together a multi-party research and development (R&D) program, linking Agriculture and Agri-Food Canada (AAFC) with National Resources Canada– National Research Council of Canada (NRC) to set in place the National Bioproducts Program (NBP) to address Canadian priorities for sustainable energy, the environment, and rural revitalization. This research program was expected to bring together stakeholders and expertise from government, academia, and industry to tackle this large-scale multi-dimensional project.

NBP identified microalgae biomass as holding the greatest potential to meet the stated objectives and set out to develop and support Canadian industries focused on the production of renewable fuels from microalgae biomass for electrical generation, land transportation, and aerospace applications. NBP's goals were to achieve biomass production capability that would be cost effective and competitive with other conventional energy sources, provide a positive impact on the environment and sustainable energy, and contribute to the economic vitality of the Canadian energy sector [49].

To achieve the desired outcomes, several significant barriers needed to be overcome. One major barrier was the identification of algae strains that demonstrate the best potential for producing biofuels. Efficient and scalable cultivation technologies for Canadian climatic conditions would need to be developed. Then, cost-effective industrial-scale processing technologies compatible with end-use applications required development.

With that context, NRC came up with a number of sub-projects. First, it would screen algae species for biofuel applications. Second, it would support commercial-scale photobioreactor cultivation technologies aimed at concentrating solar energy for algae production, heat, and power. The third focused on the development and evaluation of processing and conversion technologies. The gross steps leading from the production of algae to its conversion to biofuel were mapped out. Current solutions and process limitations were identified along with areas where research was required for cost-effective solutions to meet the overarching objectives. The fourth and final project was to evaluate the algae-derived fuels and lubricants for the aerospace industry [49].

Today, the NBP links Canada and the US under the collaborative Clean Energy Dialogue, a partnership that includes the US-DOE, the National Renewable Energy Laboratory (NREL), Sandia National Laboratories (SNL), and the Pacific Northwest National Laboratory (PNNL).

Where progress continues to be made on all four projects outlined above, considerable work remains. Of interest related to the NBP has been the development of the NRC's "Brite-Box" algae cultivation photobioreactor (PBR) as discussed below.

5. Algae Cultivation Technologies Suitable For The Canadian Climate

The following section introduces nine scalable PBR algae cultivation technologies with potential application to Canadian northern climates. Table 2 provides relevant patent information [50-70] on PBR technologies used by the companies discussed in this study.

Table 2

5.1 Open Pond Raceways (OPR) And Algae Raceway Integrated Design (ARID)

Open pond raceways (OPR) are currently the most cost-effective means of cultivating algae. A good example of this technology can be found at the University of Arizona. Although not currently considered a viable or sustainable for Canada, no known research has attempted to quantify the extent to which this technology could be employed. Its two greatest barriers are temperature and access to ambient light during winter.

Open pond raceways require large water surface areas to allow for light penetration, especially as the algae culture density increases. For this reason these ponds are generally less than a half meter deep. The large surface area enables higher use of solar photons, the energy that allows the microalgae plants to grow but during colder periods contributes to the rapid cooling of the ponds, thereby limiting metabolic activity associated with algae growth. Given the many energy-intensive industrial processes used in the province of Alberta, there may be an opportunity to harvest the associated low-grade heat and thus maintain favorable pond temperatures and increase productivity.

To counteract the fluctuating temperatures associated with OPRs, an algae raceway integrated design (ARID) was developed and continues to undergo testing, with early results showing good promise. With this approach, the ponds are drained at night into a deeper holding area. In the morning, after the sun has heated the greater pond area, media are recirculated into the cultivation ponds. The deep pond retains the heat from the day to a greater extent, resulting in a more favorable cultivation temperature. More research specific to the Canadian context is warranted.

5.2 Algae Aqua-Culture Technology (2010) MT

The Algae Aqua-Culture Technology (AACT) PBR for algae cultivation is designed for challenging climatic conditions and is part of a fully integrated production bio-cluster or closed-loop biorefinery platform. The system includes photobioreactors for algae cultivation, anaerobic bioreactors that digest the algae using benign digestive bacteria, and an Organic Carbon Engine (OCE) generating syngas from waste wood from a neighboring lumber mill to produce bio-oil and biocarbon (biochar).

The 465 m² facility run by a staff of 4 can convert 6 tonnes of waste wood to 2 tonnes of soil amendment daily, generate 2.1 GJ/hr heat and create up to 250 kW of continuous power. The associated CO_2 and nitrous oxide fuel algae growth. A patented automated computer control system ANT (Autonomous Networked Technology) keeps all of the operation components in balance and adapting to environmental changes [71].

Algae produced in a serial batch process with daily harvesting goes into to the biodigester to produce methane used in the OCE. The nutrient-rich digestate is combined with biochar to produce a dry saleable fertilizer. The approximate 370 m² of algae ponds represents some 50 m³ of growth media and uses carbon dioxide from the pyrolysis of the waste wood residue from the adjoining lumber mill [72, 73]. The system, an integrated biorefinery, can have a five-year payback in isolated regions where energy prices are high. Research continues on the extraction of other high-value products from algae biomass.

