8-14-2013

Biofuel from Microalgae – A Review on the Current Status and Future Trends

Rajee Olaganathan
Embry-Riddle Aeronautical University, olaganar@erau.edu

May Zaw Htet
James Cook University, Singapore Campus

Lim Yan Ling
James Cook University, Singapore Campus

Sek Hui Yun
James Cook University, Singapore Campus

International Journal of Advanced Biotechnology and Research
ISSN 0976-2612, Online ISSN 2278–599X,
Vol 4, Issue 3, 2013, pp 329-341
http://www.bipublication.com

BIOFUEL FROM MICROALGAE – A REVIEW ON THE CURRENT STATUS AND FUTURE TRENDS

May Zaw Htet, Lim Yan Ling, Sek Hui Yun and Olaganathan Rajee*

School of Business (Bachelor of Business and Environmental Sciences)
James Cook University, Singapore Campus, 600 Upper Thomson Road, Singapore, 574421
***Corresponding Author:** Email: rajeeom@gmail.com; rajee.olaganathan@jcu.edu.au
Tel: + 65-96513726

[Received-13/06/2013, Accepted-14/08/2013]

ABSTRACT

The constant reliance on fossil fuel energy resources is unsustainable, due to both depleting world reserves and increasing green house gas emissions associated with their use and thus there are dynamic research at the global level envisioned at developing alternative renewable and potentially carbon neutral solid, liquid and gaseous biofuels as alternative energy resources. The contemporary knowledge and technology predictions have proved that among the third generation biofuels especially those derived from microalgae are considered the best reasonable alternative energy resource compared to undeniable drawbacks of first and second generation biofuels. Moreover, its efficiency to sequester carbon from the atmosphere and industrial gases which can efficiently utilize the nutrients present in wastewater and industrial effluents. Therefore, culturing algae provide several benefits such as providing biomass for the production of biofuels to sequester the atmospheric carbon, removing the nutrients from the wastewater and is not competing with agricultural land, water resources and food crops. This study reviewed the technologies underneath the microalgae-to-biofuels processes, focusing on the biomass production, harvesting, conversion technologies, and the lipid extraction methods. The genetics and molecular biotechnology aspects have also been briefly discussed. Though the economical assessment of algal biofuels is not attractive, it suggests them to be environmentally better than the fossil fuels.
Key words: Biofuel, Micro algae, renewable energy, Alternative Energy, third generation biofuels

[1] INTRODUCTION

The world demand of fuel becomes more and more increased in recent years and there is no indication for decreasing trend of demand for fuel supply. Most of the research works have reported that the world's oil and gas supply will disappear within three decades and also other natural resources such as coal will extinct within a century [1]. The overexploitation of fossil fuels and the increasing greenhouse gases are considered to be the forthcoming crisis for human's population. Especially, carbon dioxide, which is the main component of greenhouse gas emissions, poses great challenge to the world. The inexorable usage of fossil fuels has contributed to approximately 98% of carbon emission from its combustion into the environment. Hence the fossil fuel usage has to be reduced in order to decrease the amount of carbon di oxide and other pollutants being emitted [2]. Currently, as an alternative, most of the countries have imposed regulations / legislations to use alternate/renewable sources such as wind, hydro and solar energy to replace fossil fuel usage. Other efficient sources have also been emphasized to replace fossil fuels such as oil extraction from plants to be processed into biodiesel for combustion uses. One of the great alternative energy sources is considered to be the Biodiesel and it has various applications in different fields. It has a number of advantages such as high biodegradability, minimal toxicity, minimal hydrocarbon emission, free from sulphur and aromatics. Most important is it can run internal combustion engines without any major modifications [2, 3].

The main objective of using biofuel is to stabilize carbon dioxide emissions. With the progress of research and development into new energy forms, biofuel is thought of as an effective and practical alternative transport fuel that may, in the future, play a significant role in the reduction of transportation related CO_2 emissions. The available technologies for CO_2 capture include injection into deep oceans or geological formations, physicochemical adsorption and enhanced biological fixation. Abiotic methods, such as direct injection of CO_2 into the Deep Ocean or geological formations poses significant challenges like high space requirements and potential leakage with time [4]. The adsorbent materials used in physicochemical adsorption processes are naturally nonrenewable, expensive and it is difficult to control such process. One of the environmentally sustainable way to reduce the carbon problem is carbon sequestration i.e., to reduce greenhouse gas emissions associated with energy production. Carbon sequestration is primarily a carbon sink which transforms carbon into a chemically and biologically stable form that can be stored [5].

Biodiesel is derived from either plant or animal oils by chemical trans-esterification [6]. There are many biomasses that have been proposed as biofuel feedstocks such as palm oil, jatropha and microalgae. Among these biomasses, microalgae have received notable attention because of their high photosynthetic rate, which can be more than 6.9×104 cells/ml/h. This shows that microalgae have a photosynthetic rate that is approximately 50 times higher compared to terrestrial plants. Microalgae have been reported to accumulate more than 70% lipid on a dry weight basis [4]. The lipid content of microalgae, specifically the triglyceride content, is important for biodiesel. Biomass, however, can be converted into biofuel oil through a thermochemical conversion process. Micro algal biodiesel production system involves the following steps: cultivation, harvesting, dewatering, extraction, and transesterification [7]. To achieve high oil yields and CO_2 fixation capacity during cultivation, the key process considerations are the choice of Micro algal strain, cultivation conditions, and the cultivation system (photobioreactors or open ponds). Different technologies are available for harvesting, dewatering, extraction and transesterification. However, high efficiency, energy saving and low CO_2 emission technologies are the optimum targets for full-scale industrial application of microalgae biotechnology. This paper will present a general review about the benefits of using microalgae as a biofuel feedstock, culture types, its harvest and extraction process of algal oil.

