



(REVIEW ARTICLE)



## A review on third-generation biofuels from marine diatoms

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### Abstract

Modern life increases the consumption of fossil fuels leading to a decrease in natural resources. Therefore, an alternative energy sources like biowastes - food waste, agricultural waste, municipal waste, etc. energy tree sources like edible and non-edible oily seeds; and various aquatic plants were also identified as energy sources. In recent years, many efforts have been made to determine the possibility of using algae as a source of bio-oil and biogas for energy production. Algae are considered as large and diverse group, often autotrophic organisms, ranging from unicellular to multicellular. They can produce far greater amounts of biomass and lipids per hectare than any other type of terrestrial biomass-producing organism. Due to their global presence, diatoms are known for producer of biofuels in recent years. Another energy sources is biodiesel, which is used as a sustainable form of diesel fuel derived from natural sources. Diatomic biofuels are among the third generation biofuels that ultimately contribute about 60-90% less greenhouse gas emissions than traditional fuel sources.

**Keywords:** Diatoms; Lipids; Biofuel; Energy source; Ecofriendly

### 1. Introduction

Increase in human population globally ultimately leads to an increases the needs like food, energy sources etc from natural resources to meet their demands in present and future days (Alexandratos and Bruinsma, 2012). Nowadays, scientists and researchers are mainly focusing on alternative sources for human needs through natural resources. One of the major available sources to meet the human food and others demands is the marine resources. Therefore, the use of pre-existing organisms such as diatoms is considered an extraordinary source for biofuel production.

Diatoms are known as single-celled algae surrounded by a hard shell, show rapid growth in the presence of sun light conditions, biomass concentrations double within hours, cell division by characteristic spores production, freely float on water body along with water currents. They are considered as major contributors to phytoplankton blooms in water bodies like lakes and in the sea. They are central in aquatic ecosystems and the global carbon cycle (Parker et al., 2010). Algae can capture carbon dioxide and convert solar energy into chemical energy. Diatoms are best suited for biofuel production. The presence of diatoms on a global scale (saltwater, freshwater, in soil or on wet surfaces) gives diatom cells a competitive advantage than other. The growth of diatoms can be easily controlled by silicate accessibility, one of the major advantage in production of valuable fuels and other biological products (Wang and Seibert 2017).

Biodiesel considered as renewable energy source derived from natural sources (natural fats and oils). Advantages of biodiesel such as economic growth, fuel quality, environmental friendliness, as well as energy security compared to other fuel- diesel oil. The method of use for conversation of natural resources into biodiesel is known as trans esterification. The process include the use sources such as animal fats, oils of vegetable origin and oils from microalgae, which are then subjected to alcohol esterification (either methanol or ethanol) in the presence of of a catalyst (either

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potassium sodium hydroxide or sodium hydroxide) to form more fatty esters (methyl ester or ethyl ester) (Vasudevan and Briggs 2008). History of algal biofuels