5.3 Algae Tec Limited. (2007) Australia

Algae Tec Limited (ASX:AEB) has developed the proprietary McConchie-Stroud algae cultivation system. This publicly traded company has conducted hundreds of its own research trials from laboratory scale to bench-top and pilot tests, and conducted detailed engineering evaluations of a commercial-scale plant operation. The company, reporting revenues of \$4.5 million AUD in 2014 [74], claims significant advances in product yield, productivity, and CO₂ sequestration, as well as reduced capital cost savings. It is commencing a joint commercial-scale algae plant project in India consisting of a high-yield modular PBR and harvesting system. An industrial-scale plant is also underway near Sydney to convert CO₂ from the Macquarie coal-fired power plant into valuable bio-oil. The facility is targeted to produce 50 million L (50,000 T) of algal oil per year. Oil production was scheduled for the end of 2014.

5.4 AlgaBloom Technologies (2009) BC

The focus of AlgaBloom Technologies is to develop large-scale microalgae farming solutions. The company has developed a suite of PBRs from the land-based "AlgaBioReactor" to the roofbased "AlgaRoof," the "AlgaBag," a large-scale bioreactor bag with an integrated sparging and agitation system, and the "AlgaBox," a compact multi-level bioreactor. The modular multi-layer matrix design, consisting of both thin-film and suspended components, enables control over both environmental and nutritional factors. 400 m² of growth media surface area is achieved within a 30 m^2 footprint.

AlgaBloom is also developing associated oil extraction, harvesting, and monitoring capabilities. One of the strains of algae considered is *Synechococcus PCC 7002*.

The company has established a commercial partnership with Qponics Limited based in Australia on an omega-3 oil production project.

5.5 AlgaeCan Biotech Ltd. (2009) BC

AlgaeCan Biotech Ltd. is currently financing a demonstration plant with PBRs of a scalable commercial biorefinery that includes both the cultivation and subsequent processing of the algae through to saleable products. An initial key market with the production of astaxanthin from *Haematococcus pluvailis* is the primary focus of research activities, along with reducing energy inputs. Key production achievements include:

- attaining >3% yield (wt) of dried algae biomass where open pond producers only achieve 1.5%;
- establishing the following key optimization parameters:
 - o light frequency, intensity, saturation, low energy
 - o consistent, non-shearing low energy flow
 - o dependable, simple, low-cost sterilization
 - o monitoring and control capability of 5 crucial bioreactor factors

- nutrient formulation to ensure cost-effective yields specific to their algae strain
- establishing production protocols to efficiently transition the algae from the vegetative growth phase through the induction of astaxanthin production and the extraction of this product into an oleoresin without the use of solvents.
- production of 4mg softgels using a toll processor technology.

The work of the company has progressed from lab-scale trials, to bench-top, and to 1000 L and 7,000 L PBRs. The company is currently building a 14,000 L PBR.

5.6 HyTek Bio LLC (2008) MD

HyTek Bio has developed a PBR based on cylindrical PVC bags (a mylar-like material with carbon fiber and Kevlar structural support) that provide a growth media column approximately 1 m diameter and 6-7 m high and a total growth volume of 6.8 m³. The system is housed in a protective building environment that helps control environmental temperatures. The system includes system monitoring and control capability. HyTek's technology takes down stack gas emissions from industrial processes, with algae absorbing not only CO_2 but also the other potent greenhouse gases like SO_x and NO_x . Carbon credits as well as offsets from not having to use other costly gas scrubbers and their associated maintenance are anticipated. The company is achieving algae culture densities that support relatively high production yields.

Together with the University of Maryland, the company has isolated a proprietary *HTB-1* strain of algae that shows the greatest promise for the company's commercial objectives. The algae has a 42-47% lipid content and is able to survive environments with 100% CO₂. The algae can also withstand high variability in pH from acidic to basic and temperature swings from 15^oC-43^oC. In natural conditions, algae double their mass in 22 hr. In research trials, it can double within 12 hr.

Significant research headway has been made on the monitoring and control of nutrients and lighting to optimize algae growth. Lighting regimes have reduced energy use to 10% of traditional LED lighting systems.

The cost of the 6.8 m³ 30 kg tank is approximately 25% of the cost of a similar volume stainlesssteel tank. The development plan is to construct a commercial-scale tank that holds 18m³ of growth media.