[2] BENEFITS OF ALGAE

Unlike plants, unicellular microalgae do not partition large amounts of biomass into supportive structures such as stems and roots that are energetically expensive to produce and often difficult to harvest and process for biofuel production. Microalgae have a number of advantages in CO_2 capture and bio-oil generation. These include high photosynthetic conversion efficiencies, rapid biomass production rates, year round harvest [2], the capacity to produce a wide variety of biofuel feedstock, ability to thrive in diverse ecosystems, distinguished environmental bioremediation such as CO_2 fixation from the atmosphere or flue gas, and water purification [8, 9] non-competitiveness for land with crops and non-competitiveness with the food market. Moreover, microalgae have carbon concentrating mechanisms that suppress photorespiration [10, 11]. The selection of algal strain with high lipid content is the main technical hitch in biodiesel production.

Algae is differentiated into micro and macro algae, between the two there are over 3000 species that exists and among that roughly around 200 species are available that can be used for human consumption, biodiesel, pharmaceuticals etc. [12, 13]. According to Song *et al.* [14] microalgae are the oldest living organisms on the earth and it can grow faster and double their biomass per day. Many different species can become biodiesel with optimum growing conditions; temperature, pH, carbon dioxide/oxygen and biomass consistence etc. [8]. Thus the consistence of biomass energy which is produced from plants and animal wastes are important in oil production such as carbohydrates, protein and lipids. Microalgae can produce higher amount of lipids which is part of biomass when compared with palm oil and soybean [15]. The common micro-algal species are: *Chlorella* sp, *Botryococcus* sp., and *Scenedemus* sp. which are easy to cultivate in comparison to other species and potentially contain more lipids [16, 17]. In addition, Chlorella spp. specifically *C. emersonii, C. minutissima, C. vulgaris* and *C. protothecoides* were reported to be capable of producing more than 63% lipid content on a dry biomass basis [14]. They have high flexibility to adapt to diverse culture conditions and can be cultivated under both phototrophic and heterotrophic conditions [14, 16]. The biodiesel produced from these species were acid methyl ester, linoleic acid methyl ester and oleic acid methyl ester. Unsaturated fatty acids methyl ester comprised over 82% of the total biodiesel content [9, 14]. The properties of the biodiesel produced from Chlorella conform to US Standard for biodiesel [10]. In addition to biodiesel, some microalgae were also reported as good producers of hydrogen. Hydrogen is high in energy, and an engine fuelled by pure hydrogen produces almost no pollution. Hence, Micro algal biomass is considered to be a renewable and green method of producing energy.

[3] ALGAL CULTURE

Culturing algae is not a difficult task as these plants can be planted in brackish water and non-arable land. Microalgae can be effective in other aspects such as non-fuel protein which can be used as animal feed; absorbs carbon dioxide for growth which reduces the amount of greenhouse gases available in the atmosphere; and a diverse metabolism allowing easy growth throughout the year. Furthermore, it can also grow in wastewater and utilize the nutrients from it efficiently. Though microalgae are the best solution for biodiesel, there are still challenges to prove the efficiency of algal biodiesel. Thus a generalized rule can be concluded here: should an effective irrigation be implemented, the system should provide efficient biomass ingredients which in turn can promote oil-efficiency.

For profitable biodiesel production, optimal growth and lipid maximization is needed for oil extraction. It is suggested to grow algae in optimal conditions before exposing them to unfavourable conditions [16]. Algae are highly sensitive to temperature and the optimum temperature range from 20-30°C [5, 8]. Removal of oxygen and replenishment of carbon dioxide is equally important and suggested to be a continuous process to maximize lipid content in the microalgae culture. With the balancing of oxygen and carbon dioxide, the pH changes are countered by a pH controller which processes the oxygen into a degassing zone [8]. As a result this, the culturing system provides a carbon sink reducing the amount of carbon dioxide, hence dealing with global warming.

There are three common types of algae-cultivation methods: (1) Phototrophic, (2) Heterotrophic and (3) Mixotrophic.

3.1. Phototrophic cultivation

Phototrophic cultivation is the most commonly used and the easiest method to scale-up. Microalgae takes in carbon source from air and sunlight as energy source, through photosynthesis and is converted to various forms of chemical energies such as polysaccharides, proteins, lipids and hydrocarbons. In this culture, typically microalgae can be grown in two common types of cultivation open and closed systems respectively; raceway ponds and photo-bioreactors [6, 16]. Algae can be grown in flue gas or waste water to obtain the required nutrient amount, which consequently deals with the environmental issues of today [16]. It is the most cost effective method to use in culture as it can minimize the usage of technical appliances. Phototrophic culture is the easiest assessment for microalgae production as the main energy sources can get abundance from nature [18]. However, the culture has limitations as it uses the sun as the major source of supply, it will not be easy to utilize in the area especially in temperate zone. Furthermore, as the culture is 'open system', it is necessary to specifically counter the effects of excessive contamination and evaporation aspects [19].

