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CULTIVATION SYSTEM OF GREEN MICROALGAE, BOTRYOCOCCUS BRAUNII: A REVIEW

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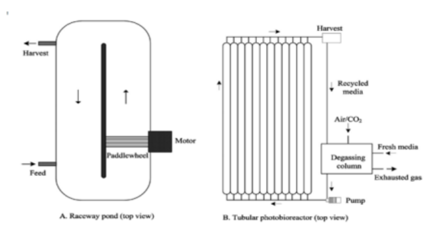
Graphical abstract



Abstract

Renewable energy by using microalgae is another route to solve fossil fuel dependence, reduce climate change and enhance food security. The microalgae has a simple structure. Photosynthesis reaction of the microalgae converts solar energy into chemical energy which later can be used as fuel. This paper reviews microalgae as a new prospective for biofuel feedstock that is produced from non - food sources which offer a long term of sustainability and energy security. The cultivation system under open and closed system are discussed in this paper. A common weakness of the systems is low lipid production. A two stages culture also known as hybrid cultivation system that is discuseed in this paper can be used to overcome the weakness. A review on the performance of the microalgae biofuel applying in the internal combustion engines is presented in the last section of this paper.

Keywords: Biofuels, microalgae, raceway ponds, photobioreactor, hybrid cultivation system

1.0 INTRODUCTION

In the new global economy, renewable biofuels has become a central issue for substitution of current petroleum fuels due to shortage of fossil fuels, increasing crude oil price, environmental issues and political concerns [1]. Hence, microalgae seem to be an alternative route to produce biofuel feedstock. Microalgae received notable attention because of their potential in producing environment-friendly and sustainable biofuel and contribute to the effect of biofixation of waste CO_2 (1 kg of dry algal biomass utilize about 1.83 kg of CO_2) because they convert CO_2 (and supplementary nutrients) into biomass via photosynthesis [2].

It is highly claimed that good yield of biofuels from microalgae depends mainly on the content of triacylglycerols (TAGs), which composed more than 70% of lipid content and the biomass productivity [1]. Most studies reported high TAGs in the lipid content refers to lipid only instead of TAG as the feedstock for the biodiesel production. Many microalgae species have a potential to accumulate substantial amount of lipids, though under specific conditions with average lipid contents vary between 20% to 50% dry weight biomass, while some species may reach 75% dry weight under certain conditions [2, 3].

Among all microalgae species, *Botryococcus braunii* is known as one of the potential microalgae species to accumulate a substantial amount of lipids. *B. braunii* is recognized as green colonial, slow growing algae and normally can be found in freshwater and brackish lakes, reservoirs and ponds. B.braunii consists of three races A, B, and L depending on the type of hydrocarbons synthesized [4]. The hydrocarbon content of strains A and B are very high, can constitute up to 60% of the dry biomass during the active growth phase in laboratory culture. Race-L produce a single tetraterponoid hydrocarbon known as lycopadiene and it constitutes up to 2-8% of the dry biomass [5].

Although in a simplistic view, *B. braunii* seems to have large advantages over other microalgae species, mainly in terms of high lipid content, the biomass productivity and lipid productivity need to be further improved. The idea of improvement can be accomplished during the cultivation stage to be economically feasible. Both biomass and lipid productivity are basically depends on the cultivation system either open or closed system *B. braunii* is identified as an untapped resource for high lipid production due to their genetics factor without dispute the significance of cultivation system. The cultivation systems under open and closed system were compared and an approach of the hybrid cultivation system by two-stage growth was highlighted in this paper.

2.0 BOTRYOCOCCUS BRAUNII CULTIVATION IN RACEWAY PONDS

Open systems is mainly consists of lakes and natural ponds, circular ponds, raceway ponds and inclined system. The system is less expensive to build and operate and it needs low maintenance costs compared with closed system [6]. Raceway pond, for example, is an oval shape, closed loop channels, maintained depth in between 20 cm to 40 cm equipped with paddle wheels for mixing. Continuous mixing is significant to provide equivalent light utilization throughout the culture and to prevent sedimentation in the pond [7]. An example of a raceway pond is shown in Figure 1. An open system is expected to expose with contaminates and other fast growing heterotrophic organism thus restricting large scale mass production of algae [7, 8].

