

Review

Achieving a Green Solution: Limitations and Focus Points for Sustainable Algal Fuels

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Abstract: Research investigating the potential of producing biofuels from algae has been enjoying a recent revival due to heightened oil prices, uncertain fossil fuel sources and legislative targets aimed at reducing our contribution to climate change. If the concept is to become a reality however, many obstacles need to be overcome. Recent studies have suggested that open ponds provide the most sustainable means of cultivation infrastructure due to their low energy inputs compared to more energy intensive photobioreactors. Most studies have focused on strains of algae which are capable of yielding high oil concentrations combined with high productivity. Yet it is very difficult to cultivate such strains in open ponds as a result of microbial competition and limited radiation-use efficiency. To improve viability, the use of wastewater has been considered by many researchers as a potential source of nutrients with the added benefit of tertiary water treatment however productivity rates are affected and optimal conditions can be difficult to maintain year round. This paper investigates the process streams which are likely to provide the most viable methods of energy recovery from cultivating and processing algal biomass. The key findings are the importance of a flexible approach which depends upon location of the cultivation ponds and the industry targeted. Additionally this study recommends moving towards technologies producing higher energy recoveries such as pyrolysis or anaerobic digestion as opposed to other studies which focused upon biodiesel production.

Keywords: algae; challenges; limitations; sustainability

1. Introduction

In the current climate there are many reasons for considering alternative fuel sources and algae has been heralded as a potential “silver bullet”, however, after initial excitement it appears that the concept will not be commercially viable for at least 10 years. The technology to produce fuel from algae is currently available and vehicles have been powered by this feedstock in a number of cases [1]. As a commercially viable alternative to fossil fuels, however, the technologies are not yet there, the energy balance of producing the fuel is high, the economics cannot compete and the overall sustainability is in doubt. The concept of algal fuels is one that has been with us since the 1950s [2]. Funding for such practices has fluctuated since the idea was conceived roughly in line with rising and falling crude oil prices. One of the main contributors in pioneering algal fuels is Professor W.J. Oswald who designed systems to cultivate algae on a large scale in the 1950s and 60s [3,4]. He developed the concept to remove nutrients from wastewater and provide a useful biomass for food or fuel. At the time however the viability of the concept appeared unachievable and as oil prices dropped so did funding for such projects. The price of oil however is continuing to rise with little sign of slowing down and it is now important to focus once again on improving the viability of fuel from algal feedstock. This paper will look at where we have come since the first research on algal cultivation and energy recovery was initiated, what is currently hindering the commercial application of the concept and where we need to go from here. Continued research will allow us to recover the maximum potential from algal biomass which will provide an increasingly important resource for us in the future as predicted by Professor Oswald [5].

The focus of this paper is to investigate the current state of energy recovery from algal biomass, focussing upon combining cultivation with wastewater treatment and the possibility of mitigating CO₂ emissions through utilisation of flue gases. The paper reviews the main processes involved in the cultivation, harvesting and processing cycle and considers which are most viable in terms of energy consumption, the environmental acceptability and practicality of use. The aim of this paper is to consider conventional and novel processes to identify sustainable approaches for algal biomass to energy.

2. Background

2.1. The Beginning for Algal Biofuels

With predictions of an ever-increasing global population in the 1940s and 1950s, many researchers were considering how feeding such vast number of people would be possible. Traditionally livestock is fed using arable crops however researchers believed that algae could play a large part in providing a high protein food source for livestock and thus a method for feeding the global population [5]. At University California at Berkley, Professor Oswald began designing pond systems to cultivate freshwater algae on a large scale. The idea was to design a low impact system (*i.e.*, low energy requirements and environmental impact) which provided conditions allowing high productivity of the cultivated algae. Oswald’s work also focussed upon combining algal cultivation with wastewater treatment providing a co-benefit [3]. The algae could therefore provide a means of improving the water quality of raw or partially treated effluent as well as providing livestock feed.

The biomass produced from the cultivation process was not restricted to livestock feed and studies were performed assessing the amount of biogas the algal biomass was capable of providing [2]. Algae was deemed a potentially valuable substrate for biogas production and various strains have been tested for their suitability up to the present day [6–8]. Further investigations led to algal biomass being assessed for alternative fuel types. Due to the high oil content of many algae species [9–14] biodiesel was considered a valuable fuel which could be extracted and processed from algal biomass. The concept of producing biodiesel from microalgae was developed considerably by the US Department of Energy's Aquatic Species Program: Biodiesel from Algae [15]. The program ran from 1978 to 1996 and was focused upon producing biodiesel from microalgae fed with CO₂ from flue gases. The program was born out of a requirement for energy security as the US relied heavily upon gasoline for transport fuel, disruption to supplies could have significant repercussions to the economy. The program provided excellent contributions to the area of algal cultivation for biofuel but when funds were diverted to alternative fuel research the program was phased out in 1996 [15].

Recently the interest in biofuels from algae has dramatically increased as a result of increased fossil fuel prices and the need to find an alternative due to the threat of climate change. Areas of studies include optimising biofuel yields, methods of reducing energy consumption, investigating alternative products and assessing environmental impacts.

2.2. Algae and Wastewater Treatment

All autotrophic algal strains require a source of nutrients. The most important nutrients, (*i.e.*, those that are needed in greatest concentrations) are nitrogen and phosphorous, but many other nutrients and trace metals are also necessary for optimal growth [16]. There are many media recipes designed to provide optimal nutrition for numerous algal strains. Nutrient rich effluents however are often capable of providing almost all of the nutrients required by several algal strains [17,18] and consequent cultivation provides two significant benefits. Firstly, direct uptake of these nutrients and metals, produces cleaner water. Secondly, the algae generate oxygen which aids aerobic bacterial growth leading to additional metal and nutrient assimilation. In the 1950s experiments were carried out by Oswald and his colleagues investigating the symbiotic relationship between algae and bacteria for wastewater treatment in oxidation ditches [4,19–22]. The experiments which were undertaken used the algae *Euglena* sp. due to their natural presence in ditches under examination. Oswald and his colleagues discovered that the bacteria and algae in the oxidation ditch develop a symbiotic relationship producing a more stable and less hazardous effluent [21]. The economic potential of the algal cells for livestock feed was identified, and the merits of using a faster growing species of algae in effluent specifically for the purpose of livestock feed were discussed [19]. Particular attention in the late 1950s was given to the design of wastewater treatment ponds and relationships between oxygen production, biological oxygen demand (BOD) removal and light use efficiency over specific periods of time as a function to a variety of species, depths of pond, treatment time, loading rates to identify optimal operational conditions [4]. The importance of algae in a heavily loaded oxidation pond to provide the necessary oxygen for sludge oxidation was highlighted, and this early research remains of particular interest as it identified optimal conditions for effluent treatment and demonstrated that oxidation ponds using the symbiotic relationship can achieve significant BOD removal (>85%) [4].

Further important work was carried out on the use of algae in wastewater treatment in the 1970s and 1980s. Of particular interest was the research lead by G. Shelef, with a focus on the growth of dominant species of algae in open ponds using raw sewage as the main source of nutrients [23]. His research indicated that *Micractinium* and *Chlorella* dominated in most cases, with retention times of around three to six days. Using an influent with concentrations of total suspended solids (TSS) *ca.* 340 mg/L and BOD *ca.* 310 mg/L, a considerable reduction to levels *ca.* 60 mg/L TSS and below 20mg/L BOD was reported. Additionally phosphate was reduced to very low levels and around 10–40 mg/L ammonia remained. This resulted in low levels of organic contamination which allowed the use of the treated wastewater for irrigation, and the residual nutrients provided a source of fertiliser as an added value. Like Oswald, G. Shelef assumed that the algae could be used as high protein feedstock for cattle feed, yet the latter mentioned the potential of anaerobic digestion of the biomass for biogas production [23].

In the 1980's the concept of growing algae was further developed however the focus turned to utilising the biomass for fuel production due to the energy crisis in the United States at the time. Oswald continued his research and was joined by Dr. Benneman and together they investigated the potential for the cultivation of algae on a large scale for fuel production. During this time most research moved away from wastewater treatment and more towards high productivity of biomass and high fuel yields with ideas related to the use of flue gas as a source of carbon dioxide. It has been recently reported in the literature that to produce algal fuel at a viable cost, it would require further benefits [24], which is in line with research conducted in the 1970s and 1980s that combined algal biomass productivity for fuel production with wastewater and flue gas treatment. At present, there has therefore once again been a revival in research conducted investigating the benefits of algae with wastewater treatment. Many different wastewater types have been investigated, most common are domestic sewage [25–28], agricultural wastewater (swine and cattle) [27,29–32] and several industrial wastewaters, e.g., carpet manufacturing [33] and distillation [34]. Previous research suggests that cultivation in tertiary treatment steps may provide the ideal conditions for good algae growth due to high residual nutrient loading and prior removal of organic contaminants [26]. In such a scenario algae can provide an effective means of nutrient polishing. Agricultural effluents in general and swine manure in particular contain very high nitrogen and phosphorous loadings, e.g., *ca.* 1210 mg/L and 310 mg/L, respectively, for dairy manure [29], providing potentially suitable media for algal growth and a method of effluent treatment where use of high nutrient effluent is not required. Various strains of algae have been shown to effectively remove nitrogen and phosphorous forms in a number of synthetic and actual wastewaters (Table 1).

Table 1 suggests that there is a significant potential for nutrient removal in wastewaters with nutrient loading using the various strains of common algae and some mixed cultures. The majority of research reported so far has been conducted at a lab scale in photo-bioreactors which provides controlled conditions (e.g., temperature, light and species control) and therefore will vastly improve the productivity of the algae and thus the uptake rate of nutrients. Limited research has been conducted investigating how this concept can be scaled up and moved into an open environment with the use of raceway ponds where it may not be possible to maintain a selected strain. Nevertheless the use of algae for nutrient removal clearly has promise for a wide variety of wastewater types and further research should prove useful in moving towards full scale viability.

Table 1. Nutrient removal efficiencies of algae in wastewaters.

Wastewater	Algae	Growth Infrastructure	N Removal (%)	P Removal (%)	Productivity rate (mg/L/day) unless specified	Refs.
Synthetic	<i>Scenedesmus obliquus</i>	PBR	70	94	-	[35]
Synthetic	<i>Chlorella vulgaris</i>	PBR	50	78	-	[36]
Synthetic	<i>Scenedesmus</i>	PBR	50–66	>50	39.3	[37]
Swine manure	Mixed species	Turf	98	76	-	[38]
Swine manure (pre-treated)	<i>Chlorella sorokiniana</i>	PBR	65 (NH ₄)	-	-	[39]
Swine manure	<i>Chlorella sorokiniana</i>	PBR	94–100	70–90	-	[40]
Municipal wastewater	<i>Scenedesmus, micratinium, chlorella,</i>	Open pond	96	99	24.4 (Lipid)	[27]
Municipal wastewater	<i>Scenedesmus</i>	PBR	99	99	250	[41]
Municipal wastewater	Cyanobacteria	PBR	88.3	64.8	10.9 g/m ² /day	[42]
Municipal wastewater	<i>Chlorella</i>	PBR	82.4	90.6	0.948/day	[43]
Dairy manure	Mixed culture	Turf scrubber	51–83	62–91	8.3–25.1	[44]

2.3. The Possibility of Carbon Mitigation

Cultivation of algal biomass could provide a method of carbon mitigation through CO₂ uptake from flue gases during photosynthesis. Providing algae can utilise industrial gases, there is the potential to remove CO₂, which would otherwise be emitted. The mitigation of CO₂ from flue gas using algae would be ideal in an industrial scenario, as targets for reducing greenhouse gas emissions are becoming tighter [45]. The improvement of biomass yields by introducing a concentrated source of CO₂ has been reported [46,47], however, there are many barriers yet to overcome; for example concentration of CO₂ in flue gas may be too high for many strains of algae resulting in toxicity, and/or the presence of other toxins in the gas may adversely affect productivity, and/or gas transport cost to algal biomass growth reactors or ponds may be unviable. Nevertheless, as mentioned, there is certainly potential for flue gases to play a part in an algal biomass cultivation system.

The atmosphere provides a CO₂ concentration of 0.038% for the growth of algae; theoretically, with a higher concentration available, higher productivity is possible [48]. Early studies found *Chlorella* sp. to be highly suitable for cultivation in flue gases due to its capacity to be grown with the injection of gas containing a CO₂ concentration of 15% [49], a concentration similar to that of most flue gases [47]. Experimentation conducted within the US aquatic species programme [15] using flue gases as a source of CO₂ indicated that local strains of algae dominated with a high CO₂ use efficiency. Single algal biomass productivity rates as high as 50 g/m²/day were recorded, although attempts to achieve

consistently high productivity rates failed during a long-term experiment for one year, provably due to low ambient temperatures [15]. In 2002 research was conducted by the National Renewable Energy Laboratory (NREL) and the US Department of Agriculture investigating uptake of CO₂ from synthetic and flue gas sources and its commercial and environmental viability. The technical feasibility and economic viability of integrating a micro-algal cultivation system with a coal fired power plant was investigated [50], using a bench scale system as a test rig. An artificial flue gas (12% CO₂; 5.5% O₂; 423 ppm SO₂; 124 ppm NO_x) based on the composition of a North Dakota power station boiler was produced and sparged into a bio-reactor tank. Two strains of algae were cultivated, *Monoraphidium* and *Nannochloropsis*, both of which grew successfully under the administered conditions. It was reported that growth rates of the microalgae varied between 15 to 25 g/m²/day and contained 41% protein, 26% lipid and 33% carbohydrate [50].