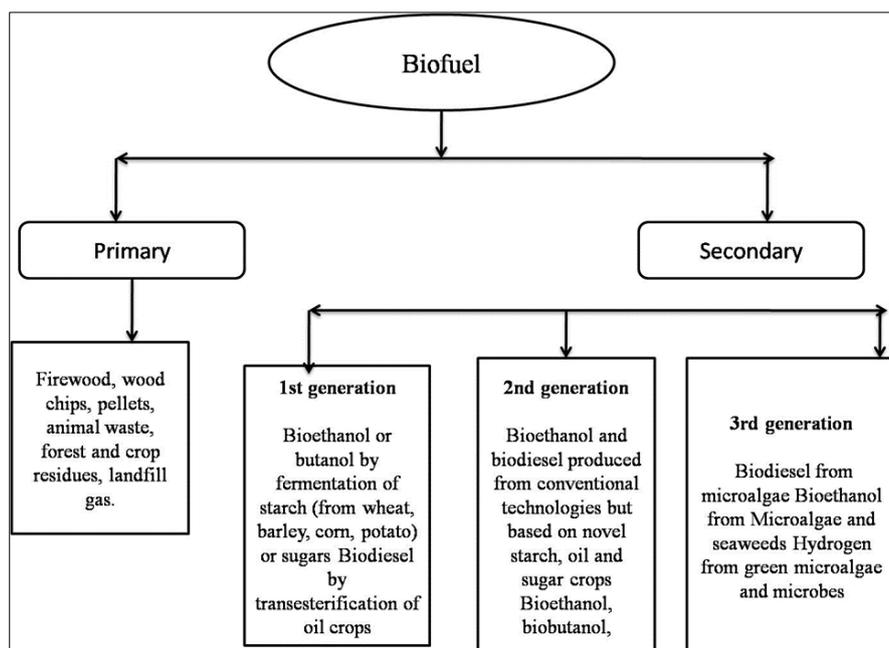
The advantages of microalgae as a feedstock for bioenergy stated since the mid-20th century. Although a commercially viable and scalable system has not yet emerged, early studies have identified the fundamental approaches and later the technologies being explored. The use of algae mainly as energy production began in the late 1950s when Meier (1955) and Oswald & Golueke (1960) proposed the usage of carbohydrate fraction of algal cells to produce methane by fission and anaerobic destruction. A detailed technical analysis given by Benemann et al. (1978), showed that microalga cell systems can produce methane at a price competitive with the expected cost of fossil fuels. The discovery that many species of microalgae can produce large amounts of lipids in the form of cellular oil droplets under certain growth conditions dates back to the 1940s. Multiple reports in the 1950s and 1960s indicated that the lack of key nutrients, such as nitrogen or silicon, can lead to this phenomenon. However, the concept of using fat as an energy source only got serious attention during the oil ban in the early 1970s and energy prices skyrocketed throughout the decade. The idea of using fat as an energy source eventually became a major benefit of the Department of Energy's Aquatic Species Program (ASP). The Aquatic Species Program (ASP) reports one of the most comprehensive research efforts to date on microalgae biofuels. The program ran from 1978 to 1996 and supported research primarily at DOE's National Renewable Energy Laboratory (NREL), formerly known as the Solar Research Institute. The Aquatic Species Program has also funded research at many academic institutions through subcontracts. Approximately \$25 million (Sheehan, 1998) has been invested in the program over 18 years. In the early years, the focus was on using algae to produce hydrogen, but the focus shifted to liquid fuels (biodiesel) in the early 1980s. Initiation was made by isolation and characterization of algae strains. In addition, studies on algal physiology, algal biochemistry, genetic engineering, process development and demonstration on large scale algal mass culture were reviewed. The technology approach, economic analysis and resource assessment are also important aspects of this programme. A detailed outline of the project was completed in 1998 (Sheehan et al., 1998). Some of the project highlights are Aquatic Species Program (ASP), where researchers collect microalgae strains, mainly green algae (Chlorophyceae) and diatoms (Bacillariophyceae), represents the diversity of aquatic environments and types of water. Many strains have been isolated from shallow saltwater environments in Iceland, which often experience dramatic changes in temperature and salinity. The isolates were screened for their tolerance to changes in salinity, pH and temperature, as well as their ability to produce neutral lipids. After selecting promising microalgae species, subsequent studies observed the ability of many microalgae strains to induce lipid accumulation under nutrient-limited conditions. Although nutrient deficiency reduces the overall rate of oil production in the culture medium (due to a concomitant decrease in cell growth), the response to nutrient restriction has led to valuable insights. The lipid biosynthesis mechanism under inducible conditions, shown to accumulate up to 60% of their dry weight as lipids, mainly triacylglycerides (TAGs) (Chisti, 2007).

### 1.1. Classification of biofuel

Biofuels are referred to as solid, liquid or gaseous fuels derived from organic materials. Biofuels are divided into primary and secondary biofuels (Fig. 1). While primary biofuels such as firewood are used in their unprocessed form mainly for heating, cooking and to generate electricity. Secondary biofuels such as bioethanol and biodiesel are derived from biomass processing and can be used in various industrial processes. Secondary biofuels can be grouped into three generations - first-generation, second-generation and third-generation biofuels depending on parameters such as type of process technology, type of materials or their growth (Hussain et al., 2010). Although biofuel processes have great potential to provide a carbon-neutral pathway for fuel production.

### 1.2. First generation biofuel

The first-generation biofuel production systems have significant limitations: economic and environmental. The most common concern with first-generation biofuels today is that as production capacity increases, so does their competition with agriculture for arable land used for food production increased. Increasing pressure on arable land currently being used for food production can lead to severe food shortages, especially in developing countries, where more than 800 million people suffer from hunger and malnutrition. In addition, intensive land use with the use of large amounts of fertilizers, pesticides and water use can cause serious environmental problems (Schenk et al., 2008).