Few years back, several researchers had conducted cultivation of *B.braunii* via raceway ponds. Dayananda *et al*. had collected a sample of green, colonial, unicellular microalgae *Botryococcus sp*. from freshwater ponds in Mahabalipuram, Tamil Nadu, India. As the sample undergone microscopic and scanning electron microscopic images, it was found to be *Botryococcus sp*. Further analysis had been carried out and the sample shown similarities with genus *B.braunii*. Hence, the Indian isolate was designated as *Botryococcus mahabali* based on the morphological features and 18s rRNA sequence analysis. The *B.braunii mahabali* was scaled up in open air raceway ponds in batch mode. As a result, the maximum biomass yields were found to be 2 g/L (w/w) on 14th day of cultivation in outdoor raceway ponds. Based on biomass analysis, *B. braunii mahabali* contain 19% protein, 18% carbohydrates and 14% lipid [8].

In 2011, Ashokkumar and Rengasamy carried out a research on the mass culture of *Botryococcus braunii* Kutz. under open raceway pond for biofuel production. The research used three different strains of colonial green algae *B.braunii* Kutz, AP103, AP104 and AP105 which were isolated from the freshwater lake Kolleru, India. Under laboratory conditions, AP-103 showed highest biomass concentration 1.7 g/L, 17% lipids, 17% protein, 32% carbohydrates and 13% hydrocarbons. Similarly, when cultivated in open-outdoor raceway pond, AP-103 showed the highest biomass concentration of 18 g/L, 19% lipids, 18% protein, 33% carbohydrates and 11% hydrocarbons. There were a presence of heptadecane, hexadecane,oleic, linolenic, almitic acids had been identified in the lipids extracted from AP-103 [9].

Rao *et al*. carried out a research on cultivation, seasonal variation in growth, hydrocarbon production, fatty acids profile of green algae *Botryococcus braunii* in raceway and circular ponds under outdoor conditions. The result showed that, race A strain (LB-572) growth maximum hydrocarbon and carbohydrate content in raceway rather than circular pond after 18th day of cultivation. Race A strain (LB-572) showed hydrocarbon content of 24% and 19% in raceway and circular ponds respectively. The fat content was found in the range of 20-24% (w/w) with palmitic and oleic acids as major fatty acids. A maximum biomass yield of 2 g/L was observed within three months during winter, which correlated to hydrocarbon production. While for race B strain (N-836), the biomass yield and hydrocarbon content was estimated at five day intervals. The maximum biomass yield of 1 g/L and 27% hydrocarbon (w/w) content were observed after the 25th day of cultivation [10].

3.0 BOTRYOCOCCUS BRAUNII CULTIVATION IN PHOTOBIOREACTOR

A closed system is mainly based on closed photobioreactor technology, which designed to overcome the limitations of open systems. They consist of tubular, flat plate and column photobioreactors. Photobioreactors offers single-species of microalgae cultivation thus eliminates the risk of contaminations and hence prolonged microalgae growth durations [6]. Photobioreactors are characterized by better control culture conditions and growth parameters such as pH, temperature, mixing, , CO_2 and O_2, eliminate evaporation, no CO_2 losses and promote high biomass productivity as well as microalgae densities. Their limitations are overheating, fouling, gradients of pH, dissolved oxygen and CO_2 along the tubes, difficult scale up and high cost of building and operation [3]. An example of a photobioreactor is shown in Figure 1. The comparative analysis of open and closed system are shown in Table 1.

