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Biodiesel from microalgae: A critical evaluation from laboratory to large scale production

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ABSTRACT

The economically significant production of carbon-neutral biodiesel from microalgae has been hailed as the ultimate alternative to depleting resources of petro-diesel due to its high cellular concentration of lipids, resources and economic sustainability and overall potential advantages over other sources of biofuels. Pertinent questions however need to be answered on the commercial viability of large scale production of biodiesel from microalgae. Vital steps need to be critically analysed at each stage. Isolation of microalgae should be based on the question of whether marine or freshwater microalgae, cultures from collections or indigenous wild types are best suited for large scale production. Furthermore, the determination of initial sampling points play a pivotal role in the determination of strain selection as well as strain viability. The screening process should identify, purify and select lipid producing strains. Are natural strains or stressed strains higher in lipid productivity? The synergistic interactions that occur naturally between algae and other microorganisms cannot be ignored. A lot of literature is available on the downstream processing of microalgae but a few reports are available on the upstream processing of microalgae for biomass and lipid production for biodiesel production. We present in this review an empirical and critical analysis on the potential of translating research findings from laboratory scale trials to full scale application. The move from laboratory to large scale microalgal cultivation requires careful planning. It is imperative to do extensive pre-pilot demonstration trials and formulate a suitable trajectory for possible data extrapolation for large scale experimental designs. The pros and cons of the two widely used methods for growing microalgae by photobioreactors or open raceway ponds are discussed in detail. In addition, current methods for biomass harvesting and lipid extraction are critically evaluated. This would be novel approach to economical biodiesel production from microalgae in the near future. Globally, microalgae are largest biomass producers having higher neutral lipid content outcompeting terrestrial plants for biofuel production. However, the viscosities of microalgal oils are usually higher than that of petroleum diesel.

Contents

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1. Introduction

Currently one fifth of the global CO_2 emissions are from the transport sector. The outlook for reduction of emissions from this sector does not look promising as the number of light motor vehicles on the roads globally is estimated to increase to over 2 billion vehicles by 2050 [1]. Due to diminishing supply, crude oil will continue to rise in cost thereby making production of fuels from alternate sources more feasible. Further climate change will be accentuated by the continued use of fossil fuels due to release of environmentally unfriendly gases such as CO_2. Biodiesel is produced from microalgal oil, thus crude fossil petroleum can be substituted by mass cultured biomass's microalgal oil for eco-sustainable biodiesel production in the near future. Biodiesel is a monoalkyl ester produced by the transesterification of triglycerides or free fatty acids with short chain alcohols and has the ability to be used in conventional diesel engines with little or no modification [2]. Biodiesel has been experimentally shown to be less eco-toxic than petro-diesel. In studies conducted by Lapinskiene and co-workers [3], it was shown that diesel fuel at concentrations greater than 3% (w/w) is toxic to soil microorganisms. Biodiesel however is non-toxic at total soil saturation. Biodiesel contributes no net carbon dioxide or sulphur and overall less gaseous pollutants to the atmosphere than petro-diesel [4–6]. With growing concern for the environment, these factors play an important role in the acceptability of biodiesel. First generation biofuels have been around for over a century [7]. Biodiesel is currently produced in the United States from soybeans [8]. Sources of commercial biodiesel include canola oil, animal fat, palm oil, corn oil, waste cooking oil, and jatropha oil. The use of plant oils for fuel production is however highly controversial and requires resources such as arable land which may not be available in large enough quantities to meet fuel requirements of a designated area and will greatly affect food security [9]. Currently 1% of the arable land available globally is used for crop based (1st generation) biofuels. This fuel is sufficient only to meet 1% of the global requirement [10]. Research into production from alternate sources has resulted in biodiesel from microalgae being hailed as the most appropriate petro-diesel alternative [8].

1.1. Microalgae

Microalgae are the largest autotrophic microorganisms of plant life taxa in the world. The biomass produces three major biochemical components by *de novo* synthesis consisting of carbohydrates,

proteins and lipids (natural oils). Microalgae are known to synthesise and can rapidly accumulate substantially higher amounts of lipids than terrestrial plants due to their high growth rates [11], concomitantly by alteration of the lipid biosynthetic pathways for storage as neutral lipids. The lipid yields and growth rates vary significantly among different species [4,8]. Microalgal oil and spent biomass are offered good potential sources as biodiesel feedstocks. Microalgal lipids contain twice the energy stored per carbon atoms than carbohydrates, which translates directly into a twofold increase in fuel energy content thereby outcompeting terrestrial plants for biofuel production. Lipid rich microalgae biomass feedstock have been the focus of attention for the scientific community for an extended period of time, for production of alternate sustainable energy to resolve the world energy fossil fuel depletion and its harmful effect on the environment i.e. increasing global warming [8,12].

1.2. Crops vs. microalgae as biodiesel feedstocks

More than 95% of biodiesel sources are first generation agricultural edible crop oils. First generation biofuels have a great impact on food security and have the potential to increase the cost of food crops such as soybean thus also making biodiesel production more expensive [13]. Second generation biofuels such as jatropha oil, waste cooking oil and animal fats do not affect food security and have significant advantages over first generation oil crops. However sustainability of second generation biofuels is not favourable. Moreover production of crop derived biofuels gives rise to challenges such as poor cold flow properties and saturated fatty acids contained in animal fats give rise to production difficulties and may constitute a bio-safety hazard due to their solid nature at room temperature [14]. Available alternative, in terms of social and economic acceptability and greater energy security, microalgal oil is regarded as third generation biofuels source. Algal productivities can be twenty times that of oilseed crops on a per hectare basis and is thus a more viable alternative [8,14–16]. Microalgae have faster growth rates than plants and are capable of growth in highly saline waters which are unsuitable for agriculture. They utilise a large fraction of solar energy making them effective solar to chemical energy converters [21]. Microalgae have greater photosynthetic efficiency than terrestrial plants and require very little simple nutrients supply for growth [17]. The lipid content of microalgae, on a dry cellular weight basis generally varies between 20% and 40%, however lipid contents as high as 85% have been reported for certain microalgal strains [18–20]. Microalgae have the potential to produce 25–220 times higher triglycerides than terrestrial plants [14,21] and can be readily converted to biodiesel by the transesterification process [19,22]. As compared to biomass from trees and crops, microalgal oil is more economical in that transportation costs are relatively low [14]. Microalgae offer significant higher yield advantage over sunflower as potential feed stock for biodiesel production.

1.2.1. Limitations of crop based biodiesel

First generation crop based biodiesel, such as rapeseed, soybean, palm and sunflower oils is severely limited in that the competition brought about by its use will result in imbalance in the global food market and thus increased cost of the edible oil as well as the biodiesel [14]. Deforestation has become a concern in certain countries as more forest land is re-designated for cultivation of commercial crops for biofuel production. Second generation biofuels, such as jatropha, mahua, jojoba oil, tobacco seed, salmon oil and sea mango are thought to be a suitable replacement to first generation biofuels in that they are non-edible and can be grown in wastelands that are not suitable for crop cultivation and can sustain reasonably high yields without the management required for production of food crops. Second generation biofuels however contain high amounts of free fatty acids which increase the energy demand of process and production costs as they require additional production steps and may reduce the overall quality of the biodiesel produced [23].

The major limitation associated with the use of second generation biofuels is the issue of sustainability. For production of biodiesel from jatropha to completely satisfy the diesel requirements of the UK (estimated 25 billion litres in 2008), half the land area of UK (17.5 Million hectares) would be required. There is also some debate as to whether a positive energy balance is attained by the use of crop based biodiesel. The total energy input for the life cycle of rapeseed and soybean is estimated to be half the total energy of the fuel [24].

Palm oil, due to its high productivity, represents one of the major raw materials used for the production of biodiesel and is ranked as the third largest biodiesel feedstock providing the source for 10% of the biodiesel produced worldwide [14]. A major challenge associated with palm oil biodiesel is poor cold flow properties. This may however be overcome by blending and the use of triglycerides are able to improve the cold flow and cloud point properties [25]. Production of palm oil in Malaysia has resulted in the clearing of vast areas of natural forest. This has indirectly increased the areas vulnerability to devastating fires and cause irreparable damage to the areas biodiversity and ecosystems. Further the over production of palm oil has resulted in production of millions of tons of solid waste and palm mill effluent that have been shown to negatively impact terrestrial as well as aquatic environments [14].

A further criticism levelled against the use of first and second generation biofuels is that the greenhouse gas balances may be affected due to indirect land use not being taken into account. This may result in zero net greenhouse gas benefit to the use of these fuels as compared to petro-diesel. Production of biodiesel from palm oil grown on dried peat marsh could be responsible for increase in greenhouse gas emissions [7].

1.2.2. Advantages of microalgae

Microalgae have very short harvesting life that are capable to allowing multiple and continuous harvesting of biomass year round unlike oilseed crops [8,26]. They have higher solar energy yields thereby giving superior lipid productivity. Lipids produced are generally neutral lipids that have a high level of saturation making it a suitable feedstock for biodiesel production. Microalgae have rapid growth rates and short generation times. Microalgae are commonly known to double their weight with respect to biomass within 24 h. Some species have doubling times as short as 3.5 h i.e. multiple biomass divisions per day. This high productivity imparts the potential for the modern high theoretical yield production of the yield of 47000–308000 L ha^{-1} $ammun^{-1}$ [27]. Comparatively palm oil has the ability to produce 5950 l of biodiesel per hectare per year [14].

Microalgae require less freshwater for cultivation than terrestrial plants. Microalgal cultivation can occur on non-arable land, in brackish water thus reducing strain on resources required for the production of food crops whilst reducing other environmental effects. There is no need for use of chemicals such as herbicides or pesticides thus reducing costs and environmental impacts [28]. In addition, these microorganisms require significantly less land, estimated 2% of the land required to produce the same amount of biodiesel from oil bearing crops [14]. The rapid growth rate of microalgae and high lipid storage capacity, far outcompete terrestrial crops [10]. Growth of microalgae can effectively removed phosphates and nitrates from wastewater, thus making it an ideal substrate for the cultivation of microalgae for biofuels production whilst acting as a tertiary treatment for wastewater. Some microalgae produce valuable by-products in the form of high value prod-

ucts in the form of proteins, pigments, biopolymers and carbohydrates such as docosahexanoic acid and carotenoids including antioxidant substances for commercial or pharmaceutical purpose [10,14,28,29].

The cost associated with harvesting and transportation is relatively low as compared to that of oil crops. Residual biomass post extraction offers methods for improving economics by using it as a fertilizer or for producing other high energy products [14]. Microalgal biodiesel is environmentally advantageous in that it is a carbon neutral fuel due to the photosynthetic fixation of atmospheric carbon dioxide. Microalgal growth actively utilises 1.83 kg of CO_2 for every 1 kg dry biomass produced [8]. Microalgal biodiesel has properties similar to those of petro-diesel. These include density, viscosity, flash point, cold flow and heating value. None of the other potential sources of biodiesel are realistic in terms of replacing petrodiesel sustainability as microalgae do. This is mainly due to the environmental impacts that occur as a result of use of other feedstocks [14]. Pyrolysis as an alternative method of bio-oil production is more cost effective for microalgae biomass as a feedstock than lignocellulosic biomass. This is due to microalgae containing higher amounts of cellular lipid, polysaccharide and protein which are more easily pyrolysed and result in higher quality bio-oil production [30].

1.2.3. Challenges of using microalgae

Harvesting of microalgae is seen as one of the major challenges of using microalgae for the production of biodiesel. Microalgae that store lipids are generally unicellular, have low densities and are found in suspension making separation difficult. Large scale extraction procedures for microalgal lipids are complex and still in the developmental stage [28] (Refer to Section 4.4). Currently research is underway to alleviate these challenges.

Microalgae grown in open pond systems are prone to contamination. Bacterial contamination actively competes for nutrients and oxidise organic matter that could lead to putrification of the culture. Control of heterotrophic bacteria may be achieved by increase in pH. Aerobic bacteria generally found in algal ponding systems have an optimum pH of 8.3. Increase in pH beyond this level gives effective inhibition thus preventing competition by influencing nitrogen efficiency [31,32]. Open systems are also susceptible to grazers in the form of protozoa and zooplankton. These organisms actively consume microalgae and can devastate algal concentration in relatively short periods of time (2–3 days). Zooplankton can reduce microalgal concentration by up to 90% of the original density in 48 h [33] and *Daphnia* can lower microalgal density by a massive 99% over a few days [32]. Several methods to control these organisms are available including filtration, centrifugation, low dissolved oxygen (DO), application of hormones and increase in free ammonia. These methods however have drawbacks in that filtration is difficult due to the size of microalgal species such as *Chlorella* sp. making separation technically difficult. Centrifugation is prohibitively expensive at large scale requiring high capital and energy inputs. Photosynthetic microalgae produce oxygen thus actively increasing the DO as a function of growth.

Increase in free ammonia as a control method may be achieved by pH elevation by volatilisation of ammonia. It has been eluded that the toxicity of high pH may actually be due to increased free ammonia levels that are brought about by the volatilisation of ammonia at high pH [34]. Thus the most appropriate method of controlling zooplankton and bacterial populations is to increase pH to 11 [32]. The range of optimal pH for algae varies with species. The optimal level of growth for many freshwater microalgae is close to 8 and deviation from this level subsequently leads to reduction in biomass [35]. Microalgae such as *Amphora* sp. and *Ankistrodesmus* sp. have been shown to grow uninhibited at pH 9 and 10 respectively [32]. pH exceeding 11 is reported to occur in high rate algal pond systems due to consumption of carbon dioxide and carbonic acid by the process of photosynthesis [31]. Microalgae are estimated to require 6–8 tons of nitrates per hectare per year, 55–111 times the requirement of field crops [36]. The associated cost may be readily alleviated by the use of wastewater as a growth substrate [28].

Other challenges with respect to the use of biodiesel as fuel is that it is susceptible to bacterial oxidation subsequently causing internal corrosion of the storage tanks [15]. Production of microalgal biodiesel can be an energy intensive process. Large amounts of glycerol are produced as a by-product and will likely flood the market thus driving prices down. Methanol used in the transesterification process is currently derived from crude sources. These challenges can be overcome by implementation of measures such as state of the art design of the biodiesel storage tanks. High energy input may be overcome by use of the bio-refinery concept, whereby biomass is converted to energy resulting in a waste-less process [28]. Glycerol produced in large quantities could be used to make higher value products or to benefit the community in the form of soap and candles in 3rd world countries. An effective use of glycerol is as a fermentation stock to produce methane as part of the bio-refinery concept. Methanol used for transesterification is currently derived from crude sources and biodiesel has the potential to be a 100% biological fuel in the future [15].

1.3. Technology for biodiesel production from plants and microalgae

Biodiesel feedstocks differ from petro-diesel in that they are highly viscous and thus not suitable for direct use in modern diesel engines and thus require conversion to meet regulatory standards. The technology for conversion of vegetable oils, animal fats and microalgal oil are primarily (1) direct use, (2) blending with petro-diesel, (3) micro-emulsions with solvents or alcohols, (4) pyrolysis, and (5) transesterification [37].

Converting biological lipid into biodiesel using transesterification is most commonly used feasible methods for many years. This was done in South Africa and other countries using converted vegetable oil (biodiesel) to power heavy vehicles before World War II [38] and has been received considerable attention in the past few decades. However the well established chemical catalysed transesterification (alcoholysis) process of biofuel production technology does have its demerits. Overall the process is highly energy intensive thus increasing in terms of production cost, the catalyst needs to be removed from the production by purification or separation steps, alkaline water from washing may cause difficulties such as saponification, water and free fatty acids result in loss of conversion yield product due to saponification because the operation stability is very sensitive and glycerol is technically difficult to remove in downstream recovery and may require easier process steps to simplify product recovery [2,39]. Biocatalysed/Enzymatic catalysed esterification is a viable process and effective method for catalysing parent oils containing high levels of free fatty acids as they can be converted to alkyl esters without presence of organic solvents. Other benefits include utilisation of moderate reaction conditions thus decrease energy consumption. More desirable enzyme activities involves improved alcohol to oil ratio requirements for production and makes product recovery much easier [28,39]. However, for large scale continuous higher volume biodiesel production, this may have challenges which need to be addresses to make it economically viable. This is not an ideal energy balance situation as the reaction proceeds using competitive high enzyme production rates, and does not run the reaction to completeness [15,39]. High-tech thermal conversion processes, maximize energy conversion of biomass into superior biofuels, has come to the vanguard as a promising neutral carbon energy processes for reshaping liquid fuels production. This contributes dramatically to

biofuels production as an essential alternative sustainable, renewable, environmentally friendly process, that is no threat to food security and has economic potential due to diminishing levels of fossil fuels [23,28]. Much data has been generated at laboratory scale as an academic exercise but not much has been published in way of technology transfer to large scale.

1.4. Evaluation of technology for scale up

The technology for large scale production of microalgae already exists. Open pond systems are easily scaled up. Two 1000 m^2 raceway ponds were used as a test facility between 1987 and 1990 in New Mexico. This was used as proof of concept for production of microalgal biomass for low cost biodiesel and was deemed technically feasible [12]. Globally, current commercial production however is for high value products only. These ventures have well established harvesting and processing methods but energy input is not of major concern due to the nature of the high value products [24]. The approach to large scale production of low value products such as lipids for biodiesel production must however be approached differently in order to make the process feasible. Scale up of microalgal biomass production for biodiesel must take into account various factors to aid in ensuring an economically feasible process. These include selection of cultivation method (open or closed system), the use of microalgae with a competitive advantage to avoid contamination, supply of nutrients and carbon dioxide and a viable source of water that has little to no environmental impact [24]. In addition, nutrients should be obtained from a cheap source e.g. using urea as a source of nitrogen or wastewater as a complete medium [28].

