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Algae as a Feedstock for Bioethanol Production: New Entrance in Biofuel World

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Abstract: Bioethanol is produced from different source, they have their own application and limitation, and their study is related to fermentation process. In this era ethanol production is not a big approach, but when using some renewable source, it may affect in fuel world. This type of studies have revealed to a novel side of innovation world. Bioethanol from algae feedstock are the "third generation" of biofuel to be known as one of the most important renewable energy source. Today, global demand of ethanol generate the opportunity to explore new renewable biomass sources. Microalgae are a promising alternative source of lipid for biodiesel production but certain algae are rich in carbohydrates of various forms. They provide viable feedstock for fermentation to ethanol. Algae have shorter growth cycle as compared to other plant, hence the algae is a very promising source of biomass for the production of biofuels and also reduce in climate change effects. The review mainly focus on researches related to the bioethanol production, where the algae have acted as a most fast growing supporter of bioethanol and also define the bioethanol production potentially higher than other crops.
Keywords: Bioethanol, Microalgae, Macroalgae, Fermentation.

1.INTRODUCTION

After the fuel crisis in 1970, worldwide ethanol production has strongly increased. The market of ethanol grew from less than a billion litres in 1975 to more than 39 billion litres in 2006, and is expected to reach 100 billion litres in 2015 [1]. The international ethanol market has been stimulated by governmental politics of incentive to the use of renewable fuels and energy source. Bioethanol can be produced from many different biomass feedstock. Nonetheless, the feasibility and economy of using lignocellulosic biomass materials as a feedstock is often limited by the low yield and the high cost of the hydrolysis process based on the current technologies [2]. Microalgae are having the capability of producing energy rich lipids, Besides this some microalgal species can also produce substantial amounts of carbohydrate related materials. For example, some microalgae and cyanobacteria have the capacity to accumulate large amounts of storage polysaccharides.These storage products include starch, glycogen, and chrysolaminarin. Furthermore, some microalgal cell walls are composed of cellulose, mannans, xylans, and sulfated glycans. These polysaccharides can be broken down chemically or enzymatically into simple sugars that can then be converted into ethanol.

In this perspective, algal biomass is secure wide attention as an alternative renewable feedstock for the production of bioethanol [3]. The mass production of first-generation liquid biofuels has resulted in a series of problems related to food prices, land usage, and carbon emissions [4] and second generation biofuels production suffers with cost effectiveness, technological barriers, and feed stock collection networks[Fig.1] [3]. Algal biofuels are an appealing choice [5] due to its rapid growth rate, high lipid content, comparatively low land usage and high carbon dioxide absorption and uptake rate [6-11].

Microalgae appear to be the a source of biodiesel that has the potential to completely displace fossil diesel[12]. Extensive research has been conducted to investigate the utilization of microalgae as an fermentative feedstock, with applications being developed for the production of bioethanol [13-15].

Figures:

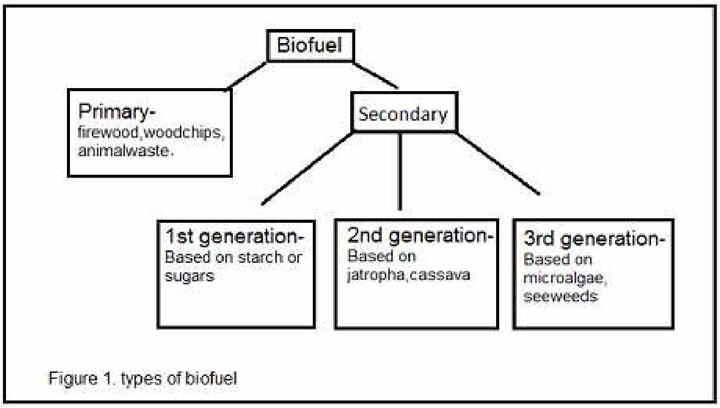

Fig.1 Types of biofuel [3]