Research using real flue gases for CO₂ uptake and cultivation of algal biomass has also been conducted [46,47,51,52]. For example, *Chlorella* sp. was cultivated using a photobioreactor system approach and the productivity of *Chlorella* sp. was investigated in presence of a flue gas (6%–8% CO₂) from a natural gas boiler and in presence of a control gas, which resulted in higher productivity in the flue than in the control gas, of 22.8 ± 5.3 g/m²/day [53]. As a result it was suggested that 50% of the flue gas could be decarbonised using that system [51]. Similar studies conducted with *Chlorella vulgaris* using a photobioreactor system approach and flue gas from a municipal waste incinerator indicated that this strain was tolerant to a concentration of 11% (v/v) CO₂ as well as to the flue gas, with a higher biomass productivity in the flue [47]. Both studies suggest that the presence of potential contaminants in the flue had little adverse impact upon the algae. Examples of various strains of algae cultivated with the addition of CO₂ with their productivity rates are summarized in Table 2. Existing research indicates that an improved growth of algal biomass has been obtained using artificial and flue gases with CO₂ concentration up to approximately 12% (Table 2). Above this concentration it appears that productivity is reduced, most likely due to acidity caused by the high CO₂ levels. It is suggested that, although most strains would benefit from an increased concentration of CO₂, testing is required to identify optimal CO₂ concentrations as this appears to vary between strains. Similarly only a limited number of flue gas sources have been investigated; if the concept is likely to be taken up across many different industries, a variety of flue gases will need to be tested.

Table 2. Examples of algal biomass cultivated in a source of CO₂ and biomass productivity.

Algae Species	Gas	CO ₂ (%)	Productivity (g/m ² /day)	Refs.
<i>Chlorella</i> sp.	Air	Air	0.68	[46]
<i>Chlorella</i> sp.	Synthetic	2	1.45	[46]
<i>Chlorella</i> sp.	Synthetic	5	0.90	[46]
<i>Chlorella</i> sp.	Synthetic	10	0.11	[46]
<i>Chlorella vulgaris</i>	Air	Air	0.04	[54]

Table 2. *Cont.*

<i>Algae species</i>	Gas	CO₂ (%)	Productivity (g/m²/day)	Refs.
<i>Chlorella vulgaris</i>	Flue gas (MSW incinerator)	10–13	2.50	[47]
<i>Spirulina</i> sp.	Synthetic	Air	0.14	[51]
<i>Spirulina</i> sp.	Synthetic	6	0.22	[51]
<i>Spirulina</i> sp.	Synthetic	12	0.17	[51]
<i>S. Obliquus</i>	Synthetic	Air	0.04	[51]
<i>S. Obliquus</i>	Synthetic	6	0.10	[51]
<i>S. Obliquus</i>	Synthetic	12	0.14	[51]

In summary, research to date suggests mitigation of CO₂ using algae cultivation is promising providing the gases are at a low concentration and contain low levels of contamination and operational conditions (e.g., pH, temperature, light) are controlled.

2.4. Comparison of Open Ponds and Photo-Bioreactors

The two main methods of infrastructure considered suitable for cultivation of algae are open (raceway) ponds or photo-bioreactors (PBRs) [55], and are compared in Table 3. Raceway ponds are similar to oxidation ditches used in wastewater treatment systems being large, open basins of shallow depth and a length at least several times greater than that of the width. Raceway ponds are typically constructed using a concrete shell lined with polyvinyl chloride (PVC) with dimensions ranging from 10 to 100 m in length and 1 to 10 m in width with a depth of 10 to 50 cm [55]. Oswald considered the open pond to be the most viable method of combining algal cultivation and wastewater treatment in the 1950s [22].

Table 3. Comparison of raceway ponds and photo-bioreactors.

	Raceway Pond	Photobioreactor	Refs.
Estimated productivity (g/m ² /day)	11	27	[55]
Advantages	Low energy Simple technology Inexpensive Well researched	High productivity High controllability Small area required Concentrated biomass	[55]
Disadvantages	Low productivity Contamination Large area required High water use Dilute biomass	High energy Expensive Less researched	[55]

Photobioreactors are more commonly used for growing algae for high value commodities or for experimental work at a small scale. Recently, however, they have been considered for producing algal biomass on a large scale as they are capable of providing optimal conditions for the growth of the algae [55,56]. A closed reactor allows species to be protected from bacterial contamination, shallow

tubing allows efficient light utilisation, bubbling CO₂ provides high efficiency carbon uptake and water loss is minimised. PBRs provide very high productivity rates compared to raceway ponds. In their life-cycle assessment (LCA) study, Jorquera *et al.* [55] estimated volumetric productivity to be at least eight times higher in flat-plate and tubular PBRs. The reason why PBRs however have not become widespread is due to the energy and cost intensity of production and operation. PBRs require a far higher surface area for the volume of algal broth compared to alternative infrastructure. Much higher volumes of material are therefore required which in turn requires a higher capital energy input and increases environmental impacts [56]. During operation algal biomass must be kept in motion to provide adequate mixing and light utilisation. These increase productivity but also require additional energy for pumping. So far in comparison to raceway ponds the benefits of PBRs do not outweigh the necessary energy requirements identified in the LCA study published by Jorquera *et al.* [55]. A net energy ratio (*i.e.*, energy produced/energy consumed) of 8.34 has been reported for raceway ponds as compared to a net energy ratio of 4.51 and 0.20 for flat-plate and tubular photobioreactors, respectively [55]. It is likely that ponds will continue to provide the most effective infrastructure for algal cultivation due to their low impact design and low energy input requirement. PBRs will continue to be important however, for laboratory work, developing cultures and producing biomass with high economic value. As research continues it may also be possible to develop infrastructure that will provide the benefits of both PBRs and open ponds together.

2.5. Biomass Processing

2.5.1. Harvesting

As the biomass cannot be utilised efficiently at low concentrations in media, the first step in the biomass processing stage is harvesting the algae for subsequent processing. The method of harvesting used depends very much upon the type of algae which is under cultivation. Microalgae require more intensive harvesting methods in comparison to macroalgae, because of their cell size. Depending upon circumstances, often a series of harvesting methods is required to produce a final biomass below a desired moisture content. Common methods of harvesting of algae are: microfiltration, flocculation, sedimentation, flotation and centrifugation [57].

One of the most effective methods of harvesting is filtration using micro-filters. This method of filtration generally uses a rotary drum covered with a filter to capture the biomass as the influent passes through from the centre outwards [58]. Initial harvesting tests in the 1960s tested micro-filters but found that the majority of algal cells simply passed through most of the filter types [59]. It was later suggested that micro-filtration was suitable for strains of algae with a cell size greater than around 70 µm and was not suitable for those species with cell sizes lower than 30 µm [60]. The size of the opening in the filter mesh dictates what percentage of biomass is captured likewise with the size of the biomass cells. The pore size also affects how much pressure is required to facilitate the flow of water through the filter which will in turn affect the energy consumption [61]. The concentration of the algae in suspension also influences the efficiency of removal as highly concentrated biomass will foul the filter very quickly causing reduced performance and a requirement for backwashing and thus further energy consumption. If filtration is to be used it is essential that the method suits the species of algae

which is being harvested, otherwise the filtration will be ineffective and provide low yields of biomass. If the cultivated algal species allows for filtration (e.g., *Spirulina*, *Spirogyra*, *Coelastrum*), the filtration method can prove very efficient and cost effective method of harvesting. Mohn [62] for example found that gravity filtration using a microstainer and vibrating screen both provided good initial harvesting of *Coelastrum* up to a total suspended solid of 6% with low energy consumption (0.4 kWh/m³). Mohn [62] also investigated pressure filtration of *Coelastrum* which provided even higher total solids of concentrate up to 27%, although requiring more than twice the energy. Clearly inexpensive and low energy harvesting of biomass is possible with filtration, providing the dominant algae being harvested is of a suitable cell size and optimal concentration level.

Sedimentation and flotation have also been proven as viable options for harvesting algal biomass with no requirement for specific cell size. Both sedimentation and flotation rely on biomass density to facilitate the process, both processes are aided by flocculation and flotation is aided additionally with bubbling. As a method of biomass removal, sedimentation was considered a viable process in the 1960s due to its prominence in wastewater treatment and its low energy requirement [59]. Due to the low specific gravity of algae, the settlement process is, however, slow but, under certain conditions, the self-flocculation of some strains of algae is possible. Nutrient and carbon limitation and pH adjustment appear to be methods of auto-flocculation of algae which may provide a low-cost solution to the initial harvesting process [58,63]. Recent studies have focussed upon bio-flocculation which occurs as a result of using several bacteria or algal strains to flocculate with the desired algal biomass to allow settlement. Gutzeit *et al.* [64] found that gravity sedimentation was possible using bacterial-algae flocs developed in wastewater for the removal of nutrients, and reported that the flocs of *Chlorella vulgaris* were stable and settled quickly. Other approaches investigated the combined use of autoflocculating microalgae (*A. falcatus*, *Scenedesmus obliquus* and *T. suecica*) to allow for flocculation of non-flocculating oil-accumulating algae (*Chlorella vulgaris* and *Neochloris oleoabundans*) [65], which resulted in a faster sedimentation as well as a higher percentage of biomass harvested. This method of harvesting appears viable due to its low energetic inputs but also because it does not rely on chemicals, thus allowing the water to be discharged or recycled without further treatment. However, it should be noted that this method of flocculation may not be suitable for all types of algae, and thus further research is required in this area.

Conventional methods of flocculation using flocculants common to wastewater treatment such as alum, ferric chloride, ferric sulphide, chitosan among other commercial products are likely to provide a more consistent and effective solution to flocculation. Much research has been conducted upon the removal of algae using flocculants with varying degrees of success (Table 4). For example, a complete removal of freshwater microalgae, *Chlorella* and *Scenedesmus*, using 10 mg/L of polyelectrolytes while 95% removal using 3 mg/L of polyelectrolytes has been reported [59]. A comparative study where alum and ferric chloride were used as flocculants for three species of algal biomass (*Chlorella vulgaris*, *I. galbana* and *C. stigmatophora*) indicated the low dosages of alum (25 mg/L) and ferric chloride (11 mg/L) were sufficient for optimal removal of *Chlorella vulgaris*, while higher dosages of alum and ferric chloride were required for the removal of marine cultures *I. galbana* (225 mg/L alum; 120 mg/L ferric chloride) and *C. stigmatophora* (140 mg/L alum; 55 mg/L ferric chloride) [66]. Additionally it has been reported that the combined use of chitosan at low concentrations (2.5 mg/L) and ferric chloride provided much quicker flocculation of the algal cells,

Chlorella vulgaris, *I. galbana* and *C. stigmatophora*, and reduced the requirement of ferric chloride [67]. The use of chitosan as a flocculant for the removal of freshwater algae (*Spirulina*, *Oscillatoria* and *Chlorella*) and brackish algae (*Synechocystis*) has been investigated [40], and chitosan has been found to be a very effective flocculant, at maximum concentrations of 15 mg/L removing about 90% of algal biomass at pH 7.0. The use of conventional and polymeric flocculants for the removal of algal biomass in piggery wastewater has been recently investigated [67]: ferric chloride and ferric sulphate were found to be effective flocculants at high doses (150–250 mg/L) providing removal rates greater than 90%; polymeric flocculants required less dosing (5–50 mg/L), although provided lower biomass recoveries; chitosan performed poorly at both low and high dosages for each of the algal species types with a maximum removal of 58% at a dose of 25 mg/L for a consortium of *Chlorella*.

Table 4. Maximum removal rates of various flocculants for the removal of algal biomass.

Flocculant	Algae	Removal (%)	Dosage (mg/L)	Media type	Refs.
FeCl ₃	<i>Chlorella</i>	98	250	Piggery wastewater	[40]
FeCl ₃	<i>S. obliquus</i>	95	100		
	<i>Chlorococcum</i> sp.	90	150		
	<i>Chlorella</i>	90	250		
	<i>S. obliquus</i>	98	150		
Fe ₂ (SO ₄) ₃	<i>C. sorokiniana</i>	98	250	Nutrient media	[68]
Chitosan	<i>Spirulina</i> , <i>Oscillatoria</i> , <i>Chlorella</i>	>90	15		
Polyelectrolyte (Puriflocs 601 & 602)	<i>Chlorella</i> , <i>Scenedesmus</i>	95	3	Sewage	[59]

2.5.2. Sedimentation

Sedimentation of algal biomass is a further method of biomass removal but generally requires prior flocculation for high removal efficiencies. Sedimentation can be carried out with some species without flocculation, but removal efficiency is generally considered poor. Flocculation can be used to increase cell dimensions allowing improved sedimentation. If carried out in conjunction with flocculation, a sedimentation tank can provide a reliable solution for biomass recovery [69].