3.2. Heterotrophic cultivation

Algae are usually grown as a monoculture to prevent infections and contaminations with the required nutrients for example nitrogen and phosphorus provided through the aqueous solution they live in [13]. Due to the expensive equipment involved in heterotrophic system such as enclosed-bioreactor, it has been debated about whether open systems or closed systems are more beneficial or profitable. Open systems requires less capital in comparison to a close system, in which a close system from beginning to end can cost from \$2.6 million to \$10.9 million which may not include maintenance costs [6]. Fortunately, a closed bioreactor requires little space in comparison to an open raceway pond that is open to contamination, infections or parasites which is not desirable for producing algae for biodiesel in which the conditions of algae need to be sanitized and clean at harvest. Currently, the heterotrophic method provides much more lipid content including sucrose, carbohydrate and glucose [20]. Evidence shows that heterotrophic growth may give rise to much better oil productivities, and this approach has therefore attracted considerable interest; a 40%-increase in lipid content was extracted in *Chlorella protothecoides*, from the change of phototrophic to heterotrophic cultivation [21]. Even though it has advantages, the main problem is the cost and energy consumptions which are unsuitable for commercial scale. Therefore, more studies need to be done to promote heterotrophic method with low cost in the future.

3.3. Mixotrophic cultivation

In mixotrophic culture, algae can be grown by using either phototrophic system with sunlight or by applying organic substitutes, as in heterotrophic system. This type of culture uses a hybrid mix of the previous two culturing methods mentioned earlier [20].

[4] HARVEST

The harvesting process is one of the difficult tasks because of size and suspension. There are still choices to harvest algae: separating algae from suspension by using natural gravity factors, bulk harvesting or by using centrifugation and filtration which is called thickening. Due to the high energy consumption and the cost involved in the first process, bulk harvesting is more efficient on a commercial scale.

Micro algal culture dewatering is a major impediment to industrial-scale processing of microalgae for biofuel and production of other high-value biochemical due to the very dilute nature of harvested Micro algal cultures which results in requirement of high energy consumption for dewatering. This also causes much CO_2 emission during dewatering, thus making microalgae-based products less attractive economically [22]. The common techniques used for dewatering the microalgae include flocculation, centrifugation, filtration such as high pressure filtration / tangential flow filtration, and gravity sedimentation. On commercial scale microalgae harvesting usually involves flocculants to reduce the time required to separate the medium from the algal cells. Flocculants are materials that have the ability to support the bridging phenomena between two molecules, leading to the coagulation process. Flocculation is a process of aggregating the Micro algal cells to promote their separation, beginning with the addition of a material, flocculants into the medium, which disturbs the stability of the particles in suspension, including microscopic cells, causing them to aggregate [23]. Flocculants with higher molecular weights are more effective as they can adsorb several particles at once, forming a three-dimensional matrix. When this occurs, the aggregated cells become easier to harvest. This is why the most effective flocculants are polymers, either natural or synthetic [24]. Flocculation is not a critical step in separating algal cells. However, the selection of inefficient or inappropriate flocculants can be costly. Organic flocculants can be obtained naturally or synthetically. Flocculants can be applied in many ways: auto flocculation, microbial flocculation and electrolytic flocculation. Auto flocculation, involves the combination of two or more different types of flocculants and works with the aid of physical process such as air sparging. While, microbial flocculation involves the addition of a minute quantity of a microbial culture (as low as 1 g/l), into the Micro algal culture that have to be separated. The microbes selected as the flocculating agent must be able to release extracellular polymeric substances when depleted of nutrients. The microbe was feed with an organic substrate such as crude glycerol, and this is less expensive compared to other flocculating agents. In addition, this technique will not damage the Micro algal cell, and allows the culture medium to be reused without further treatment. It is reported that the recovery efficiency (RE), is more than 90% [25]. On the other hand electrolytic flocculation that involves no flocculants and only requires electricity as low as 0.3 kWh/m3 was also reported in the literature [25]. This technique was typically applied to remove the taxonomic group of algae in a reservoir for drinking water and has removal efficiency as high as 90%. After the flocculation process, the separated algal cells where subjected to filtration, centrifugation, floatation or sedimentation before a further drying process. Filtration process harvests Micro algal biomass directly by using a microbial membrane which only allows algal cells to pass through. This technique appears to be the cheapest technique to harvest microalgae. However, this technique requires backwashing to maintain the efficiency of the membrane filter and is time-consuming. Micro algal harvesting using an ultrasound technique is currently under development [26]. In this method, the Micro algal cells experience a force that drives them into the planes of pressure nodes when they are exposed to an ultrasonic standing wave. When the field is switched off, the aggregated cells settle rapidly because of the gravitational forces. This technique requires further study before it can be applied on a large scale, especially in open ponds where contamination is high because this technique can not only coagulate Micro algal cells but also other sediments such as mercury [27]. Centrifugation is seen as the most efficient biomass recovery technique. However the energy and capital costs associated with it, especially for industrial-scale processing, are unattractive. The choice of dewatering technique is dependent on the microalgae species and the desired product quality. At present the efficient technique used in industries are flocculation and filtration [28, 29].