Harvesting at large scale poses major challenges. Harvesting of 25–33% of the reactor volume may be required daily for viable production of biodiesel [40]. Aquatic unicellular microalgae can be harvested throughout year. They can be used for biotransformation of wastewater as wastewater nutrients are utilised as a substrate for their growth. Thus integrated phycoremediation and biofuel technology appears to be the only source of sustainable production of biofuels. Greater microalgal biomass production effectively decreases the cost of harvesting/dewatering steps and the high cost of biomass recovery [8,10,28,32,41,42]. The most rapid and effective universally accepted method for total biomass separation is by continuous centrifugation [43,44]. However, for large scale biomass harvesting in the biofuels arena, this is generally not practiced due to process being energy intensive and not typically economically feasible [42]. Gravity sedimentation is preferred due to low cost; however the efficacy of gravity sedimentation is strongly influenced by the density and radius of algal cells [10]. Flocculation is used to enhance the settling characteristics by increasing particle density of culture that may be unsuccessfully separated due to low particle density [43]. The process may be enhanced by the use of lamella separators and sedimentation tanks. Ancient technique filtration is another commonly used method. Upgrading the two basic techniques of sedimentation and filtration may be used in combination for dewatering with flocculation. Vacuum filtration is effective for the recovery of larger algae (greater than 70 μm) when operated under required pressure combination with a filter aid. This has lead to more compact solid biomass harvesting. Microalgae of size greater than 30 μm have to be found to be effectively harvested by filtration. The potential use of membrane microfiltration or ultra-filtration is reliable for cells of smaller size [10]. This may be impacted by replacement of low-cost membrane due to rapid fouling membrane and pumping of the biomass without back flushing. Microalgal biomass harvesting is one of the major steps in upstream processing. The end results of many methods tend to be highly energy intensive and more complex [42]. Drying or dewatering of biomass is generally required as a pre-treatment prior to lipid extraction or use in various thermo-chemical conversion techniques. Moisture in the biomass will negatively interfere with the downstream processing and greatly influence the cost of product recovery [45,46]. Furthermore the biomass can spoil in a matter of hours post harvest should it not be rapidly processed. Drying may be achieved by spray drying, drum drying, freeze-drying, solar drying, as well as various forms of oven drying [12,45].

Microalgal lipid is generally extracted from biomass before conversion to biodiesel. This may be achieved by mechanical methods such as cell homogenizers, bead mills, ultrasounds, autoclave, and spray drying or non-mechanical methods such as freezing, utilisation of organic solvents, osmotic shock, acid and base as well as enzyme reactions [12]. The method of choice of lipid extraction will depend on the type of microalgal cells grown and the thickness of the cell walls impeding liberation of intracellular lipids [40]. Many of these methods are not feasible at large scale due to high energy input requirements. The most appropriate method of lipid extraction to date is the use of solvents. The inherent disadvantage of this method is that solvent extraction may deem the residual biomass unfit for use as animal feed.

Microalgal lipids require conversion to biofuels to be effectively used. The process of biodiesel production is commonly achieved by transesterification with short chain alcohols whilst mediated by acid, base or enzyme catalysis. High levels of free fatty acids in the lipid feedstock undergoing base catalysed transesterification results in losses by the formation of soap. Saponification is responsible for consumption of the base catalyst as well as making downstream recovery difficult [19] containing high levels of free fatty acids [15]. The reaction however is slow. Speeding up the acid catalysed reaction requires an increase in temperature and pressure making it prohibitively expensive at large scale [44]. Enzyme mediated catalysis is generally not suitable for large scale conversion due to high enzyme production cost and the inability of the reaction to run to completeness [28]. Selective separation and detection methods that are versatile in the downstream process of biofuels production can be achieved using both wet and dry microalgal biomass containing long chain fatty acids via transesterification. This could be performed by both direct esterification and simultaneous extraction with esterification. Biomass derived liquid extraction involves a multistep process and requires a combination of polar to non-polar solvents for rapid lipid extraction, ultra-sonication with ambient temperature, heating at high pressure (3.5 atm), filtration for separating impurities, density separation of liquids and solvent and oil recovery by gradual evaporation to dryness are highly employed [47]. Esterification processes are energy intensive and thus may be limiting due to high cost. The high viscosity of the resultant biodiesel is problematic if used directly in conventional diesel engines.

For the viable production of microalgal biodiesel significant breakthroughs are required in the processes of dewatering and lipid extraction [40]. Thermo-chemical conversion such as direct burning, pyrolysis and liquefaction has been suggested as a viable alternative to biofuels production via the production of bio-oil and alternate energy resources such as syngas [24]. Catalytic pyrolysis has potential to produce fuel with high hydrocarbon content and octane number [30].

2. Upstream processing

2.1. Isolation

Bioprospecting is a vital step in the collection of microalgal strains and the identification of potential hyper-lipid producers [14]. The selection of indigenous microalgae is crucial to the success

of large scale cultivation of microalgae especially for the production of biofuels. These organisms are specific to certain environments and prevailing climatic conditions [6,10,44]. The diversity of microalgae is a key point to the success of secondary metabolites (producing biodiesel) from microalgae in that there are reported to be between 1 and 10 million (upper limit) of algal species in nature [48]. These species, especially microalgae occur in diverse aquatic environments including freshwater, lacustrine, brackish, marine, maturation ponds and hyper-saline. A critical review of all the bioprospecting protocols has been published [44]. In addition, researchers can bioprospect and collect indigenous hyper-lipid producing microalgal strains and set up a microalgal culture bank at their research institutes. Alternatively it is also recommended to buy suitable lipid producing starter microalgal cultures from reputable and specialized culture collections such as UTEX (USA), ANACC (Australia), CCAP (UK), NIES (Japan), SAG (Germany), CPCC (Canada), etc. However the rule of thumb is that researchers should cultivate microalgal strains that adapt to their local environmental conditions. Therefore the use of endemic indigenous microalgal strains is highly recommended since these strains are already adapted to the local environmental conditions [44,49].

2.1.1. Strains from culture collections vs. indigenous strains

Culture collections contain strains from a variety of aquatic environments. These strains are acclimatised to the environment of isolation at the time of isolation. As cultures age due to continual sub-culturing to ensure viability, they become adapted to the environment of cultivation and may lose characteristics such as competitive advantages that are of major benefit to open pond culturing systems. These microalgae will generally have longer acclimatisation periods to climatic conditions. Cultures may also lose lipid productivity if stored continually under non-stressed condition. Adaptability of culture collection strains may also be impeded. The benefit of use of strains from culture collections is that much of the characterisation has been done with regard to growth requirements. Indigenous strains have an inherent adaptability that may be the competitive edge required for open pond systems. They generally are able to adapt quickly to changes in environment and climate. Indigenous strains however require isolation, establishment of optimal culturing conditions and determination of feasibility of production by experimental techniques. This is time consuming, labour intensive and is more costly than strain selection from culture collections. The benefit of adaptability however may outweigh the drawbacks in the longer term [50].

2.1.2. Selection of aquatic environments

Selection of environments similar in climatic conditions to area in which cultivation is proposed is beneficial in that the strain finally selected for cultivation becomes easily acclimatised to cultural conditions. This will aid in the ease of culturing at large scale due to shorter acclimatisation period. Understanding of environmental conditions allows mimicking of those environments to encourage growth. The simplest way of accomplishing this is measurement of abiotic factors such pH, temperature, etc., nutrient levels and other physic-chemical properties at the sampling point. It is prudent to collect the water from the microalgal sampling point and filter sterilise it and use this for media formulation in the laboratory. The use of culture enrichment for microalgal growth before purification is a particularly useful tool for isolation. Some knowledge of the taxonomic group being targeted is also useful to encourage *in vitro* growth. Addition of essential nutrients should occur as soon as possible after sample collection. This will help prevent culture death that may occur due to change in growth conditions. Collection of samples during warmer months has been shown to be more adaptable to change in temperature [51]. Contaminants in the form of bacteria, algal grazers and undesirable cultures may be eliminated by a combination of one or more techniques. Filtering of samples at collection with a 100 μm sieve is useful for the removal of debris and some zooplankton [44]. Serial dilution, streaking on agar plates and the use of single cell isolation techniques are extremely useful for elimination of contaminants. Continued sub-culturing allows determination of supply of essential nutrients. Culture death after a number of sub-cultures indicates the lack of an essential nutrient or the accumulation of metabolic wastes in the culturing environment [51].

2.2. Screening of microalgae for lipid production

Screening for oil producing microalgae among the isolates is a vital part in the optimisation of biodiesel production. Certain strains of microalgae such as *Botryococcus braunii* have high lipid storage potential (up to 75% lipid/g dry cell weight) but this is accompanied by low productivity. Cultures with moderate lipid accumulation levels (20–50%) but higher lipid productivities are preferred for mass cultivation [12,52]. Factors other than lipid productivity such as lipid profile need to be taken into account such as the ability to grow under specific environmental conditions. Lipid profiles determine the suitability of lipid for the production of biodiesel. Lipid profiles are affected by nutritional, processing and cultivation conditions. Selection criterion should be based on a number of factors including growth rate, lipid quantity and quality, strong adaptability to changes in environment and determination of preferred nutrients and nutrient uptake rates [52]. In order to gain high oil yields it is necessary to ascertain the amount of oil, if any, produced under normal conditions. This is likely to allow maximum overproduction under stress. Yield optimisation is very important from the economies of scale point of view.

The viability of a species of microalgae for use in the production of biodiesel is often dependent on the yield. Healthy, actively dividing cells usually have a low percentage of TAG. TAG may be found in elevated proportions, in some algal species during stationary phase. Stationary phase may be induced by limiting one or more variables that control growth, e.g. nitrogen, phosphorus or silicon limitation [53]. Adaptation of microalgae to environmental change is generally as a result of change in lipid patterns and synthesis of various unusual compounds [54]. This is likely the reason that microalgae overproduce fatty acids when subjected to stressful conditions. It is thus possible to induce or enhance lipid content by nitrogen starvation or other stress factors. Nutrient deficiencies such as silicon depletion may lead to an increase in cellular lipid content. A Lipid content increase of 60% is possible by silicon starvation of the diatom *Navioua pelliculosa* [55]. Lipid fractions as high as 70–85% on a dry weight basis have been reported. Such high lipid contents exceed that of most terrestrial plants [55].

Certain diatoms have the ability to rapidly switch from carbohydrate accumulation to lipid accumulation via mechanisms that are not yet understood. The application of diatoms for production of microalgal lipids is of great potential [55]. During early stages of growth, large amounts of polar lipids and polyunsaturated C_{16} and C_{18} fatty acids are produced. This differs from the dominant lipids produced on approaching the stationary phase of growth, which generally consist of neutral saturated 18:1 and 16:0 long chain fatty acids. Change in lipid composition is however species dependant as blue-green microalgae shows relatively little change in composition during the growth cycle. Temperature and light also play roles in the type of lipid produced. Polyunsaturated C_{16} and C_{18} fatty acids as well as mono- and di-galactosyl-diglycerides, sphingolipids and phosphoglycerides in *Euglena gracils* and *Chlorella vulgaris* are enhanced by light. Synthesis of polyunsaturated C_{18} fatty acids by *Monochrysis lutheri*, and changes in the fatty acid composition of *Dunaliella salina* can be brought about by growth under low temperature conditions [55].

Stress induced by changes in salt content of the aquatic environment has been shown to affect the quantity of lipid contained within microalgal cells. The intracellular lipids and triacylglycerides content of saltwater microalgal species grown in 1.0 M NaCl were markedly higher (67% and 56% lipid) than those in the culture with 0.5 M NaCl (60% and 40% lipid). Sodium chloride concentration higher than 1.0 M inhibits biomass production; however NaCl concentration below 1.0 M has no marked effect on biomass. *Dunaliella* cells were reported to secrete glycerol in response to increase in NaCl concentration [56].

Characterisation of lipids is required as different species of microalgae produce different types of fatty acids. Some fatty acids are more suitable for transesterification to biodiesel than others. Most microalgal oils are rich in polyunsaturated fatty acids with four or more double bonds. The problem associated with this degree of polyunsaturation is, these fatty acids and fatty acid methyl esters (FAME) are susceptible to oxidation during storage, thus reducing their acceptability for use in biodiesel [8]. It is imperative that the strain selected for large scale cultivation produces the appropriate lipid under the envisaged culture conditions at large scale. This is however difficult to ascertain as mimicking of open systems is dependent on a number of factors that cannot be accurately replicated in the laboratory. The cost of culturing at large scale must also be considered when selecting the strain for large scale cultivation. Microalgae growth required rich of nutrients substrates .The possibility of utilising more cheaply available substrates such as wastewater can make the process more economically viable.

2.2.1. Are we selecting the correct strains? Natural vs. stressed conditions

Determination of lipid production capacity of microalgae growing in the natural environment is near impossible. Microalgae have the ability to proliferate on minimal nutrients thus they may produce lipid within the natural environment depending upon the conditions present. Furthermore different strains of the same species of microalgae are known to react differently under the same growth conditions. The only effective method of determining stressed conditions is growth of the microalgae *in vitro* and experimentation to evaluate the effects of stress conditions on biomass and lipid productivity. Under nutrient depleted conditions, cells do not abundantly proliferate. A lower amount of light intensity is required for biomass maintenance and excess energy in the form of free electrons are directed to lipid production as an energy sink to prevent photooxidation [57]. Under normal cultural growth conditions, i.e. with no nutrient stress, photosynthesis increases with an increase in light intensity until light saturation sets in, at which point the maximum growth rate will be attained [96]. Photoinhibition and consequently decrease in microalgal growth rate is as a result of irradiance of the culture above the level of light saturation [57]. In algal historical taxa, Chlorophyceae, a diverse family of microalgae species have been reported to contain high levels of neutral lipids therefore this class may represent a large pool of lipid producers that may be useful for lipid production [58].

2.2.2. Lipid yields under natural and stressed conditions

Lipid accumulation under standard growth conditions was observed to be very low and increased after nitrogen starvation [59]. Under stressed conditions, many microalgae alter their biosynthetic pathways to the production of neutral lipids [60]. Nitrogen limitation or depletion is commonly used for the induction or increase of lipid content in microalgae and is regarded as the most effective method [10,12]. Upon reaching nutrient limited status, carbon is assimilated into cells but cell proliferation does not occur [61]. This carbon is converted to TAGs or carbohydrate within existing cells depending on species [10,16].

Optimisation of growth and lipid yield is essential to the economic viability of production of biodiesel from microalgae. Lipid accumulation occurs naturally as a mechanism for energy storage during unfavourable conditions [60]. The role of lipids in the growth of microalgae is as energy reserves and part of the structural components of the cell. Phospholipids and glycolipids are the primary components of cell wall structures and determine the fluidity of membranes under various conditions. This is achieved by being able to adapt quickly to changes in the environment by recycling of lipids and *de novo* synthesis. A large proportion of phosphate is present in the cell wall [6]. Triacylglycerols (TAGs) are the primary storage components as energy reserves [20,62]. The greater proportion of the lipids produced is TAGs which are produced as metabolic rate of microalgae slows [6]. Some microalgal species have high growth rates and the ability to produce high amounts of lipids under certain growth conditions. Lipid accumulation may be induced or effected by a variety of stress factors such as the removal or limitation of essential nutrients such as nitrogen as well as changes in inorganic carbon and light intensity [63].

Changes in cultural conditions may be used as a mechanism for the manipulation of metabolic pathways resulting in the redirection of cellular function to the production of desired products such as neutral lipid [64]. This method of metabolic manipulation is preferred over mutagenesis and the production of transgenic strains due to problems with stability of transformants and the potential impact on environmental security, especially for large scale commercial applications. Microalgal lipids are the one of the most valuable component of microalgal biomass for biofuels production. Microalgal lipids are high in energy and similar to conventional fuels [62]. Lipid accumulation generally has an antagonistic relationship to growth rate. Therefore it is important to determine the trade off between neutral lipid production and algal growth as part of the optimisation for biodiesel production [14,20]. Nitrogen limitation has variable effects on different types of microalgae in terms of growth and cellular content [65]. Amounts of lipid accumulation may be variable depending on the amount of nitrogen available [66]. The ease of achieving this trade-off under large scale conditions may be difficult to achieve as the culture reaches stability and synergistic interactions in the form of bacterial nutrient cycling occur and thus this must be taken into account. Selection of microalgae that require high nutrient concentrations may be a more viable option for cultivation at industrial scale.

2.2.3. Synergistic interactions in the environment

Microalgae occur under various nutrient conditions in nature. Much of the nutrients supplied are via the nutrient cycling brought about by bacterial degradation of organic matter. Isolation of microalgae from the natural environment may result in non-proliferation of cultures due to lack of some essential metabolites required for growth that are not supplied in artificial media. These metabolites may be produced by various organisms within the natural environment. A common bacterium, *Azospirillum brasilense* was found to promote growth of *Chlorella vulgaris* as well as induce changes in the lipid profile and pigment production [67]. Interactions within the natural environment may dictate the ability of microalgae to colonise that environment and proliferate abundantly. This in turn will determine the ability of such a strain of microalgae to be successfully isolated in the laboratory. Very few reports are available on synergistic interactions which suggest that further research is required to elucidate these associations and their mechanisms [67].

2.2.4. Selection of media for screening

Production of microalgae requires important inorganic nutrients in the forms of nitrogen and phosphorus [10,68]. Microalgae are

known to have different nutrient requirements not only by composition but also by concentration of the nutrients supplied. Microalgal growth media are therefore composed of macronutrients generally consisting of a nitrogen source, phosphate and a metal chelator. Iron is generally supplied in as a micronutrient. Some microalgal species have an inherent adaptability to cultural conditions due to the environment of isolation being in constant flux. *Chlorella* sp. are known to grow fairly well in nutrient rich media [65,69]. Illman et al. [65] showed growth of various species of *Chlorella* in Watanabe media containing 1.25 g/l KNO_3. Change in cultural conditions may be used as a mechanism for the manipulation of metabolic pathways resulting in the redirection of cellular function to the production of desired products such as neutral lipids [64]. Nutrient rich media may cause culture shock and result in death brought on by nutrient toxicity [70]. Media for screening should ideally range from low to high nutrient concentration so as not to exclude potential cultures by lack or excessive nutrient supply. Care should be taken to avoid excessive bacterial growth by addition of yeast extract and other components that may support bacterial growth. Overgrowth of bacteria can cause death of the microalgae by inducing anoxic conditions or causing culture toxicity [51].

2.3. Strain selection

The first step in this process is selection of an appropriate species for biodiesel production [14]. The species selected must meet the requirements for large scale microalgal cultivation. Selection of high lipid producers is paramount to success; however this is just one of the considerations to be taken into account. Eukaryotic microalgae are preferred to prokaryotes as they have been shown to store more lipids [6]. The species selected should have some competitive advantage such as growth in a selective environment (such as high nutrient or alkaline environment) to enable successful culturing at large scales [10,69]. The ability to adapt to various changes in conditions needs to be considered as a factor in selection as temperature fluctuations and diurnal cycles are very difficult to control in open systems [10]. The strain of choice should preferentially be isolated from an area close to the site of production. This allows for a reduction in time required for acclimatisation to climatic conditions. The strain should be able to be produced in open system to make the process economically viable. Phototrophic cultivation is the lowest cost type of cultivation as the energy and carbon sources used (light and inorganic carbon) are available in the form of freely available sunlight and atmospheric carbon dioxide as well as being able to be produced in open raceway pond systems [43].

