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REVIEW ARTICLE

A Comprehensive Review on Sustainable Bioenergy from Algae and Cyanobacteria

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ABSTRACT
Sustainable bioenergy production is critical for addressing global energy demands while minimizing environmental impact. This review paper deals with third generation bio fuels which does not compete with for food. Cyanobacteria and algae, photosynthetic microorganisms, have developed as a significant source for bioenergy because of their capacity to flourish in diverse environment, quick growth rates and ability to effectively use carbon dioxide and sunlight. This review see through the potential of cyanobacteria for bioenergy applications, focusing on their roles in biofuel production, bio ethanol, bio hydrogen, bio butanol and biomethane. Here, we discuss recent advances in metabolic engineering, strain optimization, and cultivation techniques that enhance biodiesel and bioethanol production by yielding high amount of lipid and carbohydrates. By addressing challenges such as scalability, economic viability, and technological innovation, this review underscores the capacity of cyanobacteria as a sustainable and renewable energy resource, providing the way for a greener future.
Keywords: *Cyanobacteria, bio diesel, lipid extraction, bio ethanol, yeast cell, bio hydrogen, bio butanol, bio methane.*

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INTRODUCTION

Globally, the use of fossil fuel increasing day by day while the universal reservoir constantly depleting. Increased consumption of fuels lead to the environmental pollution which include human health risk and global warming. This would be essential to explore alternative fuel from fresh, resourceful and sustainable production process known as Biofuel [3]. In past few years, the biofuel have been generated and utilised in many countries including US, Brazil, Europe and in some Asian countries [4]. Based on sources of feedstock and bioprocess technologies, four generation of biofuel have been produced. The first two generations of biofuels produced from animal and plant feed stocks. Furthermore, the third-generation biofuel is principally produced from eukaryotic algae and prokaryotic cyanobacteria which not to fight each other for available food and fertile land. This would increase the biomass production by increase the lipid accumulation for biofuel production and while the fourth generation incorporates genetic engineering to enhance the commercialisation of biofuel [1]. In the last three decades, cyanobacteria have been the subject of much research for the manufacture of biofuels. Of particular significance has been their cells' capacity to synthesise lipids and effectively accumulate biomass through photosynthesis. The lipid content of cyanobacteria is higher than the algae and plants. Thus results in increase the production of biofuel from cyanobacteria [2]. The recent annual production of biofuel is about 1.324 billion tons of oil approximately. It is expected that the value of the worldwide biofuel industry would reach record-breaking levels, rising at a compound annual progression rate of 4.92%, it will reach $246.52 billion by 2024 from $168.18 billion in 2016. The production of biofuel from Cyanobacteria is highly cost effective because it can grow fast and thrive in minimal nutrient concentrations. In addition to this, it is potential in efficient burning, reduced sulphur emissions, and absence of aromatic compounds. [25]

Algae

Algae are eukaryotic photosynthetic, unicellular or multicellular organism, they are mostly occurred in aquatic environment and less in terrestrial environment. Based on their pigments, the algae are distributed into 11 classes that are *Cyanophyceae*, *Chlorophyceae* (green algae), *Euglenineae*, *Xanthophyceae* (yellow green algae), *Chrysophyceae* (golden algae), *Bacillariophyceae* (diatoms), *Phaeophyceae* (brown algae), *Pyrrophyceaes*, *Cryptophyceae*, *Rhodophyceae* (red algae), and *Chloromonadineae*. Nearly, 50% of photosynthesis takes place by algae and they are easily cultivable in higher CO_2 concentrations [32]. All algal species can able to produce energy rich oils that also called as biofuels. The bioengineered cells of algae develop specific characteristics and produce value added products. These characteristics make a platform for high potential for creating cost effective biofuels [33].

Cyanobacteria

Cyanobacteria is a microalgae which also knows Blue green algae due to its colour and similarities with microalgae [24]. Cyanobacteria are prokaryotic organism and thus lack well developed nucleus and membrane bound organelles such as Choloroplast, Golgi bodies and etc. It is one and only bacteria which contain chlorophyll a [34]. Cyanobacteria can be found on marine and fresh water ecosystems and thus can able to grow wide range of temperatures. Under certain circumstances, cyanobacteria can develop very quickly, which can result in "blooms" that change the food chain, create anoxia, cause fish kills, and generate excessive turbidity in the water [6]. Since the Cyanobacteria are photosynthetic organism, they produce their food using sunlight and CO_2. Due to these bacteria have small genome which already sequenced completely and this is easy to modify genetically in order to enhance the biofuel production [7].