[5] PRE-TREATMENT

The drying process has to be done harvested algae which are contaminated with excess amount of water. For the purpose of drying, Solar drying might be the best and favourable process but the method is not feasible in some areas such as temperate countries since limited amount of sunlight is assessable at certain time of the year. In this case, the generated heat from using of fossil fuels is required to dry excessive water in microalgae biomass continuously to ensure optimum biomass production. However, a recent research has mentioned that using gases, fuel for drying procedure consumed nearly 69% of the overall energy input and led to a negative energy balance in producing microalgae biofuels [30]. Ranjan *et al*. [31] showed that drying or cell disruption of concentrated Micro algal culture actually enhanced the lipid and/or protein extraction. The main advantage of processing dried biomass is the better percolation of solvents or fluids through the cell to improve extraction efficiency. However, drying is not considered as an economically viable option for biomass production because of the high-energy requirements [32]. In other words, high dependency on fossil fuels for drying process will jeopardize commercial viability of microalgae biofuels and thus, new technologies or approaches (e.g. development of efficient dryers) are urgently needed to ensure the sustainability of microalgae biofuel production.

Cell rupture techniques of the harvested biomass, which could support extraction by avoiding or reducing the use of solvents, include mechanical, chemical and enzymatic treatments, and these, have been described as applied to oilseeds and microalgae by Pernet and Tremblay [33]. Other strategies for algal bio-fuel productions such as partially or without drying biomasses are through in-situ trans esterification and hydrothermal liquefaction [34]. All these process are explained in the following sections.

[6] EXTRACTION

The next step after harvesting and drying the microalgae biomass is liquid extraction i.e. algal oil extraction. Although the energy consumed in lipid extraction from dried microalgae biomass contributed a relatively small portion to the overall energy life cycle of microalgae biofuels (around 5–10%), the usage of appropriate extraction method is still vital as part of production practice [30, 35]. Using effective lipid extraction is essential in particularly for microalgae with low lipid content as reducing the lipid contents during extraction process may bring a significant impact towards the production cost of microalgae biofuels [31]. There are three different categories of extraction: Chemical, mechanical and biological [36].

6.1. Chemical Extraction

Chemical solvent extraction is the most common method used to extract lipid from microalgae biomass. This is because chemical solvent has high selectivity and solubility towards lipid and therefore, even inter-lipid can be extracted out through diffusion across the cell wall [31]. The disadvantages of using chemical solvent are mostly related to their high toxicity towards human beings and the surrounding environment. Chemical solvents such as n-hexane, methanol, ethanol and mixed methanol–chloroform (2:1 v/v) (Bligh and Dyer method) are effective to extract microalgae lipid, but the extraction efficiency is highly dependent on microalgae strains [15]. Modified Bligh and Dyer method is the most favorable method to extract microalgae lipid from various strains and relatively high extraction efficiency can be attained compared to other solvents [37]. Although n-hexane is widely used to extract oil from various seed crops, it is inefficient to extract microalgae lipid since microalgae lipid contains high concentration of unsaturated fatty acid while n-hexane is a non-polar solvent, and therefore the selectivity of lipid towards the solvent is greatly deducted [31]. Another solvent system which is widely used is chloroform-methanol 1:1 [17, 38], however the solvent chloroform-methanol-water 1:2:0.8 has the highest yielding lipid content of 93.8% [37]. Apart from that, it is worth to mention that n-hexane, methanol and chloroform are highly toxic compounds that can cause safety and health hazards if proper precaution steps are not taken. In addition, the usage of n-hexane and methanol is not a sustainable method since both solvents are conventionally derived from non-renewable fossil fuels.

On the other hand, ethanol emerged as a greener solvent since it has low toxicity level and can be derived from renewable sources such as sugar-based plant (e.g. sugar cane and sweet sorghum) and lignocellulosic material (e.g. wood and corn). However, ethanol always gives low extraction efficiency because ethanol is an azeotrop mixture (with 5% of water) and the presence of water may possibly reduce its extraction efficiency. The usage of chemical solvent in extraction, diffusion process is always a limiting factor in the overall mechanism and it becomes more serious in microalgae as the cell wall prohibits the solvent from diffusing into the inner cell for lipid extraction. Therefore, cells disruption method can be introduced to enhance solvent diffusion efficiency and consequently, to improve microalgae lipid recovery rate. As the amount of lipids obtained is again dependent on the species, it becomes important to select the highest lipid yielding species and most efficient extraction method to obtain high oil content for biodiesel production. However, in the last decades, concerted efforts have been made to increase extraction efficiency, and to reduce the use of toxic and polluting organic solvents through the development of supercritical fluid extraction [17]. Supercritical fluid extraction, though a benign technique, has a high investment and a high operating cost due to energy consumption during fluid compression.

