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Microalgae -An Emerging Option For Biodiesel

Biotechnology

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ABSTRACT

Biodiesel has received considerable attention in recent years as it is biodegradable, renewable and non-toxic fuel. It emits less gaseous pollutants than conventional diesel fuel, and can work directly in diesel engines with no required modifications. The use of microalgae as a source of biofuels is an attractive proposition from the point of view that microalgae are photosynthetic renewable resources. They produce oils with high lipid content, have fast growth rates and are capable of growth in saline waters which are unsuitable for agriculture. Green algae and diatoms are the mostly used for biofuels production, because of their high storage of lipids. This present review provides an overview in the production of biodiesel from microalgae including different systems of cultivation such as open ponds and closed Photobioreactors, the methods of harvesting biomass and extracting the oil content.

INTRODUCTION

Biodiesel is an attractive fuel for diesel engines that it can be made from any vegetable oil (edible or non edible oils), used cooking oils, animal fats as well as microalgae oils. It is a clean energy, renewable, non toxic and sustainable alternative to petroleum based fuels, and it is able to reduce toxic emissions when is burned in a diesel engine. The interest of this alternative energy resource is that fatty ester acids, known as biodiesel, have similar characteristics of petro-diesel oil which allows its use in compression motors without any engine modification. The problem is that biodiesel has viscosities approximately twice those of conventional diesel fuels. Therefore, biodiesel esters can be used directly or blended with petro-diesel. These blends are used to minimize the different properties between biodiesel and conventional diesel fuel. In order to decrease greenhouse gas emissions from industrial combustions and transports, biodiesel demand is constantly increasing, but oil crops are not able to satisfy it because of their high cost of performance. This high cost is due to the competition of biofuels with the food industry. Microalgae are photosynthetic microorganisms that convert sunlight, water and carbon dioxide to sugars, from which biological macromolecules, such lipids, can be obtained. They have been suggested as very good candidates for fuel production because of their advantages of higher photosynthetic efficiency, higher biomass production and faster growth compared to other energy crops. In table 1 it is shown the physical and chemical properties of biodiesel. (Demirbas 2008).

Table 1: Physical and chemical properties of biodiesel

Name	Biodiesel
Chemical Name	Fatty acid Methyl Ester
Chemical Formula Range	C14-C24 methyl esters
Kinematic Viscosity Range	3,3- 5,2
Density Range	860-894
Boiling point Range (K) Flash Point Range (K)	>475 430-455
Distillation Range (K)	470-600
Vapor Pressure (mmHg at 295K)	<5
Solubility in water	Insoluble in water
Physical appearance	Light to dark yellow transparent liquid
Odor	Light soapy and oily odor
Biodegradability	More than conventional diesel
Reactivity	Stable, avoid strong oxidize agents

MICROALGAE

The microalgae are recognized as one of the oldest living organisms that have chlorophyll *a* as their primary photosynthetic pigment and lack a sterile covering of cells around the reproductive cells (Brennan and Owende 2010). One of the most serious environmental problems today is the global warming caused primarily by the heavy use of fossil fuels. The CO_2 generated by power plants and industry can be recovered with technology such as chemical absorption. Microalgae are potential candidates for using excessive amounts of CO_2 (Khan et al. 2009). Since the cultivation of these organisms are capable of fixing CO_2 to produce energy and chemical compounds with the presence of sunlight. In general, microalgae can be referred as microscopic organisms that can grow via photosynthesis. Microalgae reproduction occurs primarily by vegetative (asexual) cell division, although sexual reproduction can occur in many species under appropriate growth conditions. Their photosynthetic capability is similar to land based plants, due to their simple cellular structure, and they are submerged in aqueous environment where they have efficient access to water, CO_2 and nutrients. Microalgae are generally more efficient in converting solar energy into biomass than land plants. The most abundant microalgae studied for biodiesel production are Cyanophyceae (blue green algae), Chlorophyceae (green algae), Bacillariophyceae (diatoms) and Chrysophyceae (golden-brown algae). Many microalgal species are able to switch in phototrophic and heterotrophic growth. In heterotrophic growth algae cannot synthesize their own food and depend on glucose or other utilizable carbon sources for carbon metabolism and energy. (Carlsson, 2007). Heterotrophic production is not as efficient as using photosynthetic microalgae.

Microalgae can provide several different types of renewable biofuels. They are composed mainly of carbohydrates, proteins and lipids. The lipid content of algal oil can be processed into biodiesel, carbohydrates into ethanol and proteins into animal feed or human nutritional supplements. Also, by anaerobic digestion of the algal biomass they can provide biogas and fertilizers. (Rosenberg et al. 2008). An important algal characteristic for biodiesel production is the suitability of lipids in terms of type, chain length, degree of saturation and proportion of total lipid made up by triglycerides. Microalgae have also a high technical potential to abate greenhouse gases, given their ability to use carbon dioxide in their photosynthetic efficiency, and the possibility of achieving faster growth as compared to any energy crop. They reproduce quickly and can be harvested day after day. However, the lipid content in microalgae required to be high, otherwise the economic performance would be hard to achieve.

