

Review Article

Assessment of Third Generation Bioethanol Production Using Microalgae as Feedstock

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***Corresponding author:** Sibi G, Department of Biotechnology, Indian Academy Degree College-Autonomous, Bangalore, India**Received:** May 02, 2020; **Accepted:** May 23, 2020;**Published:** May 30, 2020**Abstract**

Bioethanol from microalgal biomass have been recognized as a more promising alternative feedstock. The algal starch, cellulose or other accumulating carbohydrates can be used for the production of ethanol. Microalgae undergo a process consisting of pre-treatment, hydrolysis and fermentation to produce bioethanol. Strains having high biomass yield with high carbohydrates are taken into consideration for bioethanol production. The selection of appropriate pre-treatment depends on their cost effectiveness. The exposure of the intracellular components of algae by using hydrolysis is crucial for bioethanol production. Similarly, the recovery of bioethanol varies on the microalgal species and the growth optimization is an effective way to maximize the bioethanol production. In other words, environmental and operational factors greatly influence bioethanol generation from algal biomass. Another important strategy is using the high yielding/immobilized co-cultures during the fermentation process. Further research optimization must be guided toward the development of cost-effective scalable methods to produce high bioethanol yield under optimum economy.

Keywords: Microalgae; Bioethanol; Hydrolysis; Fermentation; Pre-treatment**Introduction**

Due to sharp increase in universal energy, there is a strong incentive to reduce the CO₂ emissions and develop other energy sources as alternatives to fossil fuels [1]. In addition, increasing global population will lead to overexploitation of the resources and drives the scarcity of arable land to its limit [2]. It is a critical concern to develop the alternative energy resources and adopt policies to minimize the utilization of fossil reserves, maintain the environmental sustainability and cost-effective, and reduce the releases of greenhouse gas. The global demand for renewable energy sources has been continuously growing. Algae would be good candidates for renewable energy sources, receiving energy from the sunlight and building their biomass by eliminating CO₂ from atmosphere through photosynthesis [3].

Biofuels are biological sources generally derived from primary fuels such as firewood, wood pellets, wood chips, animal waste, crop residues and landfill gas; while secondary fuels which consists of bioethanol, butanol, biodiesel, and biohydrogen [4,5]. First-generation fuel which used the sources of food as feedstock but the large conversion of agricultural crops to biofuels has raised controversial debates [2]. Second-generation biofuels are mainly produced from lignocellulosic materials but involved difficulty and high costs to convert lignocellulosic biomass into biofuel [6-8]. Third-generation biofuels produced from microalgae as feedstocks have been recognized as a more promising alternative feedstock that do not require arable land, not competing with food cultures, high growth rate, high photosynthetic efficiency, potentially to cultivate in offshore marine environment and easy to be cultivated in larger quantity [9,7,10].

Bioethanol is produced from biomass by the fermentation of available carbohydrates, usually simple sugars, into bioethanol and carbon dioxide. In addition to bioethanol's easy storage and distribution, the superior characteristics of bioethanol alone and blended with naturally occurring fossil fuels have made it a highly suitable automobile fuel. Un burned hydrocarbon and carbon monoxide emission levels of bioethanol combustion is significantly low when compared with gasoline combustion [11]. Bioethanol can be employed to replace gasoline, octane enhancers, and aromatic hydrocarbons, and has the advantage of being compatible with current infrastructure [12,13]. It is estimated that by 2050, liquid biofuels such as bioethanol is predicted to be on top of the 'biofuel ladder' due to their effectiveness in replacing gasoline for the transportation sector [14]. Extensive cultivation of energy crops raises concerns regarding pollution of agricultural land with fertilizers and pesticides, soil erosion, reduced crop biodiversity, biocontrol ecosystem service losses and greenhouse gas emissions. Several species of algae with high starch content are being tested to produce ethanol. Bioethanol of the third generation produced from microalgae biomass may represent an environmentally friendly fuel. It has many advantages in view of first- and second-generation biofuels produced from higher plants [15,16], mostly due to the rapid generation rate. Although plants have been used to produce bioethanol, alternative sources that do not require arable land should be considered [17]. Microalgae form a class of organisms that is likely to be adequate for producing third-generation bioethanol [18]. The algal starch, cellulose or other accumulating carbohydrates can be used for the production of ethanol after hydrolysis. Algae have higher photon conversion efficiency and can synthesize and accumulate large quantities of carbohydrate biomass for bioethanol production, from inexpensive raw materials [19,20]. The energy cost per carbon for triacylglycerol synthesis is

53% greater than for storage carbohydrate synthesis under standard conditions [21]; in this way, microalgae seem a truly viable feedstock for bioethanol production.

