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A review on microalgae as potential lipid container with wastewater treating functions

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Abstract

Microalgae are reported as potential source to produce lipids from their biomass cells. Lipid as a group of organic compound is a primary raw material used in biofuel production as well as component for foods, cosmetic products, fertilizers and animal feed. As the resources of manufacturing lipid from synthetic media are costly, the derivation of inexpensive carbon and nutritional sources from wastewater such as palm oil mill effluent (POME) is useful in massive scale. Furthermore, unique characteristic of microalgae as alternative agents to treat POME wastewater is another encouraging aspect of its application. In addition, biodiesel production from algae can produce 5,000 –15,000 gallons of biodiesel per acre/year. However, high yield production of high-lipid-content-algae biomass, determination of effective techniques in order to harvest grown algae, algal oil extraction and trans-esterification of extracted oil for converting into biodiesel are challenging issues need deep investigation. This review is focused on previous studies on POME as possible carbon and nutritional source used to treat environmental pollution caused by POME discharges and to increase the growth rate of microalgae in order to high-lipid content production.

Keywords: Lipid production, Microalgae, *POME*, Wastewater treatment

1. Introduction

Nowadays, Palm oil industry as significant agriculture-based industry in several countries especially Malaysia is growing rapidly. The number of palm oil mills has been increased tremendously starting with 10 mills in 1960 climbed to 410 operated mills in 2008.As a consequence, overproduction of Palm Oil Mill Effluent (POME)as a result of Malaysian government initiatives in order to promote palm oil industry is expected [1]. Therefore, it is required to address appropriate technique for controlling human health hazards and environmental pollution [2].

The feasible way is the use of microalgae known as potential agent to treat wastewater [3] such as CO_2 and NO_x removal [4] with high capacity of nutrient uptake [5]. The idea of using microalgae in wastewater treatment has been investigated since 1950s, started by Oswald (1957).In addition, microalgae appears to be an attractive renewable energy source especially for biodiesel production [6- 10]. It is due to its rapid growth rate about 100 times faster than land-based plant. Besides, it double sits biomass in less than one day. Furthermore, microalgae is able to divide once every 3 to 4 h, but mostly divides every 1 or 2 days under favorable growth conditions [8, 11, 12].

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Actually, the potential interest of microalgae for biodiesel production is because of high lipid content in some species. In fact, the lipid synthesis especially the non-polar TAGs, which are the best substrates to produce biodiesel, can be modulated by different growth conditions. The total content of lipids existing microalgae may vary from 1 to 85% of their total dry weight, where the value higher than 40% is typically achieved under nutrient limitation. Moreover, study on some factors such as temperature, irradiance and most markedly, nutrient availability are significant parameters affect lipid composition and its amount in microalgae [13].

Because agro-industrial wastewater consists of large amount of organic compounds and heavy metals that are significantly hazardous to environmental health, microalgae have been suggested as prime candidate to remove these pollutants and breakdown the organic complexes [15]. On the other words, culturing microalgae in wastewater offers a sustainable and inexpensive alternative method to the conventional form of wastewater treatment. At the same time microalgae can utilize the nitrogen and phosphorus compounds present in wastewater to generate microalgae biomass used for lipid synthesis and finally results in biofuel production [11].

Since late 1900's, fossil fuel has been tremendously used for transportation, power plants and heating. The contribution of petroleum to air pollution and the unpredictable growth of fossil fuel price make it untenable as a predominant source of energy. Therefore, an alternative source of energy or fuel from renewable resources could help fuel industry to be less dependent on the conventional fuel energy. For this reason, a microalga comes up to solve this problem. In this study, we reviewed the previous works on potential treating ability of microalgae as well as sustainable lipid enriched raw material for production of biofuel. In addition, Palm Oil Mill Effluent (POME) is introduced as a proper and cheaper carbon source for overproduction of lipid content in different species of microalgae.

