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Review Article

Microalgae as Sustainable Renewable Energy feedstock for bioethanol production

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Abstract: Due to rapid growth in population and industrialization, worldwide ethanol demand is increasing continuously. Microalgae have shorter growth cycle as compared to other plant; hence the algae are a very promising source of biomass for the production of biofuels and also reduce in climate change effects. Technological breakthroughs in all major aspects must be overcome before it can be a successfully large-scale and commercialized product. In the light of aforesaid issues, algae are becoming the centre of attention as an optional renewable source of biomass for production of bioethanol. They provide viable feedstock for fermentation to bioethanol production, which is grouped under 'Third generation biofuels". Extensive research is being carried out over the last three decades there has been extensive research on algal biofuels production and the use of algae for CO_2 bioremediation. Algae metabolic engineering forms the basis for 4th generation bioethanol production which can meet this need.
Keywords: Bioethanol, Biomass, Microalgae, Fermentation.

INTRODUCTION

After the fuel crisis in 1970, worldwide ethanol production has strongly increased. The market of ethanol grew from less than a billion liters in 1975 to more than 39 billion liters in 2006, and is expected to reach 100 billion liters in 2015 [1]. It is assumed that the global demand for petroleum will be amplified 40% by 2025 [2].

The depletion of fossil fuels due to continuous consumption throughout the world and their contribution in environmental pollution and global warming shifted the interests of researchers to explore sustainable, economical, and ecofriendly energy sources alternative to petroleum based fuels [3, 4]. Bioethanol is an excellent substitute to gasoline fuels as comparatively cheap, convenient, and environmentally safe transportation fuels [5, 6]. Nowadays scientists are taking keen interests in bioethanol production from renewable feedstocks [7]. Algae are photosynthetic organism producing considerable amounts of carbohydrates which can be converted to bioethanol through fermentation process [8].

The biofuel production from renewable sources is extensively considered to be one of the most sustainable alternatives to fossil fuels and a viable means for environmental and economic sustainability. [9].

Microalgae can comprise bacteria (cyanobacteria), diatoms (e.g., Chromalveolata), other protists (e.g., Chromista), and unicellular plants (e.g., Chlorophyta) [10]. However, unlike higher plants, microalgae do not require a vascular system for nutrient transport, as every cell is photoautotrophic with directly absorbing nutrients. Microalgal cells are sunlight-driven cell factories that can convert carbon dioxide (CO_2) into raw materials for producing biofuels (e.g., biohydrogen, biodiesel, and bioethanol), animal food chemical feedstocks and high-value bioactive compounds (e.g., Docosahexaenoic acid (DHA)) [11−13]. In particular, the ability of these cells to absorb CO_2 suggests microalgae cultivation as an attractive alternative for CO_2 sequestration that can be applied to fossil fuel power plant gas effluents to facilitate the reduction of greenhouse gas emissions [14].

Microalgal biomass can be used for bioethanol production through fermentation processes using fungi or bacteria. Due to the rapid rate of microalgal growth and their high photosynthetic efficiency, these organisms can be cultivated industrially. The advantages in utilizing microalgae as a bioethanol feedstock include their high capacity for CO_2 capture, large quantities of synthesized carbohydrates, and (in some organisms) great tolerance to differences in abiotic factors [15, 16].

Thus, the third generation bioethanol is focused on the use of algae as raw material. Algae can be considered as a suitable feedstock for bioethanol production due to the ease of cultivation and abundance [17]. Algae biomass would provide as advantageous substrate for production of ethanol due to its ubiquitous nature. The fermentation of carbohydrate present in algae biomass, to ethanol is achieved by *Saccharomyces cerevisiae*. Algae are considered to be most important source for the production of clean and renewable energy. The production of bioethanol from algae involves some earlier steps before hydrolysis and fermentation which involve a drying process with the aim to preserve the crude extract and prevent the algae from gelling [18] and a size reduction to increase surface area.

