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An Overview of Bioethanol Production From Algae

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1. Introduction

The depletion of fossil fuels which meet most of our energy requirements in near future and the pollutants from fossil fuels necessitates the usage of alternative renewable energy sources extensively. In this context, biomass is considered as an important alternative energy source to fossil fuels. Biodiesel and bioethanol produced from biomass sources are one of the best alternatives for petroleum-based fuels and recently, they are commonly used for transportation in many countries. Bioethanol is the most produced biofuel in the world and especially in Brazil and the United States two main producing countries with 62% of the world production. Large scale manufacture of ethanol as fuel is performed from sugar cane in Brazil, while it is produced from corn as a raw material in the United States [1]. Bioethanol production of 2013 in the countries is given in Table 1 [2].

Country	Production
USA	50,274
BRAZIL	23,690
EUROPE	5,182.38
CHINA	2,630.88
INDIA	2,060.1
CANADA	1,976.94
OTHER	2,748.06

Table 1. Bioethanol production amounts of countries in 2013 (million liter) [2]

Bioethanol is basically produced from first or second generation feedstocks. First generation bioethanol is produced from some cereals and lugumes such as corn, sugar beet, wheat and barley used for also food sources. Sugars which are obtained from first generation feedstock

such as sugar cane, molasses, sugar beet and fruits can be fermented via yeast directly. Advantages of these raw materials are high sugar yields and low conversion cost. Their disadvantage is their production in just certain periods of the year. While 25 gallons of ethanol produced from an average of 1 ton sugar beet, 20 gallons of ethanol is produced from 1 ton of sweet sorghum stalk yearly. However their production is more expensive than that produced from sugar cane due to its energy and chemical inputs [3].

Usage of this first generation feedstock for bioethanol production leads to various discussions about increasing food prices and occupation of agricultural land. These problems are solved partially by using second generation feedstocks lignocellulosic materials such as waste or forest residues. Second generation feedstocks have some advantages over first generation feedstocks due to not being used as food source and less land requirement. However their harvesting, purification and various pre-treatment needs made their production quite challenging and not economical. Algae which are the third generation feedstock for biofuels are an alternative for the first and second generation feedstocks due to their productivity, easily cultivation and convenient harvesting time [4-6]. Recently, they are mostly utilized for biodiesel production because of their high lipid content. On the other hand, they have cellulosic structure and large amounts of carbohydrate embedded in, so they can be also utilized for bioethanol production directly or with the remains which is obtained after oil extraction. Since bioethanol production from conventional feedstock is considered for emitting more greenhouse gases than fossil fuels in consequence of the production steps and applications during the process, algal bioethanol production can overcome these problems. In comparison with conventional feedstocks, algal production areas don't occupy agricultural lands and they needn't any fertilizer for cultivation. With these advantages and significant carbohydrate content, higher ethanol yields are obtained from algae. In table 2, ethanol yield values from different feedstocks including first and second generations are given [7].

Feedstock	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
Algae	5,000–15,000	46,760–140,290

Table 2. Ethanol yield values from different feedstocks [7]

Although it depends on the raw material which is used, ethanol production have three main steps: to obtain fermentable sugars, conversion of sugars to ethanol via fermentation process and distillation and purification of produced ethanol. In this chapter, these steps are presented in detail with their alternatives. All literature studies on the subject are reviewed, discussed and also new approach to pre-treatment methods of raw materials to produce bioethanol is presented.

2. Algae as bioethanol feedstock

Algae are simple organisms containing chlorophyll and they use light for photosynthesis. Algae can grow phototrophically or heterotrophically. Phototrophic algae convert carbondioxide in atmosphere to nutrients such as carbohydrate. Conversely, heterotrophic algae continue their development by utilizing organic carbon sources [8]. Algae can grow in every season and everywhere such as salty waters, fresh waters, lakes, deserts and marginal fields etc. However for their cultivation, generally open systems like ponds and photobioreactors as closed systems are used. Open ponds are the most used cultivation systems in industry. They are more preferable than other systems due to having low investment and operation costs. On the other hand difficult control of cultivation conditions and contamination risk are the main disadvantages of the open systems. Besides being cheap and low energy need, their cleaning also can be done easily. Although, open tanks have low cost and easy operation, parameters like light intensity, temperature, pH and dissolved oxygen concentration cannot be controlled easily. Most produced algae species in open systems are *Spirulina*, *Chlorella* and *Dunaliella* [9]. In comparison with open systems, photobioreactors have very high photosynthetic efficiency. Thus, photobioreactors ensures high biomass yield. Though they are expensive, they are preferred for specific algal production. Algal production which is controlled in terms of parameters like light, pH, carbon dioxide etc., can be achieved and also contamination risk is not seen mostly in photobioreactors. Since they are closed systems, evaporation doesn't occurred and they enable production of special biochemical materials. Although there are many types of photobioreactors, most commonly systems are vertical and horizontal tubular columns and flat-type photobioreactors [10]. These photobioreactors which are made of glass or plastic, can be designed in shapes of horizontal vertical, conical or curved etc [11,12].