6.2. Supercritical fluid extraction

In recent years, researches in extraction and reaction field has entered a new dynamic era with the introduction of supercritical fluid technology in which carbon dioxide gas is liquefied under the great pressure and heated until it possesses both liquid and gas properties which will be used as a solvent in oil extraction. On the down side, it requires expensive and intensive energy consuming equipment to provide high pressure in suppressing the gas [17]. At supercritical phase, thermo physical properties: density, viscosity, diffusivity and dielectric constant of a fluid will change drastically depending on the input of temperature and pressure but consequently the changes of the thermo physical properties transform the fluid into a super-solvent and thus, improve extraction and reaction efficiency. Several supercritical fluids that are currently being explored are ethylene, CO_2, ethane, methanol, ethanol, benzene, toluene and water [39, 40]. Among these, supercritical- CO_2 has received the most interest typically in extraction of pharmaceutical and health related products from microalgae [41, 42, 43, 44]. In fact, supercritical- CO_2 offers several advantages in comparison with chemical solvent extraction: (1) non-toxic and provide non-oxidizing environment to avoid degradation of extracts, (2) low critical temperature (around 31 °C) which prevent thermal degradation of products, (3) high diffusivity and low surface tension which allow penetration of pores smaller than those accessible by chemical solvents and (4) easy separation of CO_2 at ambient temperature after extraction [39, 41, 44]. However, the main disadvantages of supercritical- CO_2 are associated with high cost of operation and safety related issues. Research studies on using supercritical- CO_2 to extract microalgae lipid for biodiesel production has been explored recently. In a study, lipid from wet-paste Chlorococcum sp. biomass was extracted using supercritical- CO_2 and a lipid yield of 7.1% were attained at critical temperature of 60 °C, critical pressure of 30 MPa and extraction time of 80 min[45]. In addition, the lipid yield attained from the wet-paste is even higher than dry biomass (5.8%), suggesting that energy consumed in drying process can be reduced through supercritical technology. Since supercritical-CO_2 is a nonpolar solvent, the presence of water in the system acts as a natural polar co-solvent and thus, facilitated the extraction of polar lipids and improve total lipid yield extracted. Apart from that, sox let extraction, hexane was found to be less efficient than supercritical-CO_2 extraction, achieving only 5.8% lipid yield after an extraction time of 330 minutes.

However, a contradicting result was observed when a comparative study between supercritical-CO_2 and Bligh and Dyer method (chemical solvent extraction) which were used to extract lipid from heterotrophic cultured microalgae *C. cohnii* [46]. The lipid yield attained from Bligh and Dyer method was nearly double that of supercritical- CO_2, indicating that microalgae strains and culture conditions plays a significant role in determining the appropriate lipid extraction methods. Although the energy consumed in operating supercritical- CO_2 extraction is expected to be low due to the low critical temperature of CO_2, however, the energy required in separating pure CO_2 from atmosphere and re-compressing the CO_2 after each extraction should not be ignored. Hence, a complete analysis is urgently required to compare the feasibility of supercritical- CO_2 and chemical solvent extraction in industrial scale, typically in term of energy efficiency and cost effectiveness.

6.3. Mechanical extraction

There are several mechanical techniques to disrupt microalgae cell wall, such as autoclave, bead-beater, ultra sonication and microwave as osmotic shock**.** Among these - Ultrasonic extraction is used as commonly since lipid extraction from microalgae biomass is relatively difficult due to the presence of thick cell wall that prevents the release of intra-lipid. This method uses sound waves which create cavitation bubbles to make shock waves that break down the cell walls and release algal oil. This technique is capable of increasing the Micro algal oil yield by 50–500% compared to conventional methods. However, this yield is affected by the ultrasonic strength and solvent type. Cravotto *et al*. [47] and Lou *et al*. [48] proved that the extraction times can be reduced up to 10-fold. In addition to pure oil, other biochemical compounds in Micro algal biomass such as carotenoids and chlorophyll can also be extracted by using ultrasound technique with comparable results to the supercritical method [49]. Lee *et al*. [37] reported a negative energy balance for autoclave and ultra-sonication, because these two machines have high energy consumption and the quantity of lipid recovered is relatively low compared to bead-beater and microwave. Alternatively, bead-beater method attained the most promising result with the highest positive energy value, followed by microwave and non-disruptive method. The results simply suggest that introduction of cell disruption method in lipid extraction does not always improve the system; instead it may lead to negative net energy value. On the other hand, ultra sonication and microwave posed several safety and health hazards and need to be addressed before up-scaling to commercial stage. Although higher microalgae lipid yield can be achieved after cell disruption, care should be taken as additional energy is required. The microwave technique, which is similar to the ultrasonic technique, has also been reported to be capable of yielding higher unsaturated and essential fatty acids compared to typical extraction techniques such as water bath control. Approximately 76–77% of the total recoverable oil can be extracted within 30 min [50].

6.4. BIOLOGICAL EXTRACTION

Enzymatic extraction - The most common method in biological extraction is by using enzymes to speed up oil yield, by degrading the cell walls of algae. Disadvantage in this process is the financial costs which are estimated to be higher than hexane extraction.

Genetic engineering - Osmotic shock uses the reduction in osmotic pressure to rupture the cells, used to release cellular components thus, oil release.