Large scale production of microalgae poses a number of challenges. Strain selection is of paramount importance to the successful culturing of microalgae at large scale [8]. Ideally the species of choice should balance requirements for biofuels and production of value added co-products. This is generally a difficult task in that bioprospecting of a large number of species is required. Production systems need to be tailored towards attaining high photosynthetic activities. Contamination by undesirable algae, bacteria and grazers is often difficult or costly to control at large scale. There is a requirement for development of techniques to prevent CO_2 losses due to diffusion and high rates of evaporation. There is a severe lack of data available with regard to large scale production due to there being very few commercial production operations [7].

The criterion for selection of superior lipid producers should not simply be microalgae that produce the largest amount of lipid but the strain with the highest lipid productivity [39]. Lipid productivity is a measure of the amount of lipid produced taking into account the growth rate of the microalgae concerned. As lipid is formed as a storage product there is a general inverse relationship between lipid production and biomass yield. Microalgal lipid consists of primarily triglycerides but also contain fractions of isoprenoids, phospholipids, glycolipids and hydrocarbons. They also contain more oxygen and are more viscous than crude oil [63]. Desirable characteristics of the selected microalgal strains are (1) production of biofuels and valuable co-products (2) high photosynthetic efficiency (3) high oil productivity (4) and potential for favourable energy balance taking into account energy required for growth and processing [8,10]. The primary target towards the goal of finding a suitable strain that meets the desirable characteristics for biodiesel production from microalgae is the selection of a high lipid producing strain. Bring out clearly dependence on culturing system and associated cost...nutrient requirements and supply.

2.3.1. Evaluating freshwater and marine microalgae

Successful cultivation of marine microalgae is seen as favourable for improving the economics of biomass production of microalgae [71]. Furthermore the selective environment will serve to reduce extensive contamination. Sea water can be used directly instead of freshwater sources [52]. Choice of microalgal species for cultivation should depend on lipid and biomass productivities as well as location of the cultivation plant. Marine and freshwater species have shown similar biomass and lipid productivities omit thus making strain selection dependant on other factors [14].

A major factor to be considered is water availability [12]. Despite the cultural benefit of lower contamination, feasibility of the production is hinged on the cost of production, thus marine production plants are limited to coastal regions due to availability of seawater. This limits the sites available to set up a commercial plant and the chosen sites come at high capital cost. Furthermore seawater generally contains marine flora that consume microalgae and large amounts of water need to be filtered or marine flora be removed by some other method that will potentially negatively impact that economics of production. High evaporative rates could result in increase in salinity thus adjusting cultural conditions. Salinity needs to be monitored on a regular basis and corrected by the addition of freshwater (an additional cost). Increase in salinity results in osmotic shock and may result in the rupturing of cells under conditions that may not be suitable for lipid recovery [12]. Cultivation of freshwater microalgae with similar productivities may be more appropriate in inland areas. Furthermore cultivation of freshwater species allows a more diverse consortium of species to be propagated.

2.3.2. Comparison of lipids produced

Marine microalgal species have been shown to produce higher levels of phospholipids than triacylglycerols (TAGs) [64]. These types of lipid are unsuitable for the production of biodiesel via transesterification. Freshwater strains have been shown to produce large quantities of saturated neutral lipid making them ideal candidates for biodiesel production. *Scendesmus obliquus* contains predominantly saturated fatty acids and mono unsaturated fatty acids. This imparts the property of oxidative stability [14]. The fatty acid compositions vary between species of freshwater microalgae as well as between species of marine microalgae. Further it is possible to manipulate the type of lipid produced by adjustment of cultural conditions [41]. Each strain selected must be investigated to find the best fit of characteristics for the climatic conditions and location selected. The cost and feasibility of adjusting the cultural conditions must be taken into account. Adjustment of cultivation temperature is generally used at laboratory scale to induce changes in lipid characteristics. Large scale cultivation in the form of raceway ponds generally does not incorporate temperature control. This negates the adjustment of temperature as a viable option. Moreover, incorporation of such systems into raceway ponds increases the energy requirement for cultivation thus affecting the overall energy balance and economic feasibility.

3. Pre-pilot to demonstration plant

The move from laboratory scale experimental trials to demonstration plant scale is not an easy step. There are a lot of factors that should be considered when deciding to move to large scale. Some of these factors are; when to scale up, outdoor conditions, volumes, procedure for scaling up, seed culture preparation, cultivation method at large scale. These vital factors will be critically explored and discussed in detail below.

3.1. When to scale up?

It is imperative to do a full optimisation study at lab scale before considering scaling up. The volume at which large scale operations capacity must be established and the correct amount of seed culture for inoculation must be calculated [62]. The seed culture propagation is an important excise which can determine the success or failure of the scaling up process. The stepwise propagation of the seed culture is the methodology of choice that has been demonstrated by other researchers and the seed inocula should be 20–25% of the final culture volume [72]. The viability, robustness and vigour of the chosen microalgal strain must be routinely checked and analysed at all stages of the scaling up process. The cell density is important and it should be above 1×10^7 cells per ml to avoid a long lag phase after inoculation. In order to prevent culture shock it is recommended that the medium used for seed culture generation must be the same as that used for large scale cultivation of the same microalgal strain.

There are important physico-chemical parameters such as light intensity, pH, temperature, oxidation reduction potential (ORP), salinity, conductivity and nutrient composition *inter alia* which require rigorous optimisation before scaling up can be considered [72]. When all these important variables affecting the microalgal strain of choice are known, it is desirable to design a suitable experimental run and generate and collate enough data for system testing and analysis. The cultivation method is another deciding factor such as open system (raceway ponds) versus closed system (photobioreactors). The pros and cons of each method need to be weighed against the expected returns or products.

It is crucial to study the ecology and biology of the microalgal strain selected for cultivation under the experimental conditions for optimal product output. Some microalgae can grow easily under open growth conditions such as *Chlorella* sp. with minimal contamination from non-target microalgae. However microalgae such as *Chlorella* sp. are known to grow under diverse conditions as a mixed population with *Scenedesmus* sp. [49]. The choice of media is also important if the whole exercise is to succeed such as the use of artificial media against supplemented municipal domestic wastewater [49]. Under open growth conditions a wide variety of microalgae have been shown to show seasonal variations hence a thorough study of microalgal population dynamics is crucial. Economic feasibility of the whole exercise from upstream to downstream processing is important to establish if it is feasible and profitable.

3.2. Guidelines for scaling up

Significant amounts of screening of wild-type and genetically modified microorganisms are necessary before scaling up to commercial production of biofuel feedstock [73]. The selection of lipid producing microalgal strains is based on the assessment of all the cultures collected and therefore the selection of the best strain in terms of growth rates and lipid yield. This can only be achieved after isolating and purification of all the collected microalgal samples. Physico-chemical parameters affecting microalgal growth is strain specific therefore these factors must also be considered when selecting microalgal strains. The media selected should be able to support the growth of the selected microalgal strain. In order to avoid contamination of the purified microalgal strain, a portion of the purified strain must be stored under recommended storage conditions [44]. In order to reduce production costs, the utilisation of domestic wastewater should be initiated at lab scale to establish if the target microalgal strain can grow abundantly in this medium. If necessary, supplementation with essential macronutrients such as nitrates and phosphates can be considered. In addition, careful manipulation of pH can be done in order to prevent contamination by grazers such as zooplankton. Moreover, a detergent and phenol procedure may be employed as a prevention method for bacterial contamination [74].

The method that is going to be used for microalgal cultivation must be carefully chosen taking into account the pros and cons of open systems vs. closed systems. Also the operational mode of cultivation such as batch mode or continuous mode must be considered. Some microalgal strains grow well in the open system with minimal contamination such as *Chlorella* sp. However some strains such as *Dunaliella* sp. grow poorly under closed systems in photobioreactors. Before scaling up, it is prudent to design miniature lab scale photobioreactors and open ponds for preliminary laboratory pilot scale trials. This will furnish valuable information if the microalgal strain will be able to grow under the raceway pond or photobioreactor. Moreover this will also enable proper design and engineering of the raceway pond or photobioreactor at large scale. All the details with regard to experimental design, data collation, and parameter monitoring must be properly scheduled. At this point, the pilot scale experiments will enable the assessment of biomass and oil yields.

Microalgae can grow anywhere with minimal nutrient supply and consequently the biomass and oil yield is not much. Commercial cultivation of microalgae can be a costly venture should the necessary measures to minimise media utilisation costs and energy expenditure not be curbed whilst still providing a competitive advantage for growth of the target microalgal strain. All the physico-chemical factors affecting microalgal growth must be considered as large scale systems allow control of only a limited number of factors due to cost and culturing system considerations. The cost of media for microalgal biomass production in a 100 ton per annum is estimated to be approximately \$3000 ton^{-1} [75]. The cost of conventional media for microalgae production is not feasible for low value products such as oil thus other nutrient sources must be considered [28,76,77]. The one-factor-at-a-time approach for optimisation experiments is frequently used for optimisation studies despite being associated with drawbacks such as being time consuming and labour intensive. To date some workers have used the response surface methodology for optimisation studies and this is reported to be fast and a large set of experiments can be done simultaneously [78]. However this approach is not amenable at large scale since a number of experimental trials must be run which is nearly impossible at large scale. The optimisation of physico-chemical parameters is technically difficult under open growth conditions since it is impossible to control factors such as temperature, light intensity, quantity, spectral quality and photoperiod are prone to environmental variations and fluctuations. This is mainly aggravated by the open nature of the system which is open to all the vagaries of weather.

The most critical step is seed culture propagation. As previously mentioned, seed culture preparation is a crucial step since it decides the success or failure of the cultivation process. The stepwise seed culture propagation method is the best method whereby a series of ponds of varying sizes are constructed whereby 20–25% of the inoculum in the first pond is used to seed the next pond and so on [72]. The seed culture requires close monitoring and all the physico-chemical parameters must be routinely checked

and the viability and growth kinetics of the culture closely monitored. It is ideal to inoculate the large scale culture facility when the seed culture is still viable and still in the exponential growth phase. The vital process engineering and understanding of the stumbling blocks of scaling up supersedes all the challenges associated with microalgal cultivation. To this end, critical information on scaling up is not readily available in literature.

3.3. Media used for microalgal growth at large scale

The choice of media is fundamental for the success of microalgal cultivation at pilot scale phase. A wide range of artificial media have been formulated and the recipes are available in literature [44]. The choice of media can be made based on the findings and recommendations of other researchers in literature if the nutritional requirements of the target microalgal strain are known. However if the requirements of the selected microalgal strain are not known, it is recommended to do a trial and error approach using the different modified media that can promote the growth of a wide range of microalgae [24]. The widely used artificial media are BG-11, AF6 and Bold's Basal Medium (BBM) *inter alia* [44]. *Spirulina*, *Chlorella* and *Dunaliella* are amongst the most important commercially produced microalgal strains. Open system cultivation of these strains is possible as they grow in highly selective media and therefore can be cultivated with relatively limited contamination by other microorganisms [79]. The use of artificial media at large scale is not economically viable therefore it is desirable to formulate cheap and readily available alternative media such as the use of domestic wastewater streams for sustainable microalgal biomass accumulation [49].

Wastewater constitutes important macronutrients that are vital for the growth of a wide variety of microalgae [28,84]. Important macronutrients in wastewater are nitrates, phosphates, ammonium, urea and essential trace elements such as vitamins (biotin and thiamine) and certain trace metals. The unique nutritional composition of municipal domestic wastewater makes it a valuable medium for the growth of microalgae with the added advantage of phycoremediation of the wastewaters to avert eutrophication [8]. The pH and the dissolved CO_2 concentration in the wastewater are ideal for the growth of several microalgae. The use of post-chlorinated wastewater is more suitable at large scale cultivation of microalgae since the microbial load in the wastewater is greatly reduced consequently minimising the risks of bacterial contamination [80]. The problem of eutrophication is of global concern and is mainly exacerbated by a wide range of anthropogenic activities such as release of copious amounts of partially treated wastewater into water bodies [28]. In order to ameliorate this problem, it is desirable to aggressively use the wastewater for microalgal growth. As the wastewater provides nutrients for microalgal growth, the main pollutants in the wastewater are concurrently removed by the microalgae enabling safe disposal into the receiving water bodies in the environment. The use of wastewater as media for microalgal growth has not been well documented especially at large scale commercial cultivation of microalgae.

Wastewater is cheap and readily available and is an excellent medium whose feasibility as substrate at large scale microalgal cultivation requires serious assessment [26]. Coupling of wastewater treatment with the production of microalgae for biofuels has the potential to significantly improve the economics of biomass production. Secondary and tertiary wastewaters contain nitrates and phosphates in sufficient levels support microalgal growth with little supplementation. [81] suggested the wastewater utilisation can reduce the nitrogen by 94% and eliminate the need to the addition of elements such as potassium, magnesium, and sulfur. CO_2 rich wastewater promotes the growth rates of microalgae as it balances the ratio of carbon: nitrogen: phosphorus. This further decreases harvesting cost due to higher biomass concentrations and overall costs by increased lipid production [10]. This reduces the cost of treatment that would normally be incurred for nutrient removal by conventional methods [46,82].

The use of wastewater reduces the need for enormous amounts of freshwater in microalgae cultivation, improving the economic viability whilst being an environmentally friendly means to renewable microalgal biomass production [10,82]. Growth of microalgae on wastewater provides a means removal of organic contaminants, heavy metals and pathogens, thus saving on the costs of chemical remediation [10]. The cost of conventional removal of nitrogen and phosphorus is reported to be \$4.4 kg^{-1} N and \$3.05 kg^{-1} P removed. [83] showed that a 70–110 ton ha^{-1} $annum^{-1}$ facility using wastewater can result in a saving of \$48400 – \$74800 ha^{-1} $annum^{-1}$ for nitrogen removal and \$ 4575 – \$7625 ha^{-1} $annum^{-1}$ for phosphorus removal. The combination of saving from wastewater treatment and reduction of microalgae production costs is thus a win–win strategy when used for the production of energy or liquid fuels [46].

Despite the favorable outlook for the use of wastewater mediated biomass production, the real potential must be explored practically at large scale. A potential problem associated with wastewater utilisation is the viral and bacterial contamination that may or may not negatively affect the production process. The composition of wastewater varies and may impact on growth rates. This factor cannot be controlled and close monitoring and adjustment of nutrient levels may be required. Utilisation of wastewater will further necessitate frequent cleaning of the culturing system [46].

4. Mass cultivation of microalgae

4.1. Process configuration

In the context of large scale microalgal cultivation, process configuration is defined as the combination of economic viability, upstream processing and downstream processing. This concept will be critically evaluated in terms of the microalgal cultivation facilities commonly used, their merits and de-merits, large scale biomass production, harvesting, lipid extraction, challenges at large scale, and existing global commercial enterprises involved in biofuels production from microalgal biomass. The synergy between upstream processing and downstream processing for viable microalgal cultivation for sustainable biofuel production is currently receiving global attention.

4.2. Photobioreactors vs. open raceway ponds

There are mainly four types of microalgal cultivation techniques that are available, namely; photoautotrophic, heterotrophic, mixotrophic, and photoheterotrophic. Of these, the most dominant method commonly used for microalgal cultivation is phototrophic cultivation [43]. The two main practical and feasible methods employed for the cultivation of microalgae are tubular photobioreactors (PBRs) and open raceway ponds (ORPs) and to a lesser extent fermenters for mixo/heterotrophic cultivation [8,45,84,85]. The first two methods are widely used and are therefore discussed in detail in this section. Photobioreactors are closed systems enabling mono-specific microalgal cultivation while open raceway ponds are open system [86,87].

4.2.1. Open Pond systems

Raceway ponds the most common cultivation system used [10]. They are generally cheaper to build and easier to operate than photobioreactors [88,89]. They are generally constructed from concrete, but plastic covered earthlined ponds have been used [10].

Most paddlewheel driven raceway ponds are limited to 20 cm to 30 cm in depth. The paddle wheel prevents settling of the culture and reducing the shading effect [10,90]. Unmixed ponds may be up to 50 cm in depth. The requirement for shallow open ponds is to allow adequate penetration of sunlight [69]. Open ponds are generally regarded as the more cost effect method of microalgal biomass production [26,91].

Open ponds however have various advantages and limitations. Amongst the major limitations is low productivity as compared to photobioreactors and environmental factors to some extent cannot be controlled [8,69,88]. Low productivities in open ponds occur as a result of a number of factors. Evaporative losses result in changes to ionic composition of the media and potentially detrimental effects on culture growth. Changes in temperature, photoperiod and seasonal variation are beyond control in open systems and directly affect productivity [8]. Major contributors to low productivity are CO_2 transfer rate and light limitation due to increasing culture densities. Atmospheric carbon dioxide is usually used to satisfy the carbon requirement. Techniques to enhance CO_2 absorption into the culture media such as aerators or bubbling may improve the overall biomass productivity. Improved mixing can minimise impacts of both CO_2 and light limitation thus improving productivity [10]. Due to low productivities, large areas of land may be required to meet the desired output of cultivation [52]. Open pond systems tend to become contaminated with undesirable species fairly quickly [26,88,90,91]. Contamination by protozoa and other algae may be reduced by utilisation of highly selective culture conditions [10]. This limits the number of suitable species open pond cultivation. A few examples include (i) *Chlorella* species which require nutrient rich media, (ii) *Duniella salina* is adaptable to high salinity and (iii) *Spirulina* which grow well under high alkalinity [69].

The selection of culturing system must also take into account the intrinsic properties of the microalgal species to be cultivated. Natural climatic conditions and the cost of land and water availability also play a role in the determination of culturing system [69]. Usage of marginal and non-arable land is a major advantage. Maintenance and cleaning of open systems is easier and less energy intensive than photobioreactors [10]. The overall energy input for raceway pond operation is lower than for photobioreactors and therefore have the potential for a larger net energy production [85]. Commercially produced raceway culture of *Dunaliella salina* cost approximately $2.55 per kilogram of dry biomass in 2008, which was considered to be too high to justify production for biofuels [10]. It must be noted that the culture was grown on media for the production of high value products.

4.2.2. Closed systems

The limitations of open pond culture have lead to much research into photobioreactors, as a method of primarily overcoming contamination and low productivity [10,52]. They are generally used for culturing of microalgae for high value products such as pharmaceuticals, that cannot be grown as a monoculture in open systems. The increased utilisation of photobioreactors may be as a result of a higher degree of process control and higher biomass productivities [10]. The most popular photobioreactor configurations are tubular, vertical or column, flat plate and annular reactors [10,52]. The fundamental principal behind most photobioreactors is reduction of the light path thereby increasing the amount of light received by each cell [69]. They are generally mixed by airlift or mechanically stirring/pumping. Mixing provides a benefit in phototrophic systems in that mixing as well as aeration is accomplished simultaneously [52]. Mixing is essential for gaseous exchange within the system [10]. Photobioreactors are more versatile than open opens in that they can use sunlight, artificial light and various combinations of light sources thus giving the potential to increase photoperiod and enhance low light intensities given by sunlight variation. The stability of light intensity and photoperiod provided by artificial light has potential to enhance yearly total oil yields by 25–42% [52].