Algae are classified as microalgae and macroalgae. Microalgae as their name implies, are prokaryotic or eukaryotic photosynthetic microorganisms. They can survive in hard conditions with their unicellular or simple colony structures [13]. Because of being photosynthetic organism, they can produce high amount of lipid, protein and carbohydrate in a short time. Besides biodiesel and bioethanol there are lots of high value products and sub-products produced from microalgae such as biogas [14, 15], biobutanol, acetone [16], Omega 3 oil [17], eicosapentaenoic acid [18], livestock feed [19], pharmaceuticals and cosmetics [20, 21]. Especially sub-products are preferred for economic support of main process [22]. Chemical composition of microalgae can change according to the cultivation type and cultivation conditions. They can have rich or balanced composition of protein, lipid and carbohydrate amounts. Microalgae especially get attention due to have high lipid content [23]. Many species of microalgae accumulate a significant amount of lipids in their structure and can provide high oil yield. Their average lipid content can change between 1-70%, but this ratio can reach up to 90% of dry weight under certain conditions [13]. Macroalgae or seaweed are plants which are adapted to the marine life, often located in coastal areas. They are classified as brown seaweeds, red seaweeds and green seaweeds according to their pigments [24]. Due to have high photosynthesis capability, they have sufficient carbon source for usage in biorefinery. On the contrary of their appearances, their features of morphologic and physiological and chemical compositions are different from terrestrial plants [24]. Unlike the structure of the lignocellulosic biomass of microalgae, they comprise substances such as carrageenan, laminaran,

mannitol, alginate which are used in various sectors [25]. They are separated from microalgae with having low lipid content and different from lignocellulosic material with having less or no lignin in their structure [6].

Microalgae stand out as biodiesel feedstock with the ability of lipid production and high photosynthetic efficiency. As for macroalgae, they are utilized for biogas or bioethanol production with their carbohydrates [26]. First studies as algal biofuels are focused on biodiesel production. However, there is a potential for carbohydrates in the structure of algae which can be utilized for ethanol production after various hydrolysis processes. Algal cells in the water don't need structural biopolymers such as hemicellulose and lignin which are necessary for terrestrial plants [4]. This simplifies the process of bioethanol production. Marine algae can produce high amount of carbohydrate every year. Also it is expected that algae will meet the demand of biofuel feedstock due to harvest in a short time than other biofuel raw materials [27]. Microalgae which have high amount of starch such as *Chlorella*, *Dunaliella*, *Chlamydomonas*, *Scenedesmus* are very useful for bioethanol production. In addition to that, microalgae don't need energy consumption for distribution and transportation of molecules like starch. Like microalgae, macroalgae are also raw materials that can be used in ethanol production. Absence of lignin or having less lignin in the structure, simplifies the hydrolysis stages [4,28]. Although it changes with the algal species, they have various amounts of heteropolysaccharides in their structures. Whereas red algae contain carrageenan and agar, brown algae have laminaran and mannitol in their structure [6].

3. Algal polysaccharides

Ethanol production from algae is based on fermentation of algal polysaccharides which are starch, sugar and cellulose. For microalgae, their carbohydrate content (mostly starch) can be reached to 70% under specific conditions [29]. Microalgal cell walls are divided into inner cell wall layer and outer cell wall layer. Outer cell layer can be trilaminar outer layer and thin outer monolayer. Also there can be no outer layers as well [30]. Outer cell walls of microalgae contain certain polysaccharides such as pectin, agar and alginate. However their composition can be vary from species to species [30]. On the contrary, inner cell walls of microalgae constitute mostly cellulose, hemicellulose and other materials [30]. Due to have cellulose in their cell walls and starch, microalgae are considered as a feedstock for production of bioethanol [31]. Most of their cell wall polysaccharides and starch can be fermented for bioethanol production [32].