Microalgae Production Systems

Producing biomass from microalgae is generally more expensive than growing crops. Photosynthetic growth requires light, carbon dioxide, water and inorganic salts. Algal biomass contains generally three components: carbohydrates, proteins and lipids. Growth medium must provide the inorganic elements that constitute algal cells. These essential elements are nitrogen (N), phosphorous (P), iron, and in some cases Silica (Chisti et al. 2007). Microalgal cultivation can be done in open production systems such as lakes or ponds and in controlled closed systems called Photobioreactors (PBRs). The most important methods include batch, continuous and semi-continuous modes.

a. Batch system:
This is a closed system, volume limited, in which there is no inputs or output of materials. The microalgal population cell density increases constantly until the exhaustion of some limiting factor, whereas other nutrient components of culture medium decrease over time. Other products produced by the cells during growth also increase in culture medium. When resources are used by the cells, the cultures die unless supplied with new medium. This is done by transferring a small volume of existing culture to a large volume of fresh culture at regular intervals. With this method microalgae are allowed to grow and reproduce in closed containers. In batch mode microalgae shows a typical growth dynamic pattern consisting of a succession of five phases of growth such as adaptation, acceleration, exponential growth, stationary and decline phase.

b. Continuous system:
In continuous culture systems, resources are potentially infinite. Cultures are maintained at a chosen point on the growth curve by the regulated addition of fresh culture medium. A volume of fresh culture medium is added at a rate proportional to the growth rate of microalgae, while an equal volume of culture is removed. This method maintains the cultures very close to the maximum growth rate, because the culture never runs out of nutrients.

c. Semi-continuous systems:
In this system the fresh medium is delivered to the culture all at once, by simply opening a valve in the medium delivery line. Fresh medium flows into the culture vessel, and spent culture flows out into a collecting vessel. Once the required medium has entered the culture, the valve is closed, and the culture is allowed to grow. The culture is grown up again and partially harvested. As the culture is not harvested completely, the semi-continuous method yields more algae than batch methods for a given tank size.

Open Ponds:
Open ponds systems are "raceway" designs in which microalgae are cultivated. Nutrients can be provided through runoff water from nearby land area or by channeling the water from waste treatment plants. The ponds are kept shallow to maintain the algae exposed to the sunlight and to keep the sunlight penetrated in the limited depth of the ponds, typically from 0.25 to 1m. The water is usually kept in motion by paddle wheels or rotating structures. During daylight, the culture is fed continuously in front of the paddle wheel where the flow begins. Broth is harvested behind the paddle wheel, on completion of the circulation loop. Generally, open ponds are more susceptible to environmental conditions, not allowing control of water temperature, evaporation and lighting. Their use is limited to a few algal species. Even so, they can produce large amount of microalgae, but occupy more extensive land area and are more susceptible to contaminations from other microalgae or bacteria.(Teresa et al. 2010). Open ponds productivity is measured in terms of biomass produced per day per unit of available surface area. This biomass remains low because raceway ponds are poorly mixed and cannot sustain an optically dark zone. The necessary for a large-scale cultivation area has been pointed out as a limitation in using open ponds to grow microalgae for mitigating the CO_2 released from power plants. A common feature of most of the algal species produced commercially (i.e. *Chlorella*, *Spirulina* and *Dunaliella*) is that they can be grown in open ponds systems and still remain relatively free of contamination by other microorganisms.

Photobioreactors:
Algae can be grown in closed systems called Photobioreactors (PBRs). These devices are bioreactors which incorporates some type of light source. PBRs are flexible systems that can be optimized according to the biological features of the microalgal species that are cultivated. PBRs provide a protected environment with safety from contamination by other microorganisms and culture parameters can be better controlled. They allow more species to be grown than open systems, and permit especially single-species culture of microalgae. They also prevent evaporation and reduce water use, lower CO_2 loss and permit higher cell concentration and consequently higher productivity. As microalgae are grown, excess culture is overflowed and harvested. If sufficient care is not taken, continuous bioreactors often collapse very quickly. PBRs can be divided into four groups as Vertical tubular, Horizontal tubular, Plate and Plastic bag systems. A comparison of different parameters in open and closed production of microalgae was summarised below in table.2.

