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Review

Overview: Comparison of pretreatment technologies and fermentation processes of bioethanol from microalgae

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ABSTRACT

Continuous exploitation of natural resources especially in fossil fuels to fulfil the market demand has jeopardized the natural resources in the Earth. Bioethanol produced by microalgae is one of the promising biofuel for energy security and ecological sustainability. Yet, to date only a modest review has been reported on the bioethanol production technology from microalgae by using various pretreatment methods, fermentative microorganisms, fermentation processes, commercialization techniques and its environmental perspectives. This review paper aims to provide a comprehensive information by comparing the recent pretreatment technologies by using different types of ethanologenic microorganisms in various fermentation processes to maximize the bioethanol production. Studies of the economic viability and environmental perspectives of bioethanol from microalgae have been carried out to investigate its potentialities to eliminate environmental issues and towards commercialization. Therefore, this review is essential in providing ideas for the future researches in renewable energy resources technology to develop a more efficient way to scale-up the bioethanol production from microalgae for commercial and industrial application.

1. Introduction

Depletion of natural resources due to the gradual growth of the worldwide population has resulted in the current resource depredation with significant environmental impacts in energy shortage, worsening climate change and increasing greenhouse gasses emission [14]. Fossil fuels which are the primary energy source for the world are non-renewable and estimated to be used up by middle of the century [5,28]. In addition, increasing global population which is projected to exceed 9 billion by 2050 will lead to overexploitation of the resources and drives the scarcity of arable land to its limit [42]. Thus, 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 [8,71,132]. Recent statistical report of International Energy Agency (IEA) revealed that the total primary energy supplied by fuel showed the energy produced from biofuels and waste increased steadily from 2.3% in 1973 to 5.7% in 2016 with the total of 3740 and 5257 (Mtoe) respectively [67]. Therefore, it is expected that biofuels will emerge to one of the most strategically sustainable energy source [109]. 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 [95,137].

Biofuels are categorized into first-, second-, third-, and fourth-generation biofuels based on the type of feedstock used [121,129]. Research and developments in biofuels initiated from the first-generation fuel which used the sources of food (corn, wheat, barley and sugarcane) as feedstock has evolved to fourth-generation algae metabolic engineering [42]. The production of first-generation fuel has raised controversial debates on food supply, food security and land use changes due to the large conversion of agricultural crops to biofuels

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[83,142,147]. First-generation bioethanol not only lead to the growing concern of the environmental issue but also causes dilemma of unstable food prices in the market because they are food sources and feed crops globally [146]. Nevertheless, the growing interest in biofuels has been switched to second and third generation which have no food-fuel conflict and better environmental performance in terms of reducing greenhouse gasses emission [49]. Second-generation biofuels are mainly produced from lignocellulosic materials from forest and agriculture residues and industrial organic wastes such as straw, grass, woods, sawdust and others [88,146]. However, using the agricultural residues or industrial wastes still facing the limitations, due to difficulty and high costs to convert lignocellulosic biomass into biofuel in pretreatment process [64,88,146]. Third-generation biofuels produced from microalgae as feedstocks such as bioethanol, biodiesel, biohydrogen and biomethane have been garnering interest worldwide [25,85,146]. Microalgae 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 they are easy to be cultivated in larger quantity [18,89,159]. Algae can be grouped into prokaryotic microalgae (cyanobacteria *Chloroxybacteria*), eukaryotic microalgae (green algae *Chlorophyta*), red algae (*Rhodophyta*), and diatoms (*Bacillariophyta*) [122]. Microalgae are microscopic algae or photosynthetic unicellular microorganism that generally found in ocean and fresh water environment.

Microalgae are convincing alternative resources for bioethanol production in comparison with conventional plant crops and their carbohydrate contents are mainly in the form of starch and cellulose which are easily break down to fermentable sugars via microbial fermentation [23,44]. The cell wall composition of microalgae are also different from the lignocellulosic crops due to low content or absence of the lignin [37]. Bioethanol has been recognized as a clean and sustainable fuel because it is non-toxic, biodegradable, and produce almost zero pollutant to the environment [109,133]. In addition, the global production of bioethanol showed a rapid growth from 17.25 billion liters in 2000 to over 46 billion liters in 2007 [10] in a few years and it is projected to exceed 160 billion liters by 2020 [15]. There are many pretreatment methods to break down the carbohydrates of microalgae and convert them to bioethanol. To ensure microalgae completely break down into simple sugars or monomers, pretreatment for saccharification is done before fermentation. Among of these methods, one of the most popular method is fermentation process. The bioethanol production of microalgae biomass is greatly influenced by the degree of difficulties in pretreatments and the type of fermentation process [30]. Currently, there is no other comprehensive review reported about the pretreatment of microalgae, importance of fermentative microorganism to bioethanol production, various fermentation processes and commercialize value of bioethanol produced from microalgae. Therefore, this review aims to discuss the recent information on the pretreatment technologies, production of bioethanol with different fermentative microorganisms in various fermentation processes and the potential applications of bioethanol production from microalgae to the environmental and economic impacts.