**Figure 1** Classification of biofuels

### 1.3. Second generation biofuel

Future second-generation biofuels aim to produce fuel from lignocellulose biomass, the woody part of plants that does not compete with food production. Second-generation biofuel sources include agricultural by-products, logging byproducts or woodworking waste such as leaves, straw or wood chips as well as unused portions of corn or sugarcane. However, converting wood biomass into fermentable sugars requires expensive technologies involving pretreatment with special enzymes, which means that second-generation biofuels cannot be easily produced due to problems with economies of scale (Brennan and Owende, 2010). Therefore, third generation biofuels derived from microalgae can be considered as a viable alternative energy source without the major disadvantages associated with first and second generation biofuels. (Nigam and Singh 2010, Chisti, 2007, Li et al., 2008). Microalgae can produce 15 to 300 times more than conventional crops grown on an acreage basis. In addition, compared to traditional crops that have typically harvesting process, once or twice a year whereas microalgae have a very short harvest cycle ( $\approx 1-10$  days depending on the process), allowing for multiple harvests, continuously with significantly increased output (Liang et al., 2008).

#### 1.3.1. Forms of biofuel

Biofuel is defined as a solid, liquid or gaseous fuel obtained from non-living or living biological material. It is similar to fossil fuels, obtained from long-buried biological material. Biomass or biofuel is dead matter derived from living organisms or some fossil fuels. This includes plants, animals and their by-products. For example, manure, yard waste and crop residue are all sources of biomass. It is a renewable energy source based on the carbon cycle, unlike other natural resources such as oil, coal and nuclear fuel. The U.S. Department of Energy estimates that it would cover an area of 15,000 square miles (38,400 square kilometers) if algae biofuels replaced all petroleum fuels. Microalgae, such as *Botryococcus braunii* and *Chlorella vulgaris* are relatively easy to grow, but microalgae oil is not easy to extract. There are many approaches available, some of which work better than others.

### 1.4. Third generation biofuel

Algae fuel, also known as "third generation biofuel" is a fuel made from algae. Algae are a low-input, high-yield raw material for biofuel production. Algae produce 30 times more energy per acre than land crops like soybeans. With the rising price of fossil fuels (petroleum), algae farming (seaweed farming) is attracting a lot of attention. One of the advantages of many biofuels over most other fuels are biodegradable and relatively harmless to the environment when spilled. Third-generation biofuels has a very high growth yield as compared with classical lignocellulosic biomass (Brennana and Owendea 2010). Algal derived biofuels generally relies on the lipid content, usually, some species like *Chlorella* and *Scenedesmus* are aimed because of their high lipid content (around 60 to 70%), (Liang et al., 2009) and their high biomass productivity (7.4 g/L/d for *Chlorella protothecoides*) (Chen et al., 2011). There are many challenges relevant to the algal biomass, some of them are geographical and some are technical challenges. Typically, algae will produce 1 to 7 g/L/d of biomass in ideal growth conditions (Chen et al., 2011). This implies that large amounts of water

are needed on an industrial scale, which poses a major problem for countries like Canada, where temperatures are very low (below 00C) for most of the year. The high water content creates a problem when lipids need to be extracted from algal biomass, the extraction process requiring dehydration, either by centrifugation or filtration prior to lipid extraction. Lipids obtained from algae can be treated by transesterification or can be hydrolyzed to yield kerosene-grade alkanes that can be used as alternative aviation fuels (Tran et al., 2013).

Biofuels appear to be the only renewable biofuels capable of meeting the global demand for transportation fuels. Microalgae use sunlight just like plants to produce oil, but they are more efficient than cultivated plants. Microalgae may provide some renewable biofuels. These include the methane produced by the anaerobic digestion of microalgal biomass (Spolaore et al., 2006), biodiesel derived from microalgae oil (Roessler et al., 1994; Sheehan et al., 1998; Banerjee et al., 2009) and biohydrogen were generated by photobiology (Ghirardi et al., 2000, Akkerman et al., 2010). Many valuable and useful by-products can be extracted from algal biomass, including fatty acids, amino acids, plant hormones, pigments and oils for biofuels, nutraceuticals, metabolites and  $\beta$ -carotene. Many microalgae species are endowed with very high lipid ratios and analytical methods that make up the quality of lipids produced by many microalgae species. In short, many parameters such as lipid content, growth rate, fatty acid composition and culture conditions must be considered to identify the most promising microalgae species and maximize oil yield per acre for biofuel production. Fossil fuels, crop-based biofuels, coal, nuclear power, renewable energy, natural gas, etc. are sources of fuel in the first place. But due to the many disadvantages of these fuel sources and their limited storage tanks, they are not a promising fuel stockpile for the future.