[7] GENETIC ENGINEERING AND MOLECULAR BIOLOGY TECHNIQUES

Generally, species are not genetically programmed for optimized mass production of a particular product under large-scale operational conditions. Thus, many key parameters require careful improvement based on both genetically and non-genetically manipulated organisms (GMOs and non-GMOs). Transgenic microalgae are an emphasis of growing interest, with an opportunity to construct new and highly efficient phenotypes [51]. New strains can be developed by specifically targeting genes using reverse or forward genetics strategies. However, the lack of transformation techniques is currently a major limitation for most algae developed for biofuel production; an alternative strategy is the isolation and breeding of highly efficient non-GMO strains. Such an approach can involve high-throughput screening of libraries after chemical treatment or UV mutagenesis, which avoids the regulatory problems of using GMO strains in outdoor production systems. With automated screening techniques, this approach is becoming very attractive. Successful attempts with the haploid yeast *Pichia stipitis* [52] based on transcriptome analysis combined with backcrossing approaches will open up opportunities to probe Micro algal mutants with high bioenergy production capacity in non- GMO strains. However, this approach still requires molecular biology techniques based on a haploid, fully sequenced and annotated genome.

Successful selection, construction and molecular analysis of the genotypes of GMO and non-GMO mutant strains require comprehensive knowledge of the Micro algal genome and access to molecular and gene manipulation tools, including selectable markers, vectors and techniques for systematic insertion in screening libraries. Apart from *Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum, Chlorella kessleri, Porphyridium, Nannochloropsis and Dunaliella salina* [53] have been successfully transformed.

The idea to genetically engineered microalgae to increase their valuable compounds is very attractive. Compared with higher plants, microalgae represent a much simpler system for genetic manipulations compared with higher plants due to the absence of cell differentiation. However, the progress in the genetic engineering is extremely slow until recently little work has been done by adopting a genetic engineering approach to improve the algae. The methods successfully used for transformation in other systems failed when applied to algae. Techniques to introduce DNA into algal cells with suitable promoters, new selectable marker genes, and expression vectors have to be standardized. Currently, all these requirements have been fulfilled for the diatom *Phaeodactylum*, the green alga, *Chlamydomonas* and the blue green algae, *Synechococcus* and *Synechocystis* [54]. The development of a functional transformation system can be expected in the near future for other diatoms, blue green algae and the red alga, *Porphyridium.* The success of genetic engineering lies in the improvement of nutritional value, product yield with optimal production parameters. However, the following factors are to be considered to achieve the above features

1. The accumulation of valuable substances in algae via genetic transformation can only increase up to a point where cellular metabolism starts negatively affecting
2. Transgenic algae potentially pose a considerable threat to the ecosystem and will most likely to be banned from the outdoor cultivations otherwise be under strict regulation
3. Usually the transgenic cells exhibit less fitness than wild type and therefore cells that lose the newly introduced gene quickly outgrow the transformants.

To prevent this, a constant selection is necessary, by the addition of antibiotics, a potential public health hazard. Therefore, the prime field of genetic engineering will be an improved production of valuable products and bioactive compounds in closed culture systems. Current genetic engineering pursuits are towards microalgae that are of greater interest in industrial applications and environmental conservation. Several approaches have been developed especially to improve microalgae biomass or lipid production and CO2 capturing efficiency [55].

[8] **CONCLUSION**

It is obvious that the depletion of mineral oil reserves and increase in atmospheric CO_2 concentration requires the rapid development of carbon-neutral renewable alternatives. CO_2 fixation by microalgae provides a promising alternative for CO_2 mitigation, feedstock for biofuels, pharmaceutical by products and other high-value products. At the same time, wastewater can be treated using this system. Thus this could present a sustainable process by the integration of CO_2 capture, wastewater treatment and biofuel production. At present, there are few examples of large-scale continuous microalgae-based CO_2 capturing system [56]. However, laboratory and pilot plant studies suggest that capturing CO_2 by microalgae is a potentially viable strategy for mitigating CO_2 emissions from anthropogenic sources. To be more precise, although the advantages of algal oil are obvious, the commercial production stages have been delayed due to production costs and insufficient technology. On the other hand, we can still use other renewable energy alternatively before completing algal oil production instead of depending on microalgae. Economic assessments suggest that the costs of carbon capture and biofuel production from microalgae may attain the cost of producing petroleum-based fuels in the next few decades. Therefore, microalgae biofuels will be one of the main biofuel products.