Similarly, carbohydrate content of macroalgae is found 25-50% in the green algae, 30-60% in the red algae and 30-50% in the brown algae. Macroalgae species which have the highest polysaccharide content are *Ascophyllum* (42-70%), *Porphyra* (40-76%) and *Palmaria* (38-74%). High carbohydrate content of algal species are presented in Table 3 [32]. Polysaccharides in the cell wall of macroalgae are composed of cellulose and hemicelluloses. Cellulose and hemicelluloses content of macroalgae compose 2-10% of dry weight. Lignin is only exists in *Ulva* species and it constitutes 3% of dry weight [27]. Differently from microalgae, alginate, mannitol, glucan and laminarin are the most abundant polymers in macroalgal structure [42]. Alginates are

Algal species	Carbohydrate content (%)	Ref.
<i>C. vulgaris</i>	55.0	[33]
<i>Chlamydomonas reinhardtii</i> UTEX 90	60.0	[34]
<i>Chlorococum</i> sp.	32.5	[35]
<i>S. obliquus</i> CNW-N	51.8	[36]
<i>Tetraselmis</i> sp. CS-362	26.0	[37]
<i>Ulva lactuca</i>	55-60	[38]
<i>Ascophyllum</i>	42-70	[39]
<i>Porphyra</i>	40-76	[40]
<i>Palmaria</i>	38-74	[41]

Table 3. Carbohydrate content of algal species

polymers which are obtained from cell walls of various brown algae. They consist of mannuronic acid and L-gluronic acid monomers and they are extracted from cell walls by using sodium carbonate. Although they are usually used as stabilizer in pharmaceutical industry, they also used in paper and adhesive manufacture, oil, photography and textile industries [43-45]. Caragenan is another polysaccharide which is obtained from red algae. It is used as stabilizer in food, textile and pharmaceutical industry. Agar is also acquired from red algae. Like caragenans and alginates, it is extracted with hot water and used as stabilizer and gelling agent. 90% of produced agar is utilized in the food industry, the remaining 10% is used in microbiological and biotechnological field [44,46]. Mannitol which is a structure in brown algae is a sugar alcohol, especially found in *Laminaria* and *Ecklonia*. Mannitol content of macroalgae can change with season and environmental conditions. Mannitol can be used in pharmaceutical, paint, leather and paper manufacture. In addition to that, mannitol can be utilized in food industry as a coating material [27]. Laminarin is a polysaccharide which helps the immune system by increasing the B cells, provides protection against infection by bacterial pathogens and severe irradiation. Another polysaccharide from macroalgae is ulvan. It is mainly presented in *Ulva* sp. and it is source of sugars for production of fine chemicals [27].

4. Pre-treatment technologies for biomass

The most important difficulties encountered in the production of bioethanol are the pre-treatment of biomass. The objectives of an effective pre-treatment are obtaining sugars directly or later by hydrolysis, preventing lost or degradation of obtained sugars, limiting the toxic materials which inhibit the ethanol production, reducing energy requirement for process and minimizing the production cost. There are four pre-treatment techniques including physical, chemical, physicochemical and biological pre-treatments that are applied to biomass [1]. Pre-

treatment process is the step that forms the significant part of the cost of ethanol production. Although there is no technique that can be considered as the best option, researches and developments are carried on to reduce cost and improve performance [3].

4.1. Physical pre-treatments

4.1.1. Mechanical comminution

Chipping, grinding and milling are the most used techniques for mechanical comminution. Comminutions improve the efficiency of the process for the next steps by reducing the polymerization degree and increase the specific surface by reducing cellulose crystallinity. Energy that is need in the process depends on the initial and final dimensions of particles, moisture content and structure of the raw material [1,47]. In order to assist enzymatic hydrolysis of lignocellulosic materials various milling techniques can be used. For instance, pre-treatment of rice straw with wet disk milling gave higher hydrolysis yields than usual dry milling [48].

4.1.2. Pyrolysis

Pyrolysis is an endothermic process which is a reaction needs low energy input and treats biomass over the temperature of 300°C and degrades cellulose to char and gaseous products like CO and H₂. When the char is washed with water or diluted acid, remaining solution contains sufficient amount of carbon source to support microbial growth for the production of bioethanol. Approximately 55% of biomass weight is lost in the washing step [1]. It is reported in a study that Fan et al. [49] have performed 80-85% conversion of cellulose to reducing sugars.