Biofuels

Cyanobacteria are being a photosynthetic organism, they synthesize and store the energy in proteins, carbs, and fats [26]. The energy become feedstock for production of biofuels. Among these, lipid is the main energy source which has to be transesterified with chemical process and their hydrocarbons extracted simultaneously. Then the hydrocarbons used as transport fuel such as biodiesel. Under dark anoxic conditions, the carbohydrates converted into ethanol by fermentation [9]. Alternatively, the energy components can be transformed into bio methane under anaerobic condition. Furthermore, biofuels can be categorized into several types such as biodiesel, bio hydrogen, bio ethanol, bio methane [6,41, 27].

METHODS OF BIOFUEL PRODUCTION

Diesel

Chlorella ellipsoidea and *Leptolyngbya tenuis* were cultured in BG 11 medium (1.5 g $NaNO_3$, 0.04 g K_2HPO_4, 0.075 g $MgSO_4 \cdot 7H_2O$, 0.036 g $CaCl_2 \cdot 2H_2O$, 0.006 g citric acid, 0.006 g ferric ammonium citrate, 0.001 g EDTA, 0.02 g Na_2CO_3, and 1 ml trace metal mixture) [27]. The filamentous cultures kept at 22°C in 16:8 light-dark cycles while being shaken for two to three hours per day at 120 rpm. The cultures' biomass was assessed by centrifuging a known volume of culture for ten minutes at 10,000 rpm, and the supernatant was disposed of. Impurities gathered in the pellet was removed using deionized water and the culture was carefully relocated to pre weighed vials kept at 70°C for drying. Dry weight of the cyanobacterial culture was calculated using this formula:

Biomass productivity (mg/L/d) = Final dry biomass (mg/L) - Initial dry biomass (mg/L)/Cultivation time (d).

Additionally, the biochemical characteristics such as chlorophyll, carotenoids, proteins and carbohydrates were determined [9]. The lipids accumulate in the Cyanobacterial cells were extracted with help of binary solvent system. The dried cells of cyanobacteria added in the chloroform and methanol in the ratio of 2:1 [43] and crude extract added to the water which used to separates the lipids in lower chloroform phase, dried at 70°C. The lipids were estimated using this equation [5]:

Lipid productivity (mg/L/d)= Biomass productivity (mg/L/d) × Lipid content (%)/100

The lipids were added to the 3.5% of methyl sulphuric acid which would convert into fatty acid methyl esters (FAME) and this known to as transesterfication[35, 43], held at 65°C for 2 hours. Followed by this, the hexane-treated crude extract is used to extract the FAME, which is subsequently allowed to dry at room temperature. The total FAME yield is computed by dividing the FAME weight by the lipid weight and multiplying the result by 100 [10]. Finally, the quality of biodiesel (mixture of FAME and hexane) analysed by Gas Chromatography Mass Spectroscopy [31].

Ethanol

Cyanobacteria such as *Leptolyngbya valderiana* BDU 41001, *Lyngbya* sp. BDU 90901, *Nostoc* sp. BDU 0051,*Oscillatoria formosa* BDU 91041, *Oscillatoria salina* BDU 10142, *Phormidium tenue* BDU 46241, *Synechococcus elongatus* BDU 141741,and *Spirulina subsalsa* BDU 30311, and four marine green microalgae viz. *Chlorella* sp. BDU 10241, *Chlorella* sp. BDU 20021, *Dunaliella* sp. BDU 10113, and *Tetraselmis indica* BDU GD001 were obtained from National Facility for Marine Cyanobacteria (NFMC), Bharathidasan University, Tiruchirappalli, Tamil Nadu, India[11]. These cyanobacteria were cultivated using BG 11 medium [28] maintained at 25 ± 2 ∘C [36] and shaken three to four times a day to prevent sinking. Artificially, the fluorescent lamps illuminated at 75 μmol photon m− 2 s− 1 PAR for their rapid growth. Mass cultivated and biochemically characterized above mentioned cyanobacteria [38]. Using the most popular industrial strain called *Saccharomyces cerevisiae* AXAZ-1[38] enhanced with the biomass to investigate the bioethanol production by fermentation process. Under anaerobic conditions the fermentation process performed in 250ml Duran bottles and stirring periodically at 30 °C. Initially, to induce the cell growth the fermentation flasks were shaken at 150 rpm. Neubauer type haemocytometer used to detect the yeast cell growth in the fermentation flask. The fermentation held constant temperature at 28°C and the pH ranges from 4.4-4.6, since the yeast grow between pH 4-5. The concentration of bioethanol was measured using an HPLC (Ultimate 3000, Dionex, Germany) system fitted with an Aminex HPX-87H column (300 mm × 7.8 mm, Bio-Rad, Hercules, CA, USA) and a reflective index detector (RI-101, Shodex, Kawasaki, Japan), in which ethanol was detected [12]. The bioethanol yield can be calculated according [13]

$$\text{Bioethanol yield (\%dcw)} = \frac{\text{Bioethaol produced (mg/l)}}{\text{Biomass yield (g/l)}} \times 100$$