[9] REFERENCES

1. Raja R., Hemaiswarya S., Kumar N.A., Sridhar S. and Rengasamy R. (2008) A perspective on the biotechnological potential of microalgae. *Crit. Rev. Microbiol*. 34: 77-88.
2. Demirbas M.F., Balat M. and Balat H. (2011) Biowastes-to-biofuels. Energy Conv.Management, 52(4): 1815-1828., ISSN 0196-8904
3. Atabani A.E., Silitonga A.S., Badruddin I.A., Mahlia T.M.I. and Masjuki H.H. (2012) A Comprehensive Review on Biodiesel As An Alternative Energy Resource and its Characteristics. *Renewable Sustainable Energy Rev.* 16: 2070-2093.
4. Lackner K.S. (2003) A guide to CO2 sequestration. *Science.* 300: 1677-1678.
5. Chang T.H., Su H.M. and Chiu C.L. (2011) Value-at-risk estimation with the optimal dynamic biofuel portfolio. *Energy Economics*. 33: 264-272.
6. Scott S.A., Davey M.P., Dennis J.S., Horst I, Howe C.J., Lea-Smith D.J. and Smith A.G. (2010) Biodiesel from algae: challenges and prospects. *Curr. Opin. Biotechnol*. 21: 277–286.
7. Harun R., Danquah M.K. and Forde G.M. (2010) Micro algal biomass as a fermentation feedstock for bioethanol production. *J Chem Technol Biot*. 85: 199–203.
8. Chisti Y. (2007) Biodiesel from microalgae. *Biotechnol Adv*. 25: 294–306.
9. Wang B, Li Y.Q., Wu N. and Lan C.Q. (2008) CO2 bio-mitigation using microalgae. *Appl Microbiol Biot.* 79: 707–18.
10. Jansson J., Marell A. and Nordlund A. (2010) Green consumer behavior: Determinants of curtailment and eco-innovation adoption. *Journal of Consumer Marketing*. 27: 358 -370.
11. Spalding M.H. (2008) Micro algal carbon-dioxide-concentrating mechanisms: Chlamydomonas inorganic carbon transporters. *J Exp Bot*. 59: 1463–73.
12. Lee A.K., Lewis D.M. and Ashman P.J. (2009) Microbial flocculation, a potentially low-cost harvesting technique for marine microalgae for the production of biodiesel. *J Appl Phycol*. 21: 559–67.
13. Mata T.M., Martins A.A. and Caetano N.S. (2010) Microalgae for biodiesel production and other applications: a review. *Renewable Sustainable Energy Rev*. 14: 217–32.
14. Song D., Fu J. and Shi D. (2008) Exploitation of oil-bearing microalgae for biodiesel. *Chin J Biotechnol.* 24: 341–8.
15. Lam M.K. and Lee K.T. (2011) Renewable and sustainable bioenergies production from palm oil mill effluent (POME): win–win strategies toward better environmental protection. *Biotechnol Adv.* 29: 124–41.
16. Pokoo-Aikins G., Nadim A., El-Halwagi M.M. and Mahalec V. (2009) Design and analysis of biodiesel production from algae grown through carbon sequestration. *Clean Techn Environ Policy*. 12: 239–254.
17. Lee J.Y., Yoo C., Jun S.Y., Ahn C.Y. and Oh H.M. (2010) Comparison of several methods for effective lipid extraction from microalgae. *Bioresour Technol.* 101: S75–7.
18. Borowitzka M.A. (1999) Commercial production of microalgae: ponds, tanks, tubes and fermenters. *J Biotechnol*. 70: 313–21.
19. Schenk P., Thomas-Hall S., Stephens E., Marx U., Mussgnug J. and Posten C. (2008) Second generation biofuels: high-efficiency microalgae for biodiesel production. *Bioenerg Res*. 1: 20–43.
20. Boyle N.R. and Morgan J.A. (2009) Flux balance analysis of primary metabolism in Chlamydomonas reinhardtii. *BMC Syst Biol.* 3: 4.

21. Amaro H.M., Guedes A.C. and Malcata F.X. (2011) Advances and perspectives in using microalgae to produce biodiesel. *Appl Energy*. 88 (10): 3402-3410.
22. Danquah M.K., Ang L., Uduman N., Moheimani N. and Fordea G.M. (2009) Dewatering of Micro algal culture for biodiesel production: exploring polymer flocculation and tangential flow filtration. *J Chem Technol Biot*. 84: 1078–83.
23. Gregory J. (2006) Particles in water: properties and processes. CRC Press; p. 121–48. doi:10.1201/9780203508459.ch6.
24. Sharma B.R., Dhuldhoya N.C. and Merchant U.C. (2006) Flocculants – an ecofriendly approach. *J Polym Environ*.14:195–202.
25. Lee A.K., Lewis D.M. and Ashman P.J. (2009) Microbial flocculation, a potentially low-cost harvesting technique for marine microalgae for the production of biodiesel. *J Appl Phycol*. 21:559–67.
26. Bosma R., VanSpronsen W.A., Tramper J. and Wijffels R.H. (2003) Ultrasound a new separa-tion technique to harvest microalgae. *J Appl Phycol*. 15:143–53.
27. He Z., Siripornadulsil S., Sayre R.T., Traina S.J. and Weavers L.K. (2011) Removal of mercury from sediment by ultrasound combined with biomass (transgenic Chlamy-domonas reinhardtii). *Chemosphere*. 83:1249–54.
28. Uduman N., Qi Y., Danquah M.K., Forde G.M. and Hoadley A. (2010) Dewatering of Micro algal cultures: a major bottleneck to algae-based fuels. *J Renew Sustain Energy*. 2.
29. Halim R., Gladman B., Danquah M.K. and Webley P.A. (2011) Oil extraction from microalgae for biodiesel production. *Bioresour Technol*. 102: 178–85.
30. Sander K. and Murthy G.S. (2010) Life cycle analysis of algae biodiesel. *Int J Life Cycle Assess.* 15: 704–14.
31. Ranjan A., Patil C. and Moholkar V.S. (2010) Mechanistic assessment of Micro algal lipid extraction. *Ind Eng Chem Res.* 49: 2979–85.
32. Lardon L., Hélias A., Sialve B., Steyer J.P. and Bernard O. (2009) Life-cycle assessment of biodiesel production from microalgae. *Environ Sci Technol*. 43: 6475–81.
33. Pernet F. and Tremblay R. (2003) Effect of ultrasonication and grinding on the determination of lipid class content of microalgae harvested on filters. *Lipids.* 38: 1191–5.
34. Brennan L. and Owende P. (2010) Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable Sustainable Energy Rev.* 14: 557–77.
35. Stephenson A.L., Kazamia E., Dennis J.S., Howe C.J., Scott S.A. and Smith A.G. (2010) Life-cycle assessment of potential algal biodiesel production in the United Kingdom: a comparison of raceways and air-lift tubular bioreactors. *Energy Fuel.* 24: 4062–77.
36. Pienkos P.T. and Darzins A. (2009) The promise and challenges of Micro algal-derived biofuels. *Biofuels Bioprod Bioref.* 3: 431–440.
37. Lee J.Y., Yoo C., Jun S.Y., Ahn C.Y. and Oh H.M. (2010a) Comparison of several methods for effective lipid extraction from microalgae. *Bioresour Technol.* 101: S75–7.
38. Chen C.Y., Yeh K.L., Aisyah R., Lee D.J. and Chang J.S. (2010) Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. *Bioresour Technol.* 102: 71–81.
39. Mendes R.L., Nobre B.P., Cardoso M.T., Pereira A.P. and Palavra A.F. (2003) Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. *Inorg Chim Acta.* 356: 328–34.
40. Sawangkeaw, R., Bunyakiat K. and Ngamprasertsith S. (2010) A review of laboratory-scale research on lipid conversion to biodiesel with supercritical methanol (2001-2009). *The Journal of Supercritical Fluids*. 55(1): 1-13.
41. Jaime L,Mendiola J.A., Ibáñez E., Martin-Álvarez P.J., Cifuentes A., Reglero G., et al. (2007) β-Carotene isomer composition of sub- and supercritical carbon dioxide extracts. Antioxidant activity measurement. *J Agric Food Chem.* 55: 10585–90.
42. Kitada K., Machmudah S., Sasaki M., Goto M., Nakashima Y., Kumamoto S., et al. (2009) Supercritical CO2 extraction of pigment