2.5.3. Flotation

Flotation was a method of harvesting considered in the 1960s [59] however the recoverability of biomass was generally found to be poor with a wide range of reagents tested. It has been reported that using dissolved air flotation mixed algal species could be harvested up to a slurry of 6% total solids; using electro-flotation, which creates air bubbles through electrolysis which then attach to the algal cells, mixed algal species could be harvested up to a slurry of 5%, but this approach required a significant energy input; using dispersed air flotation which uses froth or foam to capture the algal cells resulted also in similar results [69]. Existing research indicates that flotation offers a quicker alternative to sedimentation following algal flocculation, but more energy is required and thus cost is higher whilst providing a final product with lower total solids content.

2.5.4. Centrifugation

Probably the most effective method of biomass removal, with very high recovery rates, is centrifugation. As with the other alternative methods, centrifugation was considered a feasible option in early algal biomass dewatering work in the 1960s. Golueke and Oswald [3] investigated various means of dewatering algae further to provide a biomass with a sufficiently low moisture content. One of the methods they looked at was centrifugation and three of the four centrifuges that they tested proved to be extremely effective producing a maximum removal of 79% and a biomass with solids content of 11.5% and maximum of 18.2%. Further research was conducted by Mohn [62] in the area of harvesting algal biomass using centrifugation and he focussed on suitability of algal strains, cost and energy use. In accordance with Golueke and Oswald, Mohn found centrifuges to be very effective for the removal of *Scenedesmus* and *Coelastrum*, particularly the Westfalia self-cleaning plate separator and the Westfalia nozzle centrifuge [62]. The centrifuges provided biomass with total solids content of 2%–22% with a minimum energy consumption of 0.9 kWh per m³. Table 5 provides an overview of Mohn's findings indicating the possible harvesting methods, effectiveness, energy requirements and reliability of several harvesting methods. Mohn's results suggest filtration provides the best harvesting strategy in terms of high concentration of solids with low energy requirements [62].

Table 5. Harvesting methods, effectiveness and energy requirements.

Algae species	Harvesting Method	% TSS of Concentrate	Concentration Factor	Energy Requirement (kWh)	Reliability	Refs.
<i>Coelastrum</i>	Gravity filtration	6	60	0.4	Good	[62]
<i>Coelastrum</i>	Pressure filtration	22–27	245	0.88	Very high	[62]
<i>Scenedesmus</i> , <i>Coelastrum</i>	Centrifuge (Westfalia self-cleaning)	12	120	1	Very good	[62]
<i>Scenedesmus</i> , <i>Coelastrum</i> , <i>proboscideum</i>	Centrifuge (Westfalia screw)	22	11	8	Very good	[62]

Despite centrifugation being an effective method of concentrating biomass, the energy requirements are much higher than that of filtration. However clearly the choice of harvesting depends heavily upon the biomass type, if the cell size is large enough, then filtration is likely to be the most effective and economically viable option. Otherwise it is likely that a process stream involving flocculation, sedimentation, flotation or centrifugation is necessary. There is little parallel between the effectiveness of common flocculants for harvesting algae in research conducted. It can be observed that there are many effective flocculants for algae removal however suggested optimal dosages vary significantly between studies. Ferric chloride can be considered a viable option potentially combined with chitosan to improve yield and reduce time and material input. Further research is necessary for individual scenarios to choose the most effective method of flocculation and consequent harvesting.

2.6. Fuels

Following harvesting, the algal biomass requires conversion to fuel through a variety of techniques to extract and process the sought-after products within the cells. The necessary processing depends upon the desired fuel [70]. The three fuel types that will be covered here are those that are currently considered the most suitable for energy recovery from algae: being biodiesel, bioethanol and biogas. Each of these biofuel types require a different process stream and diagrams are presented in Figures 1–3.

Figure 1. Simplified process diagram for biodiesel production.

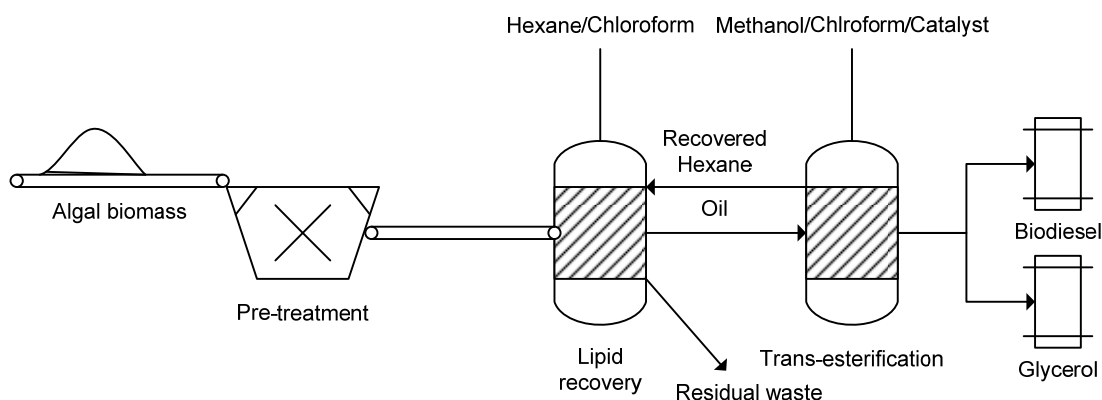


Figure 2. Simplified process diagram of bioethanol production from algal biomass.

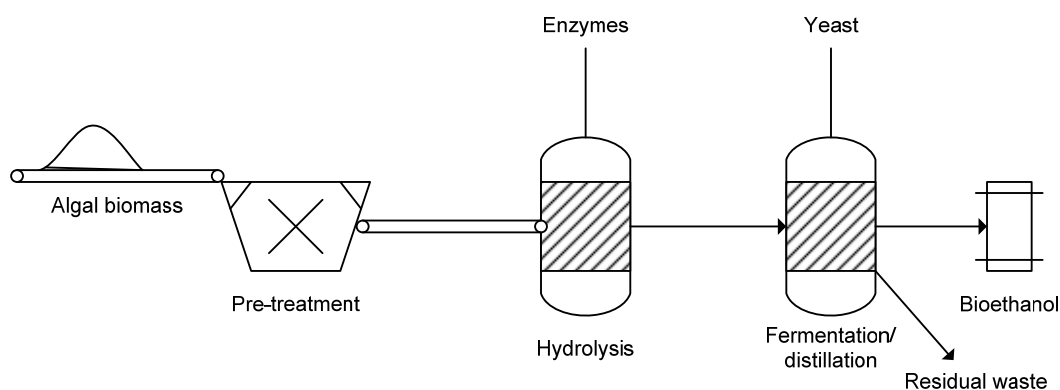
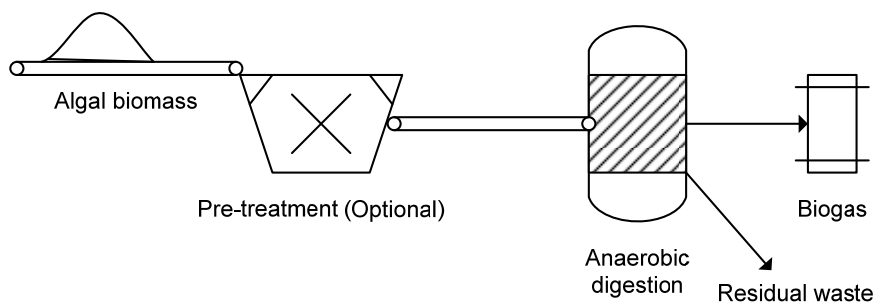


Figure 3. Simplified process diagram of biogas production from algal biomass.



2.6.1. Biodiesel

Biodiesel is the most common fuel type researched as a method of recovering energy from algae due to the high oil content of many algae strains [11,14,71]. The production of biodiesel initially requires the extraction of the lipid content of the algal cells. Most researchers follow a standard protocol written by Bligh and Dyer in 1959 [72] which uses chloroform and methanol as the extraction technique. Prior to lipid extraction the cells must be disrupted to allow access to the oils within the cell. Disruption can be achieved by homogenisation, bead beating, mechanical pressing, microwave treatment, acid/alkali treatment, sonication, lyophilisation and autoclaving among others.

Lee *et al.* [12] produced a study investigating the various methods of cell disruption and corresponding lipid extraction efficiencies. They found that for each algal strain (*Botryococcus* spp. *Chlorella vulgaris* and *Scenedesmus* spp.) microwave treatment provided the highest lipid yield. In terms of productive strains, *Botryococcus* spp. provided the highest yield using microwave treatment at 28.6% lipid recovery from the biomass. Bead-beating however, almost matched this value. Each of the disruption methods (autoclaving, bead-beating, microwaving, sonication and osmotic shock) produced lipid yields higher than a no-disruption technique.

The next step of the process is the lipid extraction and most studies extract the lipid content of the biomass using a modified version of Bligh and Dyer's method [72]. This requires the addition of methanol and chloroform, typically in proportions of approximately 1:1 methanol to chloroform mixed with the sample also at a ratio of about 1:1 methanol/chloroform mixture to sample [12]. Once the reaction is complete the oil can be separated using a centrifuge or funnelling method as the densities of the materials differ. Methanol, chloroform and a catalyst (acid or base) are then mixed with oil to allow trans-esterification to occur. The two products from the reaction are methyl esters (biodiesel) and glycerol. The products are biphasic and thus can be easily separated.

Research in the area is now looking at the possibility of improving extraction of oils from wet biomass which eliminates the energy consumption required for drying of the biomass. It is generally considered that removal of oil from dry biomass is most efficient and practical [73]. Johnson and Wen [74] investigated the use of both wet and freeze-dried algal biomass (*S. limacinum*) for the production of biodiesel. The researchers found that wet biomass produced 20% less fatty acid methyl-esters than the dried biomass, lowering the biodiesel value. Further research has been conducted by Patil *et al.* [73] who conducted experiments producing fatty acid methyl-esters from wet biomass via a supercritical methanol method. The process required only one step for extraction and trans-esterification with addition of methanol at ratio of 1:9, biomass to methanol respectively, a temperature of 255 °C and reaction time of 25 min. The results showed a Fatty Acid Methyl Ester (FAME) recovery of around 88% from *Nannochloropsis* biomass. The research suggests that high recovery is possible without the energy intensive process of drying and separate lipid extraction. Similarly positive results of direct extraction from wet biomass were produced from Wahlen *et al.* [75] who experimented with direct biodiesel production from various freshwater green algae strains, cyanobacteria and mixed wild algae. More research is required to assess the potential of recovering biodiesel from wet algae in a single stage process yet the concept appears promising. Energy costs of the process may be higher but this could well be outweighed by the reduced energy cost from drying of the biomass as was calculated by Lardon *et al.* [76] in their LCA of biodiesel from microalgae. This LCA study compared methods of

cultivating and processing algal biomass for maximum energy recovery, they investigated the energy consumption associated with producing 1 kg of biodiesel. In their study they found that drying required 81.8 MJ of heat and 8.52 MJ of electricity per kg of biodiesel with no heating requirement for wet biomass. Oil extraction required higher energy consumption for wet biomass than dry but the final energy balance for wet biomass was significantly positive, 105 MJ/kg biodiesel) compared to the negative balance for dry biomass (−2.6 MJ/kg biodiesel).

2.6.2. Bioethanol

An alternative or addition to the production of biodiesel is the production of bio-ethanol from the carbohydrates and starches in the algal cells. Depending upon the strain and composition of the algal species significant yields of ethanol can be produced from algal biomass [77–80]. Strains with filamentous cells such as *Spirulina* and *Spirogyra* are considered most promising due to the higher percentage of carbohydrate in their make-up. The conventional process of producing bioethanol using hydrolysis and fermentation is well understood for many feedstocks but optimal conversion has not yet been achieved for algal biomass. Similarly to lipid extraction, the first stage in the process is the disruption of the biomass cells which can be carried out using numerous techniques including bead-beating, autoclaving, microwaving and acid or alkali treatment. Once the cells have been disrupted the carbohydrates and starches can be converted into sugars using enzymatic or acid hydrolysis. Following hydrolysis the sugars are then be fermented with yeast (typically *S. cerevisiae* or *S. bayanus*) which will provide a broth of up to 17% (v/v) ethanol depending upon the concentration of sugars (AB Mauri, personal correspondence). The next step to produce bioethanol is to distil the broth to produce an ethanol concentration of around 98% (v/v) then further refinement of the ethanol produces a fuel which can be used as an additive to conventional engines or up to a maximum of 85% in specialised E85 engines [81].