Table 2: Comparison of opened and closed production systems

Parameter or issue	Open ponds and raceways	Photobioreactors (PBR)
Required space	High	Low
Water loss	Very high, may also cause salt precipitation	Low
CO_2 loss	High, depending on pond depth	Low
Oxygen concentration	Usually low enough because of continuous spontaneous outgas.	Closed systems requires gas exchange devices (O_2 must be removed to prevent inhibition of photosynthesis or photooxidative damage)
Temperature	Highly variable, some control possible by pond depth	Cooling often required (immersing tubes in cooling baths)
Shear	Low(gentle mixing)	High (fastand turbulent flows required for good mixing, pumping through gas exchange devices)
Cleaning	Not required	Required (wall-growth and dirt reduce light intensity)
Contamination Risk	High (limiting the number of species that can be grown)	low
Biomass quality	Variable	Reproducible
Biomass concentration	Low, between 0.1 and 0.5 g/l	High, between 2 and 8 g/l
Production flexibility	Only few species possible, difficult to switch	High, switching possible
Process control and reproducibility	Limited (flow speed, mixing,	Possible within certain tolerances
Weather dependence	High (light intensity, temperature, rainfall)	Medium (light intensity, cooling required)
Startup	6 – 8 weeks	2 – 4 weeks

Capital costs	High ~ US $ 100.000 per hectare	Very high ~ US $ 1.000.000 per hectare (plus supporting systems)
Operating costs	Low (paddle wheel, CO_2 addition)	Very high (CO_2 addition, pH-control, oxygen removal, cooling, cleaning, maintenance)
Harvesting cost	High, species dependent	Lower due to high biomass concentration and better control over species and conditions
Current commercial applications	5000 t of algal biomass per year	Limited to processes for high added value compounds or algae used in

Microalgae Oil extraction
Under optimal conditions of growth, algae synthesize fatty acids for esterification into glycerol-based membrane lipids, which constitute about 5-20% of their dry cell weight. However, under unfavorable environmental conditions, many algae alter their lipid biosynthetic pathways to the formation and accumulation of neutral lipids (20-50% DCW), mainly in the form of triglycerides (TAGs). For biodiesel production, these neutral lipids have to be extracted from microalgal biomass. Extracting microalgal oil is one of the most costly processes which can determine the sustainability of microalgae-based biodiesel. Several methods have been employed to dry microalgae, where the most common include spray-drying, drum-drying, freeze-drying and sun-drying. After drying it follows the cell disruption of microalgae. For biodiesel production, lipids and fatty acids have to be extracted from the microalgal biomass. Microalgal oil can be extracted using mechanical methods or chemical methods

Mechanical methods
These methods are classified in mechanical expeller press and ultrasonic assisted extraction.

Expeller press: microalgae are dried to retain its oil content and it can be pressed out with an oil press. Commercial manufactures use a combination of mechanical press and chemical solvents in extracting oil.

Ultrasonic extraction:. Ultrasonic waves are used to create bubbles in a solvent material, when these bubbles collapse near the cell walls, it creates shock waves and liquid jets that cause those cells walls to break and release their contents into the solvent. This method can be done with dry or wet microalgae.

Chemical Methods
Neutral lipids or storage lipids are extracted with non-polar solvents such as diethyl ether or chloroform but membranes associated lipids are more polar and require polar solvents such as ethanol or methanol to disrupt hydrogen bonding or electrostatic forces. The chemical extraction solvents are Hexane, benzene and ether. The first one is the most popular and inexpensive but is a good solvent only for lipids of low polarity. By working with chemicals care must be taken to avoid exposure to vapors and contact with the skin.

Hexane solvent method: Hexane solvent can be used together with a mechanical extraction method, first pressing the oil. After the oil has been extracted using an expeller, the remaining product can be mixed with hexane to extract all the oil content. Then, Oil and hexane are separated by distillation. Different solvents can be also used such as ethanol (96%) and hexane-ethanol (96%) mixture. With these solvents it is possible to obtain up to 98% quantitative extraction of purified fatty acids.

Soxhlet extraction: Oils from algae are extracted through repeated washing, with an organic solvent such as hexane or petroleum ether, under reflux in special glassware or Soxhlet extractor shown in figure

CONCLUSION
The demand of biodiesel production is increasing every year, and oil crops are compromising food crops. So, other sources of biodiesel such as microalgae will have to be commercialized. Microalgae are potential candidates for using excessive amounts of CO_2. Since the cultivation of these organisms are capable of fixing CO_2 to produce energy and chemical compounds with the presence of sunlight. The oil extracted from microalgae to produce biodiesel has a number of advantages over other oil crops. They are more productivity than crop plants and have the ability of carbon dioxide mitigation. Microalgae can be cultivated in open ponds systems or closed Photobioreactors (PBRs). Both methods are technically feasible. PBRs provide much greater oil yield per hectare and has more controlled environment than open ponds. However, PBRs are more expensive than open ponds systems. In Open ponds systems the strains are exposed to contamination by other microorganisms. Oil extraction from microalgae is also one of the most costly processes and determines the sustainability of algae-based biodiesel. Best costly feasible methods combine chemical extraction solvents and mechanical extraction.

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