There has been a remarkable surge in research to investigate the utilization of microalgae as an advanced energy feedstock for bioethanol production [22,23,19]. Microalgae like *Chlorella*, *Dunaliella*, *Chlamydomonas*, *Scenedesmus*, *Spirulina* are known to contain a large amount (>50% of the dry weight) of starch and glycogen, useful as raw materials for ethanol production [24]. Microalgae can also assimilate cellulose which can also be fermented to bioethanol. This review attempts to summarize a thorough understanding of the significance of bioethanol production paves away for it use as a versatile transportable fuel with excellent performance through microalgae.

A systematic search was carried out in PubMed, Scopus and Web of Sciences using a combination of Boolean operators. Peer reviewed papers in English on the bioethanol production using microalgae were retrieved and evaluated based on titles and abstracts. The retrieved papers were managed using Mendeley and the data were consolidated.

Bioethanol Production Process

Bioethanol production from algal biomass takes place by either the sugar or syngas pathway. Algae are directly fermented to produce bioethanol by the sugar pathway, while when processed via the syngas pathway, hydrocarbons of algal biomass are converted to syngas through gasification followed by fermentation of syngas to produce bioethanol. Starch-rich microalgae are extensively studied for the production of bioethanol via fermentation and different pre-treatment methods have been evaluated to release the fermentable sugars from algal biomass in order to enhance bioethanol production. The production of bioethanol involves an extensive process. Like other feedstocks, algae undergo a process consisting of pre-treatment, hydrolysis and fermentation to produce bioethanol. Each step contains many variables, many distinctive methods exist, and the optimization of these steps is required to maximize the bioethanol yield.

Pre-Treatment

The vast diversity of microalgae and derived products leads to a variety of pre-treatment. The selection of appropriate pre-treatment depends on their cost-effectiveness. As far as microalgae pre-treatment is concerned, several studies have reported benefits of physical, chemical and biological pre-treatment methods in terms of product recovery.

Microwave pre-treatment: Microwave pre-treatment promotes starch digestibility which can enhance, depending on the conditions of the pre-treatment, the accessibility of enzymes to the pre-treated substrate [25]. The heating is performed by two mechanisms: 1) by the rotation of the dipoles where the polar molecules try to align in the electromagnetic field that changes rapidly by the microwaves and 2) by the ionic conduction consisting of the instant superheating of the ionic substance due to the friction of the ionic molecules generated by the movement that produces the electric field [26]. Since microalgae are grown in water and given the ionic nature of water, microwave radiation is well absorbed by the medium and consequently, it is an

efficient and rapid way to carry out the pre-treatment.

Pyrolysis: Pyrolysis is widely used as a physical pre-treatment in which a high temperature is applied on the biomass for short time duration. However, the cost associated with its high energy consumption restricts its implementation at a commercial scale production [27]. Other physical methods including steam explosion and autoclaving rupture the microalgal cell wall, resulting in the release and recovery of bio components. The steam explosion method provides accessibility to the degradation of cellulose. Steam explosion is increasingly considered to be one of the most efficient, eco-friendly and cost-effective processes for commercial application and thus, it have been widely tested at the pilot scale for various biomasses [28].