Tubular photobioreactors are regarded as one of the most suitable for types for large scale outdoor culturing. The solar collector is generally made of glass of plastic with a diameter of 0.1 m or less to allow penetration of light through dense culture[8]. The orientation of the solar collector may be horizontal, vertical, inclined or as a helical coil around a supporting frame [10,92]. Cultures are generally reticulated by pump passing through a degasser at regular intervals in order to remove excess oxygen. Higher levels of oxygen lead to lower productivities due to photooxidative stress. Mixing within the reactor is achieved by creating turbulence. This is costly in terms of energy utilisation and wear and tear on the pumps [90]. Tubular photobioreactors have large surface area that is exposed to light and is thus regarded as suitable for outdoor cultivation [10]. Tubular reactors are currently used for the production of cultures producing high value products such as astaxanthan [90].

Flat plate photobioreactors are capable of achieving high cell densities due to the large surface area available for solar capture. To allow maximum sunlight capture, the reactors are constructed from transparent material with a layer of dense culture flowing over the flat plate [10]. Mixing is achieved by sparging with air at a rate of 1 L air per litre of reactor volume per minute [90]. Due to the high photosynthetic efficiency and low accumulation of dissolved oxygen, flat plate reactors are more suitable for large scale culture than tubular reactors but at higher mixing and installation costs [10,90].

Photobioreactors allow a larger range of species to be cultivated [93]. The main benefits of photobioreactors over open ponds are the higher productivity and level of control. Photobioreactors offer the opportunity to optimise light path length and thus improve biomass productivity [52]. They are able achieve and operate at high biomass concentrations due to their high surface area to volume ratio [92,93]. Atmospheric contamination can be avoided. Monocultures are possible in for extended periods of time if they are operated in a sterile manner [10,52]. Tubular photobioreactors can be erected in any space open space [92]. Greater control of culture conditions results in the final product of microalgal propagation being of more consistent quality and composition [69]. Scale up of photobioreactors may present engineering and design challenges. Tubular reactors are limited on the length of the tubes due to inefficient gaseous exchange. Extensive tube lengths give the potential for excess oxygen accumulation, CO_2 depletion and pH variation. All of which negatively effect biomass productivity. The photoarray is also prone to growth of culture attached to the walls and fouling [10]. Despite their advantages and superior productivity to tubular reactors, flat bed reactors are difficult to scale and maintain temperatures at desirable levels [10]. The major limitations to utilising photobioreactors for large scale cultivation of microalgae are the capital and operational costs [52,93]. Estimates of capital and production costs vary greatly. High power consumption is one of the major shortcomings of many photobioreactors. The use of artificial light adds to the power consumption and increases capital costs [52]. The use of light emitting diodes has been proposed as a more cost effective method than the use of fluorescent light. The use of optical fibres exposed to sunlight to illuminate inner regions of the culturing system has been suggested as a method of reducing power consumption [52].

4.2.3. Hybrid production systems

Considering the advantages and drawbacks of raceways and photobioreactors, the logical step in cost effective biomass production would be a combination of the technologies. Hybrid systems combine growth in bioreactors and raceway ponds [26]. Hybrid

systems have been used historically in aquaculture for the growth of inoculum. This allows production of inoculum free of contamination and provides a large enough volume to give the culture of choice a competitive advantage in the open system [26]. The use of hybrid systems for biofuels production utilises a large scale photobioreactor and open pond sequentially. The first stage of growth is undertaken within a bioreactor to maintain culture purity and achieve high biomass concentrations [52]. The second stage is undertaken in a raceway pond as this is ideal for nutrient stress [10]. Hybrid systems can produce as much as 20–30 toe ha^{-1} of lipid annually depending on climate favorability [85].

Open raceway ponds can facilitate multi-strain cultivation while photobioreactors are well suitable for single strain cultivation. Due to the open nature of the open raceway ponds, there are very high chances of contamination from non-target microalgal strains and photobioreactors can proliferate only a single strain under sterile conditions [86]. Minimal cleaning is required for open raceway ponds and photobioreactors require intermittent cleaning due to wall growth and dirt accumulation. It is technically difficult to control growth conditions in open raceway ponds. Due to the closed nature of the photobioreactors, growth conditions such as temperature, light and CO_2 concentration can be easily controlled [44,86]. Under open raceway pond cultivation system, temperature is highly variable whereas photobioreactors require cooling [8]. Cooling for open raceway ponds is not viable as compared to the inbuilt automatic cooling for photobioreactors. In addition, automatic heating system is not suitable for open raceway ponds whereas this system can be fitted to photobioreactors. Generally there is no microbiological safety in open raceway ponds whereas UV could be used for controlling microbial contamination in photobioreactors.

4.3. Biomass production

The quality of microalgal biomass produced in photobioreactors is of high quality and reproducible as compared to the variable and inconsistent biomass quality in open raceway ponds due to sporadic crashes. Biomass yield is higher in photobioreactors as compared to open raceway ponds and this is due to adequate nutritional control mechanisms and mono-specific culture growth conditions in photobioreactors [10,62]. In addition, lipid productivity is higher in photobioreactors as compared to open raceway ponds since all the growth parameters are amenable for optimisation for higher lipid productivity in photobioreactors. There is higher light utilisation efficiency in photobioreactors as compared to open raceway ponds and this is mainly due to a large surface area in the open system.There can be a great amount of variation between algal biomass production rates. These depend on the levels of inputs and can even vary between strain [7]. Biomass yields of 0.5–1 g/L are accepted as standard for raceway ponds. Photobioreactors are generally limited to 4 g/L for photobioreactors before the shading effect greatly limits further growth [93]. The theoretical maximum biomass productivity is estimated to be within the range of 77–96 g DCW m^{-2} day^{-1}. This translates to 280–350 ton DCW ha^{-1} $ammun^{-1}$ [83]. This however is generally not achievable and productivities in the order of 27 to 62 g DCW m^{-2} day^{-1} (100–227 ton DCW ha^{-1} $ammun^{-1}$) are regarded as reasonable targets [26,94]. Based on the potential oil yield of 30–50% oil yield, the theoretical yield of 47000–308000 L ha^{-1} $ammun^{-1}$ [27]. The cost of biomass production is the only relevant factor for comparison between raceway ponds and photobioreactors. The cost of recovery of oil and transesterification is not affected by the type of culturing system [8].

Algal productivities in open pond systems range from 5 to 50 g DCW m^{-2} day^{-1}. This is species, climate and operation dependant [22,77,83,95]. Raceway ponds with water depths of 15–20 cm allow for productivity of 10–25 g DCW m^{-2} day^{-1} [26]. In practise these are generally lower than projected [83]. [22] reported an average of close to 10 g DCW m^{-2} day^{-1} with a maximum productivity of 50 g DCW m^{-2} day^{-1} for biomass achieved by a pilot scale raceway pond in Roswell, New Mexico. Researcher in Spain were able to achieve 8.2 g DCW m^{-2} day^{-1} [10]. [96] reported that an average of 19–25 g DCW m^{-2} day^{-1} may be achieved in well managed ponds with peak productivities ranging from 12 to 40 g DCW m^{-2} day^{-1}. Tubular reactors are able achieve cell densities ranging from 2 g/L to 6 g/L. Higher surface to volume ratios give superior productivities [93]. Cell densities of up to 10 g/L are possible in well designed photobioreactors [94]. Reported productivities for photobioreators range from 20 to 40 g DCW m^{-2} day^{-1} [97]. The cost of closed systems is significantly higher than open ponds. Photobioreactors require 10 times the capital investment as compared to raceway ponds [22]. Reductive of the cost of construction of closed systems is imperative for the success of biodiesel production from microalgae [10]. It is possible to produce dewatered biomass at a cost ranging from $5.08 to $5.27 US/kg dry weight using tubular reactors [90,98].

4.4. Operational mode

In both photobioreactors and open raceway ponds, there is generally a built in air pump for aeration. Despite the air pump installations in these cultivation methods, CO_2 transfer rate is poor in open raceway ponds as compared to photobioreactors. Moreover, CO_2 loss is higher in open raceway ponds depending on pond depth as compared to lower CO_2 loss in photobioreactors. In addition, there is low shear in open raceway ponds in comparison to the high shear in photobioreactors. A paddle wheel is installed for purposes of mixing and circulating the suspension to avoid biomass settling and the shading effect in dense cultures [26]. However the mixing efficiency is poor in open raceway ponds as compared to photobioreactors. Due to the open nature of the ponds, water loss due to evaporation is higher as compared to photobioreactors where there is little to no evaporation at all. Oxygen concentration is low in open raceway ponds due to continuous spontaneous out gassing whereas there is oxygen build up in photobioreactors which require a gas exchange device installation. Costs of mixing may account for a significant proportion of the total cost of biomass produced. These costs are approximately $0.10, $1.61 and $3.93 US/kg for raceway, tubular reactors and flat panel reactors respectively [90]. Reduction in the costs of mixing in photobioreactors will significantly improve the production economics. Raceway pond production estimates place the cost of dried algal biomass at $0.34 US/kg. At a predicted lipid productivity of 24% lipid per gram DCW this translates to $1.42 US/kg and $209 US/bbl lipid. At a more favourable lipid yield of 40%, the cost decreases to $0.85 US/kg and therefore $126 US/barrel [26].

4.4.1. Carbon dioxide sequestration

The release of gases that cause climate change is showing little abatement though there is a serious campaign to arrest this perpetual global predicament. However the concomitant coupling of biofuel production and application of microalgae as a tool for CO_2 sequestration and mitigation is a new phenomenon and is technically feasible [26,78]. Open pond raceway ponds are more favourable for CO_2 mitigation using microalgae as compared to photobioreactors. Open pond raceway ponds can be constructed near industrial areas producing huge amounts flue gases which can be used as CO_2 source for microalgal growth. The use of CO_2 gas derived from power plants for microalgal growth is still at an infant stage where companies will be awarded carbon credits as result of mitigating release of harmful gases to the environment.

4.4.2. Key limitation for biomass productivity in culturing system

Large scale microalgal production has a number of challenges that need to be overcome in order to make it commercially viable. These include strain selection, maintaining culture integrity, photosynthetic activity, and gaseous exchange [10,97,99]. Open ponds are able to culture only certain species of microalgae [96]. Algae that grow under extreme conditions (high pH, nutrient level, etc.) provide a competitive advantage thus limiting contamination by other microalgae. Contamination is however inevitable and requires constant propagation of seed culture in order to keep the culture of choice as dominant [97,100]. Contamination by non-target microalgae is only regarded as problematic should the contaminating species not have a desired trait, have a negative impact on the culture or be capable of outgrowing the species of choice. Increased control of the growth environment is can effectively reduce contamination but increases the cost. Biofouling becomes a possibility if the microalgae adhere to the walls of the bioreactor. This effectively increases shading thereby reducing productivity. Biofouling can also impede culture flow, requiring more energy and thus increasing the productivity [100].

Supply of photosynthetically active radiation (PAR) becomes a limiting factor in dense cultures in both open and closed systems thus reducing productivity [97]. Supply of carbon dioxide is essential for the prevention of carbon limitation. Despite ambient air containing sufficient CO_2 for microalgae growth, CO_2 needs to be in solutions for uptake. Less than 10% of the CO_2 resources are available to the algae for uptake. Bubbling of air is not an effective delivery system for open ponds due to short residence time [12]. Optimisation of bubbling technology remains an engineering challenge. Removal of oxygen is imperative for the prevention photo-oxidative stress in photobioreactors. Oxygen above atmospheric concentration inhibits photosynthesis. This problem is usually remedied by sparging of air through the reactor or a section thereof in order to strip excess oxygen. This increases energy consumption and thus cost [97].

4.5. Large scale biomass production

Microalgal biomass production at large scale is almost exclusively done using open raceway ponds in the batch mode [76,79]. It must be noted that currently large scale cultivation of microalgae is carried out for products other than biofuels. [40] undertook a case study whereby they suggested that cultivation should be carried out on a continuous basis. This is a more efficient approach to microalgae cultivation for biofuels production. The viability of a continuous system is dependant upon the prevailing species, biomass productivity, culturing system and culturing conditions. Closed systems are more likely to be run as continuous systems due to higher efficiency and significantly lower amounts of contamination of undesirable algae and other organisms. The use of raceway ponds is however considered to be more viable for cultivation of microalgae due to a better net energy ratio when compared to closed systems [99].

Efficient open raceway pond designs typically consist of independent closed-loop systems. Artificial systems equipped with a paddle wheel are used to generate more simplified circulation by which flow is directed around bends by baffles placed in the flow channel to ensure desired mixing [101]. Fig. 1a illustrates the open raceway pond cultivation method and the small ponds with different volume capacities are for the stepwise seed culture propagation. Inoculum density of the seed culture is an important process parameter that requires close monitoring in order to avoid prolonged lag phase. The main advantages of large scale biomass production using the open raceway ponds are (1) simplicity and low costs, (2) use of cheap and readily available substrate alternatives such as domestic municipal wastewater streams, (3) reduced capital costs and (4) large tracks of marginal land not suitable for agricultural purposes can be used without compromising food security [62].

The optimisation of operational parameters for large scale biomass production is technically challenging and is one of the major drawbacks of this microalgal cultivation method at this scale. As mentioned previously, the upper limit of world algae biodiversity is more than one million species. Many of the characterised species are been known to produce high value secondary metabolites including lipids. Additionally, raceway pond configuration and operating procedures are extremely important for algal cultivation but have not yet been optimised for many microalgal species that have been evaluated for oil production [85,101]. The overall costs involved in large scale biomass production depend on prevailing economic conditions in the local market [45]. The design of the open raceway pond is critical for increased growth rates and consequently high biomass yield. The cascading open raceway ponds are potentially valuable since there is nutrient limitation at the end of the growth cycle and therefore increased lipid yield.

The single channel open raceway ponds are cheaper to construct and are also widely used but they are not as efficient as the cascading design. Real cultivation remains questionable worldwide due to relatively high engineering, construction, design, maintenance, operating cost and complexity of operation which are expensive. The use of photobioreactors is limited due to high capital and operation costs and is mainly used for the generation of high value products [101] (Fig. 1b). In order to mitigate problems associated with outdoor microalgal cultivation, microalgal

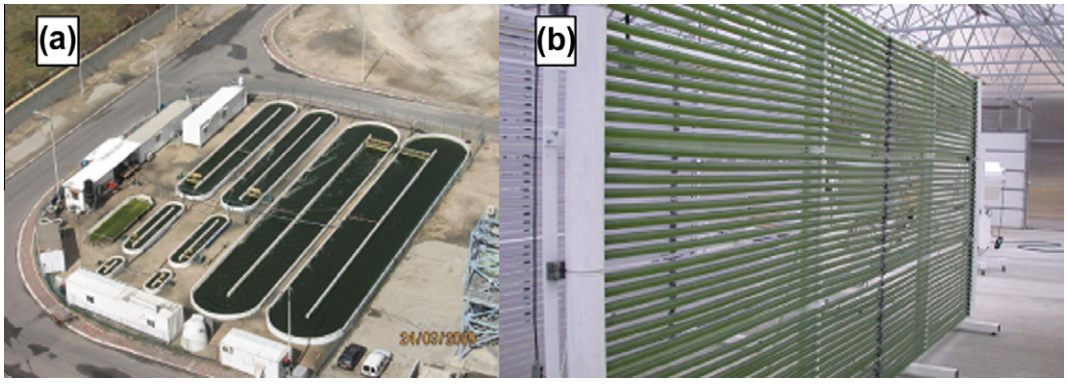

Fig. 1. (a) Image of large-scale Seambiotic *Nannochloropsis* sp. culture ponds. Image courtesy of Greenwell et al. [101] and Nature Beta Technologies Ltd., Eilat, Israel, subsidiary of Nikken Sohonsha Co., Gifu, Japan. (b) Tubular PBRs in operation. Such systems have a small path length ensuring high volumetric production coupled with a small footprint. Photograph courtesy of Greenwell et al. [101] and Varicon Aqua Solutions Ltd., UK.

biodiesel research interests are looking to address these issues including use of large size open raceways in conjunction with enclosed photobioreactors. The integrated use of PBRs and open raceways is an efficient method of production of large inoculums thus enabling short cultivation period by maximum efficiency to utilise light in outdoor raceways, in this manner decreasing opportunities for adverse events [32,101].

4.6. Biomass harvesting

Recovery of microalgal biomass is a problematic and challenging undertaking in the area of microalgal biofuel production technology largely due to the microscopic size of the microalgal cells (2–200 μm), process recovery costs of the microalgal entities existing in aqueous suspension in a relatively dilute broth (0.5–5 kg m^{-3} dry weight) and that large volumes of water are handled [45,101]. Biomass harvesting can potentially contribute to 20–30% of the total biomass production costs[12].Type of biomass recovery is dependent on the density of the biomass slurry. Dilute broth requires a combination of one or more solid–liquid separation steps. Harvesting of biomass by employing strategies such as centrifugation, filtration or in some cases, gravity sedimentation. These processes may be preceded by a flocculation step [45]. The main physical properties of microalgae targeted when choosing a strain are mainly intracellular biomass composition and yield of the desired product. Indeed processing options such as ease of microalgal biomass recovery must also be considered when selecting strains for large scale cultivation [101]. The search for suitable and cost effective microalgal biomass recovery techniques is an ongoing exercise and here we summarise the pros and cons of conventional techniques that are currently used for harvesting microalgal biomass (Table 1).