### 1.5. Biofuel Production from Diatoms

Biofuel based on diatoms can be obtained by two routes. The first method is the thermochemical conversion of the entire biomass into bio-crude oil such as crude oil and the next method directly extracts the lipid content and then sends it for processing into biofuel. Although the second method is the conventional technological way to date, the first achieves movement because it has positive advantages. Another related fact is: what percentage of oil is in diatom cells? For the oil content, the most important step is to learn all about the lipids available, including all forms of unsaturated fatty acids and forms of saturated fatty acids, followed by the extraction process, and finally is the quantification of oil content (Jha and Zi-Rong 2004). Besides choosing the best method to maximize biological products, it is very important to choose an appropriate strain that can give high yield. It is very common that by selecting specific species and manipulating the supplements provided in the growth medium, the oil production of microalgae can be affected. It has been found that under conditions where organisms grow under nitrogen deficiency, the triacylglycerol of *Chaetoceros gracilis* can account for more than 70% of the total cell volume (Syvertsen, 2001). The measurement was made because the weight is much more consistent, but it shows other possible problems associated with the common algae biofuel production process. Algae cells grown in nutrient-deficient conditions will achieve maximum lipid content, but this is only possible if we prolong the microbial growth period. This affects the overall productivity of the production process per unit area over time. Algae not exposed to stressful conditions can grow rapidly, but the lipid content obtained is limited. A major difference between lipids is established in diatoms, a few of which are extrachloroplast phospholipids and membrane bound glycolipids.

### 1.6. Microalgae-valuable biofuel over first and second-generation biofuel

Biofuel can be obtained from various sources such as sesame, rapeseed/canola, oil palm, sunflower, mustard, soybean, cotton, corn, etc. Because they are more nutrient dense and difficult to grow in adverse environmental conditions, they cannot supplement the world's dependence on fuel. Algae were once considered "aquatic plants", but they are now classified separately because of the lack of plant characteristics such as true roots, stems, leaves and embryos. Although we refer to algae as feedstock for biofuels, this definition includes all simple unicellular or multicellular microorganisms, including prokaryotes, e.g. cyanobacteria (Chloroxybacteria) and eukaryotic microalgae, e.g. green algae (Chlorophyta), red algae (Rhodophyta) and diatoms (Bacillariophyta). The main advantages of microalgae-derived biofuels over first and second generation biofuels includes the microalgae growth which can be year-round and thus the amount of oil produced exceeds that of the best oil crops (Schenk et al., 2008; Chisti 2007; Dalemans, et al 2002). The potential for rapid growth and the number of microalgae species with oil content between 20 and 50% of the dry weight of the biomass is another advantage of microalgae to be selected as potential biomass. Microalgae grow rapidly compared to terrestrial cultures. They usually double every 24 hours. The exponential growth rates can double their cell biomass in just 3.5 hours (Chisti 2007, Spolaore et al., 2006).

Second, despite growing in water, algae require less water than in terrestrial cultures, which also reduces stress on freshwater resources (Dismukes et al., 2008). That's why microalgae can also be grown in brackish water on uncultivated land, and thus may not alter the land use, minimizing associated environmental impacts (Searchinger et al., 2008), without affecting food, fodder and other products derived from terrestrial crops (Chisti 2007). According to

Chisti (2007), 1 kg of dry algal biomass uses about 1.83 kg of carbon dioxide, so the production of microalgae biomass can help biofix the carbon dioxide waste involved in maintaining and improve air quality. Cyanobacteria (blue green algae) contain a large proportion of lipids with a chemical composition similar to vegetable oils. The many fatty acids of cyanobacteria are essential components of human and animal diets and an important feed additive in aquaculture (Borowitzka 1988). Lipids of some cells are rich in fatty acids (Singh 2002).