As the concept of converting algal biomass into bioethanol is relatively under-researched most studies have simply focussed upon investigating what ethanol recoveries are possible. In an early study by Hirano *et al.* [79] a variety of freshwater and marine algae was selected for testing. *Chlorella vulgaris* was found to contain a high proportion of starch (37%) and a recovery of 65% of ethanol from the starch was obtained using enzymatic hydrolysis followed by fermentation with *S. cerevisiae*. An overall recovery of 24% from the biomass was therefore obtained. Using the strain *Chlorococum* spp., a conversion efficiency of about 38% of the ethanol was obtained [80], which can be considered promising however this was an optimal value and no consideration was given to the energy requirement of processing. What is interesting from this research is that when the lipid content of the biomass was recovered prior to fermentation, ethanol yields were far higher [80]. This suggests that biomass could provide both diesel and ethanol, maximising potential recoveries. Nguyen *et al.* [82] found in several studies that yields of up to 29% ethanol recovery efficiency were possible using *Chlamydomonas reinhardtii*. The studies mentioned above prove that high ethanol yields from algal biomass are possible but further studies are necessary to assess the viability in terms of energy balance, economics and environmental impacts.

Alternative methods of ethanol production have been investigated which focus upon intracellular ethanol production in which algae produce ethanol under dark, anaerobic conditions. The species

which are capable of the process are cyano-bacteria and include the species: *Chlamydomonas reinhardtii*, *Oscillatoria limosa*, *Microcystis*, *Cyanothece*, *Cicrocystis aeruginosa* and *Oscillatoria* spp. [83]. The process requires the algae to be cultivated in a closed environment with the addition of CO₂ under which conditions, it is believed that concentrations of between 0.5 and 5% ethanol can be produced. Hirano *et al.* [79] investigated this phenomenon using *Chlamydomonas reinhardtii* and *Sak-1* isolated from salt water, and a maximum yield of 1% , w/w produced by *C. reinhardtii* was reported. The ethanol-water mix can then be extracted and treated further to produce highly concentrated ethanol for fuel use. The benefits of the process are that no other organisms (e.g., enzymes and yeast) are required for hydrolysis/fermentation and the algae remains unaffected and can continue to grow without a requirement for harvesting. The energy requirements are likely to be lower than those necessary for conventional fermentation of biomass however the two methods need to be directly compared. Although the concept is still very much in the trial phase, a company in the United States, Algenol is currently developing the concept to produce ethanol commercially from Cyanobacteria [1]. Intracellular ethanol production is a promising concept. In their study Luo *et al.* [84] show that the whole process provides a positive energy balance with the greatest surplus of energy when the maximum ethanol concentration is produced. Additionally the greenhouse gas emissions compare well to emissions via gasoline production but to reach 20% of the emissions from gasoline (a government aim) would require further reductions in the process chain.

Bioethanol production from algal biomass is still very much in its infancy, the concept is proven but the viability is not. Further life-cycle analyses are required to understand the potential of the concept. Post lipid processing and intracellular ethanol production look promising as energy consumption is minimised, further research will establish viability.

2.6.3. Biogas

A simpler method of energy recovery may be facilitated by anaerobic digestion of algal biomass providing a promising source of bio-energy in the form of biogas. The process was considered a potential source of useful energy recovery from algal cultivation near the start of modern research [2]. Anaerobic digestion is a process that has been used for hundreds of years to provide a source of energy from low value organic matter with minor energetic inputs. In the case of algal biomass, all the carbohydrates, proteins and fats can be converted into methane and carbon dioxide, although some components provide greater methane yields than others. It follows therefore that there is slightly less necessity to cultivate particular strains of algae for increased yields.

Table 6, taken from a study by Sialve *et al.* [85], displays the methane potential of each biomass component. Research has been conducted investigating the potential of various strains of algal biomass and Sialve *et al.* [85] used the methane potential to calculate yields for a number of strains. Their results can be viewed in Table 7 which compares theoretical results with experimental results from literature. Table 7 suggests that the values of methane yield can vary between species due to compositional make-up and that the yield depends very much upon the growth conditions as this can have a great impact upon the composition of the biomass. Comparing the actual yields with the theoretical yields shows a realistic conversion efficiency loss of about 50% in the majority of cases.

It is important therefore that in further studies investigating potential yields, exaggerated or over-optimistic yields are not used as these may not reflect real performance.

Table 6. Methane potential from biomass substrate [85].

Substrate	L CH ₄ /g VS
Proteins	0.851
Lipids	1.014
Carbohydrates	0.415

As opposed to direct conversion, anaerobic digestion can alternatively be used to recover energy from the waste biomass following extraction of the more valuable components from the biomass cells. In their life-cycle assessment, Lardon *et al.* [76] calculated that the only feasible way of producing a positive energy balance of algal biodiesel was to recover further energy using anaerobic digestion of the residual waste. In fact, in normal culture conditions, they found that the energy produced from anaerobic digestion would be greater than that from extracted biodiesel. In their investigation of biogas from algae, Sialve *et al.* [85] suggest that at lipid contents below 40% it is unlikely to be worth recovering the lipids using current methods and the biomass should simply be digested to recover the maximum energy yield. In their LCA study of algae digestion Collet *et al.* [86] found the environmental impacts of biogas from algae to be poor in comparison to algal biodiesel using results from the study conducted previously by Lardon *et al.* [76]. The study compared the results for 1MJ of energy produced in a combustion engine. The difference in impacts was mainly due to electricity consumption and assuming anaerobic digestion is also applied following the biodiesel extraction in the biodiesel scenario. The figures used in the mentioned study provided high values of energy consumption which contrast with those used in other studies [87], the impacts may therefore not be as adverse as suggested. Collet *et al.* [86] concluded that the impacts can be improved with reduced energy consumption and a combined process of lipid extraction and anaerobic digestion may provide the optimal solution.

Table 7. Theoretical and actual methane from different algal species.

Algae Species	Proteins (%)	Lipids (%)	Carbohydrates (%)	CH ₄ (L/g) (Theoretical) [85]	CH ₄ (L/g) (Experimental)	Refs.
<i>Euglena gracilis</i>	39–61	14–20	14–18	0.52–0.8	-	[85]
<i>Chlamydomonas reinhardtii</i>	48	21	17	0.69	0.59	[88]
<i>Chlorella pyrenoidosa</i>	57	2	26	0.8	0.17–0.32 (<i>Chlorella-Scenedesmus</i>)	[2]
<i>Chlorella vulgaris</i>	51–58	14–22	12–17	0.63–0.79	0.24	[8]
<i>Dunaliella salina</i>	57	6	32	0.68–0.74	0.44–0.45 (<i>Dunaliella</i>)	[85]
<i>Spirulina maxima</i>	60–71	6–7	13–16	0.63–0.74	0.32–0.31 (<i>Spirulina</i>)	[85]
<i>Spirulina platensis</i>	46–63	4–9	8–14	0.47–0.69	0.32–0.31 (<i>Spirulina</i>)	[85]
<i>Scenedesmus obliquus</i>	50–56	12–14	10–17	0.59–0.69	0.17–0.32 (<i>Chlorella-Scenedesmus</i>)	[2]

The biogas produced through anaerobic digestion differs from bio-diesel and bio-ethanol in that it is not a fuel that can be used directly for combustion in vehicle engines. There are two options for biogas, one is combustion within a co-generator to produce electricity with possible heat recovery. The alternative is to refine the biogas removing the CO₂ and the methane can then be used as a fuel within a gas engine [89]. Further energy is required to upgrade the gas to a useable transport fuel and this is often ignored in studies with the energetic content of the gas is only considered. Further research is necessary to investigate the impact of downstream processing if comparison to the alternative biofuel types as a transport fuel is desired.

Anaerobic digestion is one of the methods of recovering energy that seems to provide a positive net energy balance due to the low inputs required [76]. The results may however be optimistic as real yields are much lower than theoretical calculated yields. Additionally the biogas may require further processing to be useful as a fuel and this will affect the energy consumption and environmental impacts. Nevertheless the process is capable of recovering energy from all strains of algae regardless of the composition and therefore can be very useful as part of a flexible approach.

3. Limitations

Despite having been researched for over 50 years now, there are still only a few companies that are growing algae for fuel on a large or commercial scale. The economics of producing algae for fuel do not currently justify the intensity of the numerous processing stages and current practicalities. Cultivating algae with high productivity year round is a challenging task unless grown in controlled conditions, however, this itself, is not a viable solution. Attempts have been made to cultivate pure strains of algae in environmental conditions but with little success. In most cases local strains of algae come to dominate, out-competing the selected strain. This section reviews the limitations currently facing biofuel production from algal feedstock.

3.1. Open Pond Cultivation and Species Control

As mentioned above, open pond cultivation is currently considered the most viable option for large scale cultivation of algal biomass for energy. According to LCA research conducted by Jorquera *et al.* [55], open ponds provide a much higher net energy ratio in comparison to PBRs, both tubular and flat plated. Jorquera *et al.* [55] investigated the amount of energy consumed and produced using the three different cultivation methods for a yield of 100,000 kg of biomass annually. Productivities and energy yields in PBRs were found about three times higher than ponds, due to differences in efficiency. The energy consumption of PBRs to produce the equivalent amount of algal biomass compared to cultivation in ponds was however considerably higher (*ca.* ten times for tubular PBRs). The high energy consumption of the PBRs is mainly due to the air pumping, water pumping and the caloric content of the equipment used. On the whole, it can be assumed that despite low productivity requirements, open ponds provide a higher biomass yield for the energy consumed. One limitation of this reported research [55] is that it assumed that the cultivation of algae and the estimated productivity in the pond is possible all year round.

It has been previously reported that, under environmental conditions, wild strains of algae are likely to dominate and the strain of algae will also change depending upon the season [90]. Before discussing

controlled conditions further, it is important to stress that certain strains of algae may be controlled by their requirement for extreme conditions. *Spirulina* and *Dunaliella*, for example, require a high pH and raised salinity to survive, most invasive species would be intolerant of these conditions [90]. The majority of species, however, require less extreme conditions and competition by native algae (and possibly other living microorganisms) remains a problem. During the summer seasons the dominant algae will be those that thrive in higher temperatures and conversely in winter those species that survive colder weather will dominate. Tseng *et al.* [91] found that at temperatures between 17 to 22 °C, *Chlorella vulgaris* dominated whereas at higher temperatures from 22 to 27 and even up to 32 °C, *Scenedesmus ellipsoideus*, *S. dimorphus* and *Wastella botryoides* were dominant. Alternative observations were, however, made by G. Shelef finding that in Israel, *Chlorella* and *Micratinium* were dominant in the summer whereas *Euglena* and *Scenedesmus* were the common species in winter [23]. Clearly the location of cultivation has a large impact upon which species will dominate in each season. What is most important, however, is that when growing algae in environmental conditions, selectivity of strain is not currently possible.

Recently research has investigated methods of species control in cultivation ponds. For example, two high-rate algae ponds have been compared, one which included recycling of algae and one which did not [90]. The species under investigation was *Pediastrum* spp. and algal biomass was collected every day and settled in algal settling cones. One litre of biomass was returned to one of the ponds and not the other. The pond with recycling provided over 90% dominance of *Pediastrum* sp. in one year whilst the non-recycled pond provided only 53% dominance. It is suggested that this method of recycling may provide a useful method of species control in open pond cultivation, however it may not be successful for every strain required for cultivation. Further research is required to assess whether recycling will be beneficial for any considered strains.

3.2. Water Resource Scarcity

With water becoming a scarce commodity, intensive use of water for bio-energy cannot be considered sustainable if water extraction is affecting agriculture, domestic use or causing environmental impacts. Being an aquatic species, algae require more water than terrestrial bio-energy plants and when cultivated in open ponds there are great water losses mainly through evaporation. According to Williams and Laurens [92] the dissociation of one mol of water occurs for every mol of CO₂ required in the photosynthetic process. In their study of water use in algal cultivation Murphy and Allen [93] calculate that 33.2 m³/m² of water per year is required to cultivate algae in a raceway pond in the United States. It is possible to recycle much of the water that is drained from the ponds during harvesting but there will be losses in harvesting the algae; freshwater must therefore be sourced. In the same study it was reported that the management of the water will require seven times the amount of energy that can be produced from biodiesel extracted from the algae [93]. The majority of countries around the world are becoming increasingly water stressed and therefore using extra freshwater in biofuel production is not sustainable. The use of wastewater as an alternative to freshwater provides an ideal solution however freshwater would still be necessary for downstream processing of the biomass or for dilution of highly concentrated wastewater.

3.3. Energy Consumption

Energy consumption in the production process is deemed the largest obstacle to algal biofuel production and a positive energy balance is a necessity but difficult to achieve. There are now several LCA studies which have investigated the amount of energy consumed in each of the necessary processes and comparing this to the energy recovery potential. Some studies have suggested that a positive energy balance is possible others suggest the contrary. Lardon *et al.* [76] found that if algae was cultivated purely for biodiesel, a positive balance would be unattainable. However if the residual biomass were to be anaerobically digested, a positive balance could be achieved in the scenario of growing algae in low nitrogen media and processing wet biomass [86].