Other key data include:

- Flue gas 100 scfm/PBR @ 11.8%CO₂ and 130 ppm NO_x
- Flue gas temperature 425°C stack T and 27°C PBR T
- Nutrient requirement 375L proprietary nutrient / PBR / day based on waste chicken manure
- 5% water loss due to photosynthesis
- Power: 180W lighting / PBR and 1.5kW air injection
- Gas injection / media mixing via micro-bubble full-floor sparging system
- Algae: HTB1
- Culture density: 3-5 g/L
- Production: 23-34 kg/day or 3.4-5 g/L/Day
- O₂ Production: 8.5 cfm 90% O₂
- Harvesting: 10% of media harvested when optical density reaches upper threshold.
- Dewatering: use bacterial aggregation agent. Removed water is filtered and replenished with nutrients, then returned to PBR
- Drying / packaging: remaining slurry spray dried and vacuum packaged for shipping
- Cycle repeated every 1.5 hr

• Automated control system

5.7 Industrial Plankton Inc. (2011) BC

Industrial Plankton is a recent algae cultivation technology developed in Victoria, BC. The technology is a fully automated PBR with monitoring and control capability that enables significant production yields. Industrial Plankton has recorded algae densities up to 210 million cells/ml (*Nanochloropsis*), 25 million cells/ml (*Isochrysis*), 18 million cells/ml (*Thalassiosira weissflogii*), 20 million cells/ml (*Skeletonema costatum*), and 4.5 million cells/ml (*Tetraselmis*). To date the company has developed a 100 L research-scale, 500, 1,000 and 1,250 L automated PBR system complete with sterilization. Air and water are micro-filtered and there is a UV sterilization cycle. The control system includes scale-up density, nutrient addition, light levels, harvest density, etc., complete with data logging for analytical research. Scaling up from 20 L to 1,000 L takes 7-10 days depending on the algae species. Harvesting takes place automatically and removed media is replaced with fresh water and nutrients. PBRs use LED lighting systems. The 75 L unit uses an average of 900W and the 1,000 L unit uses an average of 1,600 W. Several of this company's PBRs have been installed commercially.

5.8 National Research Council Of Canada (NRC) NS

The Brite-Box is proprietary technology owned by the NRC [75] and developed at 250, 500, and 1,000 L. Each unit is comprised of a cooling loop, fluorescent lights, and a pH probe coupled to CO₂ solenoid for sparging this gas into the growth media for pH control. A 50,000 L cultivation pilot plant is being planned.

Data published in 2010 showed *Chaetoceros mulleri* and *Isochrysis galbana* cultivated at 20 ^oC in seawater reached 0.6 gm/L/D over a 21-day trial cycle [76, 77]. The Brite-Box has been used to conduct algae cultivation studies on many algae strains [78].

From data collected by cultivating algae using this technology, valuable information has been accumulated to benchmark current state-of-the-art systems. From the R&D activities, the NRC has documented algae biomass yield data and extracted several unique algae strains. The information has also been used to evaluate the potential to scale up cultivation processes and determine carbon / energy balances for the biomass-to-fuel conversions. This empirical data has also been valuable for developing meaningful life cycle analyses (LCAs) and conducting techno-economic (TE) assessments.

The Brite-Box PBR was developed in collaboration with Carbon2Algae Solutions and Menova Energy Inc. and the biomass production capability in conjunction with Ocean Nutrition Canada.

The NRC collaborates with industrial / commercial partners including several of the companies mentioned below to conduct research advancing scientific knowledge and related technology development to support the evolution of the algae industry in Canada.

5.9 Pond Biofuels (2007) ON

Pond Biofuels came into existence in May 2007 and since its inception has filed 17 patents related to algae cultivation technology processes including factors related to scalability, handling of input and output gases, and recycling processed water [79].

This Canadian company, working with St. Mary's Cement and using pulsed red LED lighting systems, has successfully scaled up their PBR technology to two 12.5 m³ tanks (2013). The

lighting system can to inject more than 1kW of light energy per m3 of growth media. The company claims to grow between 4 and 6 generations of algae daily [80].

Pond Biofuels is Canada's largest and most publicized algae biomass company. They were recently awarded a \$19 million demonstration plant in cooperation with the Government of Canada and Canada Natural Resources Ltd. (CNRL) [81].

Their re-developed PBR system is based on injecting high-intensity light into large (10,000 L) plastic vessels with monitor and control capability using CO_2 from industry. Energy and CO_2 are provided by a natural gas-fired 4MW generation system in Bonnyville, Alberta. Nutrients, including N, P, and trace elements, are obtained from chemical processes.

5.10 Symbiotic EnviroTek Inc. (2008) AB

Symbiotic EnviroTek Inc. was established in 2008 with the primary goal to develop a commercial scale photobioreactor (PBR) that would cost-effectively cultivate algae for commercial purposes in adverse (Canadian) climatic conditions. The first test PBR fabricated by the company holds 106,000 L. Testing in 2010 and 2011 demonstrated that algae could be successfully grown at this scale. The company's initial focus was on developing mechanical technology, including all supporting systems (i.e., proprietary controllable, submersible LED lighting, mixing of the algae media, appropriate aeration for efficient CO₂ infusion, and nutrient mixing and delivery). It has expanded its research to include the entire spectrum of technologies and capabilities to take strains of algae, customize their associated growth parameters, and adjust the associated monitoring and control capabilities to effectively optimize the growth of several different algae strains.