Algae metabolic engineering forms the basis for 4th generation biofuel production. It uses recombinant DNA and other biological and bioengineering techniques for directed modification of cellular metabolism and properties through the introduction, deletion, and/or modification of algal metabolic networks to create or enhance biofuel production [19]. The carbon source essential for the cultivation of microalgae represents up to 60% of the total cost of the nutrients. Microalgae can grow rapidly and convert solar energy to chemical energy via CO_2 fixation and are now a promising 4th generation source for the commercial production of biofuel [20].

In this perspective, algal biomass is secure wide attention as an alternative renewable feedstock for the production of bioethanol [21]. The mass production of first-generation liquid biofuels has resulted in a series of problems related to food prices, land usage, and carbon emissions [22] and second generation biofuels production suffers with cost effectiveness, technological barriers, and feed stock collection networks [21]. Algal biofuels are an appealing choice [23] due to its rapid growth rate, high lipid content, comparatively low land usage and high carbon dioxide absorption and uptake rate [24].

Characteristics and advantage of the algae for bioethanol production

- Microalgae can be cultivated in brackish water on non-arable land, and therefore may not incur land use change, minimizing associated environmental impacts. Utilizing marine biomass, which can be grown in a variety of marine environments including fresh water and salt water, avoids the problem of land use change from arable to bioenergy crops [25, 26]. Utilizing the marine environment ensures a large cultivation area, limiting competition with other land uses and resources.

- Algae have the advantages of having no lignin and low hemicellulose levels, which result in an increased hydrolysis efficiency and fermentation yields [27, 28]; thus they can reduce the cost of the bioethanol production.

- The microalgal cells have a very fast productivity and harvesting cycle (1–10 days) compared with other feedstock (harvest once or twice a year) and thus provides enough supplies to meet ethanol production demands.

Ethanol Production From Microalgae

In ocean population marine microalgae, or phytoplankton, are mostly found: the best known are the diatoms (Bacillariophyta), the green algae (Chlorophyta), the dinoflagellates (Dinophyta and the blue-green algae (Cyanophyta). 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 [29].

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 [30, 31]. Recently attempts have been made (for the ethanol production) through the fermentation process using algae as the feedstocks to make it as an alternative to conventional crops such as corn and soyabean [32− 34]. A comparative study of algal biomass and terrestrial plants for the production of bioethanol has been given in **Table 1.**

Table1: Comparative study between algal biomass and terrestrial plants for bioethanol production.

Feedstock	Bioethanol	Reference
ALGAE		
Chlorococcum infusionum	260 g ethanol/Kg algae	Harun *et al.*, (2011)
Spirogyra	80 g ethanol/kg algae	Eshaq *et al.*, (2010)
Chlorococcum humicola	520g ethanol/kg microalgae	Harun and Danquah (2011a)
Chlamydomonas reinhardtii UTEX 90	235 mg ethanol g–1 Algae	Choi *et al.*, 2010
Chlorella vulgaris FSP-E strain	92.3% theoretical yield	Ho *et al.*, 2013*a*
Scenedesmus obliquus CNW-N	99.8% theoretical yield	Ho *et al.*, 2013*b*
Tetraselmis Subcordiformis	Starch 62.1% (dw)	Yao *et al.*, 2013
Chlamydomonas reinhardtii	29.2 %	Nguyen et al., 2009
Arthrospira platensis	16% g EtOH per g of dry *A. platensis* biomass.	Markou et al., 2013
TERRESTRIAL PLANTS		
Madhuca latifolia	251.1_0.012g ethanol/kg flowers	Behera et al., (2011)
Manihot esculenta	189_3.1g ethanol/kg flour cassava	Behera et al., (2014)
Sugarcane bagasse	165 g ethanol/kg bagasse	Kumar et al., 2014
Rice straw	93 g ethanol/kg pretreated rice straw	Sukumaran et al., (2008)

Due to the high content of carbohydrates produced by some species, they have been observed to be promising sources for bioethanol production [35]. In addition, microalgae have other characteristics that make them interesting for this purpose: (i) a high growth rate [36]; (ii) an absence of lignin [37]; and (iii) the capacity for growth rate and carbohydrate content to be increased through biochemical and genetic engineering [38].

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) can be produced from microalgae.

The complex composition of macroalgae makes it a difficult substrate to ferment to ethanol by one or a few strains of microbes. The complex compositions 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 [39].