Hydrogen

All of the tests employed *Chlorella* sp. [39] and the filamentous cyanobacterium *Fischerella muscicola* TISTR 8215 from Thailand Institute of Science and Technological Research (TISTR) [15]. These organisms cultured using BG 11 medium [29] and their biomass quantified by UV-Spectrophotometer. The cultures transferred to 50 ml tubes and precipitated at 8500g in room temperature; supernatant were removed. Again, the cultures were washed with fresh nutrient media twice and final concentration make up to 30ml and transferred to 50ml glass tubes which is then sealed with rubber stopper. Anaerobic condition was created in the sealed glass tubes, through the 18-gauge needle the argon (99.99) bubble was injected for 10mins (dark conditions) or 20mins (light conditions). 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), which dissolves in DSMO at different concentrations were added to the culture and left it there for 15 to 20 minutes in order to sustain anaerobic conditions before argon bubbling. DCMU which blocks the electron transfer and permit the production of molecule hydrogen. Then the cultures were placed both light and dark condition in bio shaker at 25°C. The culture bottle was covered with aluminium foil while it was dark, and it was continuously exposed to warm, white light (2700 K) at an intensity of 85–100 mmol photons m2 s1 when it was bright. The production of H2 was measured every 24 hours using gas chromatography, which was set up Using a molecular sieve and a thermal conductivity detector Five columns (60/80 mesh) at 30.0 °C 0.5 °C. The calibration done by 3.06% H2 in nitrogen and argon is used as carrier [14].

Butanol

Cyanobacteria are gram negative photosynthetic bacteria, they able to convert water, CO_2 and sunlight into sugar for producing bio butanol. Cyanobacteria attracted many researchers because of their metabolic and genetic engineering simplicity. Even though, production of bio butanol from cyanobacteria is difficult but it can reduce the CO_2 emission in the atmosphere. In *Synechococcus* PCC 7942 [40] the four genes such as alsS, ilvD, kivD, ilvC, yqhD have been introduced for the synthesis of bio butanol and thus strain produced about 450 mg/l [16]. Several pathways have designed to produce bio butanol. By overexpressing acetoacetyl-CoA synthase (NphT7), which condenses the malonyl-CoA and acetylCoA in Synechococcus, a unique ATP-driven acetoacetyl-CoA biosynthetic pathway has been developed. The 6.5 mg/L 1-butanol was generated via the co-expressing downstream NADH-dependent 1-butanol pathway. The distinct NADPH-dependent butyraldehyde dehydrogenase (Bldh) and alcohol dehydrogenase (YqhD) were then used in place of the NADH-dependent bifunctional aldehyde/alcohol dehydrogenase (AdhE2). The end result was a synthesis of ~30 mg/L of 1-butanol that was four times higher. According to a different study, the 1-butanol biosynthesis pathway's innate oxygen sensitivity and dependence on NADH prevented oxygenic cyanobacteria from producing 1-butanol [17,19].

Methane:
For isolating Cyanobacteria, the water samples were collected and stored in sterile flask at 30±2 ∘C. The collected samples were serially diluted and the concentration ranged from 10^{-1} to 10^{-2} which then used for further sampling. These samples cultured on BG 11 [30], ASN 111[37], BBM and modified ASN 111 in spread plate method and incubated at 37°C for seven days. Followed by incubation, single colony picked with using sterile inoculation loop and smeared on to the glass slide which placed under the microscope for morphological identification. In order to obtain pure culture the selected colonies were picked out and inoculated in BG 11, ASN 111, BBM [42] and modified ASN 111 and kept at 30±2 ∘C. The isolated Cyanobacterial colonies from water sample would be mass cultivated in four different types of media including BG 11, ASN 111, BBM and modified ASN 111. In conical flask, the filamentous growth of photosynthetic Cyanobacteria could be observed followed by seven days incubation at 30±2 ∘C [5]

$$20CO_2 + 8H^+ + 8e^- \longrightarrow CH_4 + 2H_2O \quad [44]$$

Harvested biomass pre-treated with several methods like thermal treatment, standard moisture heat procedure, µWAVE and sonicater. In triplicate, anaerobic digestion was performed by introducing 100 millilitres of culture to 120 millilitre serum vials. Twenty-one days were spent at 35°C incubating 50 millilitres of culture and 50 millilitres of anaerobic sludge in a shaker set at 100 rpm and sealed with butyl rubber. COD values were detected. Activated sludge without cyanobacterial biomass was kept for control. Biomethane production was observed in pre-treated culture while in the non-treated biomass culture did not produce methane period of 21 days incubation [22]. Methane can be calculated by,
BMPthAtc = 22.4 (n 2+a 8-b 4-3c 8) / 12n+ a + 16b+ 14c
Where, n- % carbon; a- % hydrogen; b- % oxygen; c- % nitrogen