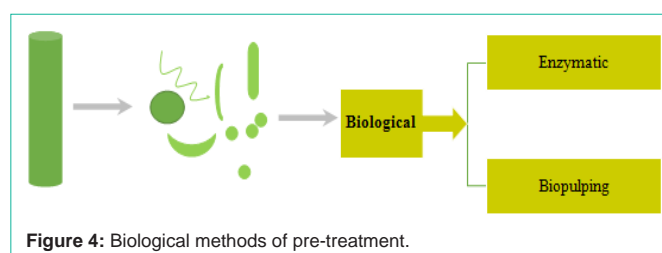
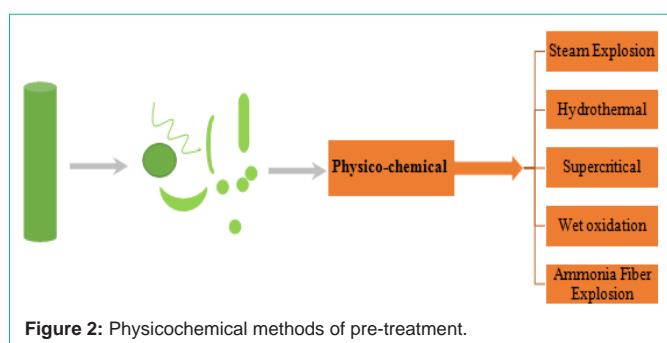
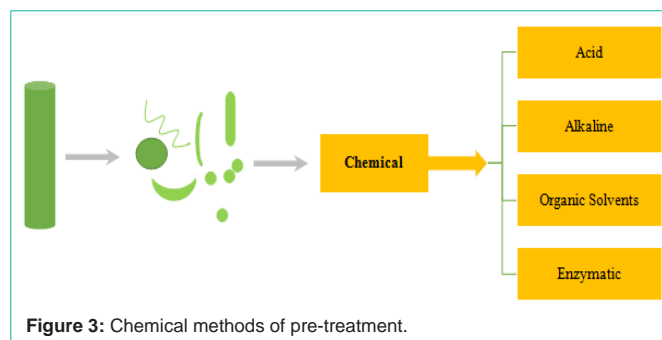
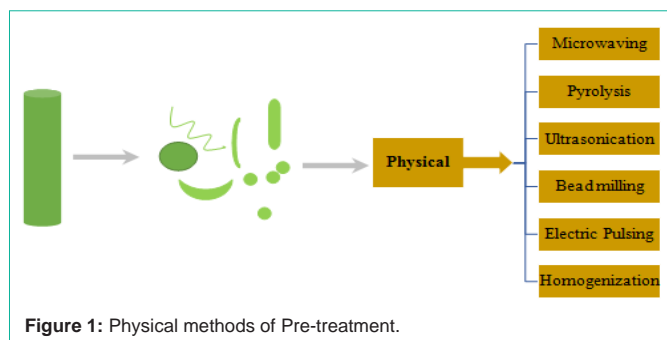
Ultrasound pre-treatment: This type of technology can help break the cell wall of microalgae because when bubbles collapse on the surface of a solid, the pressure and elevated temperature create micro jets that allow the solvent to penetrate into the raw material and a rupture of the cell wall occurs [29]. This pre-treatment method is an alternative for cell disruption where water, acid, or alkalis could be used as catalysts for cell wall disruption of microalgal biomass. Ultrasonication effectively modifies the surface structure of biomass which lead to enhanced saccharification [30,31].

Pulse electric field pre-treatment: During this treatment, an effect called electroporation or electropermeabilization occurs [32,33]. When a critical electric field is applied, the electric forces cause a dielectric break that increases the permeability together with the formation of pores that are usually irreversible. This method of pre-treatment is used to extract sugars and high added value compounds without damaging or degrading the raw materials used (Figure 1).

Mechanical methods: Under mechanical methods, cell disruption is attributed to different causes or mechanisms such as fluid shear, turbulence, shock velocity, and cavitation [34-36]. In spite of the effectiveness of these pre-treatments for cellular disruption of microalgae biomass, their main drawback is that they do not directly affect the structure of the intracellular carbohydrates, and therefore, a further step would be needed to modify the starch structure. Moreover, these methods are also very energy-intensive.

Freezing/thawing pre-treatment: This promotes the creation of ice crystals, mechanically breaking the cell walls [37]. Intracellular compounds such as carbohydrates, lipids, proteins, and pigments are released into the medium during thawing, while cell membrane compounds and water soluble organic compounds can also be extracted [38]. Nevertheless, the application of this technology is controversial since it can increase energy consumption and the time required to complete the process.