4.1.3. Microwave oven pre-treatment

Microwave oven pre-treatment is a simple method with short reaction time, high heating efficiency and low energy input. Thermal and non-thermal effects which are generated by microwaves in a liquid medium are used in this technique. The heat generated in biomass results in a polar bond vibration. This causes an explosion between the particles and degradation of lignocellulosic structure. Asetic acid is released from lignocellulosic material and an acidic medium is occurred for hydrolysis [50]. Ooshima et al. [51] investigated the effect of microwave pre-treatment on rice straw and baggase and it was found that an improvement in total reducing sugar production. In recent years, microwave pre-treatments are carried out with various chemical reagents and their potential are investigated. In the studies of alkali microwave pre-treatment, NaOH provides higher reducing sugar yields on switchgrass and coastal bermudagrass in comparison with other alkaline reagents such as Na₂CO₃, Ca(OH)₂ [52,53]. Also for pre-treatment of rice straw and its hulls, this technique made cellulose more accessible to enzymes.

4.2. Physicochemical pre-treatments

4.2.1. Steam explosion method

Steam explosion method is a technique that provides accessibility on the biomass for degradation of cellulose. This method comprise the heating of biomass under high pressure steam (20–50 bar, 160–270 °C) for a few minutes, then reaction is stopped when the pressure conditions arrive to the atmospheric conditions. Diffusion of the steam into the lignocellulosic matrix leads to the dispersion of fibers. No catalyst is used during the applied method. Levulinic acid, xylitol and alcohols are obtained after the degradation of biomass [54,55]. Many types of biomass such as poplar [56], eucalyptus [57], olive residues [58], corn stover [59], wheat straw [60], sugarcane bagasse [61], grasses [62] have been pre-treated with steam explosion method efficiently.

4.2.2. Liquid hot water method

Liquid hot water method treats biomass by using water which is kept in a liquid state under high pressure and temperature for 15 minutes without adding any chemical or catalyst. Instead of steam explosion method, this technique does not need rapid pressure drop or expansion. Pressure is used to prevent evaporation and to stabilize the water in this method [60]. Although it provides the release of hemisellulosic sugars as oligomers, it causes the formation of little amounts of undesirable components which inhibit microbial growth such as carboxylic acid, furfural [63]. Since there is no need for chemicals, it is an environmental and economic method [64]. It is reported that liquid hot water method improves the enzymatic hydrolysis by removing 80% of hemicelluloses when it is pre-treated corn stover, sugarcane bagasse and wheat straw [65].

4.2.3. Ammonia Fiber Explosion (AFEX)

Ammonia fiber explosion (AFEX) is a method that liquid ammonia and steam explosion are carried out together. In this method, biomass which has 15–30% moisture content is treated with liquid ammonia at a loading ratio of 1–2 kg NH₃/kg dry biomass. To acquire appropriate temperature, pressure over 12 atm is required. Whereas being an easy method and have short reaction time, it is not effective on raw materials that contain high lignin content [54]. Ammonia has effects such as shredding biomass fibers, partially decrystallization of cellulose and destroying carbohydrate attachments [65]. Although sugars are not released directly with this method, it enhances polymers (hemicellulose and cellulose) to be attacked enzymatically. Thus, low amount of enzyme is enough for enzymatic hydrolysis after AFEX. In order to improve the process economically, ammonia must be recover after the pre-treatment. Ammonia loading, temperature, high pressure, moisture content of biomass, and residence time are the basic parameters which effect AFEX process. Up to 90% cellulose and hemicelluloses conversions can be acquired with this technique [3].

4.2.4. CO₂ explosion

CO₂ explosion is similar to AFEX method. However this method has low process cost due to need low temperature. Also formation of inhibitors in the steam explosion is not occurred in

this technique. In addition to that, its conversion yields are very high compared to steam explosion [50,66].

4.2.5. *Wet oxidation*

Wet oxidation method is based on the treatment of biomass with water and air or oxygen as a catalyst over the temperature of 120 °C. Although solubility of hemicellulose and lignin are increased with this method, free hemicelluloses molecules do not hydrolyze. Whereas sugar monomers are formed in steam explosion and dilute acid pre-treatment, sugar which released in wet oxidation method are oligomers [67,68]. In a study performed by Pederson [69] et al. 40% glucose yield was obtained for wet oxidation of wheat straw.

4.3. Chemical pre-treatments

Chemical pre-treatments include dilute acid, alkaline, ammonia, organic solvent pre-treatments and methods that use other chemicals. These processes are easy to perform and also good conversion yields are achieved in a short time [1].