4.6.1. Filtration

Conventional filtration may be insufficient due for biomass recovery. Filtration aided by suction or optimal pressure net energy for relatively smaller size algal cells can be relatively sluggish, tedious and time consuming especially if large volumes of microalgal suspension are to be processed [45]. Furthermore, the use of membranes is not suitable for large scale processing due to frequent membrane fouling and clogging. The recovery of biomass in smaller scale production via alternative methods such as membrane microfiltration and ultrafiltration show higher performance than conventional filtration. Microfiltration has been shown to be more efficient and suitable for harvesting fragile microalgal cells [12,45].The use of filter presses come highly recommended for use as a harvesting tool larger microalgae species such as *Coelastrum proboscideum* and *Spirulina platensis.* Cultures such as Scenedesmus, *Dunaliella* and *Chlorella* are more difficult to recover due to their small size (5–20 μm) [10,42,45]. This method is moderately effective for large microalgae whereby a chamber filter press can achieve a phenomenal concentration factor of 245, thereby producing a cake of 27% solids for *C. proboscideum* [45].Various filtration methods can be used for segregation of microalgal biomass. The use of adequate vacuum with ideal pressure could improve efficiency of the process [101]. Depending on the machine and make employed, pressure filters are generally more reliable, cost effective and more efficient than vacuum filters [45]. The major disadvantage of membrane filtration processes is intermittent membrane replacement and energy intensive pumping which contribute to elevated production costs. By comparison to the centrifugation technique, a well developed technique such as membrane filtration results in lower operational costs making the technology increasingly attractive [101]. For this separation technique to be effective for microalgal biomass recovery, membrane technology is critical to improve the membrane longevity, membrane durability, performance, increased scale of operation and overall improved system conditions.

4.6.2. Centrifugation

The use of centrifugation as a biomass recovery and dewatering method is typically effective though application of this technique at large scale is problematic due to increased power consumption, consequently pushing up production costs. The principle of this method is that there is phase separation of microalgal biomass from the aqueous solution when a centripetal force is applied in a centrifuge. The main advantage of this technique is that microalgal cell separation is achieved more rapidly by increasing the gravitation field subjected to the microalgal suspension thereby concentrating the biomass into a cake with >95% cell harvest efficiency at 13000 × *g* [101]. In addition this technique is rapid, easy, efficient and is not disruptive such that intact cells are recovered for the extraction of high value intracellular products.

4.6.3. Gravity sedimentation

The most common the rapid intensively used method for harvesting is sedimentation for separation of microalgal biomass from larger volumes of water and wastewater. Despite the simplicity of sedimentation it is a very slow process (0.1–2.6 cm h^{-1}) and dete-

Table 1
Summary of pros and cons of techniques that are used for harvesting microalgal biomass.

Technique	Pros	Cons	References
Filtration	Low cost, water reuse	Slow, membrane fouling and clogging, limited volume, cell damage	[12,101,102]
Centrifugation	Rapid, easy, efficient	Very high energy input	[45,101]
Gravity sedimentation	Low cost, potential for water recycling	Slow process, product deterioration, separation depends on cell density	[43,101]
Chemical flocculation	Low cost, low cell damage	Biomass toxicity, no water reuse, inefficient, potential to remove lipids, produces large quantity of sludgethat increases the difficulty to dehydrate the biomass	[45,46,103]
Dissolved air flotation (DAF)	Low cost, easy application at large scale	Needs flocculants, water reuse and product extraction may be negatively affected	[43,45,46]
Foam fractionation	Small footprint, no addition of chemicals	Low yield due to inefficient floatation	[104]
Ozone fractionation	Small footprint, cell disruption required for extraction occurs simultaneously	Ozone generation is expensive, Loss product	[105]
Microstrainers	Easy operation, low cost construction, high filtration ratios	Strongly cell concentration dependant, smaller cells may undergo incomplete removal, difficulty and handling solids fluctuations	[43]
Bio-flocculation	High efficiency, no damage to cells	No water reuse, higher energy input than other flocculants	[46,102,106]
Electrolytic flocculation	High efficiency	High energy input (up to 16KWh/kg biomass), increased temp may damage system, fouling of cathodes	[43,107]
Cross-flow membrane	Water reuse, removal of pathogens, protozoa	Membrane fouling, requirement for frequent use	[102]
Submerged membrane microfiltration	Low cost, less shear stress, less membrane fouling than conventional cross-flow	Membrane fouling, scale up potentially has problems	[108]

rioration of the biomass may occur during the separation due to high temperature. However the process is largely accepted as a viable harvesting method for large microalgae cells such as *Spirulina* [43,45]. The advantage of this technique is that it is not expensive; process control is easy with only a requirement of a settling tank and is amenable for large scale biomass harvesting. Minimal costs may be incurred for the energy consumption for pumping the suspension from the growth chamber to the settling tank. The recycling of the water rich in nutrients to the growth chamber is advantageous since no potentially toxic chemicals are used for biomass recovery as compared to the flocculation technique which use compounds such as aluminium sulphate ($Al_2(SO_4)_3$), ferric chloride ($FeCl_3$) and ferric sulphate ($Fe_2(SO_4)_3$) which may hamper microalgal growth and also affect the final products.

4.6.4. Chemical flocculation

Multivalent cations or cationic polymers are useful for the neutralisation of microalgal negative charge that prevents aggregation in aqueous suspension [45]. The microalgal cell-surface negative charge becomes decreased by increasing the pH to 8.5 and above, and may cause auto-flocculation to occur [101]. Various flocculation techniques harness this important physical property (negative cell-surface charge) to achieve microalgal cell aggregation by increasing floc size enabling efficient centrifugation, sedimentation and filtration. Coagulation of cells resulting in precipitation or floating to the surface may be induced by the addition of multivalent metal salts such as aluminium sulphate and ferric chloride. Recovery of the microalgal biomass is then accomplished by siphoning off the supernatant or skimming cells off the surface respectively. These multivalent metal salts e.g. aluminium sulphate are effective coagulants for *Scenedesmus* and *Chlorella* and are widely used in microalgal biomass flocculation in wastewater treatment processes [45].

The use of polyferric sulphates is more effective than the non-polymerised traditional metal salt flocculants by producing flocs that can be easily dewatered [45]. In addition the use of cationic polymers (polyelectrolytes) is a potential alternative to the use of traditional flocculants. Low dosages of polyelectrolytes (1–10 mg/L) can effectively neutralise and reduce the surface charges of microalgal cells and in addition they bring microalgal cells together by physically linking the cells by the mechanism of bridging though their application is adversely affected by high salinity water from marine habitats [45]. The flocculants utilised must be cheap, non-toxic, and effective in low application dosages. Moreover, the flocculants used should be chosen so that further downstream processing and water reuse for microalgae proliferation are not negatively affected by the residues of the flocculants. The use of metal flocculants for biomass recovery for use in aquaculture and other sensitive applications is not recommended due to possible toxicity. However, flocculation caused by alkaline adjustment has been used to effectively remove *Dunalliella testolata* and *Chaetoceros* sp. from fluids [101]. Recently advanced novel methods are at developmental stage such as the efficient electroflocculation technology integrated with dispersed-air flotation for harvesting microalgae [109]. Eletroflocculation gave a biomass recovery efficiency of 93.6% after 30 min and this technique was integrated with dispersed-air flotation to give a phenomenal recovery efficiency of *Botroyococcus braunii* of 98.9% after 14 min.

4.6.5. Dissolved air flotation (DAF)

The microalgal cells are subjected to flocculation using cationic polyelectrolytes to increase the floc size before applying DAF. Dissolved air flotation involves the generation of fine bubbles produced by a decompression of pressurized fluid [101]. The fine bubbles (<10 mm) adhere to the flocs making them very buoyant and This buoyancy causes them to rise rapidly to the surface of a separation tank resulting in a concentrated cell foam (7–10% dry weight) that is then removed as slurry. The main advantage of this method is that it is cheap and also that it can be applied for the recovery of microalgal biomass at large scale (commercial plants handling more than 10000 $m^3\,day^{-1}$. The main disadvantage of this method is that there is possible contamination of the microalgal biomass with the floc agent, which may significantly decrease the value of the extracted high valued products [45].

4.7. Lipid extraction

Neutral lipids are produced by a large number of microalgae isolated from diverse aquatic environments. These lipids are favourable candidates for conversion to biodiesel and therefore are essential for biodiesel production due to their lower degree of unsaturation [76]. The biochemistry of microalgal lipid biosynthesis is well documented and the pathway for their biosynthesis has been thoroughly explored [101]. These lipids are produced at the end of the microalgal growth phase and also under nutrient limiting conditions such as nitrate starvation. Lipid extraction is costly and is one of the widely debated processes in biodiesel production. Due to the sensitivity and competition in this area of research most processes employed at large scale biodiesel production are kept confidential for proprietary reasons for possible ground breaking breakthrough for novel patentable lipid extraction method. Before lipid extraction can be attempted, the cells must be disrupted either by mechanical means or by chemical treatments. For large scale operations, mechanical methods for microalgal cell disruption such as homogenisation and bead milling are reported to be efficient [101].

A wide range of lipid extraction methods are available and the choice of each method is based on efficiency, accuracy, cost effectiveness, easy to carry out, high throughput capability, robustness and most importantly precision and reproducibility [76]. Widely reported methods for the extraction of lipids from microalgal cells include the following: Folch method, gravimetric method, and Bligh and Dyer method. Two frequently used conventional methods by researchers involve solvent extraction (n-hexane) and gravimetric determination, the former from whole microalgal cells with or without cell disruption [76]. Denaturation of cellular material can occur as result of solvent usage for lipid extraction. Removal of such material can often be difficult [101]. Addition costs are associated with solvent usage at large scale in order to meet the very high standards required for plant design and operation in terms of risk prevention such as fire, explosion and hazardous materials storage and handling [101]. Other analytical methods such as TLC, HPLC, GC and GC–MS are used to identify and quantify the microalgal lipids [73]. The method used for lipid analysis obliges that the recovery of lipid extracts, while avoiding decomposition of lipids and/or lipid components.

The classical conventional lipid extraction procedures have major limitations due to the process being time consuming and labour intensive making it difficult to be screen large numbers of microalgal samples. The most common lipid determination method is conventional gravimetric analysis which has many complicated steps. Biomass harvesting, lipid extraction, separation and concentration can result in loss of some lipids [73,76]. Hence to get around this problem, increasing attention is focused on *in situ* measurement of lipid content such as Nile red (NR) staining, time-domain nuclear magnetic resonance (TD-NMR) and Bodipy [44].

4.8. Challenges at large scale

Commercial microalgal cultivation at large scale has its attendant problems. Grobbelaar, [72], outlined 6 challenges they experienced at their large scale *Spirulina* cultivation facility in Musina,

South Africa. The challenge of deviations from design and raceway specifications is a result of construction companies with little experience in raceway pond construction as well as biologists having little or no engineering skills. This causes significant delays to the inception of the project as well as successful completion according to the project time frames. Furthermore, time constraints and requirements due to insufficient pre-construction planning may lengthen optimisation time thereby seriously delaying the whole exercise. According to Grobbelaar [72], commissioning, start-up, and basic optimisation require time and a 3-year period is the minimum.

The question of scale and seed culture preparation is a real challenge for a large scale microalgal handling facility of up to 300000 L. The equipment for handling large volumes of broth must be available. Harvesting, seed culture preparation and inoculation facilities must be well planned in advance of the project inception. Lack of skills in microalgal cultivation operations is a major stumbling block for successful operations. Therefore all personnel involved in the project must be fully trained and provided with all the essential skills on handling microalgal growth, parameter monitoring and the use of equipment such as pH meters, spectrophotometers, and probes to avoid system failures. Site specific problems can also be a major challenge such as unexpected power and water outages, seepages, contamination, water evaporation, staff absenteeism and possible red tape from the investors. Last but not least, the challenge of product quality and consistency must be addressed for successful operation and execution of the project.

Daily culture sampling and analyses is required to quality and check product consistency as this is an ongoing challenge [72]. A lot of work has been done to avert system crashes and it is critical to keep an eye on all possible sources of system failure. A lot of money is injected in microalgal cultivation and any slight mishap may put all these resources to waste if there is no careful planning, sound scientific approach, balanced chemical and mechanical engineering principles and good time management [101].

5. Downstream process

For the viable production of biodiesel Chisti.,2007 [8] stated that microalgal biodiesel has to be cost effective to become a significant source of energy by 2040. The ASTM (American Society for Testing Materials) published biodiesel standards, assure that microalgae oil produced biodiesel is having similar properties to the standard biodiesel. Microalgal biodiesel has a high cetane number and several other beneficial properties. The downstream process for biodiesel production commences with microalgal biomass harvesting and dewatering, oil extraction and subsequently transesterification of the lipids into biodiesel. As already discussed in previous sections, there are several methods that can be employed for biomass separation such as filtration, centrifugation, flocculation, screening, gravity sedimentation and dissolved air floatation techniques etc. [45,71]. Biomass separation is the first crucial step for downstream process for the recovery of PUFAs from the harvested microalgae. Several methods can be used for the extraction of both biofuel and intracellular metabolites such as high value co-products. Historically, the three most common processes for recovering oil from biological materials are mechanical pressing [96,110], solvents [47,111,112] and supercritical fluid extraction [113].

5.1. Mechanical pressing

Mechanical pressing is widely used for oil extraction from oil seeds such as soybean and sunflower and can also be used for oil extraction from microalgae for biofuel production. The percent yield of total recovered oil from the biomass depends on the efficiency of the extraction method. Previously, calculations for theoretical oil yields from microalgae were made solely because dried microalgal biomass retains the oil content that depends on the microalgal strain. The lipid generated from certain microalgae is known to reach content of up to 80% (w/w), making microalgal biomass a promising candidate for biofuel feedstock [114,115]. Currently, extraction of microalgal oil is a hotly debated topic as this is one of the most expensive and technically challenging processes. The simplest method applied for microalgal oil extraction is mechanical crushing. Mechanical technologies for extracting microalgal oil include the screw press or piston, extruder and expander, and pulverization in a mortar. In the mechanical expulsion process, oil is expelled from dried microalgal cells by one or combination of these methods. Machines that combine these technologies for increased extraction efficiencies are also available. Process inputs are basically electricity to power the machinery. According to Kleinschmidt [110], simple mechanical pressing of the biomass separates the biomaterial cake and the oil component. Cooney et al. [40], reported that the main drawback of mechanical pressing is the unicellular nature of microalgal cells and that some microalgal strains have rigid cell walls. Crushing of microalgal cells will not be readily achieved as some cells could rather flow with the water through the thousands of water micro-channels that exist in the pressing equipment. The extraction of oil from microalgae biomass using mechanical pressing equipment is not easily achieved as some cells could flow on moisture of many water micro- channels. Improvement of pressing equipment is required to avoid loss of biomass and improve efficacy of the process. The suitability of extraction technology is heavily dependent of the microalgal strain selected and needs to be optimised as has been done for many higher plants [40]. In the future, possible innovations could help to overcome the inefficiencies of mechanical pressing technologies. These may include genetically modifying algal strains to have weaker cell walls that can be broken under lower pressures or low-heat pretreatment [116]. Mechanical pressing for microalgal process engineering can be applied at both small scale as well as large scale for biodiesel production.

5.2. Solvent extraction

Oil extraction using mechanical pressing can give oil yields in the range of 70–75% of the dried biomass. The microalgal oil can be extracted using chemical such as *n*-hexane, chloroform, benzene, diethyl ether and ethanol. *n*-Hexane is the most commonly used solvent which is primarily mixed with microalgae biomass paste then is distilled to obtain the microalgal oil [112]. After extracted microalgae oil has been fractionated to get different classes of lipids or pure components of individual lipids or fatty acids. Extraction of hydrocarbons from the microalgae strain *Botryococcus* sp. using ethyl acetate is the another example.

Crude lipids can be efficiently extracted from dried microalgal biomass by the Folsch and Soxhlet extraction methods using solvents. EPA, arachidonic acid (AA) and docosohexaenoic acid (DHA) can be extracted from various microalgae using different solvent such as chloroform, ethanol, n-hexane, and diethyl ether [45]. The advantage of using these solvents for lipid extraction is that they are inexpensive and very efficient and are commonly used for oil extraction. Extraction and refining oil from microalgal biomass with some modification using n-hexane as a solvent is now being explored for its efficiency in recovering oil from algal cells at industrial scale. Cell disruption generates rather a wide distribution of cell debris, of various particle sizes, that need to be removed. Adjustments in equipment design and operating conditions are necessary in order to process for single cell oil produc-

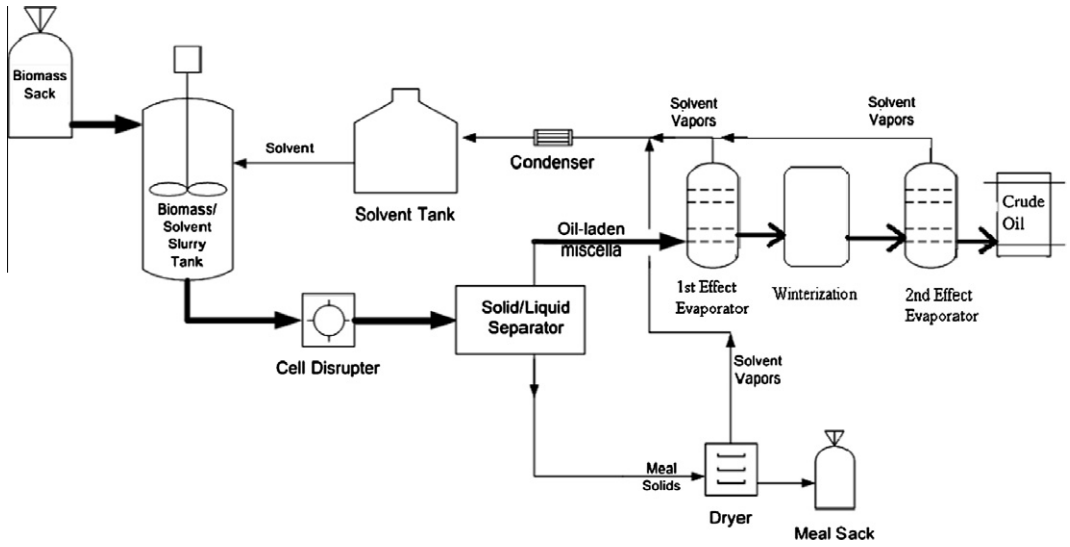

Fig. 2. Schematic diagram illustrating the downstream processing, crude oil extraction, and purification of microalgal lipids [117].

tion (Fig. 2) [117]. The oil dissolves in n-hexane and the biomass can be filtered out from the medium through distillation by either modified Folsch method or soxhlet extractor methods. In both methods, a mixture of solvents such as methanol and chloroform/hexane are generally used in varying proportions. The combination of polar and non-polar solvents enhances the extraction of both polar and non-polar lipids. For separation of microalgae metabolites such as beta-carotene, astaxanthin and essential fatty acids from microalgae biomass solvent extraction is the widely used method [45]. Microalgae species *Porphyridium cruentum, P. Tricoruntum, I. galbana, M. subterraneous* and other microalgae species has been described for fatty acid extraction [47,111].