Hydrothermal pre-treatment: Hydrothermal processing is an alternative method to break down microalgae cell walls and to gelatinize the intracellular starch. Temperatures ranging from 60-180°C and short reaction times below 60 min are used in this method. In this pre-treatment, acids, alkalis or only water can be used as reaction catalyst [39,40]. A better gelatinization is induced when temperature and pressure are increased. It is important to note that the treatment used would result in a broad spectrum of other compounds in addition to simple sugars and therefore, it is essential



to select the best pre-treatment conditions to most efficiently break the cell wall, modify the structure of carbohydrates, and obtain added-value compounds (Figure 2).

Acid and alkaline hydrothermal pre-treatment: Higher concentrations of concentrated or diluted acid and alkaline solutions decrease reaction times, avoid the use of enzymes, while the use of low concentrations makes necessary higher temperature and pressure values to achieve favourable hydrolysis efficiencies [32,41,42]. The need to neutralize the samples once treated and the consequent increases in the overall cost of the operation is also among the disadvantages associated with the application of concentrated reagents.

Hydrolysis

Algal carbohydrates comprise a mixture of neutral sugars, amino sugars, and uronic acids and the composition differs depending on species and growth conditions [43]. The exposure of the intracellular components of algae by using hydrolysis is crucial for bioethanol production. During the conversion, the polysaccharide will be hydrolyzed into free monomer molecules which can be readily fermented to bioethanol [44].

Acid hydrolysis: The release of simple sugars from the polysaccharides component can be significantly improved by using chemicals such as acid. In this type of hydrolysis, a wide range of acids have been used in which sulfuric acid (H_2SO_4) is the most preferred one. The acid role in hydrolysis can be seen in its ability to break the bonds which connect the long chains of polysaccharides. During the hydrolysis, occurrence of hydrogen bonds destruction in order to rupture the polysaccharide chains turning it into a completely amorphous state. The polysaccharide is extremely susceptible to hydrolysis at this point. Then, the acid will serve as catalyzer where it will cleave polysaccharide by hydrolyzing the glycosidic bonds. At the end of the process, any addition or dilution with water at

moderate temperature will provide complete and rapid hydrolysis of the hydrolysate into monosaccharide. Acid pre-treatments are preferable over alkali treatments, as they provide higher convertibility of cellulosic materials into reducing sugars [45]. During the acid pre-treatment, several parameters such as acid concentration, treatment time, temperature, and amount of substrate loading influence the process efficiency (Figure 3).

Alkaline hydrolysis: Since microalgal carbohydrates are mostly entrapped in the cell wall, the pre-treatment process is required to free and breakdown complex carbohydrates into fermentable sugars for bioethanol production [27]. Alkaline pre-treatment has been reported to be promising in treating various biomass feedstocks [45]. This method of pre-treatment is preferable due to the lower temperature and pressure involved [46]. In addition, alkaline pre-treatment reduces the degree of inhibition during fermentation and provides a lower production cost compared with other pre-treatment methods. As microalgae contain no lignin, it is assumed that during alkaline pre-treatment, the fermentable sugars stored inside the cell walls are released and readily available to be converted to bioethanol [47].

Enzymatic hydrolysis: Enzymatic hydrolysis (saccharification) is one of the most important steps to obtain essential sugars such as glucose and mannose for the subsequent fermentation and bioethanol production [48,49]. The enzymatic hydrolysis of the sugars present in microalgae biomass offers many advantages over chemical hydrolysis with acids or alkalis. Enzymatic hydrolysis is performed using different enzymes resulting in more efficient cell lysis and extraction of target compounds. The main advantages of the enzymatic hydrolysis are its high specificity, no severe conditions requirements, and that it is easy to carry out at industrial scale. It is important to mention that depending on the type of microalgae, its cell wall may or may not be permeable to other compounds, whereby specific enzymes are not often required to break the cell wall since these (the enzymes) can reach up intracellular carbohydrates [50] (Figure 4).

Table 1: Microalgae used in Bioethanol production.