FERMENTATION

There are different groups of microorganisms like yeast, bacteria, and fungi, which can be used for the bioconversion of sugars into bioethanol by microbial fermentation of the pre- treated and saccharified algal biomass, have the capability to produce bioethanol by intracellular anaerobic fermentation process for the production of bioethanol [40]. Through this auto-fermentation, the stored carbohydrates are degraded and ethanol is produced [41, 42].

In addition to choosing the appropriate method, it is of paramount importance to choose the right fermenting organism. Some performance parameters need to be taken into consideration: temperature range, pH range, alcohol tolerance, growth rate, productivity, osmotic tolerance, specificity, yield, genetic stability, and inhibitor tolerance [43]. The most used ethanologen, which is currently also the most efficient for processing major feedstocks, is the yeast *Saccharomyces cerevisiae* [44].

Chlorella vulgaris cake was enzymatically hydrolyzed and fermented with *Saccharomyces cerevisiae* to produce the reducing sugars and ethanol. The fermentation of carbohydrate present in algae biomass, to ethanol is achieved by *Saccharomyces cerevisiae.* The yields obtained for reducing sugars and ethanol was 0.55 and 0.17 g/g of cake, respectively [45]. *Schizochytrium* sp. was separated into sugars (mainly Dglucose and L-galactose), lipids and proteins. The separated sugars were then converted to ethanol by *Escherichia coli* KO11, resulting in 11.8 g of ethanol/L being produced from 25.7 g/L of glucose [46].

Ethanol is produced from the incomplete metabolism of glucose in plants, called alcoholic fermentation. Most commercial-scale ethanol fermentation is by yeast, one of *Saccharomyces cerevisiae* that produce ethanol and the bacterium *Zymomonas mobilis* [47]. The production of ethanol through alcoholic fermentation is shown in

Figure-1 [48]. Cultivated *Chlorella vulgaris* and *Scenedesmus obliquus*, *Spirulina sp.* respectively, under carbohydrate-enriched biomass was used for bioethanol production [49, 50].

$$2CO_2 + 3H_2O \longrightarrow C_2H_5OH + 3O_2$$

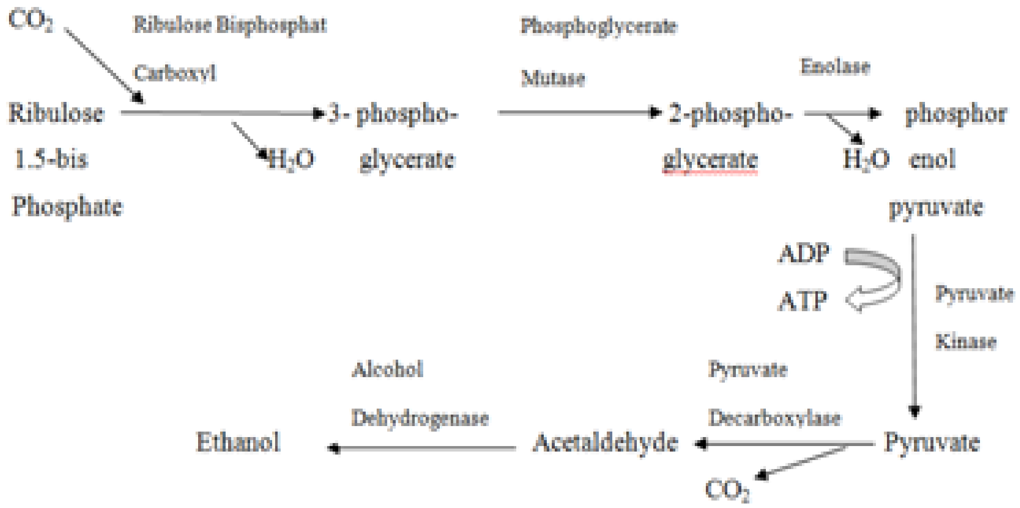

Figure1. The production of ethanol through alcoholic fermentation (Emma and Rosalam, 2012).