5.3. Supercritical fluid extraction

Supercritical fluid extraction has proved effective method in the separation of oil extraction [113]. The fundamental difference between this and other methods for lipid extraction from microalgae is the lack of catalyst requirement. CO_2 is first heated and compressed until it reaches up to the liquid–gas state or above its critical point. It is then added to the harvested microalgae, and acts as a solvent. Mendes et al. [113], reported super critical extraction method to obtain hydrocarbons from *Botryococcus braunii* and lipid from *Skeletonema*.

The liquefaction of a solvent for supercritical extraction is often an energy-intensive process. The temperature and pressure (critical point) at which the fluid liquefies varies depending on the type of solvent used omit and incoporate, which would determine respective energy inputs. The process has the efficiency and ability to isolate oil components leading to the extraction of almost 100% of the oils [22,118].

5.4. Transesterification

Fatty acid methyl esters obtained by transesteritication can be used as an alternative fuel for diesel engines. Transesterification is a simple chemical reaction commonly used to make bio-oils less viscous, turning them into biodiesel. Crude microalgal oil is especially high in viscosity, thus requiring conversion to lower molecular weight constituents in the form of fatty acid alkyl esters. Transesterification converts raw microalgal lipid (triacyleglycerols/free fatty acids) into renewable, non-toxic and biodegradable biodiesel for direct consumption by unmodified diesel engines [28]. The reaction occurs as an equilibrium reaction and thus requires the supply of excess alcohol to maintain equilibrium shift towards the product and improve reaction rate [119].

The amount of resources available for transesterification depends on two factors: biomass yield and lipid content. Biomass yield provides a measure of photosynthetic efficiency in converting sunlight into organic matter. Lipid content, as a fraction of total biomass, represents the amount of hydrocarbons and triglycerides formed as storage products resulting from photosynthetic metabolism [120]. Transesterification also known as alcoholysis is a reaction involving the parent oil with a short chain alcohol by displacement of alcohol to form an ester and glycerol. During transesterification the common alcohols generally used include methanol, ethanol, propanol, butanol, and amyl alcohol. Generally methanol and ethanol are utilised; however methanol is preferred as it has physical and chemical advantages and low-cost. Fatty acid methyl esters (FAM) and Glycerol are the reaction products of transesterification [80].

5.4.1. Catalyst

Catalysts that are used in the transesterification reaction are acids, bases or enzymes. Base catalysis is a faster reaction but is limited by the free fatty acids content. Kaieda et al. [121] have reported on the kinetics of triglyceride transesterification with methanol, i.e. methanolysis, catalyzed by Ryzopus oryzae lipase appears to be in accordance with a successive reaction mechanism. Transesterification of triglycerides and partial glycerides are reacted in the presence of an effective water soluble lipase to produce partial glycerides and free fatty acids. Monoglyceride, Diglyceride are inter mediates, FAME are synthesised from free fatty acids and methanol. In enzyme catalyzed methanolysis free fatty acids can be converted easily to methyl esters. Free fatty acids content in the region of 20–50% is responsible for saponification during base catalyzed transesterification [122].

5.4.2. In situ algal biomass transesterification

In situ transesterification is an alternative to the conventional process, which is emerging technique that has the potential for the reduction of processing units and costs of the fuel conversion process. The *in situ* process facilitates the conversion of the biomass oil to fatty acid alkyl esters (FAAE) directly from the oil bearing biomass [123]. Harrington and D'Arcy-Evans [124] were the first researchers to demonstrate this method with sunflower seeds

as feedstock. Increasing biodiesel production yields compared to conventional method up to 20% increased through *in situ* transesterification was achieved by Ehimen et al. [123]. Haas et al. [125], have reported that the use of *in situ* transesterification eliminates the solvent extraction steps required as compared to the conventional method.

In situ transesterification is carried out by adding dried microalgae samples to a methanol/potassium hydroxide solution and then ultrasonicating the mixture. The above mentioned step minimises the transesterification reaction time. The reacted biomass sample could be separate for biodiesel from the cell debris, glycerin and excess of methanol using the centrifugation method. This biodiesel production method can thus potentially reduce the overall process cost thereby aiding in the simplification of the fuel conversion process. The final fuel product cost will also be lowered as compared to physical methods. The comparative biodiesel yields from *in situ* transesterification are higher than the conventional route biodiesel production [124]. The *in situ* transesterification method controls process wastes and pollution [125].

Microalgal biodiesel production is still in the research and development stages and as previously mentioned the oils extracted from microalgal cells have been extensively investigated for fuel production. It is desirable to reduce the viscosities and increase the fluidity of microalgal oil using downstream process like transesterification. Vital steps need to be critically analysed at each stage (Fig. 3). Direct esterification may pose some difficult challenges but however, laboratory scale *in situ* transesterification gave better results as previously investigated [123]. Some of the challenges experienced are: (a) increasing the volume of reacting alcohol, (b) for improved conversions of fatty acid methyl ester (FAME) temperature control is needed (c) stirring speed in the reaction vessel for biodiesel formation, (d) a strong negative influence on the equilibrium of FAME yield by moisture content of microalgae biomass, (e) inhibited the *in situ* transesterification process when the biomass water content is greater than 115% w/w (based on oil weight). This design and modelling could give effective results at industrial scale biodiesel production from microalgae.

5.4.3. Pyrolysis

Currently biodiesel production based on transesterification has been of major interest for biodiesel production. Pyrolysis is another alternative effective option for processing biomass into biofuels. Pyrolysis is a thermal cracking gasification method involving large amounts of energy for thermal degradation. Thermal degradation and/or cracking are promising routes to obtain the triglycerides and other organic compounds from the biomass feedstock. The process uses heat in the absence of oxygen to give simpler molecules, which are alkanes, alkenes, aromatics, carboxylic acids and among others [126]. Pyrolysis of microalgal biomass to produce liquid fuel was first demonstrated in Germany in 1986. It has been reported that the catalytic pyrolysis method could yield a mixture of different hydrocarbon molecules (gasoline) with high content of aromatic hydrocarbon and octane number [127]. Raveendran and Ganesh [128], reported that various biomasses have been considered as good renewable sources for potential feedstock for pyrolysis to produce large amount of fuels including oil and gas for internal combustion engines, major source of energy for power stations and heating suppliers. The advantage of biofuels is the low sulfur emission as compared to fossil fuels. Microalgae biomass can be converted into bio-oil using pyrolysis processes [129]. Much

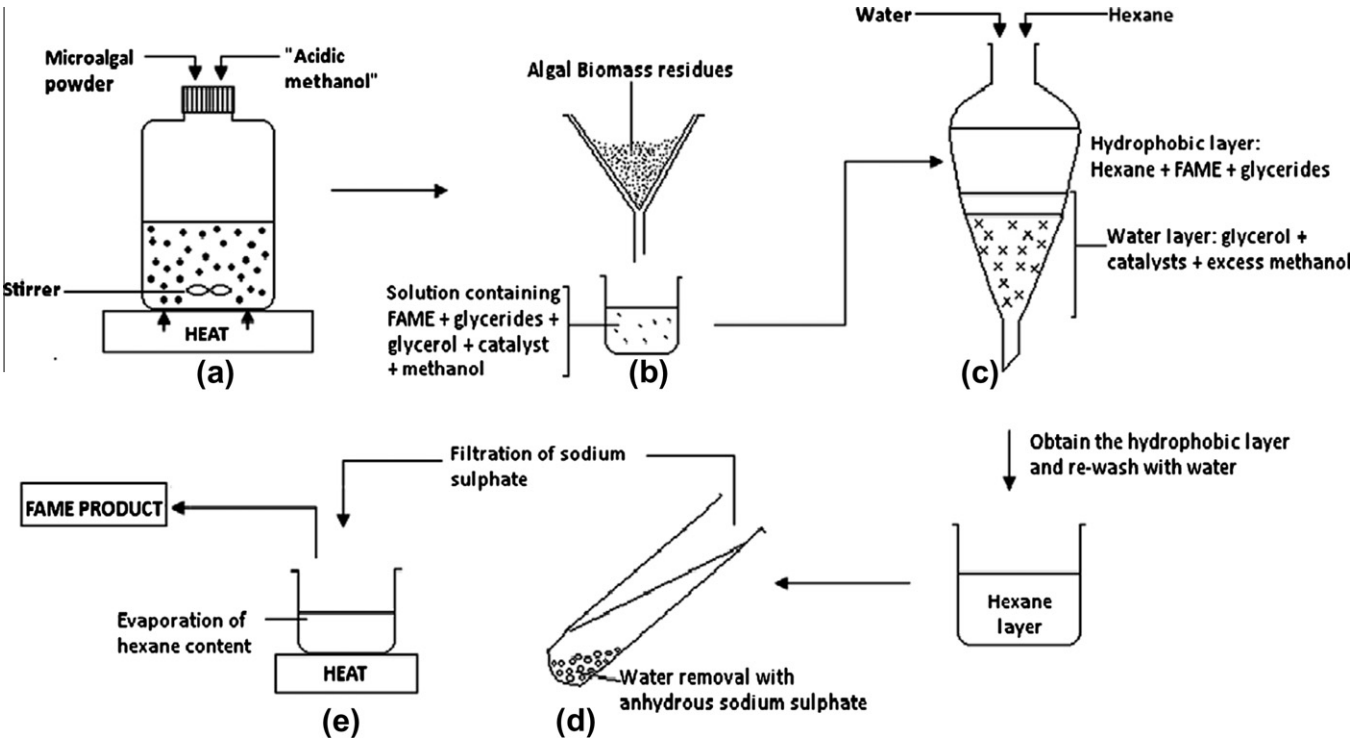

Fig. 3. Schematic presentation of the *in situ* transesterification [123]. (a) The reaction vessel is allowed to stand for 1 h to enable its contents to settle. The reaction mixture is filtered and the residues washed twice by re-suspension in methanol (30 ml) for 10 min to recover any traces of FAME product left in the residues. (b) Water (50 ml) is added to the filtrate, to facilitate the separation of the hydrophilic components of the extract, and then transferred to a separating funnel. (c) Further extraction of the FAME product is achieved by extracting three times for 15 min using 30 ml of hexane and the pooled hexane extracts are washed with water (to remove left-over traces of the acidic catalyst and methanol), separated, and then dried over anhydrous sodium sulphate. (d) The FAME product is then filtered and evaporated to obtain the FAME yields. (e) Duplicate experiments are carried out for each parameter investigated. The SG of the extracted FAME products are then determined and compared with that of the microalgae oil to monitor the extent of the conversion process.

Table 2
Comparison of properties of biodiesel from microalgal oil and diesel fuel and ASTM biodiesel standard (adapted from [131]).

Properties	Biodiesel from microalgal oil	Diesel fuel (EN 590:1999)	ASTM D6751 07b	EN 14214:2008
Density (kg/l)	0.864	0.838	–	0.86–0.90
Viscosity (mm^2/s, cSt at 40 °C)	5.2	2.0–4.5	1.9–6	3.5–5
Flash point (°C)	115	>55	>93	>101
Solidifying point (°C)	−12	−50 to 10	–	–
Cold filter plugging point (°C)	−11	*	*	*
Acid value (mg KOH/g)	0.374	Max 0.5	Max 0.5	
Heating value (MJ/kg)	41	40 – 45	–	–
H/C ratio	1.81	1.81	–	–
Cetane number		51 min	47 min	51 min

* Country specific.

research on microalgae biomass pyrolysis has been carried out during the past two decades, including *Oscillatoria tenuis* (Blue green algae) and *Chlorella protothecoides* (Green algae) [130]), *Chlorella protothecoides* (Green algae) and *Microcystis aeruginosa* (Blue green algae) [129,131], *Spirulina platensis* and *Chlorella protothecoides* [132], *Dunaliella teriolecta* [133]. *Chlorella muelleri* and *synechococcus* [134]. Mohan et al. [135], reported that the heating rate, temperature and retort atmosphere are the deciding factors for different fuel and chemical properties of pyrolysis products. Bio-oil yield is maximized by faster heating rates to a temperature of about 500 °C. Microalgae have higher photosynthetic efficiency, larger biomass, faster growth and higher content of components thus preferred over plants for pyrolysis. There are still some problems in the process of producing fuels from microalgae by pyrolysis [129]. As previously discussed, there are challenges to microalgal biomass harvesting at an economically feasible cost (both laboratory and large scale production). The search for cost-effective and efficient biomass separation is ongoing. Commercial fast pyrolysis installations using microalgae may significantly reduce the current cost of fuels production. A thorough understanding of thermal behaviour and biomass pyrolysis kinetics is required for proper design and operation of pyrolysis conversion systems. This may be attained by the use of Thermo gravimetric analysis.

5.5. Biodiesel quality

5.5.1. ASTM and EU standards

The acceptability of biodiesel from microalgae as a substitute of fossil diesel fuel is strongly dependent on compliance with existing standards. The benchmark standards currently are those of the American Society of Testing and Materials (ASTM D6751 07b) and the European Union (EN 14214:2008). Many researchers have been studied the fuel properties of biodiesel such as viscosity, cetane number, cold filter, heating value, density, viscosity, flash point, plugging point and solidifying point [19,136–139]. Table 2 shows the ASTM biodiesel standard a comparison of biodiesel from microalgal and diesel fuel properties [137]. Biodiesel produced from microalgal oil, has a cold filter plugging point of −11 °C, which is much lower than the diesel fuel. The physical properties of microalgal oil in general are comparable to petro diesel fuel (Table 2). Biodiesel production using chemical transesterification with feedstock oil is a simple, straight-forward process and glycerol formed as a by-product.

The extracted biodiesel or fatty acid methyl esters after the reaction can be considered as raw because it mixed with alcohol and soap-like numerous contaminants. Consequently it needs to be further concentrated and processed to remove these contaminants in order to must meet the American Society of Testing Materials and materials (ASTM) standards quality fuel for biodiesel. Biodiesel of a poor standard may negatively affect the diesel engine as well as nullifying engine manufacturer warranties.

6. Biorefinery approach and utilisation of residual biomass

According to Niles [140], the biorefinery concept is an emerging research field which require scientists to contribute scientific knowledge. The biorefinery approach is an essential combination of technologies to give a zero waste process by conversion of biological raw materials into useful the industrial intermediates and final products. The main objectives are exploration of different perspectives and understanding of the biodiesel production and its key components. This provides the foundations of the next objective, which is to critically discuss the biorefinery approach from laboratory to large scale production concept. The energy intensive process of green biorefineries combines production of sustainable energy fuels and economical valuable chemical products are recovery from biomass resources. This concept is a collection of processes that result in an energy efficient process. Residual biomass post lipid extraction can be significantly improved by using the biorefinery concept to produce an end product, or products of value.

Microalgal species produce biofuels and other chemical compounds by harvesting sunlight and fixing CO_2. The fixed CO_2 within these cells, through various metabolic pathways leads to the formation of lipids which after esterification produce biodiesel [141]. Residual biomass can be used in a variety of ways such as food industry, aquaculture, pharmaceutical, natural antioxidants, nutritional, enzymes, protein, carbohydrate, pigments, biogas, bioethanol and biohydrogen production and bio-oil production via pyrolysis. Microalgae are the most efficient primary producers of biomass. They produce a variety of novel compounds of which more than 15,000 novel compounds have been chemically determined [142]. Microalgae have high physiological diversity. A microalgal biorefinery can simultaneously produce biodiesel, animal feed, biogas and electrical power.

Biorefinery products improve cost effectiveness of biodiesel production. The low biomass concentration in the microalgal culture due to a number of factors such as insufficient light, nutrients supplement, space, insufficient oil content, harvesting, biochemical separation from biomass and drying of harvested algal biomass from high volume of water would be an energy-consuming process are the major obstacles between microalgae biodiesel production from laboratory to large scale production [143]. Biodiesel production integrated with the biorefinary approach is desirable due the ability of production of a wide range of chemicals and fuels. Hi-tech industrial photo-bioreactors, with high photosynthetic activities and the utilisation of low cost harvesting and drying methods such as chemical flocculation, biological flocculation, low pressure shelf drying, drum drying and spray drying are being utilised for biomass processing post cultivation.

Large scale integrated biorefinery has been categorised into 3 groups: (1) flexibility of inputs (2) processing capabilities and (3) product generation. The main aim of biorefinery from large-scale

production is to produce high-value products [144]. Recycling waste streams is also a key aspect of the biorefinery concept [145]. Microalgae biomass is rich in nutrients especially nitrogen, phosphorus, carbohydrates and proteins after biodiesel production. These nutrients can be recovered and converted by biorefinery to produce fertilizers or animal feeds [146]. Macro nutrients in the form of phosphorus and nitrogen can be attained from anaerobic digestion of waste biomass in the biorefinery process. These nutrients may be recycled to support the microalgal growth [147]. Anaerobic digestion process is appropriate in 70–80% high moisture organic waste form biorefinery production. Wet microalgal biomass can be digested aerobically to produce methane and biogas [10].

Microalgae have historically been used for variety of purposes. The production of astaxanthin from microalgae is an example of a mature industry that has evolved from prior uses. The large product range of microalgae makes it a perfect candidate feedstock for a biorefinery concept. The biorefinery products derived from residual microalgae biomass utilisation have potential to produce various products such as biomass-based chemicals. It is likely that these feedstocks will be a primary source of future industrial chemicals [148]. Many valuable products extracted by integrated microalgal product system from the same microalgal biomass thus making biodiesel production viable. Extensive research work has been conducted for protein therapeutics such as antibodies and cytokines from microalgae [149]. The considerable research, development, demonstration and commercialization are required to make large scale biorefineries a reality. This biorefinery approach in large scale production will reduce cost in future biodiesel production. The biorefinery approach has the potential of contributing to a favourable techno-economic status when evaluating the production of biodiesel from microalgae.

7. The significance of life cycle assessment (LCA) for microalgal biodiesel production

Because of its ability to exhibit maximum photosynthetic efficiency and high growth rates, the use of lipid rich microalgae with diverse lipid profiles as a possible alternative, sustainable feedstock for biodiesel production has become a global phenomena. Biomass cultivation, harvesting, drying, oil extraction and transesterification are high energy consuming processes which may work against this advantage. The energy production capacity of microalgae needs to be determined in order to assess the process has a positive energy balance. Since the 1970s many algal trial studies have evaluated energy balances. The results have varied considerably due to complexity of algal cultivation and insufficient field data. Furthermore life cycle analysis technology was still not a mature technology at time which many of the studies were carried out [150].