2. ALGAE: BASIC CONCEPTS

Algae belong to a large group of simple photosynthetic organisms. They are subdivided into two major categories based on their size. Microalgae, are small free-living microorganisms that can be found in a variety of aquatic habitats. Algae, considered as the third generation biomass have proved to be superior to any other biomass due to its environmental and economic sustainability, security of supply, absence of lignin, high photosynthetic efficiency, fast-growing rate and role in reduction of greenhouse gas emissions. They have the potential to convert atmospheric carbon dioxide into useful biomass by growing even in wastewaters and can yield biofuels without much harm to food supplies, biodiversity and agriculture. Algae can thus play a major role in the treatment/utilization of wastewater and reduce the environmental impact and disposal problems [16]. They can be grown on saline/coastal sea water or artificial sea water and on non agricultural lands (desert, arid and semi-arid land)[7, 17] and will create a food–fuel competition. Comparing in other advanced feedstock based on cellulose for biofuels production, algal genomics and basic research are more advanced and gaining in momentum[16] .

Algae have high photon conversion efficiency and can synthesize and accumulate large quantities of carbohydrate biomass for bioethanol production, from in expensive raw materials [16, 18]. Aquatic algal cells are buoyant, avoiding the need for structural biopolymers such as hemicellulose and lignin that are essential for higher plant growth in terrestrial environment. This simplifies the process of bioethanol production by eliminating the chemical and enzymatic pre-treatment steps [2].

Moreover,algal cells can be harvested within a short span of time compared to other feedstock and hence can meet the increasing demand of feedstock for ethanol production [19]. Algae biofuels is a rapidly advancing area with many studies focusing on production, harvesting, increasing carbohydrate and lipid content and processing technologies. However, there are relatively some studies on the longterm and wide sustainability and life cycle analysis of the algae to biofuels pathways[20].

The algal photosynthesis is mainly based on Calvin cycle in which ribulose-1,5-bisphosphate (RuBP) combines with CO_2 to produce two 3-phosphoglyceric acid (3-PGA) which is utilized for the synthesis of glucose and other metabolites [2]. Attempts were carried out to redirect 3-PGA to ethanol by introducing ethanol producing genes (pyruvate decarboxylase and alcohol dehydrogenase). An ethanogenic recombinant of *Rhodobacter sp.* was developed for carbon redirection from the Calvin cycle to ethanol [21]. The recombinant algal strain could produce ethanol in presence of light but required oxygen free condition as it was an anaerobe [2].

Microalgae do not need to generate elaborate support and reproductive structures, they can devote more of their energy into trapping and converting light energy and CO_2 into biomass. Microalgae can convert roughly 6 percent of the total incident radiation, into new biomass[22]. By comparison, terrestrial crops have generally lower photosynthetic conversion efficiencies. Sugar cane, one of the most productive of all terrestrial crops, for example has a photosynthetic efficiency of 3.5-4 percent[23]. Based upon this distinguishing feature, microalgae have become a target for scientific studies on biomass energy production for biofuels.

The extremely large arable land utilization for biomass production to be used as a raw material for production of bioethanol as well as biofuels could result in shortages in basic foods, such as corn, cereals, soy, mustard, barley, etc.[Table.1]. Thus, they have brought much controversy and debate on their sustainability[24]. In this respect, cultivation of algae at sea water or industrial or other wastewater provides a possible solution for this energy issue.

Table 1. Water footprint, land use and bioethanol yield of various energy crops[10]

Crops	Water footprint (m3 GJ-1)	Land use (m2 GJ-1)	Energy (GJ ha-1 a-1)	Biofuel yield (L ha-1 a-1)
Cassava	148	79	126	6,000
Wheat	93	305	33	1,560
Paddy rice	85	212	47	2,250
Corn grain	50	133	75	3,571
Potatoes	105	114	88	4,167
Sugar cane	50	81	124	5,882
Sugar beet	46	95	105	5,000
Sorghum	180	386	26	1,235
Soybean	383	386	26	1,235

Algae are considered as the only alternative to current bioethanol crops such as corn and soybean as they do not require arable land [25].The arable land could be used efficiently to grow food crops rather than biomass and oil seed crops for the production of biofuels. Water filled areas that are not utilies for growing food crops and industrial waste water can be used for the cultivation of algal biomass without any compromise with land and water resource for the production of bioethanol that will also not show adversely affect the food cost and the economy [10]. In addition, algae can be converted directly into bioenergy, such as biodiesel, bioethanol and biomethanol and therefore can be a source of renewable energy.