The characteristics and composition of microalgae significantly depend on the cultivation conditions [15]. Table 1 summarizes the lipid content, lipid productivity and microalgal biomass production under different cultivation conditions for various microalgae species.

2. Microalgae cultivation

Table 1. The lipid content of different microalgae species under different cultivation condition [15]

Microalgae species	Cultivation condition	Biomass productivity (g/L.day)	Lipid content (% of CDW)	Lipid productivity (mg/L.day)
*Chlorella minutissima (*UTEX 2341)	Phototrophic[a]	0.02 – 0.03	31.0 – 57.0	9.0 – 10.2
*Chlorella protothecoides (*CCAP 211/8D)	Phototrophic[a]	0.002 - 0.02	11.0 – 23.0	0.2 – 5.4
Dunaliellatertiolecta (IPIMAR)	Phototrophic[b]	0.12	16.7	20.0
Dunaliellatertiolecta (ATCC 30929)	Phototrophic[a]	0.10	60.0 – 67.8	60.6 – 69.8
Ellipsoidionsp(F&M-M31)	Phototrophic[a]	0.17	27.4	47.3
Isochrysissp(T-ISO CS 177)	Phototrophic[a]	0.17	22.4	37.7
Isochrysissp(F&M-M37)	Phototrophic[a]	0.14	27.4	37.8
Monodussubterraneus(UTEX 151)	Phototrophic[a]	0.19	16.1	30.4
Nannochlorissp(UTEX LB 1999)	Phototrophic[a]	0.04 – 0.35	29.9 – 40.3	15.6 – 109.3
Nannochloropsis(CS 246)	Phototrophic[a]	0.17	29.2	49.7
Pavlovaiutheri(CS 182)	Phototrophic[a]	0.14	35.5	50.2
Pavlovasalina(CS 49)	Phototrophic[a]	0.16	30.9	49.4
Scenedesmusobliquus(FCTU Coimbra)	Phototrophic[b]	0.09	17.7	15.9
Scenedesmusobliquus	Phototrophic[b]	0.06	12.7	7.14
Tetraselmissp(F&M-M34)	Phototrophic[a]	0.30	14.7	43.4
Tetraselmissuecica(F&M-M33)	Phototrophic[a]	0.32	8.5	27.0

[a] CO_2,
[b] Air,
[c] Glucose
[d] Acetate
[e] Glycerol
[f] Jerusalem artichoke hydrolysate (JAH)
[g] Corn powder hydrolysate (CPH)

3. Importance of Microalgae

3.1 Microalgae as unique raw material for biodiesel production

Microalgae appears to be as the solely source used for biodiesel production with the potential to completely replace fossil-based diesel [7, 16]. It should be mentioned that microalgae biomass has been already proven itself as one of the promising source of renewable biodiesel. Furthermore, microalgae used for biodiesel production is not comprised for the production of food, fodder and general products derived from crops [7, 11]. In addition, microalgae are able to produce 30 times more the amount of oil per unit area of land compared to terrestrial oilseed crops. A comparison between different sources currently employed for crop oil production is presented in Table 2 [7].

One unique aspect of microalgae compared to other plants is the variety of species available for safe biofuel production. Different species may be selected in order to optimize the production of biofuel. It is likely to mention that microalgae as promising alternative source for biofuel production also offers a diverse range of valuable products such as food, nutritional compounds, omega-3 fatty acids, animal feed, energy sources (including jet fuel, aviation gas, biodiesel, gasoline, and bioethanol), organic fertilizers, biodegradable plastics, recombinant proteins, pigments, medicines, pharmaceuticals, and vaccines [17].