In 2012 and 2013, R&D activities included developing protocols for specific algae strains by using agricultural waste nutrient sources, and testing specific light frequencies to optimize yields and minimize energy/cost of inputs. The company anticipated it would demonstrate sustained growth at levels above 4 gm/L/d in 2015.

Symbiotic's system was designed to be scalable for deployment and integration / co-location at existing waste industrial / agricultural waste streams at source to minimize the GHG footprint associated with an overall bio-cluster operation. An envisioned bio-field consisting of 64 modules each having 106 m³ of growth media situated on 2 acres is estimated to use over 45 tonnes of CO_2 daily and produce 25 tonnes of algae biomass.

6 Technology Assessment

Given the limited information on the technologies in the public domain, it is difficult to predict a technology best suited for the Canadian context. Table 3 provides comparative data between the technologies discussed here. From an economic perspective, success is achieved in part by minimizing the total costs of several key factors including the aggregation of a suite of related technologies that comprise the algae biomass production platform. Decisions are made for capital, down-time, operating, nutrient, media (including water), and maintenance costs. Economic success is also coupled directly to species selected for cultivation and to optimized biomass yields in both quantity and composition. However, without reliable and accurate algae production platform data, we cannot make a meaningful economic comparison and assessment.

Table 3

7 Factors Affecting Economic Viability Of Photobioreactors

Interestingly, all of the PBR designs are the result of unique perspectives, with each company choosing to focus on specific aspects of their PBR design that they believe to be most crucial. PBR design optimization studies generate reference data useful for adjusting design parameters for enhanced yield outcomes [82].

The National Research Council, academic colleges and universities, and independent commercial laboratories continue to isolate algae strains that show high concentrations of desired compounds of commercial interest. A few noteworthy strains include *Chlorella protothecoides* and *Scenedesmus obliquus* (51% lipid concentration) [34, 83] for biofuel production and *Haematecoccus pluvialis* for astaxanthin production [84, 85].

Chlorella protothecoides has been demonstrated to grow at densities of up to 17 g/L in heterotrophic conditions compared with 0.87 g/L in autotrophic conditions under 12:12 hour light:dark cycles [86]. For *Scenedesmus sp.*, recent growth trials achieved 1.3 g/L dry biomass at a density of 1.5 million cells/L and a growth rate of 0.62 div/day under 12:12 hour light:dark cycles [87].

Haematococcus pluvialis, known to synthesize high value astaxanthin, has been documented (2003) to grow at a rate of 0.7 div/day and 0.228-258 mg/L at cell densities between 200 and 250 thousand cells/ml [84]. A more recent study achieved astaxanthin accumulation of 18.21 g/m³ (3.63% by dry weight), reaching a growth rate of 0.52 div/day with a cell density of 330,000 cells/ml and an estimated production cost of \$1000/kg astaxanthin [88]. A 2009 study demonstrated the complexity of interactions in the algae growth platform based on the effects of light and pH [85].

A 2011 conceptual model comparing commercial-scale (100 ha plant) open pond raceways, tubular PBRs, and flat panel PBRs based on current exchange rates estimated costs of \$6.96, \$5.85, and \$8.38, respectively, per kg of dewatered algae biomass. When optimized for location, irradiation, zero costs for CO₂ and nutrients, these costs dropped to \$1.80, \$0.98 and \$0.96 per kg [33]. A recent review of bio-oil production from fifteen algae research reports provided a range of cost estimates from \$0.82-\$10.93 /L of oil produced [38].

In 2008, over \$350 million was invested in algae projects [89]. In 2009, Exxon Mobil Corp planned to invest some \$700 million, anticipating the development of algae fuels within 10 years. In 2013, after spending \$120 million, the company determined that it was unsuccessful in achieving commercial viability and that it would likely take at least another 15 years to reach its objective [91]. Industry reports continue to point to the significant challenges to be overcome for algae cultivation to achieve commercial viability [10, 35]. In the US alone more than \$1 billion has been invested in the algae industry.[90].

These investment figures provide useful reference points when assessing technologies introduced above with consideration to their respective economic viability. However, little information on their operational performance is available in the public domain.

The production of algae requires the monitoring and control of many variables. Different algae strains have different growth rates, and composition of the resulting biomass varies significantly and in turn determines the value of the saleable product. Each company is focused on different product outputs. In some cases, a single relatively low-value, high-volume market is targeted (i.e., biofuels). In other cases, high-value nutraceuticals are the focus (i.e., astaxanthin).

Although each company has on a primary output product, in every case consideration is given to a biorefinery approach to derive economic benefits from one hundred percent of output products to create a favorable economic output [92]. Each company has independent approaches to sourcing CO₂ and infusion, light sources, wavelengths, light-dark cycles, and intensity regimes. Sourcing lower cost nutrients and more energy efficient dewatering processes will help improve profitability.