3.PROCESS OF BIO-ETHANOL PRODUCTION

The simplified superstructure flow sheet for the integrated production of bio-ethanol from algae. First, microalgae cultivation using sunlight energy is carried out in open or covered ponds or closed photobioreactors, based on tubular or other designs. In the next step, the biomass needs to be concentrated by an initial factor of at least about thirty-fold, requiring very low-cost harvesting processes. The starch can be extracted from the cells with the mechanical tools (e.g., ultrasonic, explosive disintegration, mechanical shear, etc.) or by dissolution of cell walls using enzymes[2]. The starch is then separated by extraction with water or an organic solvent and used for fermentation to yield bioethanol. Both saccharification and fermentation processes can be simultaneously carried out in a single step if an amylase producing strain can be used for ethanol fermentation. Utilization of starch degrading ethanol producers can preclude the cost incurred for acid or enzymatic saccharification of starch. Fermentation of microalgal biomass involves minimum input of energy and the whole process is less complicated compared to biodiesel production[Fig.2]. Recently,[19] investigated the suitability of lipid extracted microalgal debris for fermentation with a yield of bioethanol about 4–10 gl^{-1} of the substrate. Besides starch, several algae, mostly green algae and red algae show different type of sugar, like green algae can accumulate cellulose as the cell wall carbohydrate, which can also be used for ethanol production. The biomass from red alga can be depolymerised to yield mixed monosugars such as glucose and galactose [2].

Identification of suitable pretreatment methods and environmental factors with enhanced enzymatic hydrolysis followed by improved fermentation processes that will help to obtain high ethanol yields from algae.

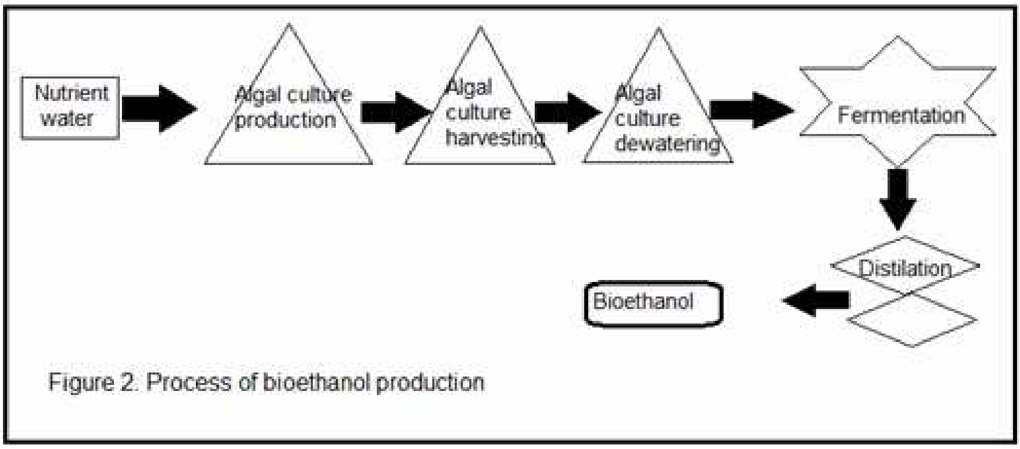

Fig.2 Process of bioethanol production from microalgae.

4.MICROALGAE AS A FEEDSTOCK FOR ETHANOL PRODUCTION

The unicellular marine microalgae were considered to be an abounding resource for carotenoids, lipids, and polysaccharides, and were widely investigated in the fields of food supplements and bio-fuel production [26]. Microalgae like *porphyridium, Chlorella ,Dunaliella, Chlamyd-omonas, Scenedesmus, and Spirulina* are known to contain a large amount (>50% of the dry weight) of starch, cellulose and glycogen, which are raw materials for ethanol production [27, 28] .

Certain species of microalgae have the ability of producing high levels of carbohydrates instead of lipids as reserve polymers like *spirulina, porphyridium purpureum*. These species are ideal candidates for the production of bioethanol as carbohydrates from microalgae can be extracted to produce fermentable sugars. It has been estimated that approximately 5000–15,000 gal of ethanol/acre/year (46,760–140,290 L/ha)[Table.2] can be produced from microalgae.