4.3.1. *Acid pre-treatment*

Acid pre-treatments are methods that acid is used as catalyst to make cellulose more accessible to the enzymes. These processes are divided into two groups as using concentrated acid or diluted acid. Using concentrated acid is less preferable than dilute acid because of forming high amount of inhibiting components and causing corrosion in the equipments [68]. Generally sulphuric acid, hydrochloric acid, nitric acid and phosphoric acid are used in these pre-treatments. Dilute acid are applied at moderate temperatures to convert lignocellulosic structures to soluble sugars [54]. Nowadays biomass is pre-treated with dilute sulphuric mostly to hydrolyze hemicelluloses and facilitate enzymatic hydrolysis [70]. Dilute sulphuric acid hydrolyzes biomass to hemicelluloses, and then hydrolyzes to xylose and other sugar and break xylose down to furfural. Furfural which is a toxic component in ethanol production process, is recovered by distillation [54]. Miranda et al. have investigated the effect of acid pre-treatments with the concentrations between 0.05-10 N, and have obtained the highest sugar yield under the condition of 2 N acid pre-treatment. In their experiments, 2 N to 10 N acid pre-treatments, it is reported that a decrease have been observed in sugar yields [71]. Larsson et al. also mentioned that in an experiment about acid pre-treatment of soft wood, a decrease in ethanol yields have been observed with an increasing acid concentration. In addition to this, it is indicated that formic acid which is a toxic molecule, is presented in the media and inhibits the fermentation [72].

4.3.2. *Alkaline pre-treatment*

These processes are carried out at low temperature and pressure compared to other techniques. Unlike acid pre-treatments, lignin can be removed without major effects on the other components. However there are limitations such as transformation of some alkaline to unrecoverable salts. In addition to that, solubility of hemicelluloses and cellulose are less in this pre-treatment compared to solubility in acid pre-treatment [73]. Alkaline pre-treatment reduces the lignin and hemicelluloses content of biomass and improves the surface area and helps water

molecules for breaking bonds between hemicelluloses and lignin [54]. The most used catalysts in this method are sodium hydroxide, potassium hydroxide, calcium hydroxide and ammonia [74]. Effects of alkaline pre-treatments are varies according to biomass. In an alkaline pre-treatment of coastal bermudagrass, reducing sugar yields are decrease with an increasing alkaline concentration [75]. However, Wang et al. reported that under the conditions of increasing alkaline concentrations, glucose yields were increased [76]. Like dilute acid pre-treatments, dilute alkaline pre-treatments also can form inhibitory by-products such as furfural, hydroxymethylfurfural and formic acid [77]

4.3.3. *Organosolv pre-treatment*

Organosolv pre-treatment is a process that uses organic solvents such as methanol, ethanol, acetone, ethylene glycol. Catalysts are also can be added to the process along with solvents. Hydrochloric acid, sulphuric acid, sodium hydroxide and ammonia are the catalysts used in the process. Besides bonds of lignin and hemicellulose can be broken, pure and high quality lignin can be obtained as a by-product [78]. Removal of lignin improves the surface area and provides accessibility of enzymes to cellulose. After the pre-treatment, cellulosic fibers, solid lignin and liquid solution of hemicellulose sugars are obtained. This method has some disadvantages like oxidation, volatilization and creating high risk in process at high pressure. Also solvents must be recovered due to formation of significant amounts of furfural and soluble phenols and to reduce operation cost [50,67].

4.4. Biological pre-treatments

Compared to the above methods applied to the production of bioethanol, using fungi in pre-treatments is considered environmentally friendly because of not using chemicals, less energy input, not required reactors that resistant to corrosion and pressure, and minimum inhibitor formation [79]. Fungi which are used in biological pre-treatments are generally brown, white and soft mold. These fungi can be degrade lignin, hemicelluloses and cellulose partially. Despite of its advantages, long process time, large production are and need of control continuously for growth of microorganisms ensue as disadvantages for commercial productions[50].

Enzymatic hydrolysis is the step of hydrolysis of cellulose by specific cellulase enzymes. Obtained products after hydrolysis are reducing sugars that include glucose. Cost of the enzymatic hydrolysis are less than acid or alkaline hydrolysis due to reaction is carried out under mild conditions (4.8 pH, temperature of 45-50 °C) [50]. Cellulase enzymes that are used in hydrolysis can be produced by bacteria and fungi. These microorganisms can be aerobic, anaerobic, mesophilic or thermophilic. Bacteria which produce cellulase can be exemplify as *Clostridium*, *Cellulomonas*, *Bacillus*, *Thermomonospora*, *Ruminococcus*, *Bacteriodes*, *Erwinia*, *Acetovibrio*, *Microbispora* and *Streptomyces*. *Trichoderma*, *Penicillium*, *Fusarium*, *Phanerochaete*, *Humicola* and *Schizophillum sp.* are identified as cellulase produced fungi among the fungi [1]. Although there are anaerobic bacteria which produce cellulase with high specific activity, these bacteria are not suitable for commercial productions. Cellulase enzymes consist of mixture of endoglucanase, exoglucanase and b-glucosidase. While endoglucanase attacks the regions where cellulose fibers have low crystallinity, exoglucanase removes the cellulose units from released chains with the effect of endoglucanase and then degrades the molecule. B-glucosi-