Species	Bioethanol yield g ethanol/g algae	Reference
<i>C. vulgaris</i>	0.178	Ho et al., [6]
<i>C. vulgaris</i>	0.214	Ho et al., [6]
<i>Chlamydomonas fasciata</i>	0.194	Asada et al., [52]
<i>Chlamydomonas reinhardtii</i>	0.235	Choi et al., [53]
<i>Chlorella sp.</i>	0.47	Zhou et al., [54]
<i>Chlorella vulgaris</i>	0.233	Ho et al., [6]
<i>Chlorella vulgaris</i>	92.3% theoretical yield	Ho et al., [6]
<i>Chlorococcumhumicola</i>	52% (g ethanol g ⁻¹ algae)	Harun and Danquah [48]
<i>Chlorococcumhumicola</i>	0.48	Harun and Danquah [48]
<i>Chlorococcuminfusionum</i>	26.1% (g ethanol g ⁻¹ algae)	Harun and Danquah [48]
<i>Chlorococcuminfusionum</i>	0.261	Harun et al., [55]
<i>Chlorococum sp.</i>	0.383	Harun et al., [56]
<i>Dunaliellatertiolecta</i>	0.14	Lee et al., [57]
<i>Scenedesmus abundans</i>	0.103	Guo et al., [58]
<i>Scenedesmus obliquus</i>	99.8% theoretical yield	Ho et al., [59]
<i>Schizocytium sp.</i>	0.055	Kim et al., [60]
<i>Tetraselmissubcordiformis</i>		Yao et al., [61]

In enzymatic hydrolysis, cellulases are the enzymes that mostly employed to degrade the polysaccharides. The physical structure of the feedstock or substrate and its interaction with the enzymes are some factors that need to be addressed properly during the process. The enzymes will break down the cell wall of the algae in order to release more monosaccharide of the feedstock. In the reaction, the binding of the enzymes to the algal feedstock will rupture the bonding of polysaccharides, consequently, enzyme concentration decreases and conversion proceeds into bioethanol in the fermenting step [51] (Table 1).

Fermentation

Various fermentation paths commonly utilized to convert algal biomass into bioethanol. The simple sugars released in hydrolysis step can be easily converted to bioethanol. The conversion of glucose and galactose into ethanol involves Embden-Meyerhof pathway of glycolysis and Leloir pathway respectively. Environmental and operational factors such as nutrient levels, alkalinity and concentration of toxic substances, temperature, and pH optimum of the fermenting microorganism greatly influence bioethanol generation from algal biomass [62].

Separated Hydrolysis and Fermentation (SHF): The basic mechanism of SHF is based on separation of hydrolysis and fermentation into two distinct processes. As in normal bioethanol production process, hydrolysis will be conducted first to degrade the feedstock into monomer sugars by utilization of enzyme. This process is followed by the fermentation reaction which will utilize the sugars formed in hydrolysis stage [63]. One problem can be a negative aspect of SHF is accumulation of glucose and cellobiose in the hydrolysis step inhibits the activity of the cellulases.

Simultaneous Saccharification and Fermentation (SSF): Microalgae-rich carbohydrates have high reducing sugars, thus their

saccharification is much easier, making algae a sustainable feedstock for bioethanol production. In SSF, the hydrolysis and fermentation process are conducted simultaneously in a single step which involves a single reactor. During the reaction, the feedstock, enzyme and yeast are put together in an orderly manner so that glucose released due to cellulases activity is directly converted to ethanol by fermentative microorganism. SSF can limit the end-product inhibition by removing the residual sugar [64]. Higher bioethanol yield can be obtained if the conditions are appropriate during the SSF reaction.

Purification

Purification step in bioethanol involve several types of techniques such as rectification, distillation and dehydration which greatly influence the end products [65,66]. Among these techniques, distillation is the most widely used in purification stage despite its high energy consumption. A distillation unit normally consists of 1) Feed (ethanol to be purified), 2) Energy source (usually steam), 3) Overhead, 4) Bottom product and 5) Condenser [67]. The distillation process facilitates mass transfer between different components moving in a counter-current fashion [65]. Two different zones will be formed based on the volatility of the components where more volatile components will be in vapour rich region while the less volatile components can be found in the liquid rich region. At the end of this stage, the end product will be drawn off from the system and can be blended with gasoline fuel or directly used as fuel on its own [68].

Conclusion

In order to select the desirable algae strains for commercial bio fuel production, high biomass yield with high carbohydrates and lipid contents are taken into consideration. Also, to exploit algae for bioethanol production optimization of mass cultivation, effective pre-treatment, successful fermentation strategies, and high product recovery is required. The viability of algal biomass as an alternative feedstock has been assessed adequately, and further research optimization must be guided toward the development of cost-effective scalable methods to produce high bioethanol yield under optimum economy.

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