Zymomonas mobilis is growing in importance for ethanol production. It is an unusual facultative anaerobic Gram-negative bacterium that utilizes a very efficient ethanol fermentation pathway [51]. Glucose, fructose, and sucrose are the main substrates used by this organism, which metabolizes these molecules to pyruvate through the Entner-Doudoroff pathway [52].

The production of bioethanol fermentation pathway is relatively straightforward. Both pdc and adhII genes from *Zymomonas mobilis* were first transformed into *Escherichia coli* for the expression of pyruvate decarboxylase (PDC, EC: 4.1.1.1) and alcohol dehydrogenase II (ADH, EC: 1.1.1.1) under the control of the lac operon [53]. More recently, [54] have reported their metabolic engineering work on *Synechocystis sp.* PCC 6803 that can photoautotrophically convert CO_2 to bioethanol (**Fig. 2**).

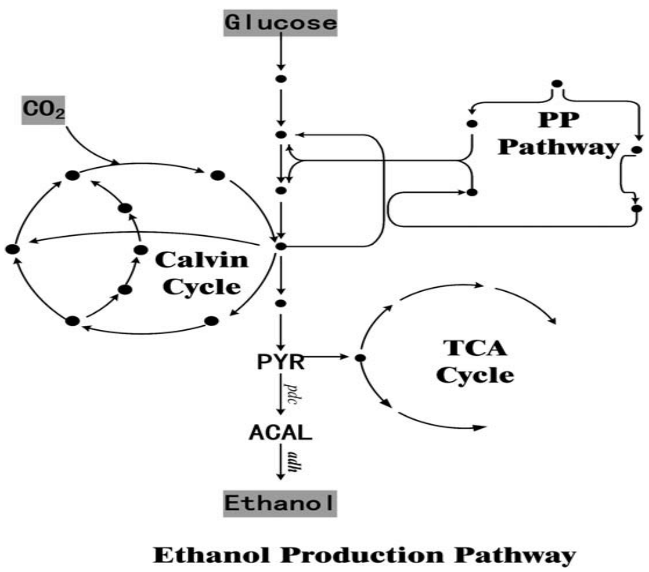

Fig-2. Bioethanol production pathway [54].

The bioethanol fermentation from microalgae involves less intake of energy, and the process is much simpler compared to the biodiesel production system. In addition, the undesired CO_2 byproduct can be recycled as a carbon source to cultivate additional microalgae, resulting in the reduction of greenhouse gas emissions. However, the commercial production of bioethanol from microalgae is still being investigated [55, 56].

Challenges for Ethanol Production:

Although bioethanol used as renewable fuel is one of the positive trends for sustainable development, there are some difficulties in production and consumption. 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 [57, 58].

Unfortunately not all production strain can survive in extreme conditions, and there is still the possibility for an extremophile contamination to arise. The issue about how to decrease harvesting and recovery process cost determines the possibility of the whole cost reduction.

CONCLUSION:

Microalgae played an important role in bioethanol production. The microalgae bioethanol production can be economical either by integrated bioethanol of high value products by enhancing biomass productivity by recombinant DNA technology, algal biology and metabolic engineering culture conditions and production system.

Studies on algal turf scrubber (ATS) has revealed that the algae can capture around 60-90% of N(nitrogen) and 70--100% of K(potassium) from manure effluents runoff water thus reducing eutrophication of water bodies. Algal waste can be utilized as nutrient rich biofertilizer (e.g. ATS), animal fodder or poultry feed. Algae derived biofuels have minute impact on the world food supply as compared to conventional biofuel producing crops.

Microalgal bioethanol presents an excellent example of technically feasible and carbon–neutral renewable alternatives. Conversion of algal biomass into biofuel can perform better developmental activities along with climate co-benefits. Future research in this area is wide open to various direction and promising aspects such as,

- Isolation and selection of proper microalgae strains, culturing and harvesting process
- Substantially reduce the cost of biomass production
- New effective yeast strain improvement of Most difficult thing in ethanol production is selection a proper strain indicating a need for greater species of microbes for high fermentation efficiency
- High capacity for CO_2 capture and High capability of growth rate
- Fourth generation microalgae strains improvement and improve of carbohydrate content

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