The algae biorefinery concept is relatively new and first appeared in technical journals in 2008 (based on a Scopus search). Of 310 published articles on the subject at the end of 2016, 241 were released between 2013 and 2016. Because of the complexity of multiple pathways, including technologies and processes from cultivation to oil upgrading, there is a body of analytical research and simulation modelling that compares biorefinery pathways and provides recommendations for large-scale algae biofuel production. A recent study explores a process "superstructure" of carbon capture for wet biomass use. In the study, four technology alternatives are considered for off-gas purification, algae cultivation, harvesting, dewatering, lipid extraction, remnant treatment, and biogas and algal oil use [92, 93].

The impact of environmental parameters like light intensity, wavelengths, and photoperiods are not included in many studies. Yet, lighting regime and photoperiod are considered key factors related to algae growth rates and biomass production [94, 95]. A recent study focused on light using *Scenedesmus obliquus* achieved cell concentrations of up to 114 million cells/ml, a growth rate of 0.86, and a density of 3.3 gm/L [96] under a specific pulsed fluorescent lighting regime.

Another area requiring further research is the correlation between cell weight and algae biomass composition to parameters like temperature, dissolved oxygen, dissolved carbon dioxide, electrical conductivity, specific nutrient concentrations, pH, and light intensity. The findings to this point appear inconclusive [96].

Successful commercialization of algae technology platforms will depend on adherence to regimented operational protocols controlling multiple parameters involved, and emphasize ongoing research and development activities that further optimize production (see Table 4).

Table 4

8 Delivery Cost Of Algae Biomass In Canada

For the algae industry globally to become a meaningful and potentially dominant economic force, the costs of cultivating, harvesting and processing algal biomass must be significantly lower than current market prices for products extracted from biomass.

To date, relatively little research has been conducted on either life cycle assessments [36, 97] or techno-economic analyses of the inclusion of technologies and processes from algae cultivation to the production of valued products. There is also skepticism among some authors about whether there is less environmental impact in producing products from algal biomass than from conventional feedstocks and petroleum resources. Research is required to provide better information. From a sustainability perspective, research must also include water use as part of an environmental impact analysis.

How algae cultivation platforms are operated and integrated with other industries will have significant impacts not only on commercial but also on environmental outcomes. For example, algae cultivation could be co-located with municipal wastewater treatment facilities, landfill operations, agricultural effluent streams (i.e., feedlots, breweries, sugar beet, corn, and potato processors), conventional energy extraction/refineries, co-generation facilities, etc. Association with such operations could result in favorable symbiotic commercial and environmental outcomes (see Table 4). With existing algae cultivation systems, there are challenges with access to accurate costing information. This challenge is made more difficult given that the process of algae cultivation leading to the delivery of dry biomass to industry generally involves many steps.

Given that this is an emerging industry requiring significant resources, the developers of algaerelated technologies generally focus on single steps within the overall production platform. Technology coupling throughout the platform will provide a complete and integrated solution. There are few integrated algae production platforms in Canada and even fewer that provide plausible scalability for industrial purposes. What may work as a prototype may not work meaningfully at a larger scale.

For some companies, algae biomass production has been focused on delivering high value compounds rather than simply generating biomass. Operation and production data are confidential. Because of the high value of the end product, meaningful revenues can be achieved even with very modest amounts of biomass produced. Costs for the production of biomass for astaxanthin, which may have a street value of \$2,500/kg [98], although important, are less a consideration than producing a million tonnes of biomass for the extraction of algal oil for biofuels (i.e., biodiesel at \$1/L(kg)).

From a production perspective, comparative costing is meaningful. Palm oil, viewed to yield the lowest cost bio-oil, has a reference production cost of \$603/tonne (\$0.61/L). To be competitive,

algae biomass (with 30% lipid content) through to oil extraction should cost \$164/tonne or less. If the reference is soybean, which in the US is the dominant source for biofuel production, with a commodity price of \$623/tonne (based on 20% lipid content), then algae biomass through to extracted oil should cost \$169/tonne or less. Where the reference is crude oil priced at \$118/barrel, the same algae biomass should cost \$204/tonne or less [97]. See Table 5 for a summary of equivalent required pricing for algae to compete with other feedstocks.

Table 5

Current cost estimates for algae biomass as a feedstock for electricity generation are \$233/tonne for open algae systems and \$17,292/tonne for closed environmentally controlled production platforms for health foods. For high value products, production costs increase to \$30,704/tonne in open systems and \$40,895/tonne in closed systems. Reported production costs vary tremendously from \$3,118 to \$19,486/tonne for open systems based on raceway ponds and \$3,774 to \$94,430/tonne for closed systems. Interestingly, 100 tonne/yr algae biomass operations had production costs of \$4,930/tonne for open systems and \$3,828/tonne for closed systems [97]. Furthermore, for algae production platforms, economies of scale do not appear to work well when going from 50 ha to 500 ha to a 5,000 ha production facility since very little cost reduction appears possible [97]. See Table 6 for a summary of production cost variability in OPR and PBR technologies found in the literature.