Life cycle assessment (LCA) or 'cradle to grave' analysis attempts to quantify the environmental impacts of all the processes that form part of provision of goods or services, in line with the ISO14040 standard. Life cycle assessment gives us an overall picture of the superior quality of energy dynamics reliability and environmental impacts and hence, can help in the decision-making process for implementation of the potential algae biodiesel production [151]. Total greenhouse gas emissions and energy utilisation potential are the two categories of greatest relevance to biofuels production. LCA attempts to quantify actual energy inputs in the growth of the biomass including energy all the energy used to make the nutrients, harvest, extract oil and transesterification. The energy used to construct and run photobioreactors are also taken into consideration [24]. The LCA is therefore a "compilation and evaluation of the energy inputs and outputs and the potential environmental impacts of a product system such as microalgal biodiesel production throughout its life cycle" [152]. An evaluation of energy and emission balance for biodiesel production and use is essential to the identification of energy utilisation and emission reductions. This can only be done using a systematic approach to investigate the entire production cycle such as LCA [153].

Microalgae offer great potential for value added products, including the production of biodiesel, but the process requires a great deal of work at pilot scale, ensuring carbon neutrality and commercial viability. Globally, LCA pilot scale studies are scarce, a consequence of a lack of multidisciplinary approach and insufficient novel technologies available in the public domain. Current biological research should aim to increase our understanding of algal biosynthetic pathways and the corresponding genomics to be applied in the new biotechnological paradigm. The work of engineers on the other hand, would develop industrial bioprocessing systems to achieve an economically feasible value added product. In doing this, integration of biology, engineering [24] and agricultural economics will be essential for the commercialization of biodiesel. Literature is of the general opinion that the energy balance is only slightly favourable for the production and processing of microalgae [109]. Harvesting and drying of biomass are the largest energy sinks in the bioprocessing of algae for diesel. Some energy saving mechanisms may be implemented by the use of nutrient rich wastewater [80] or filtered seawater rather than freshwater which is a scarce global resource.

According to the literature, one of the most energy intensive processes in algal culture is the dewatering of microalgae. In the model of Lardon et al. [154], the energy required for the dewatering process accounted for 85% of the total energy consumption [109]. According to Sheehan et al. [95], a dryer requires 3556 kJ/kg (850 kcal/kg) of water removed. Dry algae have a density of 1 g/mL and filter press capture is about 90% efficient [155]. Life cycle analysis has given rise to debates regarding the impact of water usage amongst others. Utilisation of water per unit area as well as availability and of water in a particular area play a large part in this debate considering the criticism that large scale algae culturing utilised significant amounts of freshwater. It must be considered however that microalgae consume significantly lower amounts of freshwater than conventional feedstock when cultivated on wastewater or seawater [81,156]. To offset the extensive energy costs, several life cycle analyses of algal biodiesel from virtual production facilities have outlined several assumptions that have been utilised. Some of which include the addition of fertilizers and carbon dioxide to achieve high algal yields in open ponds. Contrary to the general assumption that capture of carbon dioxide from flue gas from power stations for the production of biofuels from microalgal cultivation, carbon credits cannot be gained due to the release of captured carbon dioxide in burning of the algae derived fuel. Carbon credits are however obtained indirectly in the saving of fossil fuels that would have been utilised in the absence of a biofuel alternative. Production of electricity by utilisation of spent biomass would accrue additional carbon credits as a result of not using fossil sources for electricity generation. GHG emissions at each stage of production must be calculated to allow determine CO2 mitigation as opposed to purely sequestration [7]. The net energy ratio (NER) is defined as the ratio of the total energy produced (energy content of the oil and residual biomass) over the energy content of cultivation system construction and material plus the energy required for all plant operations. Jorquera et al. [99] found that horizontal tubular photobioreactors have negative NERs and are thus economically unfeasible. Flat bed photobioreactors were found to have the best NER. The study assumed lipid content of 29.6% (dry wt. Lipid/dry wt. biomass). However did not consider the cost of harvesting and oil extraction. Fig. 4 illus-

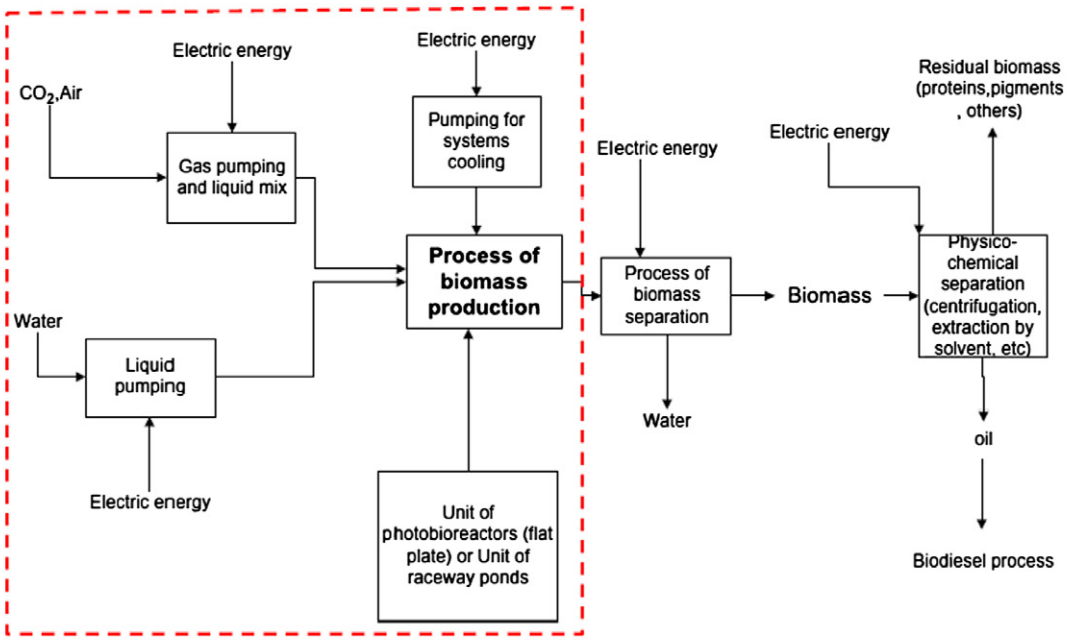

Fig. 4. Diagram illustrating process inputs and outputs plus the energy consumed by each system. The red line indicates the boundaries of the system [99]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

trates the process inputs and outputs plus the energy consumed by each system.

The US Department of Energy's National Algal Biofuels Technology Roadmap states that the commercial future of algae biofuels appears promising. Successful commercial algal biofuels production will however, depend strongly of the production of high value products. The improvement of technology for biofuels production is ongoing thereby bringing commercial production of biofuels ever closer to fruition [62].

A review of current literature leads to the conclusion that LCA studies are scarce. Furthermore a complete energy balance for closed microalgae to biofuel concept is not available. Further studies are required especially at pilot scale, to systematically record all energy input and the total quantity of biodiesel and value added products derived from the system. To offset the high costs of microalgal biodiesel production, and integrated alternative energy grid may supply and store energy to drive energy dependent equipment. Secondly, value added products can be extracted alongside the production of biodiesel probably from a second algal strain. An alternative to life cycle analysis is the mass balance/unit operation approach. According to Pfromm [152], the sustainability of algal biodiesel production is feasible; however the energy balance needs to be improved. This may be achieved by a less energy intensive method of nitrogen-based fertilizer production. Replacement of 0.1% of the US diesel demand could be replaced by cultivation of microalgae using a pond area of about 11 square miles (28,490,000 m^2) at a growth rate of 50 g m^{-2} (dry biomass) day^{-1}. For microalgal biodiesel to become a dependent source of green fuel, commercialization is dependent on information derived from life cycle assessment and related models which needs to be clearly defined and standardised for future comparative studies.

8. Conclusion

The global energy crisis has necessitated the search for alternative environmentally friendly energy sources. In the last 20 years attention had shifted to research in developing and optimising technology in biofuels as a sustainable alternative. In this regard biodiesel from microalgae has shown potential as a suitable alternative with added advantages over crop based biodiesel with one major comparative advantage being no impact on food security in addition to other advantages. However, translating laboratory and pilot scale findings to full scale commercial application still appears to be a hurdle with few credible reports of successes. Most open pond systems and photobioreactors for large scale commercial production was limited to culturing microalgae for high value products and not biodiesel. Some of the imperatives that need to be optimised for large scale application include strain selection and seed culture preparation, biomass and lipid yield optimisation, bioreactor configuration, physic-chemical parameters and most importantly harvesting and extraction of the lipid from the biomass.

Although choice of media is fundamental for the success of microalgal cultivation at pilot scale, the cost factor lends itself to investigation of waste substrates such as wastewater for the microalgal propagation. In this regard post-chlorinated wastewater of domestic origin has shown potential for large scale application. The two most common process configurations that are used for large scale application include raceway ponds and photobioreactors which each system having its inherent advantages and disadvantages. However, for large scale application open raceway ponds appear to be more favourable with one of the added advantages being the potential for CO_2 sequestration. With biomass harvesting being one of the major challenges at full scale application for producing

biodiesel from microalgae, some of the recovery methods being considered include filtration, centrifugation, gravity sedimentation, chemical flocculation and dissolved air flotation. However, the method of choice has to be selected based on a positive techno-economic evaluation also taking the energy balance into consideration. With regards to commercial ventures and claims thus far, most of the companies surveyed use closed systems (52%) for microalgal cultivation with the rest being open ponds (26%) and natural settings (22%).

The extraction of lipids from the biomass has also been another challenge when considering large scale application. Although mechanical pressing works well for oil seeds, currently the method of choice still involves solvent extraction. Other techniques such as supercritical fluid extraction are still being evaluated for large scale application. *In situ* transesterification is an emerging technology that has shown potential for processing unit and cost reduction at large scale. However the technology is still in its early stages and necessitates optimisation. A defining aspect of successful application of producing biodiesel from microalgae is to ensure that the final product quality is in compliance with existing global standards. In addition, adopting a biorefinery approach will certainly improve the techno-economics of the process.

References

[1] Balat M, Balat H. Progress in biodiesel processing. Appl Energy 2010;87:1815–35.
[2] Du W, Li W, Sun T, Chen X, Liu D. Perspectives for biotechnological production of biodiesel and impacts. Appl Microbiol Biotechnol 2008;79:331–7.
[3] Lapinskiene A, Martinkus P, Rebzdaite V. Eco-toxicological studies of diesel and biodiesel fuels in aerated soil. Environ Pollut 2006;142:432–7.
[4] Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M. Microalgal triacylglycerols as feedstocks for biofuels production: perspectives and advances. Plant J 2008;54:621–39.
[5] Hulatt CJ, Thomas DN. Productivity, carbon dioxide uptake and net energy return of microalgal bubble column photobioreactors. Bioresour Technol 2011;102:5775–87.
[6] Williams PJlB, Laurens LML. Microalgae as biodiesel & biomass feedstocks: review & analysis of the biochemistry, energetics & economics. Energy Environ Sci 2010;3:554–90.
[7] Campbell PK, Beer T, Batten D. Life cycle assessment of biodiesel production from microalgae in ponds. Bioresour Technol 2011;102:50–6.
[8] Chisti Y. Biodiesel from microalgae. Biotechnol Adv 2007;25:249–306.
[9] Lee DH. Algal biodiesel economy and competition among bio-fuels. Bioresour Technol 2011;102:43–9.
[10] 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;14:557–77.
[11] Metting F. Biodiversity and application of microalgae. J Ind Microbiol Biotechnol 1996;17:477–89.
[12] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. Renew Sustain Energy Rev 2010;14:217–32.
[13] Demirbas MF. Biofuels from algae for sustainable development. Appl Energy 2011;88:3473–80.
[14] Ahmad AL, Yasin NHM, Derek CJC, Lim JK. Microalgae as a sustainable energy source for biodiesel production: a review. Renew Sustain Energy Rev 2011;15:584–93.
[15] Antoni D, Zverlov VV, Schwarz WH. Biofuels from microbes. Appl Microbiol Biotechnol 2007;77:23–35.
[16] Feng Y, Li C, Zhang D. Lipid production of Chlorella vulgaris cultured in artificial wastewater medium. Bioresour Technol 2011;102:101–5.
[17] Gordon JM, Polle JEW. Ultrahigh bioproductivity from algae. Appl Microbiol Biotechnol 2007;76:969–75.
[18] Luque R, Lovett JC, Datta B, Clancy J, Campelo JM, Romero AA. Biodiesel as feasible petrol fuel replacement: a multidisciplinary overview. Energy Environ Sci 2010;3:1706–21.
[19] Ma F, Hanna MA. Biodiesel production: a review. Bioresour Technol 1999;70:1–15.
[20] Mairet F, Bernard O, Masci P, Lacour T, Sciandra A. Modelling neutral lipid production by the microalga Isochrysis aff. galbana under nitrogen limitation. Bioresour Technol 2011;102:142–9.
[21] Huber GW, Iborra S, Corma A. Synthesis of transportation fuels from biomass: chemistry, catalysts, and engineering. Chem Rev 2006;106:4044–98.
[22] Demirbas A, Demirbas MF. Importance of algae oil as a source of biodiesel. Energy Convers Manage 2011;52:163–70.
[23] Demirbas A. Biofuels sources, biofuel policy, biofuel economy and global biofuel projections. Energy Convers Manage 2008;49:2106–16.
[24] Scott SA, Davey MP, Dennis JS, Horst I, Howe CJ, Lea-Smith DJ, et al. Biodiesel from algae: challenges and prospects. Curr Opin Biotechnol 2010;21:1–10.
[25] Sarin R, Kumar R, Srivastav B, Puri SK, Tuli DK, Malhotra RK, et al. Biodiesel surrogates: achieving performance demands. Bioresour Technol 2009;100:3022–8.
[26] Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH, Posten C, et al. Second generation biofuels: high-efficiency microalgae for biodiesel production. BioEnergy Res 2008;1:20–43.
[27] Demirbas A. Competitive liquid biofuels from biomass. Appl Energy; in press, doi:http://dx.doi.org/10.1016/j.apenergy.2010.07.016.
[28] Rawat I, Ranjith Kumar R, Mutanda T, Bux F. Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. Appl Energy 2011;88:3411–24.
[29] Ranjith-Kumar R, Rao PH, Subramanian VV, Sivasubramanian V. Enzymatic and non-enzymatic antioxidant potentials of Chlorella vulgaris grown in effluent of a confectionery industry. J Food Sci Technol 2011.
[30] Huang G, Chen F, Wei D, Zhang X, Chen G. Biodiesel production by microalgal biotechnology. Appl Energy 2010;87:38–46.
[31] Craggs RJ. Advanced integrated wastewater ponds. In: Shilton A, editor. Pond treatment technology, IWA scientific and technical report series. London: IWA; 2005. p. 282–310.
[32] Park JB, Craggs RJ, Shilton AN. Wastewater treatment high rate algal ponds for biofuel production. Bioresour Technol 2011;102:35–42.
[33] Oswald WJ. Algal production – problems, achievements and potential. In: Shelef G, Soeder CJ, editors. Algae Biomass. Amsterdam, Netherlands: Elsevier North/Holland/Biomedical Press; 1980. p. 1–8.
[34] Oswald WJ. Micro-algae and waste-water treatment. In: Borowitzka MA, Borowitzka LJ, editors. Microalgal Biotechnology. New York, USA: Cambridge University Press; 1988. p. 305–28.
[35] Kong QX, Li L, Martinez B, Chen P, Ruan R. Culture of microalgae Chlamydomonas reinhardtii in wastewater for biomass feedstock production. Appl Biochem Biotechnol 2010;160:9–18.
[36] Sialve B, Bernet N, Bernard O. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnol Adv 2009;27:409–16.
[37] Zhang J, Chen S, Yang R, Yan Y. Biodiesel production from vegetable oil using heterogenous acid and alkali catalyst. Fuel 2010;89:2939–44.
[38] Nylund NO, Aakko-Saksa P, Sipilä K. Status and outlook for biofuels, other alternative fuels and new vehicles. Finland. Helsinki: Finland: Julkaisija-Utgivare; 2008.
[39] Benemann JR. Overview: Algae Oil to Biofuels. NREL-AFOSR Workshop, Algal Oil for Jet Fuel Production. Arlington: Benemann Associates; 2008.
[40] Cooney MJ, Young G, Pate R. Bio-oil from photosynthetic microalgae: case study. Bioresour Technol 2011;102:166–77.
[41] Griffiths MJ, Harrison STL. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. J Appl Phycol 2009;21:493–507.
[42] Pittman JK, Dean AP, Osundeko O. The potential of sustainable algal biofuel production using wastewater resources. Bioresour Technol 2011;102:17–25.
[43] Chen CY, Yeh KL, Aisyah R, Lee DJ, Chang JS. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Bioresour Technol 2011;102:71–81.
[44] Mutanda T, Ramesh D, Karthikeyan S, Kumari S, Anandraj A, Bux F. Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production. Bioresour Technol 2011;102:57–70.
[45] Grima EM, Belarbi E-H, Fernández FGAn, Medina AR, Chisti Y. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol Adv 2003;20:491–515.
[46] Lam MK, Lee KT. Microalgae biofuels: a critical review of issues, problems and the way forward. Biotechnol Adv 2012;30:673–90.
[47] Belarbi EH, Molina E, Chisti Y. A process for high yield and scaleable recovery of high purity eicosapentaenoic acid esters from microalgae and fish oil. Enzyme Microbial Technol 2000;26:516–29.
[48] Norton TA, Melkonian M, Anderson RA. Algal biodiversity. Phycologia 1996;35:308–26.
[49] Xin L, Hong-Ying H, Jia Y. Lipid accumulation and nutrient removal properties of a newly isolated freshwater microalga, Scenedesmus sp. LX1, growing in secondary effluent. N Biotechnol 2010;27:59–63.
[50] Munoz R, Guieysse B. Algal–bacterial processes for the treatment of hazardous contaminants: a review. Water Res 2006;40:2799–815.
[51] Anderson RA, Kawachi M. Traditional microalgal isolation techniques. In: Anderson RA, editor. Algal culturing techniques. London, UK: Elsevier Academic Press; 2005. p. 83–100.
[52] Amaro HM, Guedes AC, Malcata FX. Advances and perspectives in using microalgae to produce biodiesel. Appl Energy 2011;88:3402–10.
[53] Mansour MP, Frampton DMF, Nichols PD, Volkman JK, Blackburn SI. Lipid and fatty acid yield of nine stationary-phase microalgae: applications and unusual C24–C28 polyunsaturated fatty acids. J Appl Phycol 2005;17:287–300.
[54] Guschina IA, Harwood JL. Lipids and lipid metabolism in eukaryotic algae. Prog Lipid Res 2006;45:160–86.
[55] Miyamoto K. Oil Production. Renewable biological systems for alternative sustainable energy production Osaka, Japan: FAO; 1997.
[56] Takagi M, Karseno, Yoshida T. Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae Dunaliella Cells. J Biosci Bioeng 2006;101:223–6.