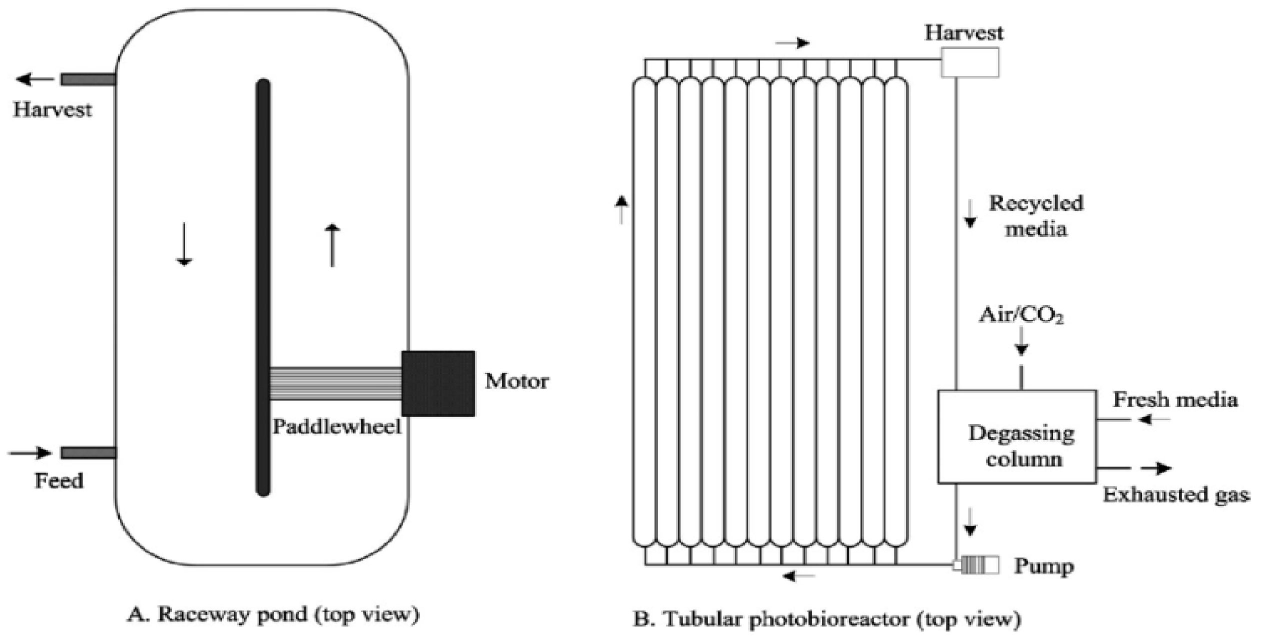


Figure 1 An illustration of an example of an open and a closed system [1]

Several researches had been carried out over the past few years for cultivation of *B. braunii* via photobioreactors. In 1999, Kojima and Zhang used *B.braunii* strains (race-B) which was obtained from the National Institute for Resources and Environment (Japan) as a sample of the research. The algae cells were cultivated in bubble column photobioreactor. The objective of the research is to relate the quantitative relationship between growth rate and hydrocarbon production in optimal operating conditions, and secondarily to evaluate the effects of photoinhibition on growth rate and hydrocarbon productivity. The result showed that the algae cell concentration reached more than 7 kg/m^3 and the hydrocarbon content was 50% based on cell dry weight when exposed to high irradiance (10 klux) in the preculture [11]

Ge *et al*. cultivated *B.braunii* strain 765 with flue gas containing high CO_2 concentrations in the range of 2% to 20% for 28 days using batch mode photobioreactor. The photobioreactor was 10 cm diameter, 50 cm length and 3 L working volume. The photobioreactor were subjected to temperature of 25°C and continuous cool white fluorescent light (150 ± 10 μmol/m^2.s^1). Maximum biomass were achieved at 2.31 g/L after 25 days of cultivation at 20% CO_2 concentration with an aeration rate of 0.2 vvm and without culture pH adjustment (ranged from 6.0 to 8.0) [12].

Xu *et al*. cultivated *B. braunii* in both 2 L and 40 L airlift bioreactors. The aim of the research was to optimize the inner structure of a cylindrical airlift bioreactor using a two-dimensional axisymmetric CFD model. This was achieved by designing an effective mixing process to improve the growth rate and increase the hydrocarbon content (up to 75% of its dry biomass). The result showed that CFD simulation were validated with experimental data collected from microalgae cultures in 2 L and 40 L airlift bioreactors. Hence allowed the optimization of the inner structure of bioreactor suitable for microalgae cultivation without required numerous time-consuming bioreactor experiments [13].

4.0 HYBRID CULTIVATION SYSTEM IN TWO-STAGE GROWTH

The idea of the hybrid cultivation system is to overcome both weaknesses in open and closed-culture system. The hybrid cultivation system comprises of two-stage cultures which manipulates the culture conditions and nutrient feed in terms of the period and concentration to increase the cell reproduction rate and lipid content of the microalgae.

Microalgae are capable of using many types of metabolism, such as photoautotrophic and heterotrophic for growth. Photoautotrophic culture requires sunlight for the microalgae cells to reproduce via photosynthesis. In photosynthesis microalgae cells absorbed energy from sunlight and stored in the form of ATP and NADPH, which will be further used in Calvin cycle to produce glucose. In the event of insufficient sunlight, the photosynthesis will be inhibited, thus affect the productivity of glucose. Artificial lights could help, however it requires higher cost. Thus, photoautotrophic culture seems to be impractical to support higher biomass productivity and lipid content of microalgae cells [1]. Heterotrophic culture, however, requires organic carbon sources such as sugars or organic acids in the absence of sunlight for the microalgae cells to derives their energy [14]. Heterotrophic culture achieved higher biomass and lipid production.