Table 6

In the context of Canada, given the relatively short growing season, open systems have not been considered as a viable commercial option and therefore only closed systems need be considered.

For many algae production platforms, a large negative gap remains between actual production costs and pricing for commercial products derived from the biomass. To bridge this gap, several companies that were initially focused on a single, large, and subsidized biofuel commodity market shifted their primary focus to high value byproducts with residual oils going to biofuel production. This shift leads to operational changes including a potential shift in algae strains used, cultivation practices including nutritional and environmental factors, and the addition of production steps that enhance the expression of desired compounds.

Other opportunities for overcoming the costing challenge include research to lower energy input, incorporating existing "waste" streams that can offset fertilizer costs and potentially provide an add-back value from the deferral waste disposal transportation and landfill costs, the uptake of amines to significantly reduce operating costs in these industrial applications, as well as the potential for CO_2 mitigation credits.

Research may provide techno-economic data on processes leading to biomass at a laboratory scale but these may have little relevance to commercial scale costing. Other research has used powerful software modeling capability (i.e., ASPEN), but results are scrutinized and questioned because of the great assumptions and parameters that need to be considered. Like challenges related to economies of scale, these modelling tools may have value for thoroughly understood commercial operations currently found in industry but may prove inadequate for the meaningful evaluation of processes related to the operation of an algae production platform. The algae industry is evolving and involves complex micro-biological, physical, botanical, marine, biochemical, and environmental interactions with thousands of strains. Moreover, cultivating, harvesting, and processing these single cell organisms introduce other technological challenges.

There have been many attempts to increase algae biomass yield and reduce costs. There is large opportunity to discover other new and innovative approaches that will undoubtedly lead to breakthroughs in cost-effective algae production strategies.

Vocal commercial, environmental, and political interest groups that ask daunting questions related to energy and carbon balances distract the research initiatives. Environmentalists and politicians are looking for meaningful solutions to climate change and reducing airborne greenhouse gas (GHG) emissions. It is well understood that algae, one of the world's fastest growing plants, can more than double their mass in a single 24-hour period. Every tonne of biomass created will take down 1.8 tonnes of CO₂ [1, 99]. Hence, significantly scaling up algae biomass production to offset GHG emissions is more than plausible. The "fly in the ointment" is: what do you plan to do with the biomass? Turning biomass to biochar is seen to be a great CO₂ mitigation strategy that locks up carbon in one of its most stable forms. Any positive net difference between GHGs produced and GHGs sequestered in the cultivation and processing of algae would qualify for carbon credits. However, this would ignore economic considerations. Furthermore, to be awarded carbon credits, a quantitative life cycle assessment that meets the International Standards Organization (ISO) guidelines must be conducted to validate results. Currently, there are concerns that the LCA results may be misleading because rarely are parameters in an LCA calculation identical [36].

With respect to the production of biofuel from algae, environmental interest groups ask similar probing questions: What is the net energy ratio? Is more energy produced through algae cultivation than consumed through associated processes? How do these compare to using conventional non-renewable energy resources? There are concerns that in many cases more input

energy is required than produced through the algae cultivation process. The same logic would hold true for CO₂ emissions.

9 Strategies And Opportunities For Sustainable Algae Cultivation In Canada

Algae companies have recently shifted focus to producing high value biomass-derived products since it shows the greatest promise of economic sustainability and can do so without government subsidies. Having determined a primary product, these companies selected algae strains known to synthesize meaningful amounts of the desired compound. Research has followed to optimize axenic algae growth by carefully conducting multiple tests to determine a combination of best lab-scale nutritional and environmental conditions and processes. Once an optimized regime has been documented, stringent protocols are established. Rigorous data logs are maintained for analysis and become part of a company's intellectual property. Based on favorable yield results, an economic model for scaling the production platform to the next stage is constructed [2]. This is essential for attracting investment funding to build the platform to each successively larger scale [100].

Scalability poses a further challenge [101]. Shifting from a laboratory setting to increasingly larger demonstration and production platforms requires a multi-disciplinary design team to ensure that axenic conditions are maintained and that the multi-variant conditions associated with growth through each production phase can be controlled, thus eliminating adverse operating effects. Controlling these conditions is necessary for a flourishing, closed growth environment (PBRs) but not possible to achieve in open cultivation systems.

As noted in this paper, there are companies and research institutions conducting primary research related to algae cultivation. Some entities compare algae composition data and others are focused on optimizing growth parameters for specific strains of algae. Yet others focus on technologies that will support the algae cultivation platform. Because of the challenges associated with each step in an algae cultivation platform, each research group focusses narrowly on a specific aspect. It is therefore prudent for research teams to find collaborative strategies for integrating and coupling strains, cultivation regimes, and associated technologies with other research groups to find more cost-effective and efficient technologies and processes to apply to their own work. These collaborations would lead to meaningful production volumes and thus early incomes of high value products. Using a business model to supply a specific product will help ease commercial transactions.