The majority of other life-cycle analyses conducted recently suggest that algal biofuel can be produced with a positive energy balance though possibly not as positive as some alternative biofuels. Clarens *et al.* [87] modelled the growth of algae in raceway ponds and compared the energy consumption and environmental impacts of the fuel produced to fuel from corn, canola and switchgrass. In terms of energy consumption it was found that algal biodiesel required a far higher input (at least four times as much) as the next highest, and a sensitivity analysis revealed that the energy consumption was mainly a result of fertiliser use and carbon dioxide production [88]. Sander and Murthy [94] conducted a LCA comparing the difference between harvesting methods of filter pressing and centrifugation, and a positive energy balance for both methods was reported, with a higher net energy yield for the filter press (almost double that of the centrifuge). The mentioned study did not provide details of the modelled strain nor likely productivity rates; it assumed that the algae contained 30% lipids, which would be difficult to achieve for an outdoor cultured strain; and did consider year round production, which would also be a challenge. In a study by Stephenson *et al.* [95] air-lift tubular photobioreactors and raceway ponds were compared in terms of energy consumption and yield, and their results suggested that the majority of energy was consumed in the cultivation stage (*i.e.*, the cultivation stage in bio-reactors required approximately 10 times more energy than raceway ponds), which is in contrast to previous studies [76,96]. This study has similarities with that of Jorquera *et al.* [55] who showed raceway ponds provided a far greater energy balance than bioreactors. A high energy consumption in the bio-reactors was attributed to the manufacture of the PVC material and circulation of the culture [55]. The majority of energy consumed from cultivation in raceway ponds was due to circulation using a paddlewheel. It was suggested that for raceway cultivation the anaerobic digestion of the residual biomass could offset the energy required from cultivation, however this was not the case for the tubular photo-bioreactors [55].

In a further study conducted by Clarens *et al.* [24], different process chains and how these affect the energy balance or Energy Return on Investment (EROI) were compared, particularly the study looked at the various end-products from the algae (*i.e.*, anaerobic digestion (AD) to electricity, biodiesel and AD to electricity, biodiesel and combustion to electricity and direct combustion) as well as source options for CO₂ (*i.e.*, virgin CO₂, carbon capture, flue gas) and nutrients (wastewater supplementation). In each case direct combustion of the biomass to electricity produced the highest EROI and the best option was direct combustion of the biomass with direct compression of flue gas providing a source of CO₂ [24]. The EROI for this scenario was 4.10, a similar scenario using flue gas and wastewater supplementation provided an EROI of 4.09. According to Clarens *et al.* [24] a value greater than 3 is

considered sustainable, comparing well to canola (2.73), but not to switchgrass (15.90). Table 8 provides a summary of the best energy balances produced through the main studies conducted investigating energy recovery from algae.

Current production of biodiesel from algae without some other form of energy recovery will usually give a negative energy balance. To overcome this, it is necessary to include another form of energy recovery such as anaerobic digestion or combustion. It may even be far more beneficial to ignore biodiesel and to recover energy directly from anaerobic digestion or combustion as their input requirements are significantly lower. Energy reduction measures in each process will further improve the viability of biofuel from algae whatever the process stream used. Further work is required to find the optimal recovery method that can compete with the energy balance of conventional biofuels.

Table 8. A comparison of LCA results of energy balances calculated in algae-biofuel studies.

LCA Study	Energy Balance	LCA Method	Comments	Refs.
Algae-biodiesel	0.95	Well to fuel	Not taking into account wastewater treatment or CO ₂ from flue gas, both of these contributing the most energy use, cultivation in ponds	[96]
Algae-biodiesel	6.7	Well to pump	Co-product allocation provides greatest energy recovery, wastewater assumed to provide nutrients, harvesting greatest energy consumer, cultivation in ponds	[94]
Algae-biodiesel	1.34	Well to fuel	Wet biomass processing and low nitrogen addition for high lipid content, anaerobic digestion of oil cake essential for positive energy balance, cultivation in ponds	[76]
Algae-biodiesel	3.05	Cultivation	Considers just the cultivation stage and energy content of the oil in the biomass, cultivation in ponds	[55]
Algae-bioethanol	5	Well to wheel	80% heat exchange efficiency	[97]
Algae-bioelectricity (combustion)	4.10	Well to wheel	Use of flue gas for CO ₂	[24]

3.4. Fertilisers

To achieve the highest productivity, it is necessary to add a source of nutrients to produce an effective medium. The fertiliser requirements can be calculated using the stoichiometric requirements of the algae. In their LCA, Clarens *et al.* [96] used triangular distributions to calculate minimum, maximum and most likely dosing rates for nitrogen and phosphorous. These dosing rates were found to be just under two times the stoichiometric requirement providing a surplus but with the excess allowing other reactions to remove the surplus. The fertilisers used were assumed to have been sourced from urea and superphosphate. Stephenson *et al.* [95] estimated a nitrogen requirements of 59 kg per ton of biodiesel produced which contrasts significantly with the 6 kg per ton estimated by Lardon *et al.* [76]. Collet *et al.* [86] in their LCA study assumed a nitrogen dosing of 221 kg per day which equates to 5.74 kg per ton of biomass. The study assumed the biomass if processed to biogas so to compare the studies it is possible to calculate the nitrogen requirement for the energy produced. In which case the requirements in the study by Stephenson *et al.* [98] is 1.56 kg N/GJ, for the study by Lardon *et al.* [76]

it is 0.16 kg N/GJ and for the study by Collet *et al.* [86] the value is 0.65 kg/GJ (assuming the energy content of biodiesel and biogas is 37.8 MJ/kg and 0.036 MJ/L respectively). Clearly each study makes different assumption and thus the fertiliser requirement estimates vary, it's most likely that higher dosing is required to provide an abundance of nutrients thus avoiding nutrient limitation.

The major drawback to the use of fertilisers is their energy input, cost and environmental impact. Lardon *et al.* [76] found fertilisers to be one of the major contributors to energy consumption and to the negative energy balance of the whole process system. When the low nitrogen scenario was considered, the energy consumption was far lower. Clarens *et al.* [96] came to similar conclusions as nutrient-derived energy consumption accounted for the greatest energy use in algae production. Similar observation were made by Shirvani *et al.* [99]. Not only do fertilisers negatively affect the energy balance, but they also provide a significant source of environmental impacts to the system. Fertiliser production requires a high energy input from both electricity and fossil fuels, both of which are high emitters of greenhouse gases. Some studies have ignored the impact of fertilisers however results in the study by Clarens *et al.* [96] suggested that using alternative sources of nutrients (wastewater) could in fact uptake CO₂ and return a positive energy balance in the best case (using source-separated urine as a nutrient source).

3.5. Carbon Dioxide

Increased concentrations of carbon dioxide (above atmospheric concentration) have been proven to improve the productivity [46–48] of algal cultivation. Production of synthetic CO₂ however is too energy-intensive to generate and a source of waste carbon dioxide is required. Many studies have proven the advantages of using CO₂ injection combined with algal cultivation [46,48,51,52,100]. As producing CO₂ synthetically is not sustainable, it is necessary for an existing source of CO₂ to be situated near to the algae growth ponds. Researchers have considered the plant flue gas from coal-fired power stations as an ideal source of CO₂ [49,51] and flue gases have been shown to be successful as a source of CO₂. Nevertheless barriers would need to be overcome to implement the concept in a scaled-up system. It is evident from literature that CO₂ concentrations that are too high (above 15%) will cause a decrease in biomass productivity and potentially death of the cells. This may limit the number of possibilities for use of flue gas, although it must be noted that generally flue gases contain CO₂ concentrations lower than this [5]. It is not only the CO₂ that could be lethal to the cells: other toxins may also negatively impact the biomass. SO₂ can have a great impact upon the biomass and the pH of the water and high SO₂ concentration cause the pH to drop to very low levels. pH can be adjusted using NaOH but this requires additional materials and energy. In addition, the temperature of flue gas is generally above that of normal culture conditions and is likely to be too high to allow biomass growth. Cooling would be necessary to reduce the temperature to an acceptable level thus requiring water and additional energy for pumping.

Clearly there are many issues related to the use of waste flue gas as a source of CO₂ that must be addressed to allow implementation on a larger scale. It may be the case that transporting and treating flue gas prior to injection would require too much energy compared to the benefit that could be gained.

3.6. Environmental Impacts

As with any production process, algal biofuel will undoubtedly have an impact on the environment relating to land use, water use, atmospheric emissions and terrestrial/water emissions. One of the key aims of biofuel production is to produce a fuel with fewer environmental impacts than conventional fossil fuels [101]. The intensive processing of the biomass, however, could result in a fuel with greater environmental impacts.

When considering environmental impacts of a product, many factors are taken into account. One of the main impact categories which is considered is the greenhouse gas emissions (GHG) in kg CO₂ equivalent, effectively the benchmark for how “green” a product is. In the best case, a biofuel can have a negative greenhouse gas emission in that during its production more carbon dioxide is taken up than is released during production and use of the fuel. Many studies have been carried out assessing the greenhouse gas emissions of various fuel types from different feedstocks. Recent studies which have investigated the production of algal biofuel have found that, under most circumstances, algal biofuels are likely to have a net positive greenhouse gas emissions [76,95,96]. This is in contrast with many other biofuels produced from conventional first and second generation feedstocks which are produced uptaking more greenhouse gases than are emitted in the process [96,102–104]. A comparison of carbon dioxide emissions from algal biofuel and alternative feedstocks is shown in Table 9. The table shows the CO₂ emissions per MJ of energy recovered as biofuel. The LCA method is included showing at which point the study stopped *i.e.*, at fuel production (well to fuel) or at combustion (well to wheel). The data displayed in table 8 exhibits how poorly algal biofuel currently performs when compared to alternative feedstocks whether they are processed to bioethanol or biodiesel. One of the studies finds algal biodiesel to provide a negative GHG balance [94], nevertheless this is in contrast to the majority [76,95,96]. The different termination points of the study make comparison more difficult as predictably there are GHG emissions associated with the transport and combustion of the fuel.

The majority of greenhouse gases in algal biofuel production are emitted as a result of energy production. Clarens *et al.* [24,96] for example demonstrated that CO₂ procurement demands 40% of total energy consumption and 30% of GHG emissions. Any electricity required will create GHG emissions at the point of generation. In their more recent study Clarens *et al.* [24] compared the greenhouse gas emissions of two scenarios: algal biodiesel with bioelectricity generated from residual biomass and just bioelectricity generated from the biomass. The results were compared with biodiesel and bioelectricity from canola and bioelectricity from switchgrass. The energy from algae scenarios both performed well with direct bioelectricity from algae producing the least GHG emissions. The process stream configuration greatly affects the energy requirements. The greater the number of processes (particularly those including lipid extraction and digestion) required more energy and thus also produced greater greenhouse gas emissions.

GHG emissions may be the most common impact category yet there are many others that also require consideration including eutrophication potential, global warming potential, land use and human toxicity. In their life-cycle analysis Lardon *et al.* [76] investigated the environmental impacts of their algal-biofuel best-case scenario (low N, wet processing) to alternative feedstocks (rapeseed, soybean, palm, diesel). In some areas the algal biofuel performed well (such as in land use and eutrophication) however it did not compare well for the majority of categories, particularly for photochemical

oxidation, ionizing radiation, marine toxicity, acidification and abiotic depletion. In the study published by Clarens *et al.* [96] a fewer number of categories were investigated but the results are similar for eutrophication and land use, both of which are favourable in comparison to corn, canola and switchgrass. Clearly improvements need to be made to minimise the adverse impacts that would be caused by the production and combustion of algal biofuels. These impacts are unlikely to ever be non-existent but it is important that the concept can perform favourably in comparison to alternatives regarding environmental impacts.

Table 9. GHG emissions from various biofuels from different feedstocks.

Feedstock	Biofuel	Cultivation	LCA Method	GHG Emissions (CO _{2e}) kg CO ₂ /MJ	Refs.
Algae	Biodiesel	PBR	Well to wheel	0.32	[95]
Algae	Biodiesel	Raceway pond	Well to fuel	0.057	[96]
			Well to wheel	0.18	[95]
			Well to pump	0.2	[76]
			Well to fuel	−0.021	[94]
Canola	Biodiesel	Agricultural	Well to fuel	−0.05	[96]
Soy bean	Biodiesel	Agricultural		0.030	[102]
Corn	Bioethanol	Agricultural	Well to fuel	−0.082	[96]
Switchgrass	Bioethanol	Agricultural	Well to fuel	−0.076	[96]
			Well to fuel	−0.024	[103]
Poplar	Bioethanol	Agricultural	Well to fuel	−0.024	[103]

4. A Sustainable Vision

To be considered sustainable, as a fuel source, it is essential that the overall process provide a positive energy balance with minimal environmental and social impacts whilst maintaining economic viability. Improving the energy balance is likely to improve the other areas. For example, reducing energy consumption requires less electricity generation which in turn will reduce environmental impacts whilst lessening production cost. If optimal process configurations can be designed for the production of algal biofuels maximising energy yield whilst minimising consumption the concept, could in the future, become a method of producing a sustainable fuel. As a result, many current studies are focussing upon grand scale systems centred around large power plants for CO₂ with potential utilisation of wastewater if available for nutrient provision/water treatment. Although research is heading in the right direction by reducing energy consumption through combining wastewater treatment for nutrient provision and carbon abatement with the use of flue gases, perhaps the future lies in more flexible, localised solutions which are adaptable to unique conditions.