Table 1 Comparative analysis of open an closed system[1]

Issue	Open system[a]	Closed system[b]
Surface to volume ratio	Moderate	High
Control of mass and gas transfer	Difficult	Easy
Evaporation rate	High	Low
Preheating	Low	High
Irradiance supplied (MJ) [c]	13.4	28.7
Biomass productivity (t/ha/yr)	20	20–33
Volumetric productivity (kg/l/d)	0.035	0.27–0.56
Total energy consumption (GJ/yr)	450	729
Total energy content in 100 MT (GJ/yr)	3155.3	3155.3
Energy recovered as biomass (MJ)[d]	1.2	2.7
Energy produced as oil (GJ/yr)[e]	1155.49	1155.49
NER of oil production [f]	2.56	1.58
NER of biomass production	7.01	4.33
Cost estimation of TAG produced ($/kg) [g]	7.5	33
Cost estimation of FAME produced ($/kg)	4	25
Cost estimation of FFA produced ($/kg)	1	29

[a] Open system was based on race-way pond.
[b] The analysis of closed system was mainly based on tubular and flat-plate photobioreactor.
[c] Results was based on 50% photosynthetically active radiance (PAR).
[d] based on estimation that 1 g C contains 47.7 J.
[e] Energy produced as oil which was estimated based on 29.6% oil yield.
[f] Net energy ratio (NER) was estimated based on energy produced per energy requirements.
[g] The cost estimation is the highest gap between photobioreactor and open pond that ever reported in literature. The high cost form photobioreactor cultivation was estimated mostly from the processing equipment such as LED-lit photobioreactor with an LDPE dispensing tube, LED arrays, motorized stirrer and educator for nutrient supply. The price is in US Dollar.

First stage of cultivation is to control the culture conditions and minimize contamination from other organisms which will favor the development of the cell numbers during the zoospore settlement also known as mixotrophic. The first stage of cultivation is via photobioreactor. Mixotrophic is the ability of microalgae to carry on photoautotrophic by fixing CO_2 to derived energy and utilize organic carbon sources simultaneously when exposed to light [14]. In mixotrophic, sunlight will not be a limiting factor for photosynthesis cell growth as either light or organic carbon substrates can support the growth continuously without risked of biomass loss during the dark phase. During the light hour, photosynthetic metabolism utilizes light for growth while during dark phases; aerobic respiration uses an organic carbon source to continue the cell growth [2].

Second stage of cultivation is by exposing the cells to nutrient stress which increase the lipid content during synchronous growth via open pond. Example of nutrient is nitrogen compound. Microalgae that cultivated in nitrogen deficient growth condition could increase lipid content. Ammonia, nitrate, nitrite and urea are some examples of nitrogen sources that can be used for growing microalgae.

In previous research, Huntley and Redalje cultivated *Haematococcus pluvialis* to produce oil and astaxanthin (used in salmon feed) using a two-stage system. As a result, the annual average microbial oil production rate was more than 10 toe ha-1 per annum with a maximum rate of 24 toe ha-1 per annum [14].

5.0 PERFORMANCE OF MICROALGAE BIODIESEL IN DIESEL ENGINE

Microalgae biodiesel are reported to be better than fossil fuels in terms of life-cycle energy performance. Energy return on investment (EROI) of algae cultivate in an open system such as open pond can be in the range from 0.13 to 7.0 based on a literature review reported by National Research Council of the National Academies (NRC). If EROI reading is less than 1.0, this means the energy consumed to make the fuel is much higher than the energy contained in the product fuels. While according to the analysis by Argonne National Laboratory (ANL), microalgae biodiesel is expected to reduce life-cycle energy consumption by 55% and carbon emission by 45% if compared to conventional diesel [15].

Although microalgae biodiesel has low cetane number, it's satisfied European Biodiesel Standards (EN 14214). Due to the low cetane number, it can be compensated by mixing microalgae biodiesel with diesel fuel. Engine which fueled with microalgae biodiesel, produced low brake power and torque output compared with conventional diesel most probably because of incomplete combustion due to low cetane number. However in terms of CO and NOx emission, lower emission was reported with microalgae biodiesel [16].

6.0 CONCLUSION

This paper has given a discussion of the microalgae *B. braunii* as a new prospective for the biofuel feedstock by taking the microalgae species and cultivation technique into consideration. The Hybrid cultivation system via two-stage growth cultures offers high biomass production and gives the desired lipid productivity as compare to the open system and photobioreactor in batch mode. The microalgae biodiesel can be considered to be used as substitution to conventional diesel engines because of improvement in emission values as much as 45% although it has small reduction of the engine torque and brake power.

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