[57] Packer A, Li Y, Andersen T, Hu Q, Kuang Y, Sommerfeld M. Growth and neutral lipid synthesis in green microalgae: a mathematical model. Bioresour Technol 2011;102:111–7.
[58] Chen W, Sommerfeld M, Hu Q. Microwave-assisted Nile red method for in vivo quantification of neutral lipids in microalgae. Bioresour Technol 2011;102:135–41.
[59] Pruvost J, Van Vooren G, Le Gouic B, Couzinet-Mossion A, Legrand J. Systematic investigation of biomass and lipid productivity by microalgae in photobioreactors for biodiesel application. Bioresour Technol 2011;102:150–8.
[60] Li Y, Han D, Sommerfeld M, Hu Q. Photosynthetic carbon partitioning and lipid production in the oleaginous microalga Pseudochlorococcum sp. (Chlorophyceae) under nitrogen-limited conditions. Bioresour Technol 2011;102:123–9.
[61] Meng X, Yang J, Xu X, Zhang L, Nie Q, Xian M. Biodiesel production from oleaginous microorganisms. Renew Energy 2009;34:1–5.
[62] Singh J, Gu S. Commercialization potential of microalgae for biofuels production. Renew Sustain Energy Rev 2010;14:2596–610.
[63] Johnson DA, Sprague S. Liquid fuels from microalgae. Intersociety energy conversion engineering conference. Philadelphia, Pennsylvania: US Department of Energy; 1987.
[64] Singh A, Nigam PS, Murphy JD. Renewable fuels from algae: an answer to debatable land based fuels. Bioresour Technol 2011;102:10–6.
[65] Illman AM, Scragg AH, Shales SW. Increase in Chlorella strains calorific values when grown in low nitrogen medium. Enzyme Microbial Technol 2000;27:631–5.
[66] Ge Y, Liu J, Tian G. Growth characteristics of Botryococcus braunii 765 under high CO_2 concentration in photobioreactor. Bioresour Technol 2011;102:130–4.
[67] de-Bashan LE, Hernandez JP, Morey T, Bashan Y. Microalgae growth-promoting bacteria as "helpers" for microalgae: a novel approach for removing ammonium and phosphorus from municipal wastewater. Water Res 2004;38:466–74.
[68] Suh IS, Lee CG. Photobioreactor engineering: design and performance. Biotechnol Bioprocess Eng 2003;8:313–21.
[69] Borowitzka MA. Commercial production of microalgae: ponds, tanks, tubes and fermenters. J Biotechnol 1999;70:313–21.
[70] Watanabe MM, Kawachi M, Hiroki M, Kasai F. NIES collection list of strains. Sixth Ed., 2000, Microalgae and Protozoa. 6th ed. Japan: National Institute for, Environmental Studies; 2000. p. 159.
[71] Uduman N, Bourniquel V, Danquah MK, Hoadley AFA. A parametric study of electrocoagulation as a recovery process of marine microalgae for biodiesel production. Chem Eng J 2011;174:249–57.
[72] Grobbelaar JU. From laboratory to commercial production: a case study of a *Spirulina (Arthrospira)* facility in Musina, South Africa. J Appl Phycol 2009;21:523–7.
[73] Elsey D, Jameson D, Raleigh B, Cooney MJ. Fluorescent measurement of microalgal neutral lipids. J Microbiol Methods 2007;68:639–42.
[74] Abu GO, Ogbonda KH, Aminigo RE. Optimization studies of biomass production and protein biosynthesis in a *Spirulina* sp. isolated from an oilpolluted flame pit in the Niger Delta. Afr J Biotechnol 2007;6:2550–4.
[75] Chisti Y. Biodiesel from microalgae beats bioethanol. Trends Biotechnol 2008;26:126–31.
[76] Viswanath B, Mutanda T, White S, Bux F. The microalgae – a future source of biodiesel. Dynamnic Biochem, Process Biotechnol Mole Biol 2010;4:37–47.
[77] Park JBK, Craggs RJ, Shilton AN. Wastewater treatment high rate algal ponds for biofuel production. Bioresour Technol 2011;102:35–42.
[78] Bilanovic D, Andargatchew A, Kroeger T, Shelef G. Freshwater and marine microalgae sequestering of CO_2 at different C and N concentrations – response surface methodology analysis. Energy Convers Manage 2009;50:262–7.
[79] Radman EM, Reinehr CO, Costa JAV. Optimization of the repeated batch cultivation of microalga *Spirulina platensis* in open raceway ponds. Aquaculture 2007;265:118–26.
[80] Mutanda T, Karthikeyan S, Bux F. The utilization of post-chlorinated municipal domestic wastewater for biomass and lipid production by Chlorella spp. under batch conditions. Appl Biochem Biotechnol 2011. http://dx.doi.org/10.1007/s12010-011-9199-.
[81] Yang J, Xu M, Zhang X, Hu Q, Sommerfeld M, Chen Y. Life-cycle analysis on biodiesel production from microalgae: water footprint and nutrients balance. Bioresour Technol 2011;102:159–65.
[82] Zhou W, Li Y, Min M, Hu B, Chen P, Ruan R. Local bioprospecting for high-lipid producing microalgal strains to be grown on concentrated municipal wastewater for biofuel production. Bioresour Technol 2011;102:6909–19.
[83] Zamalloa C, Vulsteke E, Albrecht J, Verstraete W. The techno-economic potential of renewable energy through the anaerobic digestion of microalgae. Bioresour Technol 2011;102:1149–58.
[84] Hosikian A, Lim S, Halim R, Danquah MK. Chlorophyll extraction from microalgae: a review on the process engineering aspects. Int J Chem Eng 2010;2010:1–12.
[85] Rodolfi L, Zittelli GC, Bassi N, Padovani G, Biondi N, Bonini G, et al. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol Bioeng 2009;102:100–12.
[86] Harun R, Singh M, Forde GM, Danquah MK. Bioprocess engineering of microalgae to produce a variety of consumer products. Renew Sustain Energy Rev 2010;14:1037–47.
[87] Pulz O, Gross W. Valuable products from biotechnology of microalgae. Appl Microbiol Biotechnol 2004;65:635–48.
[88] Zeng X, Danquah MK, Chen XD, Lu Y. Microalgae bioengineering: from CO_2 fixation to biofuel production. Renew Sustain Energy Rev 2011;15:3252–60.
[89] Rawat I, Kumar RR, Mutanda T, Bux F. Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. Appl Energy 2011;88:3411–24.
[90] Norsker N-H, Barbosa MJ, Vermuë MH, Wijffels RH. Microalgal production — a close look at the economics. Biotechnol Adv 2011;29:24–7.
[91] Ashokkumar V, Rengasamy R. Mass culture of Botryococcus braunii Kutz. under open raceway pond for biofuel production. Bioresour Technol 2011.
[92] Amin S. Review on biofuel oil and gas production processes from microalgae. Energy Convers Manage 2009;50:1834–40.
[93] Davis R, Aden A, Pienkos PT. Techno-economic analysis of autotrophic microalgae for fuel production. Appl Energy 2011;88:3524–31.
[94] Stephens E, Ross IL, Mussgnug JH, Wagner LD, Borowitzka MA, Posten C, et al. Future prospects of microalgal biofuel production systems. Trends Plant Sci 2010;15:554–64.
[95] Sheehan J, Dunahay T, Benemann J, Roessler P. A look back at the US Department of Energy's aquatic species program—biodiesel from algae. Report NREL/TP-580–24190. National renewable energy laboratory, Golden, CO.; 1998.
[96] Richmond A. Principles for attaining maximal microalgal productivity in photobioreactors: an overview. Hydrobiologia 2004;512:33–7.
[97] Christenson L, Sims R. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnol Adv 2011. http://dx.doi.org/10.1016/j.biotechadv.2011.05.015.
[98] Chisti Y, Yan J. Energy from algae: current status and future trends Algal biofuels – a status report. Appl Energy 2011;88:3277–9.
[99] Jorquera O, Kiperstok A, Sales EA, Embiruçu M, Ghirardi ML. Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. Bioresour Technol 2010;101:1406–13.
[100] Day JG, Slocombe SP, Stanley MS. Overcoming biological constraints to enable the exploitation of microalgae for biofuels. Bioresour Technol 2011. http://dx.doi.org/10.1016/j.biortech.2011.05.033.
[101] Greenwell HC, Laurens LML, Shields RJ, Lovitt RW, Flynn KJ. Placing microalgae on the biofuels priority list: a review of the technological challenges. J Am Oil Chem Soc 2010;7:703–26.
[102] Ahmad AL, Mat Yasin NH, Derek CJC, Lim JK. Crossflow microfiltration of microalgae biomass for biofuel production. Desalination 2012;302:65–70.
[103] Borges L, Morón-Villarreyes JA, D'Oca MGM, Abreu PC. Effects of flocculants on lipid extraction and fatty acid composition of the microalgae Nannochloropsis oculata and Thalassiosira weissflogii. Biomass Bioenergy 2011;35:4449–54.
[104] Wu Z, Zhu Y, Huang W, Zhang C, Li T, Zhang Y, et al. Evaluation of flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium. Bioresour Technol 2012;110:496–502.
[105] Cheng Y-L, Juang Y-C, Liao G-Y, Tsai P-W, Ho S-H, Yeh K-L, et al. Harvesting of Scenedesmus obliquus FSP-3 using dispersed ozone flotation. Bioresour Technol 2011;102:82–7.
[106] Lam MK, Lee KT. Immobilization as a feasible method to simplify the separation of microalgae from water for biodiesel production. Chem Eng J 2012;191:263–8.
[107] Granados MR, Acien FG, Gomez C, Fernandez-Sevilla JM, Molina Grima E. Evaluation of flocculants for the recovery of freshwater microalgae. Bioresour Technol 2012;118:102–10.
[108] Bilad MR, Vandamme D, Foubert I, Muylaert K, Vankelecom IF. Harvesting microalgal biomass using submerged microfiltration membranes. Bioresour Technol 2012;111:343–52.
[109] Xu L, Brilman DWF, Withag JAM, Brem G, Kersten S. Assessment of a dry and a wet route for the production of biofuels from microalgae: energy balance analysis. Bioresour Technol 2011. http://dx.doi.org/10.1016/j.biotech.2011.01.066.
[110] Kleinschmidt P. Methods and micro economy of biodiesel production – new. Example through a business plan analysis for a biodiesel plant. Slovak University of Technology; 2010.
[111] Medina AR, Giménez A, Camacho FG, Pérez JAS, Grima EM, Gómez E. Concentration and purification of stearidonic, eicosapen taenoic, and docosahexaenoic acids from cod liver oil and the marine microalga Isochrysis galbana. J Am Oil Chem Soc 1995;72:575–83.
[112] Sazdanoff N. Modeling and simulation of the algae to biodiesel fuel cycle. Ohio, USA: The Ohio State University; 2006.
[113] Mendes RL, Fernandes HL, Coelho JP, Reis EC, Cabral JMS, Novais JM. Supercritical CO_2 extraction of carotenoids and other lipids from Chlorella vulgaris. Food Chem 1995;53:99–103.
[114] Liu ZY, Wang GC, Zhou BC. Effect of iron on growth and lipid accumulation in Chlorella vulgaris. Bioresour Technol 2008;99:4717–22.
[115] Song D, Fu J, Shi D. Exploitation of oil-bearing microalgae for biodiesel. Chin J Biotechnol 2008;24:341–8.
[116] Ryan C. Cultivating clean energy: the promise of algae biofuels. In: NRDC, editor. Washingtonl 2009. p. 89.
[117] Ratledge C, Streekstra H, Cohen Z, Fichtali J. Down-stream processing, extraction, and purification of single cell oils. In: Cohen Z, Ratledge C, editors. Single cell oils: microbial and algal oils. Champaign: AOCS Publishing; 2010. p. 179–97.
[118] Demirbas A. Production of biodiesel from algae oils. Energy Sources 2009;31:163–8.

[119] Singh SP, Singh D. Biodiesel production through the use of different sources and characterization of oils and their esters as the substitute of diesel: a review. Renew Sustain. Energy Rev 2010;14:200–16.
[120] Hill AM, Feinberg DA. Fuel from microalgae lipid products. Portland, Oregon Solar Energy Research Inst., Golden, CO; 1984. p. 17.
[121] Kaieda M, Samukawa T, Matsumoto T, Ban K, Koudo A, Shimada Y, et al. Biodiesel fuel production from plant oil catalyzed by Rhizopus olyzae lipase in a water-containing system without an organic solvent. J Biosci Bioeng 1999:627–31.
[122] Fukuda H, Kondo A, Noda H. Biodiesel fuel production by transesterification of oils. J Biosci Bioeng 2001:405–16.
[123] Ehimen EA, Sun ZF, Carrington CG. Variables affecting the + transesterification of microalgae lipids. Fuel 2010;89:677–84.
[124] Harrington KJ, D'Arcy-Evans C. A comparison of conventional and in situ methods of transesterification of seed oil from a series of sunflower cultivars. J Am Oil Chem Soc 1985;62:1009–13.
[125] Haas MJ, Scott KM, Foglia TA, Marmer WN. The general applicability of in situ transesterification for the production of fatty acid esters from a variety of feedstocks. J Am Oil Chem Soc 2007;84:963–70.
[126] Babu BV. Biomass pyrolysis: a state-of-the-art review. Biofuels Bioprod Biorefin 2008;2:393–414.
[127] Milne TA, Evans RJ, Nagle N. Catalytic conversion of microalgae and vegetable oils to premium gasoline, with shape-selective zeolites. Biomass 1990;21:219–32.
[128] Raveendran K, Ganesh K. Heating value of biomass and biomass pyrolysis products. Fuel 1996;75:1715–20.
[129] Miao X, Wu Q, Yang C. Fast pyrolysis of microalgae to produce renewable fuels. J Anal Appl Pyrol 2004;71:855–63.
[130] Wu Q, Zhang B, Grant NG. High yield of hydrocarbon gases resulting from pyrolysis of yellow heterotrophic and bacterially degraded Chlorella protothecoides. J Appl Phycol 1996;8:181–4.
[131] Miao X, Wu Q. Biodiesel production from heterotrophic microalgal oil. Bioresour Technol 2006;97:841–6.
[132] Peng W, Wu Q, Tu P, Zhao N. Pyrolytic characteristics of microalgae as renewable energy source determined by thermogravimetric analysis. Bioresour Technol 2001;77:1–7.
[133] Shuping Z, Yulong W, Mingde Y, Chun L, Junmao T. Pyrolysis characteristics and kinetics of the marine microalgae Dunaliella tertiolecta using thermogravimetric analyzer. Bioresour Technol 2010;101:359–65.
[134] Grierson S, Strezov V, Ellem G, Mcgregor R, Herbertson J. Thermal characterisation of microalgae under slow pyrolysis conditions. J Anal Appl Pyrol 2009;85:118–23.
[135] Mohan D, Pittman CU, Steele PH. Pyrolysis of wood/biomass for bio-oil: a critical review. Energy Fuels 2006;20:848–89.
[136] Al-Widyan MI, Al-Shyoukh AO. Experimental evaluation of the transesterification of waste palm oil into biodiesel. Bioresour Technol 2002;85:253–6.
[137] Antolin G, Tinaut FV, Briceno Y, Castano V, Perez C, Ramirez AI. Optimisation of biodiesel production by sunflower oil transesterification. Bioresour Technol 2002;83:111–4.
[138] Lang X, Dalai AK, Bakhshi NN, Reaney MJ, Hertz PB. Preparation and characterization of bio-diesels from various bio-oils. Bioresour Technol 2001;80:53–62.
[139] Vicente G, Martinez M, Aracil J. Integrated biodiesel production: a comparison of different homogeneous catalysts systems. Bioresour Technol 2004;92:297–305.
[140] Niles D. Biorefining Concepts Lift Off. Ethanol Producer Magazine 2005.
[141] Verma NM, Mehrotra SS, Shukla A, Mishra BN. Prospective of biodiesel production utilizing microalgae as the cell factories: a comprehensive discussion. Afr J Biotechnol 2010;9:1402–11.
[142] Cardozo KH, Guaratini T, Barros MP, Falcao VR, Tonon AP, Lopes NP, et al. Metabolites from algae with economical impact. Compar Biochem Physiol Toxicol Pharmacol: CBP 2007;146:60–78.
[143] Li Y, Horsman M, Wu N, Lan CQ, Dubois-Calero N. Biofuels from microalgae. Biotechnol Prog 2008;24:815–20.
[144] Taylor G. Biofuels and the biorefinery concept. Energy Policy 2008;36:4406–9.
[145] Ragauskas AJ, Williams CK, Davison BH, Britovsek G, Cairney J, Eckert CA, et al. A path forward for biofuels and biomaterials. Science 2006;311:484–9.
[146] Gouveia L, Oliveira AC. Microalgae as a raw material for biofuels production. J Ind Microbiol Biotechnol 2009;36:269–74.
[147] Phang SM, Miah MS, Yeoh BG, Hashim MA. Spirulina cultivation in digested sago starch factory wastewater. J Appl Phycol 2000;12:395–400.
[148] Haveren JV, Scott EL, Sanders J. Bulk chemical from biomass. Biofuels Bioprod Biorefin 2008;2:41–57.
[149] Pavlou AK, Reichert JM. Recombinant protein therapeutics-success rates, market trends and values to 2010. Nat Biotechnol 2004;22:1513–9.
[150] Sturm BSM, Lamer SL. An energy evaluation of coupling nutrient removal from wastewater with algal biomass production. Appl Energy 2011.
[151] Kadam KL. Environmental implications of power generation via coal microalgae cofiring. Energy 2001;27:905–22.
[152] Pfromm PH, Amanor-Boadu V, Nelson R. Sustainability of algae derived biodiesel: a mass balance approach. Bioresour Technol 2011;102:1185–93.
[153] Pant D, Singh A, Van Bogaert G, Gallego YA, Diels L, Vanbroekhoven K. An introduction to the life cycle assessment (LCA) of bioelectrochemical systems (BES) for sustainable energy and product generation: relevance and key aspects. Renew Sustain Energy Rev 2011;15:1305–13.
[154] Lardon L, Helias A, Sialve B, Steyer JP, Bernard O. Life-cycle assessment of biodiesel production from microalgae. Environ Sci Technol 2009;43:6475–81.
[155] Sander K, Murthy GS. Life cycle analysis of algae biodiesel. Int J Life Cycle Assess 2010;15:704–14.
[156] Groom MJ, Gray EM, Townsend PA. Biofuels and biodiversity: principle for creating better policies for biofuel production. Conserv Biol 2008;22:602–9.