4.1. Integrated and Localised Solutions

In many industrial processes there is often a source of effluent as well as flue gases. Wastewater treatment plants, farms with AD plants, breweries, distilleries and oil refineries all have the potential to offer both materials. The basic requirements of a system to cultivate algae are an area for infrastructure, a source of nutrients (most importantly N and P), a source of concentrated carbon dioxide, freshwater and a consumer for the products obtained. Table 10 displays a number of areas

where algal cultivation may be appropriate and the advantages and disadvantages of such a system. Table 10 shows a variety of potential industries where cultivation of algae could be possible. The majority of these industries provide a wastewater stream with sufficient nutrient loading for the growth of algae. Oil refinery wastewater may have a nutrient concentration too low for optimal growth nevertheless if necessary additional fertiliser could be supplemented. The flue gases found in each of the industries are likely to contain a CO₂ concentration of up to 15% and this value is considered to be near the maximum level that will allow algal growth before it becomes toxic [50]. Each of the flue gases mentioned is likely to boost algal growth whilst sequestering carbon simultaneously.

Depending upon the industry, there are likely to be many problems to overcome. High nutrient loadings (farm effluent, distillery effluent) would lead to poor treatment or toxicity and therefore require dilution with freshwater. To dilute such high concentrations would require significant sources of freshwater possibly needing expensive transportation costs and environmental issues if located in a water stressed area. Wastewaters, particularly those from chemical industries such as oil refining and bioethanol production, could potentially contain toxic contaminants. Similarly flue gases may contain toxins that could affect the growth of the algal biomass. It is evident that there are many different opportunities for the implementation of algal cultivation in industry. Nevertheless it is not possible to have one fixed solution. Every scenario will have different wastewater characteristics, available water and land, varying flue gas characteristics, energy needs and problems related to implementation. Every approach to implementation may be different but the concept allows flexibility.

Table 10. Applicability of various industries for implementation of algal cultivation.

Industry	Total N (mg/L)	Total P (mg/L)	Flue Gas Source	Advantages	Disadvantages
WWTP ^a	15 ^b (NH ₄)	11.5 ^b (PO ₄)	AD co-generator	Provides tertiary treatment Abatement of CO ₂ from co-digester AD of biomass available	Land requirement Contamination of wastewater could affect algae
Farm	1210 ^c 5600 ^d	303 ^c 1600 ^d	AD co-generator Composting facility	Treatment of excess nutrients Treated biomass for feed Available land	Potentially no CO ₂ source High nutrient loading may require dilution
Brewery/ distillery	56.5 ^e (NH ₄) 51 ^f 560–834 ^g (TKN) 3–106 ^h (NH ₃)	177–215 ^e 57–325.8 ^h (PO ₄)	Fermentation process Boiler flue gas	Wastewater treatment Biomass for co-generator produced Sustainability targets	Land area requirement low pH wastewater
Oil refinery	8 ⁱ (NH ₃)	0.1 ⁱ	Flue gases	Abatement of GHGs Sustainability targets	Wastewater/flue gas may be too toxic Low nutrient loading

^a Wastewater treatment plant; ^b Secondarily treated wastewater [25]; ^c Raw dairy manure [29]; ^d Raw swine manure [105];

^e Bioethanol distillery [106]; ^f Distillery stillage [107]; ^g Grape distillery [108]; ^h Brewery wastewater [109]; ⁱ [110].

4.2. Algal Species

Selecting an algal strain because of its beneficial properties is unlikely to be the most successful method of recovering energy from its cultivation. As recent studies have shown, producing high value biofuel from algae may not be the most effective means of energy recovery. Instead it seems that anaerobic digestion or combustion may be more appropriate. Given this situation, it may be more important to utilise strains of algae that are most suited to individual scenarios (wastewater type, climate, *etc.*). It is also likely in many locations with climatic variation in seasons that the species of algae dominating will change as temperatures and amount of sunlight vary. There are examples of such species change in the literature, G. Shelef in his study of algal cultivation in raw wastewater in open ponds found that in Spring *Micratinium* dominated, in Summer *Chlorella* was most common and in autumn and winter *Euglena* became dominant [23].

The alternative to allowing various strains to dominate naturally is to select a strain that is capable of tolerating extreme conditions or recycling the favoured algae. *Spirulina* is a species of algae renowned for good biomass control due to high pH requirements [90,111,112]. Conditions could be manipulated to promote the growth of species such as *Spirulina* by adjusting pH in wastewater streams. In a study conducted by Olguin *et al.* [113] *Spirulina* was cultivated in piggery wastewater and seawater. In the study, continuous cultivation of *Spirulina* was achieved with no issues relating to contamination. Calculations would be necessary to understand whether or not promoting specific strain dominance would be worthwhile from an energy recovery perspective. It may be more productive to simply allow a naturally dominant strain to develop requiring fewer inputs. As studied by Park *et al.* [90], it is also possible to recycle algae improving dominance of selected strains. This may provide a robust method of selectivity and could allow for improved productivity with little input required.

4.3. Cultivation Methods

As discussed, the only potentially sustainable cultivation method currently available is the use of open ponds. This is because of their lower energy requirements compared to PBRs. Open ponds require a far greater area of land for the mass of biomass produced and area requirements need to be considered for individual cases. The necessary area will be dependent upon the volume of wastewater that requires treatment, pond depth, nutrient loading, discharge limits and hydraulic retention times (HRT).

If the focus of algal cultivation is for wastewater treatment the treatment efficiency will have a great impact upon the pond area required. Treatment of water with algae depends upon the productivity of the algae, the higher the productivity the greater the nutrients assimilated. Hydraulic retention times of around 10 days are most common [55,95] and as the HRT increases, the area required increases proportionally. It may be important to minimise the area requirements by reducing cultivation time but nevertheless if the wastewater discharged from the system is above the required limits then the time is likely to be too short. The HRT of each system will depend upon the influent nutrient loading and limits of discharge and therefore must be calculated accordingly.

4.4. Low Energy Harvesting

Harvesting of the algal biomass is one of the greatest energy consumers in the process chain for algal biofuel production. Many options exist for extracting the algae with each having their own advantages and disadvantages. Low energy harvesting is favoured but options are limited by cell sizes. If the algae being harvested are of a large 70 μm [60] the algae can be filtered. Ideally, if the conditions allow, gravity filtration is possible and this requires very little energy input. This would be the optimal solution economically and environmentally due to low energy requirements. Alternatively, should cell size allow, the biomass can be pressure filtered requiring slightly more energy but providing a higher removal efficiency.

In most cases it is likely that flocculation would be required to allow the biomass to settle or float more readily. Conventional flocculants appear favourable in terms of harvesting yield yet may cause issues downstream due to contamination. Ideally if flocculation is necessary, bio-flocculation could be carried out using bacteria or other algae strains to obtain flocs, but further research is required to fully understand in which conditions this is possible. Alternatively organic flocculants such as chitosan could provide a more sustainable option but, again, efficiency of biomass removal using chitosan requires further study. It is likely that if flocculation is necessary, a combination of flocculants would be required for the most sustainable solution. Following flocculation, sedimentation or flotation of the biomass should be a successful method of harvesting without significant energy input.

Centrifugation of algal biomass could be necessary if biomass of high solids content is required. Due to the energy requirement, centrifugation should, where possible, be avoided but due to the high and rapid recovery it could provide a necessary step. The least energy-intense processes for harvesting algae are sedimentation/flotation and gravity filtration as there is little energetic input. Ideally biomass would be filtered or settled using sedimentation due to low inputs however with the majority of strains this may not be practical without prior treatment. Flocculation with the least intensive and damaging flocculants should be used if necessary and centrifugation a last option for dewatering.

4.5. Suggested Conversion Techniques

Following biomass harvesting it is then necessary to extract the maximum energy possible from the biomass to provide the best return. The three main fuel types that have received the majority of research related to algal biomass are biodiesel, biogas and bioethanol. Due to the potentially high oil content of certain strains of algae, the ease of extraction and value of the end product biodiesel has received the most attention. Algal strains such as *Chlorella* are noted for being able to produce up to 70% oil content within their cell walls [14]. This scenario however requires very specific conditions (low nitrogen and no contamination) and would be very difficult to obtain in practice. It has been suggested by Sialve *et al.* [85] that it would not be economically viable to extract lipids from algae containing an oil yield any less than 40%, and therefore for the majority of algal species anaerobic digestion would provide the highest positive energy balance due to low input requirements. Similarly in their environmental study Clarens *et al.* [24] found that direct combustion of biomass to produce electricity provided the highest energy return on investment when compared to anaerobic digestion, biodiesel production plus anaerobic digestion and biodiesel production plus direct combustion.

Given the high energy consumption required to produce biodiesel from algae, the process does not seem beneficial to be used for a flexible system where the algae cultivated are likely to be a mix of species with low lipid content. Bioethanol has potential and in such a system the algae is likely to contain a high proportion of convertible carbohydrates but the energy balance of such a process is untested and is unlikely to yield great efficiencies in the near future and is likely to require high inputs (enzymes and yeast). It seems far more likely that anaerobic digestion or combustion of the biomass will provide the maximum energy recovery. Another benefit of such a concept is that facilities to carry out the digestion or combustion are likely to be already operating on site with no requirement for new development.

4.6. Resource Conservation and Recycling

The proposed concept utilises a variety of waste streams from industry thus saving energy and environmental impacts by avoiding manufacture of raw materials. Furthermore as a method of wastewater treatment, energy and associated impacts will be saved by avoiding alternative methods of treatment. The energy recovered through anaerobic digestion or combustion can be returned to the system, powering the units which require an energy source (paddlewheel or centrifugation). The waste heat can be used to dry the biomass if required or alternatively used to heat the ponds if the temperature falls below optimal conditions. The residual waste from the energy recovery system can be fed back into the treatment ponds supplying additional nutrients if required or alternatively sold as a fertiliser.

4.7. Current State of Concept

It is well known that many strains of algae are capable of growing in wastewater and by doing so providing a form of treatment. Outdoor productivities are, however, difficult to find in the literature as most studies are performed in the laboratory and therefore conditions are less realistic. Likely strains to dominate in specific scenarios and locations are not known and therefore it is hard to speculate what type of strain would be dominant. It is therefore also difficult to know what harvesting technique would be most appropriate for the strain cultivated and how much energy could be expected to be recovered from conversion to biodiesel, bioethanol, biogas or from combustion.

Each of the processes studied have been tested and are considered practically viable. Each of the harvesting methods considered are currently used for harvesting algal biomass regardless of their energy use and overall viability. Conversion techniques have been shown to be feasible, again regardless of how viable they are in real situations. What are missing are pilot-scale studies of the whole system to give valuable information about applicability to different situations.

4.8. Where to Go from Here

Much of the research reported so far is based on laboratory work and speculation while testing a full system would allow realistic life-cycle assessments to be carried out investigating similar systems in a number of industrial scenarios. Data related to energy consumption and yield would prove the viability of the concept. Setting up pilot scale infrastructure within most industries with suitable wastewater

would be a simple undertaking with great research benefits. The ponds would need to be inoculated with a mix of local strains and the dominance of those strains monitored. Suitable harvesting techniques would need to be tested for the algal mix, cultivated within the ponds to identify the most effective and sustainable method for each case. Further research is required to optimize energy recovery from conversion techniques which provide the maximum energy yield, most likely anaerobic digestion or combustion.

5. Conclusions

The review of the current state of knowledge and technology suggests that it is unlikely that there is one solution to biofuel recovery from algal biomass. Production of energy from algae is most likely to be successful on a case by case basis based on applicability to the particular industry and the site under question. The majority of wastewaters from common industries have shown capacity to support the cultivation of various strains of algae. Allowing natural domination of algal strains means that algae which are most effective for that particular situation should develop. If a preferred algal strain is required, the pond could be seeded with the algae and recycled continuously to promote growth.

The biomass processing stages can use existing technologies which are tested for many strains of algae. Harvesting can be optimised for each individual scenario. Optimal recovery of energy for maximum efficiency is likely to be similar for each industry. Literature suggests that recovery through anaerobic digestion or combustion provides the highest energy return for mixed strains. As the strains are likely to be mixed and varying there is little point in designing systems for specialised biofuels (biodiesel or bioethanol) which require specific biomass characteristics. Therefore a system which is flexible for numerous industries is possible. Pilot scale tests of such systems will be essential for implementation to optimise systems individually.