components with pharmaceutical importance from Chlorella vulgaris. *J Chem Technol Biotechnol*. 84: 657–61.

43. Macías-Sánchez M.D., Serrano C.M., Rodríguez M.R., de la Ossa E.M., Lubián L.M., and Montero O. (2008) Extraction of carotenoids and chlorophyll from microalgae with supercritical carbon dioxide and ethanol as cosolvent. *J Sep Sci*. 31: 1352–62.
44. Ota M., Watanabe H., Kato Y., Watanabe M., Sato Y., Smith Jr RL., et al. (2009) Carotenoid production from *Chlorococcum littorale* in photoautotrophic cultures with downstream supercritical fluid processing. *J Sep Sci*. 32: 2327–35.
45. Halim R., Gladman B., Danquah M.K. and Webley P.A. (2011) Oil extraction from microalgae for biodiesel production*. Bioresour Technol*. 102: 178–85.
46. Couto R.M., Simões P.C., Reis A., Da Silva T.L., Martins V.H. and Sánchez-Vicente Y. (2010) Supercritical fluid extraction of lipids from the heterotrophic microalga Crypthecodinium cohnii. *Eng Life Sci*. 10: 158–64.
47. Cravotto G., Boffa L., Mantegna S., Perego P., Avogadro M. and Cintas P. (2008) Improved extraction of vegetable oils under high-intensity ultrasound and/or microwaves. *Ultrason Sonochem*.15: 898–902.
48. Lou Z., Wang H., Zhang M. and Wang Z. (2010) Improved extraction of oil from chickpea under ultrasound in a dynamic system. *J Food Eng*. 98: 13–8.
49. Macias-Sancheza M.D., Mantell C., Rodrigueza M., de la Ossaa E.M., Lubian L.M. and Monterob M. (2009) Comparison of supercritical fluid and ultrasound-assisted extraction of carotenoids and chlorophyll a from Dunaliella salina. *Talanta*. 77: 948–52.
50. Balasubramaniana S., Allena J.D., Kanitkara A. and Boldor D. (2011) Oil extraction from Scenedesmus obliquus using a continuous microwave system – design, optimization, and quality characterization. *Bioresour Technol*. 102(3): 3396–403.
51. Hallmann A. (2007) Algal transgenics and biotechnology. *Transgenic Plant J*. 1, 81–98.
52. Smith D.R. et al. (2008) Rapid whole-genome mutational profiling using next-generation sequencing technologies. *Genome Res*. 18: 1638–1642.
53. Feng S.Y. et al. (2009) Improvement of efficiency of genetic transformation for Dunaliella salina by glass beads method. *Mol. Biol. Rep*. 36: 1433–1439.
54. Boussiba S.,Wu X.Q., Ben-Dov E., Zarka A. and Zaritsky A. (2000) Nitrogen fixing cyanobacteria as gene delivery system for expressing mosquitocidal toxins of *Bacillus thuringiensis* ssp. israelensis. *J. Appl. Phycol*. 12: 461– 467.
55. Radakovits R., Jinkerson R.E., Darzins A. and Posewitz M.C. (2010) Genetic engineering of algae for enhanced biofuel production. *Eukaryot Cell*. 9: 486–501.
56. Zeng X., Michael K., Danquah., Chen X.D. and Lu Y. (2011) Microalgae bioengineering: From CO2 fixation to biofuel production. *Renewable and Sustainable energy Rew*. 15: 3252-3260.