This yield is several orders of magnitude larger than yields obtained for other feedstocks (Table 2). Blue-green algae including *Spirogyra species* and *Chlorococum sp.* have been shown to accumulate high levels of polysaccharides both in their complex cell walls. This polysaccharides accumulation can be used in the production of bioethanol [29,30].The blue-green algae *Chlorococum sp.* produces 60% higher ethanol concentrations for samples that are pre-extracted for lipids versus those that remain as dried intact cells.

Table 2. Ethanol yield from different sources[54]

Sources	Ethanol yield (gal/acre)	Ethanol yield (L/ha)
Corn stover	112–150	1,050–1,400
Wheat	277	2,590
Cassava	354	3,310
Sweet sorghum	326–435	3,050–4,070
Corn	370–430	3,460–4,020
Sugar beet	536–714	5,010–6,680
Switch grass	1,150	10,760
Microalgae	5,000–15,000	46,760–140,290

The microalgae *Chlorella vulgaris*, particularly, has been considered as a promising feedstock for bioethanol production because it can accumulate up to 37% (dry weight) of starch. However, higher starch contents can also be obtained for optimized culture conditions [31]. *Chlorococum sp.* was also used as a feedstock for bioethanol production under different fermentation conditions. Results showed a maximum bioethanol concentration of 3.83 g/L obtained from 10 g/L of lipid-extracted microalgae debris [29].

Production of ethanol by using microalgal as raw material can be performed according to the following procedure. In the first step, the starch of microalgae is released from the cells with the aid of mechanical equipment or an enzyme. When the cells begin to degrade, yeast is added to the biomass to begin fermentation process. The end product of fermentation is ethanol. The ethanol is drained from the tank and pumped to a holding tank to be fed to a distillation unit. Ethanol was produced with microalgal photosynthesis and intracellular anaerobic fermentation [31-34]. The complex compositionof macroalgae make it a difficult substrate to ferment to ethanol by one or a few strains of microbes. The complex composition of seaweeds are containing several different carbohydrates. Finding a microorganism that can ferment all the different carbohydrates to ethanol is not very likely. So the microalgae are more beneficial than macroalgae. This indicates that microalgae can be used for the production of both lipid based biofuels and for ethanol biofuels from the same biomass as a means to increase their overall economic value [35].

5.MACROALGAE AS A FEEDSTOCK FOR ETHANOL PRODUCTION

Macro-algae (the large sized algae) can also be utilized for ethanol fermentation [36]. Seaweeds are classified into three groups: green, brown, and red, and they contain various types of glucans which are polysaccharides composed of glucose, though the concentration of these glucans is known to be relatively low .The absolute absence or near absence of lignin makes the enzymatic hydrolysis of algal cellulose simple. Macroalgal genera, such as, *Alaria*, *Saccorhiza* and *Laminaria* are belonging to brown algal group and grows up to meters and their main energy storage materials are laminarin and mannitol [36, 37].

The red algae such as *Gelidium amansii* also contribute in ethanol production. which is composed of cellulose, glucan and galactan, can also serve as a potential feedstock for ethanol production [38] . Macro-algae can be cultivated on nets or string, and can be seeded onto thin weighed strings suspended over a larger horizontal rope [36]. *Oleaginous* algal residue after extraction of oil also can be used for obtaining fermentable sugar for bioethanol synthesis [2] , the possibility of ethanol production from *Laminaria hyperborean* extracts was evaluated, focusing on the yeast *Pichia angophorae* and its potential of utilising both mannitol and laminaran as substrates.

Initial experiments with seaweed extract showed that *Pichia angophorae* was able to utilise both mannitol and laminaran for ethanol production[39]. Ethanol can be produced from the mannitol and laminaran contained in extracts from *L. hyperborea* fronds. *Z. palmae* was able to produce ethanol from mannitol, while *P. angophorae* could utilise both carbohydrates for ethanol production. Seaweeds are known to contain a low concentration of lignin or no lignin at all, While having a low lignin content, macroalgae contain significant amount of sugars for ethanol production but complex structure of seaweed is major problem in biofuels world.