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References

1. Solazyme and algenol to make more algae. *Chem. Eng. News* **2011**, *89*, 20–21.
2. Golueke, C.G.; Oswald, W.J.; Gotaas, H.B. Anaerobic digestion of algae. *Appl. Microbiol.* **1957**, *5*, 47–55.
3. Golueke, C.G.; Oswald, W.J. Power from solar energy-via algae-produced methane. *Sol. Energy* **1963**, *7*, 86–92.
4. Oswald, W.J.; Gotaas, H.B.; Golueke, C.G.; Kellen, W.R. Algae in waste treatment. *Sewage Ind. Wastes* **1957**, *29*, 437–455.
5. Oswald, W.J. My sixty years in applied algology. *J. Appl. Phycol.* **2003**, *15*, 99–106.

6. Saxena, V.K.; Tandon, S.M.; Singh, K.K. Anaerobic-digestions of green filamentous algae and waterhyacinth for methane production. *Natl. Acad. Sci. Lett.* **1984**, *7*, 283–284.
7. Cecchi, F.; Vallini, G.; Pavan, P.; Bassetti, A.; Mataalvarez, J. Management of macroalgae from Venice lagoon through anaerobic co-digestion and co-composting with municipal solid-waste (msw). *Water Sci. Technol.* **1993**, *27*, 159–168.
8. Ras, M.; Lardon, L.; Bruno, S.; Bernet, N.; Steyer, J.P. Experimental study on a coupled process of production and anaerobic digestion of *Chlorella vulgaris*. *Bioresour. Technol.* **2011**, *102*, 200–206.
9. Vijayaraghavan, K.; Hemanathan, K. Biodiesel production from freshwater algae. *Energy Fuel* **2009**, *23*, 5448–5453.
10. Weyer, K.M.; Bush, D.R.; Darzins, A.; Willson, B.D. Theoretical maximum algal oil production. *Bioenergy Res.* **2010**, *3*, 204–213.
11. Rodolfi, L.; Zittelli, G.C.; Bassi, N.; Padovani, G.; Biondi, N.; Bonini, G.; Tredici, M.R. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnol. Bioeng.* **2009**, *102*, 100–112.
12. Lee, J.Y.; Yoo, C.; Jun, S.Y.; Ahn, C.Y.; Oh, H.M. Comparison of several methods for effective lipid extraction from microalgae. *Bioresour. Technol.* **2010**, *101*, S75–S77.
13. Demirbas, A. Production of biodiesel from algae oils. *Energy Source A* **2009**, *31*, 163–168.
14. Chisti, Y. Biodiesel from microalgae. *Biotechnol. Adv.* **2007**, *25*, 294–306.
15. Sheehan, J.; Dunahay, T.; Benemann, J.; Roessler, P. *A Look Back at the U.S. Department of Energy's Aquatic Species Program—Biodiesel from Algae*; Golden: New York, NY, USA, 1998.
16. Chen, M.; Tang, H.Y.; Ma, H.Z.; Holland, T.C.; Ng, K.Y.S.; Salley, S.O. Effects of nutrients on growth and lipid accumulation in the green algae *Dunaliella tertiolecta*. *Bioresour. Technol.* **2011**, *102*, 1649–1655.
17. Sturm, B.S.M.; Peltier, E.; Smith, V.; Denoyelles, F. Controls of microalgal biomass and lipid production in municipal wastewater-fed bioreactors. *Environ. Prog. Sustain. Energy* **2012**, *31*, 10–16.
18. Ruiz-Marin, A.; Mendoza-Espinosa, L.G.; Stephenson, T. Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. *Bioresour. Technol.* **2010**, *101*, 58–64.
19. Ludwig, H.F.; Oswald, W.J.; Gotaas, H.B.; Lynch, V. Algae symbiosis in oxidation ponds. 1. Growth characteristics of *Euglena gracilis* cultured in sewage. *Sewage Ind. Wastes* **1951**, *23*, 1337–1355.
20. Oswald, W.J.; Gotaas, H.B. Photosynthesis in the algae-discussion. *Ind. Eng. Chem.* **1956**, *48*, 1457–1458.
21. Oswald, W.J.; Gotaas, H.B.; Ludwig, H.F.; Lynch, V. Algae symbiosis in oxidation ponds. 2. Growth characteristics of *Chlorella pyrenoidosa* cultured in sewage. *Sewage Ind. Wastes* **1953**, *25*, 26–37.
22. Oswald, W.J.; Gotaas, H.B.; Ludwig, H.F.; Lynch, V. Algae symbiosis in oxidation ponds. 3. Photosynthetic oxygenation. *Sewage Ind. Wastes* **1953**, *25*, 692–705.
23. Shelef, G. *Combined Systems for Algal Wastewater Treatment and Reclamation and Protein Production*; Technion: Haifa, Israel, 1981.

24. Clarens, A.F.; Nassau, H.; Resurreccion, E.P.; White, M.A.; Colosi, L.M. Environmental impacts of algae-derived biodiesel and bioelectricity for transportation. *Environ. Sci. Technol.* **2011**, *45*, 7554–7560.
25. Orpez, R.; Martinex, M.E.; Hodaifa, G.; El Yousfi, F.; Jbari, N.; Sanchez, S. Growth of the microalga *Botryococcus braunii* in secondarily treated sewage. *Desalination* **2009**, *246*, 625–630.
26. Delanoue, J.; Laliberte, G.; Proulx, D. Algae and waste-water. *J. Appl. Phycol.* **1992**, *4*, 247–254.
27. Woertz, I.; Feffer, A.; Lundquist, T.; Nelson, Y. Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. *J. Environ. Eng.* **2009**, *135*, 1115–1122.
28. Patel, A.; Zhu, J.; Nakhla, G. Simultaneous carbon, nitrogen and phosphorous removal from municipal wastewater in a circulating fluidized bed bioreactor. *Chemosphere* **2006**, *65*, 1103–1112.
29. Wilkie, A.C.; Mulbry, W.W. Recovery of dairy manure nutrients by benthic freshwater algae. *Bioresour. Technol.* **2002**, *84*, 81–91.
30. Shalaru, V.M.; Shalaru, V.V.; Dudnicenco, T.I.; Ichim, M.D. The use of algae in wastewater treatment from animal farms. *Phycologia* **2005**, *44*, 93–93.
31. Pizarro, C.; Mulbry, W.; Bliersch, D.; Kangas, P. An economic assessment of algal turf scrubber technology for treatment of dairy manure effluent. *Ecol. Eng.* **2006**, *26*, 321–327.
32. Park, K.Y.; Lim, B.R.; Lee, K. Growth of microalgae in diulted process water of the animal wastewater treatment plant. *Water Sci. Technol.* **2009**, *59*, 2111–2116.
33. Chinnasamy, S.; Bhatnagar, A.; Claxton, R.; Das, K.C. Biomass and bioenergy production potential of microalgae consortium in open and closed bioreactors using untreated carpet industry effluent as growth medium. *Bioresour. Technol.* **2010**, *101*, 6751–6760.
34. Travieso, L.; Benitez, F.; Sanchez, E.; Borja, R.; Leon, M.; Raposo, F.; Rincon, B. Performance of a laboratory-scale microalgae pond for secondary treatment of distillery wastewaters. *Chem. Biochem. Eng. Q.* **2008**, *22*, 467–473.
35. Fierro, S.; del Pilar Sanchez-Saavedra, M.; Copalcua, C. Nitrate and phosphate removal by chitosan immobilized *Scenedesmus*. *Bioresour. Technol.* **2008**, *99*, 1274–1279.
36. Aslan, S.; Kapdan, I.K. Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecol. Eng.* **2006**, *28*, 67–70.
37. Voltolina, D.; Gomez-Villa, H.; Correa, G. Nitrogen removal and recycling by *Scenedesmus obliquus* in semicontinuous cultures using artificial wastewater and a simulated light and temperature cycle. *Bioresour. Technol.* **2005**, *96*, 359–362.
38. Kebede-Westhead, E.; Pizarro, C.; Mulbry, W.W. Treatment of swine manure effluent using freshwater algae: Production, nutrient recovery, and elemental composition of algal biomass at four effluent loading rates. *J. Appl. Phycol.* **2006**, *18*, 41–46.
39. Gonzalez, C.; Marciniak, J.; Villaverde, S.; Garcia-Encina, P.A.; Munoz, R. Microalgae-based processes for the biodegradation of pretreated piggery wastewaters. *Appl. Microbiol. Biotechnol.* **2008**, *80*, 891–898.
40. De Godos, I.; Gonzalez, C.; Becares, E.; Garcia-Encina, P.A.; Munoz, R. Simultaneous nutrients and carbon removal during pretreated swine slurry degradation in a tubular biofilm photobioreactor. *Appl. Microbiol. Biotechnol.* **2009**, *82*, 187–194.

41. Di Termini, I.; Prassone, A.; Cattaneo, C.; Rovatti, M. On the nitrogen and phosphorus removal in algal photobioreactors. *Ecol. Eng.* **2011**, *37*, 976–980.
42. Su, Y.; Mennerich, A.; Urban, B. Municipal wastewater treatment and biomass accumulation with a wastewater-born and settleable algal-bacterial culture. *Water Res.* **2011**, *45*, 3351–3358.
43. Wang, W.; Kondrad, S.; Pizarro, C.; Kebede-Westhead, E. Cultivation of green algae *Chlorella* sp. in different wastwaters from municipal wastewater treatment plant. *Appl. Biochem. Biotechnol.* **2008**, *162*, 1174–1186.
44. Mulbry, W.; Kondrad, S.; Buyer, J. Treatment of dairy and swine manure effluents using freshwater algae: Fatty acid content and composition of algal biomass at different manure loading rates. *J. Appl. Phycol.* **2008**, *20*, 1079–1085.
45. Guine, J.B.; Heijungs, R.; Van Der Voet, E. A greenhouse gas indicator for bioenergy: Some theoretical issues with practical implications. *Int. J. Life Cycle Ass.* **2009**, *14*, 328–339.
46. Chiu, S.Y.; Kao, C.Y.; Chen, C.H.; Kuan, T.C.; Ong, S.C.; Lin, C.S. Reduction of CO₂ by a high density culture of chlorella sp in a siemicontinuous photobioreactor. *Bioresour. Technol.* **2008**, *99*, 3389–3396.
47. Douskova, I.; CDoucha, J.; Livansky, K.; Machat, J.; Novak, P.; Umysova, D.; Zachleder, V.; Vitova, M. Simulataneous flue gas bioremediation and reduction of microalgal biomass production costs. *Appl. Microbiol. Biotechnol.* **2009**, *82*, 179–185.
48. Brune, D.E.; Lundquist, T.; Benemann, J. Microalgal biomass for greenhouse gas reductions: Potential for replacement of fossil fuels and animal feeds. *J. Environ. Eng.* **2009**, *135*, 1136–1144.
49. Maeda, K.; Owada, M.; Kimura, N.; Omata, K.; Karube, I. CO₂ fixation from the flue-gas on cola-fired thermal power-plant by microalgae. *Energy Convers. Manag.* **1995**, *36*, 717–720.
50. Stepan, D.J.; Shockey, R.E.; Moe, T.A.; Dorn, R. *Subtask 2.3—Carbon Dioxide Sequestering Using Microalgal Systems*; U.S. Department of Energy: Pittsburgh, PA, USA, 2002.
51. de Morais, M.G.; Costa, J.A.V. Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. *Energy Convers. Manag.* **2007**, *48*, 2169–2173.
52. De Morais, M.G.; Costa, J.A.V. Biofixation of carbon dioxide by *Spirulina* sp. and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. *J. Biotechnol.* **2007**, *129*, 439–445.
53. Doucha, J.; Straka, F.; Livansky, K. Utilization of flue gas for cultivation of microalgae (*chlorella* sp) in an outdoor open thin-layer photobioreactor. *J. Appl. Phycol.* **2005**, *17*, 403–412.
54. Scragg, A.H.; Illman, A.M.; Carden, A.; Shales, S.W. Growth of microalgae with increased calorific values in a tubular bioreactor. *Biomass Bioenergy* **2022**, *23*, 67–73.
55. Jorquera, O.; Kiperstok, A.; Sales, E.A.; Embirucu, M.; Ghirardi, M.L. Comparative energy lifecycle analyses of microalgal biomass production in open ponds and photobioreactors. *Bioresour. Technol.* **2010**, *101*, 1406–1413.
56. Soratana, K.; Landis, A.E. Evaluating industrial symbiosis and algae cultivation from a life cycle perspective. *Bioresour. Technol.* **2011**, *102*, 6892–6901.
57. Grima, E.M.; Belarbi, E.H.; Fernandez, F.G.A.; Medina, A.R.; Chisti, Y. Recovery of microalgal biomass and metabolites: Process options and economics. *Biotechnol. Adv.* **2003**, *20*, 491–515.

