



PRODUCTION OF BIO ETHANOL BY ENZYMATIC HYDROLYSIS & FERMENTATION FROM ALGAL BIOMASS

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Abstract:

Because of rapid growth in population and industrialization, worldwide ethanol demand is increasing continuously. The first-generation and second-generation biofuels are unable to meet the global demand of bioethanol production because of their primary value of food and feed. Therefore, algae are among the most potentially significant sources of sustainable biofuels in the future of renewable energy because of the accumulating high starch/cellulose and because they are widely distributed in nature. Bioethanol is more environment friendly because it is renewable energy source, and it is considered as one of the most promising replacements of gasoline to act as a transport fuel. Despite the many developments made in the recent years, commercialization of algal bioethanol remains challenging chiefly because of the techno-economic constraints.

Introduction:

With the expansion of the world population and increase of industrial prosperity, global energy consumption also has increased gradually. The major energy demand is still supplied from conventional fossil fuels such as oil, coal, and natural gas. In addition, the usage of fossil fuels is now widely accepted as unsustainable because of the accumulation of greenhouse gas (GHG) in the environment that has already exceeded the 'dangerously high' threshold of 450 ppm CO₂-e [1]. Therefore, depletion of fossil fuels, global warming, and increasing price of petroleum-based fuels are gaining great concern and have led to a move toward alternative, sustainable, renewable, efficient, and cost-effective energy sources with lesser GHGs emissions. Unlike fossil fuels, bioethanol is more environment friendly because it is renewable energy source, and it is considered as one of the most promising replacements of gasoline to act as a transport fuel. Although the first-generation bioethanol production from food crops such as corn, grain, or sugar cane is well established and the industry is growing throughout the world, the use of these staple food crops as feed stocks is not ideal because of the high price of raw materials, which accounts for almost 40–75% of total ethanol production cost [2]. It has raised doubts about its possible impact on food supply and security, which is mainly reduced if its residues are used for bioethanol production, as studied here [3]. In contrast, the second-generation bioethanol is derived from lignocellulosic feedstock. They are a better option for addressing the food and energy security and environmental concerns. However, conversion of lignocelluloses to bioethanol is more challenging than the first-generation bioethanol process because of the complex structure of plant cell walls. Currently, no commercial-scale cellulosic ethanol plants are in operation largely because of the high price of production, which is almost twice that of corn ethanol [4]. In view of the aforementioned issues, algae are gaining wide attention as an alternative renewable source of biomass for production of bioethanol, which is grouped under 'third-generation bioethanol' [5,6]. To a greater extent, the major drawbacks of first-generation and second generation bioethanols are overcome by the algae bioethanol.

The objectives of this review paper are the published investigations on algae conversion to bioethanol and present the culture of algae, the available and accessible technologies for bioethanol production using algae biomass throughout the entire process, that is hydrolysis and fermentation stages, challenges encountered, and future perspective.

Brief Description of the Algae:

Algae are photosynthetic, eukaryotic organisms that do not develop multicellular sex organs. All algae contain green chlorophyll; however, they are masked by photosynthetic pigments that give them a distinguishing color that is used to identify key divisions [7]. Based on their morphology and size, algae are typically divided into two major categories macroalgae and microalgae. Macroalgae, commonly known as seaweed, are usually found in coastal areas in both intertidal and subtidal habitats and are composed of multiple cells, which organize to structures resembling roots, stems, and leaves of higher plants; some species have gas-filled structures to provide buoyancy [8]. They are classified into three broad groups based on their pigmentation: (i) brown seaweed (Phaeophyceae), which includes the large kelps; (ii) green seaweed (Chlorophyceae) such as sea lettuce; and (iii) red seaweed (Rhodophyceae), the most diverse group of all [10]. In contrast, microalgae are a large group of microscopic photosynthetic organisms, many of which are unicellular [8]. Unlike higher plants, microalgae do not have roots, stems, and leaves. Microalgae, capable of performing photosynthesis, are important for life on earth; they produce approximately half of the atmospheric oxygen and use simultaneously

the GHG carbon dioxide to grow photoautotrophically. The three most important classes of microalgae in terms of abundance are the diatoms (Bacillariophyceae), the green algae (Chlorophyceae), and the golden algae (Chrysophyceae) [9].

Source of Algae:

The distribution of macroalgae is worldwide. They are abundant in coastal environments, primarily in near-shore coastal waters with suitable substrate for attachment [11]. Another primarily natural source is floating seaweeds, and floating seaweeds are considered one of the most important components of natural materials on the sea surface [12]. In 1995, about 3.6 million tonnes wet weight were collected globally from natural stocks. This was about 48% of the total global seaweed biomass harvested with the balance produced by aquaculture.

In 2010, the production of seaweeds and other aquatic algae reached 19.9 million tonnes, of which nature produced 0.9 million tonnes (0.5%). Predictably, aquatic seaweed will be the most prominent in the production of bioethanol. There are at least 30,000 known species of microalgae, and they are widely distributed in marine waters, freshwater lakes, and others. As their small size makes subsequent harvesting more complicated, cultivation is the main way to generate biomass from microalgae.