6.OTHER COMMERCIAL BENEFITS FROM ALGAE

Commercialy high-level production of microalgae started in the early 1960s in Japan with the culture of *Chlorella* as a food habituate, which was followed in the 1970s and 1980s by expanded world production in countries such as USA, India, Israel, and Australia [40-42] .

In 2003 recorded production of *Chlorella*, which has a nutrient value and high protein content, was 2000 tonnes per annum. *Chlorella* is also used for medicinal value such as protection against renal failure and growth promotion of intestinal *lactobacillus* [43].There are health concerns over the ingestion of *cyanobacteria* (e.g. *Spirulina*).In studied over 50 strains of cyanobacteria and found that nearly all the strains produced the neurotoxin b-N-methylamino-L-alanine (BMAA). BMAA is linked to amyptrophic lateral sclerosis–Parkinsonism dementia complex, Lou Gehrig’s disease (ALS) and Alzheimer’s disease.

Algae are the natural food source of many important aquaculture species such as molluscs, shrimps and fish [40].The main applications for algal biomass in aquaculture are: fish feed [44] including larval nutrition for molluscs or peneid shrimp[45];colouring for farmed salmonids [45]; stabilization and improvement of quality of culture medium (‘green-water’ technique) [46];inducement of essential biological activities in bred aquatic species [45]; and enhancement of the immune systems of fish [47].

Polyunsaturated fatty acids (PUFAs) are essential for human development and physiology [48]. Among other things, PUFAs have been proven to reduce the risk of cardiovascular disease [49, 50].

Most notably pyrolysis, result in the formation of the solid charcoal residue ‘‘biochar’’, that has potential agricultural applications as a biofertiliser and for carbon sequestration [51, 52] .

7. IMPEDIMENT AND CHALLENGES FOR ETHANOL PRODUCTION

Bioethanol production is a commercial thing and is not as such easily produce, many thing are involve in this process, and also various things are hinder this process. Bioethanol fuel is one of the positive trends for sustainable development, The commercial approach for this will depend on the condition of the origin, on the temperature and light conditions of the region and on the selected microalgae species. Ethanol is an intermediate in the complete digestion of organic material and is produced by specific microbial strains. An obvious practical problem with ethanol production is that the microbial culture may have to be protected against contamination of other microbes. Thus, ethanol production should take place under controlled conditions to prevent contamination problems [37] .

8.INFERENCE AND FUTURE PROSPECTS

Microalgae played an important role in ethanol production. It shows significant improvements in the efficiency, cost structure and ability to produce large scale lipid extraction, algal growth and biofuel production. Biofuels based on algal biomass will play a role in the future energy systems. Different species of algae may be better suited for different types of fuel. Most difficult thing in ethanol production is selection a proper strain of microb, that they fermented the algal biomass very effectively.

In macroalgae case, the complex composition of seaweeds makes it a difficult feedstock to produce ethanol by one or a few strains of microorganism. Selection of microorganism is very difficult. Microalgae are unicellular marine algae, don’t have any complex structure and also have high photoconversion efficiency and shows suitability to ethanol production.

The species belong to the saccharomycatina covering over 200 million year of the yeast evolutionary history and including different genera, they are very useful in fermentation process. Different yeast species were studied for their carbon metabolism and the results are presented as specific glucose consumption rate specific ethanol production rate and specific growth rate.

Many species they highly contributein ethanol production for ex. *saccharomyces crevisiae*, production rate (EtoH/biomass/hour) is 0.84g/gDW,h. As such many species are present,they have very effective production rate for ex. *Kazachstania lodderae, Nakaseomyces delphensis, Naumauozyma castellii,Kaz. exiguus, Vanderwaltozyma polysporus* etc, [53]. They are play a role as a helper in ethanol production.

Production of ethanol from new effective yeast strain as a utilisation of algal biomass is an eco-friendly and sustainable approach for renewable bio-fuel production. Future research in this area is wide open to various direction and promising aspects such as,

- Increase the carbohydrate content of microalgae
- Isolation and selection of potential algal strains
- Optimization of their growth in various environments
- proper selection of yeast strain for improved fermentation
- proper knowledge and identification of biochemical triggers, and pretreatment methods and enzymatic methods, they help to improved fermentation process, so that automatically improvement in ethanol yield from algae.

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