Once a successful and profitable algae business is established, there is opportunity to consider complimenting business revenues with other sources of value derived from the residual components of algae biomass, including biofuels and carbon credits.

When we understand the mechanics and processes of algae cultivation in a specific, profitable niche, doors will open to cost-effective production of algae feedstock for commodities like biofuels and to carbon credits.

Scientific engineering advances may be incorporated to enhance yields of specific products including biofuels [102]. Photosynthetic research may provide important clues to maximizing yield by taking advantage of maximal irradiance [103-105]. In general, algae growth has been associated with C3 photosynthesis. More recent studies suggest that C4 photosynthesis may also take place and have implications for improving growth yields [106].

We recommend that governments set in place integrated algae cultivation and biomass production / processing platforms like or in association with ATP3 (Algae Testbed Public Private Partnership) that leads to commercial products and establishing production benchmarks. In facilities like AzCATI (the Arizona Center for Algae Technology and Innovation), innovators and companies integrate specific technologies and test systems against the existing benchmarks. When new technologies prove more efficient and effective, they will set new production standards and thereby improve efficiency.

10 Conclusions

Much of the impetus for the recent renewed interest in algae biomass is related to the acknowledgement that algae biomass has the potential to address pressing global challenges, i.e., it may help reduce atmospheric CO_2 and reliance on conventional fossil fuels and provide a good source for food and cleaner water. Climate change is and will continue to affect the entire global community. Access to energy is fundamental to maintaining a productive and healthy economy. Given that energy is a commodity, cost will always be a factor, and therefore the cheapest products are favored. Government incentives in the form of subsidies for renewable energy are important in signaling to industry that change is required and promote the adoption of alternative energy forms.

In the case of algae biomass, past government subsidies created a frenzy of commercial activity given the implications of legislation demanding that increasing percentages of petroleum fuels be from renewable sources. Over the past 20 years, over a billion dollars were spent attempting to cultivate algae biomass that would deliver a more cost-effective feedstock from which to produce renewable fuels. Although significant investments have been made the singular focus to

deliver cheap biofuels has been an elusive objective. The recent shift to cultivate algae for highvalue products to established markets and / or develop a bio-refining model to deliver multiple algae-based products is a more pragmatic approach to launch the algae industry sustainably and economically.

Future government incentives should factor in not only the development and delivery of cheap biofuel feedstocks but also the capability to mitigate GHG emissions. In order to both facilitate more rapid commercialization and to track the progress within the industry, metrics need to be established. Any government subsidies should be tied to the release of key operating and production data for both progress tracking and collaborative research purposes. Given the multi-disciplinary complexities associated with algae production platforms, access to quality data is imperative for overcoming multiple challenges still associated with algae cultivation. Open access to research data sets would enable advancement of the overall algae knowledge base for the benefit of the greater community and accelerate the transition into commercial viability for the entire industry. This would facilitate more effective technology evaluation and enable better commercial decisions to be made.

For the Canadian context, the reviewed algae cultivation technologies show tangible progress toward delivering cost-effective algae biomass for downstream bio-refining applications. In order to achieve this outcome along with the national objective of GHG mitigation, further research needs to demonstrate improved crop yields with sustained and consistent growth; more efficient dewatering, processing, extraction and refining capability; cost-effective scalability of related technologies; and reduced energy inputs in the algae production platform.

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Parameter	NS	ND	Units
Ash	10.9	14.3	% w/w _{db}
С	52.1	53.9	% w/wdb
Н	7.2	7.5	% w/wdb
Ν	7.8	3.8	% w/wdb
S	0.7	0.6	% w/wdb
Ο	21.3	19.6	% w/w _{db}
Total Lipid	23	45	% w/wdb
Heating Value (High)	25.8	25.5	MJ/kg _{db}
Heating Value (Low)	24.3	23.9	MJ/kg _{db}

 Table 1: Sample microalgae composition [29]