2. Microalgae and cyanobacteria cells

The advantages of algae, microalgae and cyanobacteria for the production of third-generation biofuels are higher than agricultural crops or lignocellulosic biomass in view of producing first- and second-generation biofuels [21]. Algae are primitive plants (thallophytes) which have no sterile covering of cells around the reproductive cells but they have chlorophyll *a* as their primary photosynthetic pigment [19]. Microalgae have cell size between 2 to 200 μm and considered as a photosynthetic microorganism which capable of producing large amounts of biomass containing lipids, proteins, or carbohydrates [5,149]. Table 1 shows the carbohydrates content in different species of microalgae. In contrast, cyanobacteria commonly referred to as blue-green algae which is a division Cyanophyta are also considered as photosynthetic protists which contain a group of oxygenic bacteria that obtain energy by photosynthesis process [21,91]. The smallest cell size of cyanobacteria can be found on picoplankton (0.2–2 μm) and the largest unicellular organisms being of about 500 μm [91]. Most studies of cyanobacteria and microalgae have been investigated to produce efficient sustainable energy like hydrogen (direct synthesis in cyanobacteria), lipids for biodiesel, and carbohydrates for ethanol production [113]. Table 2 shows the composition of protein and carbohydrates of 13 cyanobacterial species, n = 3 [107].

Prokaryotic cells (cyanobacteria) are more similar to bacteria cell because they lack of membrane-bound organelles such as plastids, mitochondria, nuclei, Golgi bodies and flagella. Eukaryotic cells (microalgae) which consist of these organelles can control the functions of the cell, allowing it to survive and reproduce [19]. In the cell of microalgae, carbohydrates usually existed in the outer cell wall (pectin, agar, alginate), inner cell wall (cellulose and hemicellulose) and inside the cell as

Table 1
Carbohydrates content in different species of microalgae (all results are presented in % dry weight).

Biomass	Carbohydrate	References
Anabaena cylindrica	25–30	[41]
Aphanizomenon flos-aquae	23	[102]
Chlamydomonas reinhardtii	17	[41]
Chlorella sp.	19.5	[116]
Chlorella sorokiniana	35.67	[24]
Chlorella vulgaris	20.99	[154]
Chloroccum sp.	32.50	[57]
Dunaliella salina	32.00	[41]
Dunaliella tertiolecta	21.69	[130]
Euglena gracilis	14–18	[41]
Isochrysis zhangjiangensis	23.2–47.7	[47]
Isochrysis galbana	7.7–13.6	[48]
Isochrysis sp.	5.2–16.4	[98]
Nannochloropsis oceanica	22.70	[26]
Nannochloropsis oculata	8	[16]
Pavlova lutheri	28.25	[118]
Porphyridium cruentum	40	[16]
Prymnesium parvum	25–33	[41]
Scenedesmus dimorphus	21–52	[41]
Scenedesmus obliquus	10–17	[41]
Spirulina platensis	31.20	[70]
Spirogyra sp.	33–64	[102]
Spirulina sp.	20	[16]
Tetraselmis maculate	15	[98]
Tetraselmis suecica	15–50	[17]
Tetraselmis sp.	24	[125]

Table 2
Composition of protein and carbohydrates of 13 cyanobacterial species, n = 3 [107].

Species	Protein (% DW)	Carbohydrates (% DW)
Calothrix crustacea (HF)	21.50 ± 0.40	7.60 ± 0.50
Calothrix contarenii (HF)	27.43 ± 0.47	8.23 ± 0.65
Gloeocapsa crepidinum (C)	56.46 ± 0.25	7.63 ± 0.55
Lyngbya martensiana (F)	18.86 ± 0.65	5.43 ± 0.41
Lyngbya semiplena (F)	27.50 ± 0.45	8.93 ± 0.15
Phormidium corium (F)	49.56 ± 0.55	16.46 ± 0.45
Phormidium tenue (F)	62.96 ± 0.55	15.46 ± 0.40
Spirulina subsalsa (F)	70.76 ± 0.90	16.63 ± 0.56
Spirulina labyrinthiformis (F)	68.03 ± 0.85	14.73 ± 0.66
Synechococcus sp. (C)	63.56 ± 0.60	8.56 ± 0.56
Oscillatoria formosa (F)	50.85 ± 0.79	9.46 ± 0.45
Oscillatoria salina (F)	41.80 ± 0.81	11.20 ± 0.36
Oscillatoria subbrevis (F)	45.16 ± 0.41	11.53 ± 0.68

(HF) Heterocystous filamentous cyanobacteria; (F) Non-heterocystous filamentous cyanobacteria; (C) Unicellular cyanobacteria.