Steps of Bioethanol Production from Algae Biomass:

Like cellulosic ethanol, bioethanol production from algae requires four major unit operations including hydrolysis and fermentation. In order to produce sugars from the algae biomass, hydrolysis is designed to help separate cellulose, hemicellulose, and lignin so that the complex carbohydrate molecules in the algae cell can be broken down by enzyme-catalyzed hydrolysis into their constituent simple sugars. Then the fermentable sugars can be fermented into ethanol by ethanol-producing microorganisms and finally recover and purify the ethanol to meet fuel specifications.

Enzymatic Hydrolysis:

Saccharification is the critical step for bioethanol production where complex carbohydrates are converted to simple monomers. Compared with other process enzymatic hydrolysis requires less energy and mild environment conditions (e.g., pH 4.5–5.0 and temperature 40–50 °C) and does not have a corrosion problem [13,14]. Therefore, one may expect low utility consumption and low toxicity of the hydrolyzates as the main advantages of this process [15].

Cellulose hydrolysis is catalyzed by a class of enzymes known as cellulases [16]. These enzymes can be produced by both bacteria and fungi. Among the various cellulolytic microbial strains, *Trichoderma* is one of the well-studied cellulase-producing and hemicellulase-producing fungal strains [16, 17, 15]. During enzymatic hydrolysis, cellulose is degraded by the cellulase to reduce sugars that can be fermented by yeasts or bacteria to ethanol. The action of cellulolytic enzymes occurs through three steps of adsorption, biodegradation, and desorption [16]. After enzymatic hydrolysis, the concentrations of sugars are much higher than those of chemical hydrolysis. Additionally, different algae contain different compounds, so cellulose enzyme supplemented with other enzymes can raise the rate of enzymatic hydrolysis. Several factors influence yields of monomer sugars through enzymatic hydrolysis; temperature, pH, substrate concentration, and enzyme loading are the main factors. The optimum pH of cellulase enzyme is 4.5–5, so before enzymatic hydrolysis, the hydrolysed should be neutralized by acid and alkali. In addition, in the process of enzymatic hydrolysis, it often tends to generate acidic substances, which will affect the stability of the system operation. Researchers often join buffer solutions to address the issues, such as citrate buffer and acetate buffer [18,19,20]. However, adding to the commercial constraints is the fact that costs of enzymes are almost exclusively accounted for and reported in terms of dollars per gallon of bioethanol [21]. And the high cost of starch/cellulose depolymerising enzymes for pretreatment of algal biomass makes the cost of algal bioethanol several folds higher. So we must decrease the cost of enzymes to ensure commercial algal bioethanol production.

Fermentation:

Fermentation using microorganisms will utilize all five of the major biomass sugars, glucose, xylose, mannose, galactose, and arabinose, which are from enzymatic hydrolysis of algae biomass. The best known microorganisms for ethanol production from hexoses are the yeast *Saccharomyces cerevisiae* and the bacterium *Zymomonas mobilis* [16]. Sulphari et al. [22] investigated bioethanol production by means of fermentation using *Z. mobilis* and *S. cerevisiae*. The results showed that *Z. mobilis* was able to survive at high concentrations compared with *S. cerevisiae*. Some researchers applied ethanogenic *E. coli* KO11 to algae fermentation. Kim et al. [18] concluded that acid hydrolysis of *L. japonica* biomass followed by simultaneous treatment of the lysate with hydrolytic enzymes and fermentation with ethanogenic *E. coli* KO11 resulted in an ethanol yield of 0.4 g ethanol/g of sugars. This yield was achievable because *E. coli* KO11 was able to utilize mannitol, which is present in high amounts in the hydrolysate. However, some researchers used the ethanogenic strain *E. coli* KO11 to ferment the invasive marine macroalgae *G. salicornia*; ethanol yield of this study was relatively low. Recently, some researchers developed surface aeration to improve bioethanol production from the hydrolysate containing C5 monosaccharides such as xylose. Yeon et al. [23] designed a surface-aerated repeated-batch fermentor culture, in which the aeration was finely controlled at 100 mL/min and delivered into the headspace

of a 2.5-L fermentor. When the medium was replaced every 48 h, bioethanol was continuously produced for 200 h under repeated-batch fermentor culture, where the level of bioethanol production was about 9 g/L. Lee et al. [24] The optimal medium replacement time during the repeated operation was determined to be 36 h, and the surface aeration rates were 30 and 100 mL/min. Under these conditions, the repeated-batch operation was successfully carried out for six runs (216 h), in which the maximum bioethanol concentrations reached about 11–12 g/L at each batch operationally. In addition, the bioethanol yields were 0.43 (about 84% of theoretical value) and 0.44 (about 86% of theoretical value) when the surface aeration rates were 30 and 100 mL/min, respectively.

Conclusion:

Although the raw materials of lignocellulosic bioethanol are cost effective, renewable, and abundant, the production of the second-generation bioethanol is costly because of the high recalcitrance of lignocellulosic raw materials. So as the sustainable development of modern society is hindered seriously by the energy crisis and environmental pollution, the algal biomass as a raw material for bioethanol production is undoubtedly a sustainable and eco-friendly source for renewable biofuel production. Although more than 100 algae-to-fuel companies have been set up around the world mostly in the last couple of years, not a single commercial facility has been built so far. So like any other industrial process, the commercialization of algae bioethanol is also dependent on the economics of the process. A biorefinery approach can be developed in the production chain of algae bioethanol. We can extract useful chemical from the algae, and residue contained rich cellulose that can be utilized as raw material for bioethanol production.

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