dase hydrolyzes the cellulose units and enables the formation of glucose [64]. Enzymatic hydrolysis can be affected by certain factors which are enzyme-related and substrate-related factors. Substrate-related factors have a directly influence on enzymatic hydrolysis. These factors are connected to each other and effect the enzymatic conversion. These factors can be defined as *degree of polymerization and crystallinity of cellulose, accessibility of the substrate, lignin and hemicelluloses content and pore size*.

Hydrolysis rates of biomass depend on the degree of polymerization and crystallinity of cellulose. Degree of polymerization is related to crystallinity. Cellulase enzymes can hydrolyze the crystalline structure of cellulose. Endoglucanase enzymes decrease polymerization degree of cellulosic component by cutting the internal sites of cellulose chains in the enzymatic hydrolysis [80]. Accessibility of the substrate is another main factor effect hydrolysis rate. The rate of hydrolysis increases with increasing substrate accessibility because of being surface area more available for enzymatic attack [80]. Lignin and hemicellulose are complex structures to hydrolyze in lignocellulosic materials. Due to have a role like cement, lignin acts as physical barrier and prevents the digestible parts of cellulose to hydrolyze and it becomes very difficult for enzymes to access cellulose. For this reason, they reduce the efficiency of hydrolysis. Removal of hemicellulose enhances the pore size and provides accessibility to cellulose for enzymes in order to perform hydrolysis efficiently [81,82]. Pore size of the substrate is one of the limiting factors in enzymatic hydrolysis process. In many lignocellulosic material, external area of the biomass is smaller than internal area and this situation causes cellulase enzymes to entrap in the pores of the material. In order to increase hydrolysis rate, porosity of the biomass should be increased [83].

5. Fermentation

Fermentation is a process that based on disciplines of chemistry, biochemistry and microbiology and which fermentable sugars are converted to ethanol by microorganisms [84]. Process consists of conversion of glucose to alcohol and carbon dioxide:



In this process 0.51 kg bioethanol and 0.49 kg carbon dioxide are obtained from per kg of glucose in theory maximum yield. However practically, microorganisms also use glucose for their growth, the actual yield is less than 100% [85]. Microorganisms used in fermentation are utilized from 6-carbon sugars in ethanol production. Therefore, cellulosic biomass which have high amount of glucose are the materials that have easiest conversion capability. One of the most effective yeast which produces bioethanol is *Saccharomyces cerevisiae*. Besides having high bioethanol production yields, it has a resistance to high bioethanol concentration and inhibitor components which can be occurred after acid hydrolization of lignocellulosic biomass. Because reaction occurs under anaerobic conditions, oxygen molecules must be removed with nitrogen gas as a swept gas. Yeast and fungi can tolerate 3.5-5.0 pH ranges [86]. *S.cerevisiae* has high

osmotic resistance and can tolerate low pH levels like 4.0. *Zymomonas* stands out with rapid bioethanol production and high productivity compared to other traditional yeasts. However *Z.mobilis* cannot tolerate the toxic effects of asetic acid and various phenolic compounds in the lignocellulosic hydrolysate [87]. Bioethanol yields of microorganisms are depend on temperature, pH level, alcohol tolerance, osmotic tolerance, resistance for inhibitors, growth rate and genetic stability [54]. Fermentation processes generally are carried out with two basic processes as *simultaneous saccharification and fermentation* and *separate hydrolysis and fermentation*, yet new production processes have been developed [1].

5.1. Separate Hydrolysis and Fermentation (SHF)

Enzymatic hydrolysis is performed separately from fermentation in this process. Liquid which comes from hydrolysis reactor first converted to ethanol in a reactor that glucose fermented in, and then ethanol is distilled and remained unconverted ksilose is converted to ethanol in a second reactor. Advantage of the process is performing reactions in optimum conditions. On the other hand, usage of different reactors is increasing the cost. Also glucose and cellulose units that obtained after hydrolysis, inhibit activity of the enzyme and decrease hydrolysis rate [3,54].