DISCUSSION
[9] *Chlorella ellipsoidea* and *Leptolyngbya tenuis* were cultivated as monoculture and co-culture in BG 11 medium for 21 days. The total number of lipids is slightly higher in co-cultures than in the monocultures. It was found that there were eighteen fatty acids: nine SFA, three MUFA, and six PUFA. Moreover, maximum amount of unsaturated fatty acids was identified in co-cultures. Here, the European-EN 14214, American-ASTM D6751, and Indian Standard Diesel biofuel qualities are hypothetically compared. In this research, they concluded that besides biomass and lipid productivity co-cultures were considered to produce high quality biodiesel that suggests their utility in sustainable development. [20] This study aims to uncover potential sources of biodiesel and other value-added products by isolating cyanobacteria from Sri Lanka's freshwater ecosystem. A total of seventy-four cultures were shown to be an effective source of high lipid, with a sp. of 31.9 ± 2.01% in Oscillatoriales. Gas chromatography verified that cyanobacteria contained FAME, which may be used as a feedstock for the biodiesel industry [12] The cyanobacteria obtained from NFMC, Bharathidasan University, Tiruchirappalli. The culture biomass of cyanobacteria become feedstock for production of bioethanol due to those contain high amount of carbohydrate. All cyanobacterial biomass cultures were supplemented with *S. cerevisiae* strain AXAZ-1 which can able to reduce the complex sugar molecules such as carbohydrate into ethanol under anaerobic conditions and this were determined using alcoholic fermentation. Theoretically, total amount of bio ethanol yield between 73.0–76.5%. [38] The purpose of this study to explore bio ethanol producing cyanobacteria. Here, they were isolated filamentous marine cyanobacteria and characterized as *Leptolyngbya valderiana* chosen for bio ethanol production. The biomass of this particular cyanobacterium is about 34% dcw which is rich in sugar (glucose) that converted into ethanol that lower the cost production of bio ethanol. [14] The synthesis of bio hydrogen is completely depended on the electron donors and energy present in the microorganisms. The hydrogen was produced in two different methods such as dark and artificial light conditions with and without DCMU. The addition of DCMU has a positive response which enhance the production of bio hydrogen while without DCMU reduce the production in both dark and artificial light conditions. [21] In this study, mainly focus to tackle the climate change and find alternative energy source. The potential of five fresh cyanobacterial isolates from rice paddies to generate H2 was examined. With the maximum activity, strain A. variabilis A-1 produced 15.2 mmol ethylene mg1 DW h1. Consequently, the strain generated 3.7 times more H2 in the dark than in the light and 43 times more H2 than *Phormidium tenue* P-1. [19] In this study, genetically engineered hydrolytes of cyanobacterial strains were used. The genes such as alsS, ilvD, kivD, ilvC, yqhD and butanol producing enzymes also incorporated and that produce high amount of bio butanol from photosynthetic cyanobacteria. [18] There are still challenges in producing bio butanol. The genetically modified cyanobacteria under photosynthetic conditions, the quantity yielding bio butanol is low when compared with ABE fermentation process. Further, the researcher using thermophile fast growing cyanobacteria for producing industrial scale bio butanol.[22] Based on CHNS composition theoretical Biomethane

production was calculated. Sonicator and µWAVE pre-treatments indicated that less amount of biomethane production and standard temperature procedure yield high bio methanol. [23]In this study, they proved that acid or alkali pre-treatments not increase the bio methane yield less than of 20%. Only, the enzymatic and thermal pre-treatment increase the bio methane produced.

CONCLUSION

Third-generation bioenergy, derived from algae and cyanobacteria, represents a promising and sustainable approach to renewable energy production. The key advantages of algae and cyanobacteria include rapid growth rates, high lipid content, and the ability to fix atmospheric CO_2, which helps mitigate greenhouse gas emissions. Theoretically, all cyanobacteria and algae isolates produce biofuel which is nearly identical. Compared to butanol and hydrogen, the commercialisation of diesel, methane, and ethanol is technically feasible and easily accessible. Furthermore, genetically modified organisms used to improve biofuel yield and efficiency.

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