58. Benemann, J.; Koopman, B.; Weissman, J.; Eisenberg, D.; Goebel, R. *Development of Microalgae Harvesting and High-Rate Pond Technologies in California*; Elsevier/North-Holland Biomedical Press: Amsterdam, The Netherlands, 1980.
59. Golueke, C.G.; Oswald, W.J. Harvesting and processing sewage-grown planktonic algae. *Water Pollut. Control Fed.* **1965**, *37*, 471–498.
60. Brennan, L.; Owende, P. Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. *Renew. Sustain. Energy Rev.* **2010**, *14*, 557–577.
61. Uduman, N.; Qi, Y.; Danquah, M.K.; Forde, G.M.; Hoadley, A. Dewatering of microalgal cultures: A major bottleneck to algae-based fuels. *J. Renew. Sustain. Energy* **2010**, *2*, 12701–12716.
62. Mohn, F.M. Experiences and strategies in the recovery of biomass from mass cultures of microalgae. In *Algal Biomass*; Shelef, G., Soeder, C.J., Eds.; Elsevier: Amsterdam, The Netherlands, 1980.
63. Spilling, K.; Seppala, J.; Tamminen, T. A potential low-cost method for harvesting microalgae using high pH and regulation of particle encounter rate. *Water Sci. Technol.* **2009**, *52*, 9–18.
64. Gutzeit, G.; Lorch, D.; Weber, A.; Engels, M.; Neis, U. bioflocculent algal-bacterial biomass improves low-cost wastewater treatment. *Water Sci. Technol.* **2005**, *52*, 9–18.
65. Salim, S.; Bosma, R.; Vermue, M.H.; Wijffels, R.H. Harvesting of microalgae by biofloculations. *J. Appl. Phycol.* **2011**, *23*, 849–855.
66. Sukenik, A.; Bilanovic, D.; Shelef, G. Flocculation of microalgae in brackish and sea waters. *Biomass* **1988**, *15*, 187–199.
67. de Godos, I.; Gonzalez, C.; Becares, E.; Garcia-Encina, P.A.; Munoz, R. Simultaneous nutrients and carbon removal during pretreated swine slurry degradation in a tubular biofilm photobioreactor. *Appl. Microbiol. Biotechnol.* **2009**, *82*, 187–194.
68. Divakaran, R.; Pillai, V.N.S. Flocculation of algae using chitosan. *J. Appl. Phycol.* **2002**, *14*, 419–422.
69. Shelef, G.; Sukenick, A.; Green, M. *Microalgae Harvesting and Processing: A Literature Review*; Report Number: SERI/STR-231-2396; Solar Energy Research Institute, US Department of Energy: Oak Ridge, TN, USA, 1984.
70. Antizar-Ladislao, B.; Turrion-Gomez, J.L. Decentralized energy from waste systems. *Energies* **2010**, *3*, 194–205.
71. Demirbas, A. Microalgae as a feedstock for biodiesel. *Energy Educ. Sci. Tech. A* **2010**, *25*, 31–43.
72. Bligh, E.G.; Dyer, W.J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Phys.* **1959**, *37*, 911–917.
73. Patil, P.D.; Gude, V.G.; Mannarswamy, A.; Deng, S.G.; Cooke, P.; Munson-McGee, S.; Rhodes, I.; Lammers, P.; Nirmalakhandan, N. Optimization of direct conversion of wet algae to biodiesel under supercritical methanol conditions. *Bioresour. Technol.* **2011**, *102*, 118–122.
74. Johnson, M.B.; Wen, Z.Y. Development of an attached microalgal growth system for biofuel production. *Appl. Microbiol. Biotechnol.* **2010**, *85*, 525–534.

75. Wahlen, B.D.; Willis, R.M.; Seefeldt, L.C. Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed cultures. *Bioresour. Technol.* **2011**, *102*, 2724–2730.
76. Lardon, L.; Helias, A.; Sialve, B.; Stayer, F.P.; Bernard, O. Life-cycle assessment of biodiesel production from microalgae. *Environ. Sci. Technol.* **2009**, *43*, 6475–6481.
77. Hirayama, S.; Ueda, R.; Ogushi, Y.; Hirano, A.; Samejima, Y.; Hon-Nami, K.; Kunito, S. Ethanol production from carbon dioxide by fermentative microalgae. *Adv. Chem. Convers. Mitig. Carbon Dioxide* **1998**, *114*, 657–660.
78. Choi, S.P.; Nguyen, M.T.; Sim, S.J. Enzymatic pretreatment of *Chloamydomonas reinhardtii* biomass for ethanol production. *Bioresour. Technol.* **2010**, *101*, 5330–5336.
79. Hirano, A.; Ueda, R.; Hirayama, S.; Ogushi, Y. CO₂ fixation and ethanol production with microalgal photosynthesis and intracellular anaerobic fermentation. *Energy* **1997**, *22*, 137–142.
80. Harun, R.; Danquah, M.K.; Forde, G.M. Microalgal biomass as a fermentation feedstock for bioethanol production. *J. Chem. Technol. Biotechnol.* **2010**, *85*, 199–203.
81. Hromadko, J.; Hromadko, J.; Miler, P.; Honig, V.; Sterba, P. Use of bioethanol in combustion engines. *Chem. Listy* **2011**, *105*, 122–128.
82. Nguyen, M.T.; Choi, S.P.; Lee, J.; Lee, J.H. Hydrothermal acid pretreatment of *Chlamydomonas reinhardtii* biomass for ethanol production. *J. Microbil. Biotechnol.* **2009**, *19*, 161–166.
83. Luo, L.; van Der Voet, E.; Huppes, G. An energy analysis of ethanol from cellulosic feedstock corn stover. *Renew. Sustain. Energy Rev.* **2009**, *27*, 409–416.
84. Luo, L.; van der Voet, E.; Huppes, G. An energy analysis of ethanol from cellulosic feedstock—Corn stover. *Renew. Sustain. Energy Rev.* **2009**, *13*, 2003–2011.
85. Sialve, B.; Bernet, N.; Bernard, O. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnol. Adv.* **2009**, *27*, 409–416.
86. Collet, P.; Helias, A.; Lardon, L.; Ras, M.; Goy, R.A.; Steyer, J.P. Life-cycle assessment of microalgae culture coupled to biogas production. *Bioresour. Technol.* **2011**, *102*, 207–214.
87. Clarens, A.F.; Resurreccion, E.P.; White, M.A.; Colosi, L.M. Environmental life cycle comparison of algae to other bioenergy feedstocks. *Environ. Sci. Technol.* **2010**, *44*, 1813–1819.
88. Mussnug, J.H.; Klassen, V.; Schluter, A.; Kruse, O. Microalgae as a substrate for fermentative biogas production in a combined biorefinery concept. *J. Biotechnol.* **2010**, *150*, 51–56.
89. Fredriksson, H.; Baky, A.; Bernesson, S.; Nordberg, A.; Noren, O.; Hansson, P.A. Use of on-farm produced biofuels on organic farms—Evaluation of energy balances and environmental loads for three possible fuels. *Agric. Syst.* **2006**, *89*, 184–203.
90. Park, J.B.K.; Craggs, R.J.; Shilton, A.N. Recycling algae to improve species control and harvest efficiency from a high rate algal pond. *Water Res.* **2011**, *45*, 6637–6649.
91. Tseng, K.F.; Huang, J.S.; Liao, I.C. Species control of microalgae in an aquaculture pond. *Water Res.* **1991**, *25*, 1431–1437.
92. Williams, P.J.L.; Laurens, L.M.L. Microalgae as biodiesel and biomass feedstocks: Review and analysis of the biochemistry, energetics and economics. *Energy Environ. Sci.* **2010**, *3*, 554–590.
93. Murphy, C.F.; Allen, D.T. Energy-water nexus for mass cultivation of algae. *Environ. Sci. Technol.* **2011**, *45*, 5861–5868.

94. Sander, K.; Murthy, G.S. Life cycle analysis of algae biodiesel. *Int. J. Life Cycle Ass.* **2010**, *15*, 704–714.
95. Stephenson, A.L.; Kazamia, E.; Dennis, J.S.; Howe, C.J.; Scott, S.A.; Smith, A.G. Life-cycle assessment of potential algal biodiesel production in the United Kingdom: A comparison of raceways and air-lift tubular bioreactors. *Energy Fuel* **2010**, *24*, 4062–4077.
96. Clarens, A.F.; Resurreccion, E.P.; White, M.A.; Colosi, L.M. Environmental life cycle comparison of algae to other bioenergy feedstocks. *Environ. Sci. Technol.* **2010**, *44*, 1813–1819.
97. Luo, D.X.; Hu, Z.S.; Choi, D.G.; Thomas, V.M.; Realff, M.J.; Chance, R.R. Life cycle energy and greenhouse gas emissions for an ethanol production process based on blue-green algae. *Environ. Sci. Technol.* **2010**, *44*, 8670–8677.
98. Stephenson, A.L.; Kazamia, E.; Dennis, J.S.; Howe, C.J.; Scott, S.A.; Smith, A.G. Life-cycle assessment of potential algal biodiesel production in the United Kingdom: A comparison of raceways and air-lift tubular bioreactors. *Energy Fuel* **2010**, *24*, 4062–4077.
99. Shirvani, T.; Yan, X.Y.; Inderwildi, O.R.; Edwards, P.P.; King, D.A. Life cycle energy and greenhouse analysis for algae-derived biodiesel. *Energy Environ. Sci.* **2011**, *4*, 3773–3778.
100. Packer, M. Algal capture of carbon dioxide, biomass generation as a tool for greenhouse gas mitigation with reference to New Zealand energy strategy and policy. *Energy Policy* **2009**, *37*, 3428–3437.
101. Antizar-Ladislao, B.; Turrión-Gómez, J.L. Second-generation biofuels and local bioenergy systems. *Biofuel Bioprod. Biorefin.* **2008**, *2*, 455–469.
102. Hu, Z.Y.; Tana, P.Q.; Yan, X.Y.; Low, D.M. Life cycle energy, environment and economic assessment of soybean-based biodiesel as an alternative automotive fuel in China. *Energy* **2008**, *33*, 1654–1658.
103. Adler, P.R.; Del Grosso, S.J.; Parton, W.J. Life cycle assessment of net greenhouse gas flux for bioenergy cropping systems. *Ecol. Appl.* **2007**, *17*, 675–691.
104. Reinders, L.; Huijbregts, M.A.J. Life cycle greenhouse gas emissions, fossil fuel demand and solar energy conversion efficiency in European bioethanol production for automotive purposes. *J. Clean. Prod.* **2007**, *15*, 1806–1812.
105. Karakashev, D.; Schmidt, J.E.; Angelidaki, I. Innovative process scheme for removal of organic matter, phosphorus and nitrogen from pig manure. *Water Res.* **2008**, *42*, 4083–4090.
106. Qiu, C.S.; Jia, X.Q.; Wen, J.P. Purification of high strength wastewater originating from bioethanol production with simultaneous biogas production. *World J. Microbiol. Biotechnol.* **2011**, *27*, 2711–2722.
107. Douskova, I.; Kastanek, F.; Maleterova, Y.; Kastanek, P.; Doucha, J.; Zachleder, V. Utilization of distillery stillage for energy generation and concurrent production of valuable microalgal biomass in the sequence: Biogas-cogeneration-microalgae-products. *Energy Convers. Manag.* **2010**, *51*, 606–611.
108. Musee, N.; Trerise, M.A.; Lorenzen, L. Post-treatment of distillery wastewater after UASB using aerobic techniques. *South Afr. J. Enol. Vitic.* **2007**, *28*, 50–55.
109. Raposo, M.F.D.; Oliveira, S.E.; Castro, P.M.; Bandarra, N.M.; Morais, R.M. On the utilization of microalgae for brewery effluent treatment and possible application of the produced biomass. *J. Inst. Brew.* **2010**, *116*, 285–292.

110. Moreno, C.; Farhbakhshazad, N.; Morrison, G.M. Ammonia removal from oil refinery effluent in vertical upflow macrophyte column systems. *Water Air Soil Pollut.* **2002**, *135*, 237–247.
111. Kim, C.J.; Jung, Y.H.; Ko, S.R.; Kim, H.I.; Park, Y.H.; Oh, H.M. Raceway cultivation of *Spirulina platensis* using underground water. *J. Microbiol. Biotechnol.* **2007**, *17*, 853–857.
112. Celeckli, A.; Yavuzatmac, M.; Bozkurt, H. Modeling of biomass production by *Spirulina platensis* as function of phosphate concentrations and pH regimes. *Bioresour. Technol.* **2009**, *100*, 6325–6329.
113. Olguin, E.J.; Galicia, S.; Mercado, G.; Perez, T. Annual productivity of *Spirulina* (*Arthrospira*) and nutrient removal in a pig wastewater recycling process under tropical conditions. *J. Appl. Phycol.* **2003**, *15*, 249–257.

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