Table 2.Thecomparison of source for biodiesel [7]

Crop	Oil yield (L/ha)	Land area needed (M.ha)	Percent of existing US cropping area[a]
Corn	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Coconut	2689	99	54
Oil palm	5950	45	24
Microalgae[b]	136,900	2	1.1
Microalgae[c]	58,700	4.5	2.5

[a] For meeting 50% of all transport fuel needs of the U.S
[b] 70% oil (by wt) in biomass
[c] 30% oil (by wt) in biomass

A microalga grows rapidly and it is exceedingly rich in oil. Its biomass doubling time during exponential growth was reported as short as 3.5 h [7]. Oil content in microalgae exceeds 80% by weight of dry biomass with oil level between 20 to 50% (Table 3) [18, 7, 16]. Not 100% oil extracted from algal are successfully applied for biodiesel production, but the specification of extracted oil are completely competitive compared to other crops [7].

Table 3.Oil content of microalgae [7, 16, 19]

Microalgae	Oil Content (% dry wt)
Botryococcusbraunii	25-75
Chlorella sp	28-32
Crypthecodiniumcohnii	20
Cylindrothecasp	16-37
Dunaliellaprimolecta	23
Isochrysissp	25-33
Monallanthussalina	>20
Nannochlorissp	20-35
Nannochloropsissp	31-68
Neochlorisoleoabundans	35-54
Nitzschiasp	45-47
Phaeodactylumtricornutum	20-30
Schizochytriumsp	50-77
Tetraselmissuecica	15-23

3.2 Lipid Production from Microalgae

Hundreds of microalgal strains are able to produce high lipid content, have been characterized recently [20]. Depending on the species of microalgae, different types of lipid, hydrocarbon and other oil complexes are expected [7]. Besides, either inorganic carbon (CO_2) or organic carbon sources (e.g. glucose, acetate and fructose) can be utilized by microalgae for lipid production [11] while the quantity and quality of lipids within the cell can be vary as a result of changes in the growth conditions (e.g. temperature and light intensity) or nutrient media characteristics (e.g. concentration of nitrogen, phosphates, and iron) [9]. Some researchers have been focused on the lipid triggers under environmental stress [20, 21, 22]. It is generally accepted that the depletion of nitrogen from medium induces lipid accumulation resulting in sharp growth on the stationary phase [23, 20].

3.3 Lipid characterization

The traditional analysis of lipid content in biological samples has been performed by solvent extraction and gravimetric determination. Recently, lipid characterization has been performed using Gas Chromatography (GC)and High-performance Liquid Chromatography (HPLC) [24]. The lipid classes basically are divided into neutral lipids (e.g., triglycerides, cholesterol) and polar lipids (e.g., phospholipids, galactolipids). Triglyceride as neutral lipid is the main material for biodiesel production [11]. Thirty microalgal strains as potential lipid factories were studied through the evaluation of their biomass productivity and lipid content in 250 mL flask laboratory cultures as presented in Table 4.The strains with the best combination of biomass productivity and lipid contents exhibited best activity among others. As instance, three members of marine genus, *Nannochloropsis,* with a lipid content of 30% or higher and productivity from 55 to 61 mg/L/day were found as most effective strains in this area [16].

Table 4. Biomass productivity, lipid content and lipid productivity of microalgal strains [13]