5.2. Simultaneous Saccharification and Fermentation (SSF)

In this process, pre-treatment and enzymatic hydrolysis steps are carried out with fermentation step in the same reactor. It is very efficient when dilute acid or hot water at high temperature is applied in the process. High bioethanol yields can be achieved with SSF process. Also inhibiton of enzyme activity is very low due to fermenting glucose and cellulose units in the same media by yeast. Therefore, this process needs low amount of enzyme. In addition to that, process cost is reduced because of the reactions are carried out in one reactor. As a disadvantage, temperatures differences between saccharification and fermentation cause various effects in growth of microorganisms. *Saccharomyces* cultures are used in pH of 4.5 and temperature of 37 °C this process [3,54,88].

5.3. Simultaneous Saccharification and Co-Fermentation (SSCF) & Separate Hydrolysis and Co-Fermentation (SHCF)

Saccharomyces cerevisiae which used in fermentation cannot convert carbohydrates like pentos under moderate conditions and this causes impurity for biomass and decreases bioethanol yield. In order to overcome this, recombinant yeasts can be used to convert residues such as pentose to ethanol. In SSCF, recombinant yeasts and cellulase enzyme complex are fed to the same vessel to convert biomass to ethanol. This system is generally the same as SSF process. SCHF process is a combination of SSCF and SHF. In this process, fermentation and hydrolysis are carried out in different vessel. This process can produce ethanol with high productivity in comparison with SHF process [88].

Due to their simple structure and being a new raw material for bioethanol production, most of these pre-treatment techniques have not applied to algal biomass yet, and just few studies have been found in literature which is presented in Table 4.

Table 4. Studies of ethanol production from micro and macroalgae

Algae	Classification	Pre-treatment	Fermenting organism, process type and process time	Yield (%)	Ref.
<i>Chlorococum</i> sp.	Microalgae	Supercritical CO ₂ lipid extraction	<i>Saccharomyces bayanus</i> , SHF, 60 h	38.30	[28]
<i>Chlorococum infusionum</i>	Microalgae	0.75% (w/v) NaOH pre-treatment in 120 °C for 30 min	<i>Saccharomyces cerevisiae</i> , SHF, 72 h	26.00	[89]
<i>Chlamydomonas reinhardtii</i> UTEX 90	Microalgae	3% H ₂ SO ₄ pre-treatment in 110 °C for 30 min	<i>Saccharomyces cerevisiae</i> S288C, SHF, 24 h	29.10	[90]
<i>Chlamydomonas reinhardtii</i> UTEX 90	Microalgae	a-amylase (90 °C, 30 min) and glucoamylase (55 °C, 30 min) enzymatic hydrolysis	<i>Saccharomyces cerevisiae</i> S288C, SSF, 40 h	23.50	[91]
<i>Chlorella vulgaris</i>	Microalgae	3% H ₂ SO ₄ pre-treatment in 110 °C, for 105 min	<i>Escherichia coli</i> SJL2526, SHF, 24 h	40.00	[92]
<i>Schizochytrium</i> sp	Microalgae	Hydrothermal degradation and a-enzymatic hydrolysis	<i>Escherichia coli</i> KO11, SSF, 72 h	5.51	[93]
<i>Chlorococum humicola</i>	Microalgae	H ₂ SO ₄ pre-treatment	<i>Saccharomyces cerevisiae</i> , SHF, 50 h	48	[94]
<i>Chlamydomonas reinhardtii</i> cw15	Microalgae	12 N H ₂ SO ₄ pre-treatment	<i>Saccharomyces cerevisiae</i> , SHF, 48 h	44	[95]
<i>Kappaphycus alvarezii</i>	Macroalgae	0.9 N H ₂ SO ₄ pre-treatment in 120 °C, for 60 min	<i>Saccharomyces cerevisiae</i> NCIM 455, SHF, 96 h	15.4	[96]
<i>Kappaphycus alvarezii</i>	Macroalgae	0.2% H ₂ SO ₄ pre-treatment in 130 °C, for 15 min	<i>Saccharomyces cerevisiae</i> SHF, 4h	1.31	[97]
<i>Gracilaria salicornia</i>	Macroalgae	2% H ₂ SO ₄ pre-treatment in 120 °C for 30 min and enzymatic hydrolysis with cellulose in 40 °C	<i>Escherichia coli</i> KO11, SHF, 48 h	7.90	[98]
<i>Gelidium elegans</i>	Macroalgae	Meicelase pre-treatment in 50 °C for 120 h	<i>Saccharomyces cerevisiae</i> IAM 4178, SHF, 48 h	36.7	[99]
<i>Sargassum sagamianum</i>	Macroalgae	Thermal liquefaction in 15 MPa 200 °C for 15 min	<i>Pichia stipitis</i> CBS 7126, SHF, 48 h	10.0	[100]
<i>Laminaria japonica</i>	Macroalgae	0.1 N HCl acid pre-treatment in 121 °C for 15 min and enzymatic hydrolysis with Celluclast 1.5 L, Viscozyme L	<i>Escherichia coli</i> KO11, SSF, 72 h	16.1	[101]
<i>Laminaria hyperborea</i>	Macroalgae	Disruption and washing in water in pH 2 65 °C	<i>Pichia angophorae</i> , SHF, 48 h	0.86	[102]
<i>Saccharina latissima</i>	Macroalgae	Shredding and saccharification pre-treatment	<i>Saccharomyces cerevisiae</i> Ethanol Red, SSF, 48 h	0.47	[103]
<i>Laminaria digitata</i>	Macroalgae	Shredding and saccharification pre-treatment	<i>Pichia angophorae</i> , SSF, 96 h	13.2	[104]
<i>Laminaria japonica</i>	Macroalgae	0.1 M H ₂ SO ₄ acid pre-treatment in 121 °C for 1 h and enzymatic hydrolysis with cellulose and cellobiase	<i>Saccharomyces cerevisiae</i> , SHF, 36 h	11.3	[105]
<i>Laminaria japonica</i>	Macroalgae	Milling and otoclave in 120 °C for 15 min	<i>Pichia stipitis</i> KCTC7228	2.9	[106]
<i>Gracilaria verrucosa</i>	Macroalgae	Enzymatic hydrolysis with cellulose and β-glucosidase	<i>Saccharomyces cerevisiae</i> , SHF, 48 h	43	[107]
<i>Gelidium corneum</i>	Macroalgae	0.5–1% acid pre-treatment in 121 °C for 30 and 60 min	<i>Saccharomyces cerevisiae</i> , SHF, 96 h	5.8	[108]
<i>Sargassum</i> spp.	Macroalgae	3.4–4.6% H ₂ SO ₄ acid pre-treatment and enzymatic hydrolysis	<i>Saccharomyces cerevisiae</i> , SHF, 48 h	65	[109]
<i>Ulva lactuca</i>	Macroalgae	2 N acid pre-treatment	<i>Saccharomyces cerevisiae</i> , SSF, 48 h	24.48	[38]