NS = Nitrogen Surplus ND = Nitrogen Deprived db = dry basis

Company Name	Patent Number	Date	Patents/Comments	Ref.
Algae Aqua Culture	WO2014015184	1/23/2014	Biorefinery system, components therefor, methods of use and products	
Technology			derived therefrom	[68]
recimology	WO2014018785	3/20/2014	Biorefinery control system,	[08]
	W02014010703	5/20/2014	components therefor, methods of use	[69]
	WO2012100093	10/26/2012	Biorefinery system, components	[07]
	W02012100075	10/20/2012	therefor, methods of use and products	
			derived therefrom	[70]
AlgaBloom	20140315290	10/22/2012	Low-Cost Photobioreactor	
Technologies				[66]
Industrial	WO2014006551A	1/9/2014	Photobioreactor for liquid cultures	[67]
Plankton			•	[07]
National Research	CA 2394518A1	1/23/2003	Photobioreactor	[50]
Council of Canada Pond Biofuels,	20140199639	7/17/2014	Dracess for monoping photobiorecetor	
Inc.	20140199039	//1//2014	Process for managing photobioreactor exhaust	[60]
IIIC.	20140186931	7/3/2014	Process for operating a plurality of	[60]
	20140180951	//3/2014	photobioreactors	[65]
	20140113275	4/24/2014	Recovering off-gas from	[05]
	20140113273	4/24/2014	photobioreactors	[61]
	20130316439	11/28/2013	Biomass production	[64]
	20130183744	7/18/2013	Producing biomass using pressurized	[0+]
	20130103744	//10/2015	exhaust gas	[59]
	20120276633	11/1/2012	Supplying treated exhaust gases for	[57]
	20120270055	11/1/2012	effecting growth of phototrophic	
			biomass	[57]
	20120202281	8/9/2012	Light energy supply for	
	20120202201	0/9/2012	photobioreactor system	[58]
	20120156669	6/21/2012	Biomass production	[63]
	20110283618	11/24/2011	Supplying bioreactor gaseous effluent	[]
			to combustion process	[52]
	20110287405	11/24/2011	Biomass production	[62]
	20110287507	11/24/2011	Process for growing biomass by	[•=]
			modulating supply of gas to reaction	
			zone	[53]
	20110287522	11/24/2011	Producing biomass using pressurized	[]
			exhaust gas	[54]
	20110287523	11/24/2011	Recovering make-up water during	
			biomass production	[55]
	20110287525	11/24/2011	Diluting exhaust gas being supplied to	с Ј
			bioreactor	[56]
Symbiotic	WO2011050472A1	5/5/2011	apparatus, method and system for	[51]
EnviroTek Inc.			algae growth	[51]

Table 2: List of Patents on PBR Technologies

Technology				Associated				
Supplier	Туре	Size	Process	Processes	CO ₂ source	N_2O	Output Products	1
Generic ATP3 Demonstration ASCATI ATP3	OPR	125 m ³			air		research facility	
Demonstration	ARID	30 m ³			air		research facility	
Algae Aqua-Culture Technology (2009), MT	EPR	370 m ²	coupled serial batch with waste wood pyrolysis	AD, pyrolysis	Pyrolysis	Pyrolysis	6 T/d wood waste to 2 T/d soil amendment fertilizer with biochar, biofuels ethanol, biodiesel, jet	
Algae Tec Limited (2007), Australia	PBR				atmosphere, stack gases		fuel, EPA/DHA nutraceuticals	
AlgaBloom Technologies (2009)	PBR	400 m ²					food, omega 3	S
Algaecan Biotech Ltd (2009)	PBR	7.5 m ³	batch multi- phase approach				astaxanthin	h
Hy-Tek Bio LLC (2008)	PBR	6.8 m ³	batch / continuous flow	HTL, enzyme conversion to biodiesel	natural gas engine exhaust	exhaust, chicken manure	methane, biodiesel, jet fuel	
Industrial Plankton (2010)	PBR	1.25 m ³	batch / continuous flow				algae biomass research facility,	Na
National Research Council	PBR	1 m ³	batch / continuous flow	HTL	natural gas		algae biomass, biodiesel	
Pond Biofuels Inc.			batch /		engine exhaust, cement	chemical	alma hiomana hio	
(2007)	PBR	10 m ³	continuous flow	HTL	production gas emissions	processes	algae biomass, bio- fuels	
Symbiotic EnviroTek	1.510	1.0 111	batch /			waste	algae biomass, bio-	
Inc (2008)	PBR	103 m ³	continuous flow	HTL	bottled gas	streams	fuels	

Table 3: Algae Cultivation Technologies Suitable for Canada's Northern Climate

EPR - Enclosed Pond Raceway, OPR - Open Pond Raceway, ARID - Algae Raceway Integrated Design, PBR - Photo Bio-Reactor

Table 4: Factors affecting algae biomass production

- Climate
- Solar irradiance
- Capital costs
- Nutrient source and cost
- Algae species / composition
- Energy costs
- Operating costs
- Colocation with symbiotic industry partners
- Ability to control production factors
- Optimization of biomass yield
- SCADA / Automation
- Active research and development access to highly qualified multi-disciplinary scientific community
- Integration of advanced production platform technologies i.e. dewatering, extraction of active ingredients, processing, etc.
- Analytic data modeling

Table 5: Comparative and Competitive Algae Production Pricing [97] Feedstocks Production Cost (\$/T) Algae Required Equiv				
Palm oil	603	164		
Soybean oil	623	169		
Crude oil	752	204		

Table 5: Comparative and Competitive Algae Production Pricing [97]

Table 6: Cost to Produce Algae Biomass						
Technology	For Biofuels (\$/T)	For High Value Products (\$/T)	Literature Variability in Pricing (\$/T)		100 T/yr Scaling (\$/T)	
			Minimum	Maximum		
OPR	233	30,704	3,118	19,486	4,830	
PBR	17,292	40,895	3,774	94,430	3,828	