Lipids play an important role as the structural component of most cell membranes. They also have an important role in tolerating a number of physiological stresses in a diverse group of organisms, including cyanobacteria. There is a dual potential for the treatment of organic wastewater from the agri-food industry for algae culture (Lan et al., 2012). In addition to providing a growth medium, key nutrients for its cultivation, e.g. nitrogen and phosphorus, can also be obtained from wastewater. A significant environmental benefit is that growing algae does not require the use of herbicides or pesticides (Rodolfi et al., 2009). Furthermore, they can also produce valuable by-products such as protein and biomass remaining after extraction of algal oil, which can be used as animal feed or fertilizer (Spolaore 2006), or can perform fermentation to produce bioethanol or biomethane. The biochemical composition of algal biomass can be altered by adjusting the growth conditions and thus significantly improving oil yield. In addition, microalgae are capable of producing “biohydrogen” (Ghirardi et al., 2000). Therefore, it becomes quite urgent that the combination of biofuel production potential, CO<sub>2</sub> sequestration, biohydrogen production and biological wastewater treatment; as summarized previously, to highlight the potential use of microalgae as feedstock for biofuels.

### 1.7. Algal fuel versus other biofuels

Algae oil can be used to produce biodiesel after treatment and is often accumulated as a membrane component, storage product, metabolite and energy source under some special conditions. Recently, microalgae has attracted considerable attention from researchers and entrepreneurs as an alternative to non-food biodiesel due to its high oil content and rapid biomass production, for example: *Spirulina*, *Westiellopsis*, *Chlorella*, *Anabaena*, *Calothrix* and some other eukaryotic algae, etc. Algae could be the preferred feedstock for high-energy production and an alternative liquid fuel for transportation. Several aspects of algae biofuel production have combined to attract the interest of researchers and entrepreneurs around the world. Certain favorable condition single species of algae grow rapidly results in blooms (Madhavi and Kondal Rao, 2004). Algal biomass production can be high per acre cultivated.

- Algae farming strategies can avoid competition with arable land and nutrients used for traditional agriculture.
- Algae can use wastewater, production water and saltwater to reduce competition for limited freshwater supplies.
- Algae can recycle carbon from CO<sub>2</sub> rich emissions from fixed sources, including power plants and other industrial emissions.
- Algae biomass is in line with the integrated vision of the biorefinery to produce a wide range of fuels and valuable co-products.

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## 2. Conclusion and future Aspect

The world has recognized that first and second generation biofuels, mainly produced from land crops, are not enough to meet global energy demand, so the researchers are looking for alternative fuel sources for biofuel production. In addition, for the cultivation of terrestrial crops, the demand for agricultural land will increase and the demand for fertilizers will also increase. The third generation biofuels that can be obtained from microalgae are attracting much attention due to their ability to spread on uncultivated land and also for high oil yield per acreage. Morphological and physiological differences between diatoms allow these cells to respond rapidly to any type of stress. The oil content of diatom cells depends on the selection of strain or species used for cultivation purposes, cultivation parameters, such as nitrogen stress conditions provided, light intensity required for growth, the framework was chosen to recover the biomass concentration, and especially the protocol was chosen for oil extraction. The effectiveness of the withdrawal strategy depends on various factors that influence the selection of the best cell lysis technique, as well as the strain selected and the nature of their cell wall, whether polar fats or polar substances, in addition to operating costs and energy costs.

The main method for developing a biofuel production strategy from diatom lipids is to obtain it at low cost through selecting the best strain to cultivate and developing a better farming strategy that allows for better harvest i.e maximum lipid yield. Soil yields such as corn, rapeseed and soybean act as the main feedstock for biodiesel production. Unfortunately, the use of food production to produce biodiesel leads to competition between agricultural land use and land use for biofuel production, which in turn increases costs.

To achieve sustainable and cleaner diatomaceous earth fuels at a cost-feasibility, special attention should be paid to environmentally friendly extraction processes, using less solvent, increasing biofuel quality learning and limiting energy as well as the use of time and steps. Therefore, the production of bio-alcohol and biodiesel can be coordinated. In this way, biodiesel can be obtained from microalgal fatty acids and bioethanol can be obtained from defatted biomass (methyl solvent is replaced by ethyl solvent during ester conversion.), instead of using petroleum-based solvents. Therefore, the use of green solvents for microalgae extraction and biofuel production is critical to economic and ecological success, and such methods can be useful in social production.

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## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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