6. Distillation and purification

A distillation process is necessary for separation of ethanol from mixture and purification of ethanol after fermentation process. Process is performed simply with boiling ethanol-water mixture. Because of boiling point of water (100°C) is higher than boiling point of ethanol (78 °C), ethanol vaporized before water [110]. However, due to being an azeotrop mixture, high amount of energy is used for distillation [42]. In order to separate azeotrop mixtures, an agent which changes the azeotrop structure must be added to the mixture. Added substance changes the volatility of mixture by effecting the molecular attractions in the mixture. Various separation agents such as benzene, pentane, cyclohexane, hexane, acetone, and diethyl ether can be used in this process [111]. Distillation column which has two streams as top and bottom, separates most of the bioethanol from the mixture. While top stream is rich in bioethanol, bottom stream is rich in water. 37% bioethanol then concentrated in rectifying column to approach concentration of 95% [78]. Product which is remained in the bottom is fed to stripping column in order to remove excess water [112]. Mostly in plants, recovery of bioethanol in distillation columns is fixed to be 99.6% due to decrease bioethanol loss [54].

7. Conclusion

Today, demand for fossil fuels cannot be met with current reserves and increasing oil prices with economical and political crisis and effects of global warming are led countries to use renewable energy sources. Algae as third generation feedstock have a great potential because of their characteristics. Different valuable products can be obtained from algae such as biodiesel, bioethanol, biogas, pharmaceuticals and nutraceuticals. Nowadays algae are mostly utilized for biodiesel production due to their high lipid content. However algae have also high carbohydrate content that cannot be ignored. Thus they can be utilized for bioethanol production directly or with the remains which are obtained after oil extraction. In this study, potential of algae as a bioethanol feedstock, important steps of bioethanol production especially pre-treatment techniques have been mentioned. In production sections, pre-treatment techniques and fermentation processes are explained in details. Recently, bioethanol production from algae is very new technology and open to development. Innovative and efficient fermentation processes and pre-treatment techniques are needed to make ethanol production preferable. In conclusion, algae will with their huge potential will outclass the first and second generation feedstocks and lots of improvements for usage of it will carried out in the future.

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