Algal group	Microalgae strains	Habitat	Biomass productivity g/L/day)	Lipid content (% biomass)	Lipid productivity (mg/L/day)
Diatoms	*Chaetocerosmuelleri* F&M-M43	Marine	0.07	33.6	21.8
	Chaetoceroscalcitrans CS 178	Marine	0.04	39.8	17.6
	P. tricornutum F&M M40	Marine	0.24	18.7	44.8
	Skeletonomacostatum CS 181	Marine	0.08	21.0	17.4
	Skeletonoma sp. CS 252	Marine	0.09	31.8	27.3
	Thalassioriapseudonana CS 173	Marine	0.08	20.6	17.4
	Chlorella sp. F&M-M48	Freshwater	0.23	18.7	42.1
	Chlorella sorokiniana IAM-212	Freshwater	0.23	19.3	44.7
	Chlorella vulgaris CCAP 211/11b	Freshwater	0.17	19.2	32.6
	Chlorella vulgaris F&M-M49	Freshwater	0.20	18.4	36.9
Green algae	*Chlorococcum sp.* UMACC 112	Freshwater	0.28	19.3	53.7
	Scenedemusquadricauda	Freshwater	0.19	18.4	35.1
	Scenedemus F&M-M19	Freshwater	0.21	19.6	40.8
	Scenedemus sp.DM	Freshwater	0.26	21.1	53.9
	T.suecica F&M-M33	Marine	0.32	8.5	27.0
	Tetraselmis sp. F&M-M34	Marine	0.30	14.7	43.4
	T.suecica F&M-M35	Marine	0.28	12.9	36.4
	Ellipsoidion sp. F&M-M31	Marine	0.17	27.4	47.3
	Monodussubterraneus UTEX 151	Freshwater	0.19	16.1	30.4
	Nannochloropsis sp. CS 246	Marine	0.17	29.2	49.7
Eustigmatophytes	*Nannochloropsis sp.*F&M-M26	Marine	0.21	29.6	61.0
	Nannochloropsis sp. F&M-M27	Marine	0.20	24.4	48.2
	Nannochloropsis sp. F&M-M24	Marine	0.18	30.9	54.8
	Nannochloropsis sp. F&M-M29	Marine	0.17	21.6	37.6
	Nannochloropsis sp. F&M-M28	Marine	0.17	35.7	60.9
	Isochrysissp(T-ISO) CS 177	Marine	0.17	22.4	37.7
	Isochrysissp F&M-M37	Marine	0.14	27.4	37.8
Prymnesiophytes	*Pavlovasalina* CS 49	Marine	0.16	30.9	49.4
	Pavlovalutheri CS 182	Marine	0.14	35.5	50.2
Red algae	*Porphyridiumcruentum*	Marine	0.37	9.5	34.8

3.4 POME as rich carbon and nutritional source for lipid production by microalgae

POME is a colloidal suspension originated from mixture of sterilizer condensate with separator sludge plus hydrocyclone wastewater at the ratio of 9:15:1 respectively [1]. Generally, around 2.5 to 3.0 tons of POME per tones of produced crude palm oil collected from the extraction procedures [25, 26]. Fresh POME is a thick brownish colloidal mixture of water, oil and fine suspended solids. It is normally hot (80-90^{0}C) and possesses high Biochemical Oxygen Demand (BOD_3) which is 100 times more toxic than domestic sewage [1]. However, effluent is non-toxic, as no chemicals are added to extraction process [27] and also acidic with a pH around 4.5 as it contains organic acids in complex forms that are suitable to be used as carbon sources [28].

The utilization of POME as nutrient source for culturing microalgae is not a new scenario in Malaysia. Most palm oil millers favor the culture of microalgae as a tertiary treatment before POME is discharged. Therefore, most of the nutrients such as nitrate and ortho-phosphate that are not removed during anaerobic digestion can be further treated in a microalgae pond. Meanwhile, nitrogen source (usually appears in nitrate form) plays an important role in promoting microalgae growth. In order to grow microalgae effectively, the basic nitrate concentration required is in the range of 200–400 mg/L [29].Other minerals such as Fe, Zn, P, Mg, Ca and K, which are required for microalgae growth, also exist in POME. Accordingly, POME emerged to be an alternative option as a chemical remediation to grow microalgae for biomass production and to increase lipid production [19].

4. Conclusion

The multiple-function of microalgae is a new approach and still needs more investigation. Particularly in Malaysia, there are some manufacturers operating small-scale cultivation of microalgae using different substrates, nevertheless no large-scale cultivation are available. Nowadays, microalgae are promising agents for halting industrial wastewater issues along with its unique characteristic as a lipid container used for biodiesel production. The ability of taking up the nutrients or toxic heavy metals from wastewater is the significant treating activity of microalgae. However, some parameters such as microalgae strain, cultivation mode, culturing parameters (e.g. light intensity, pH, temperature, salinity and nutrients) and type of substrate directly affect the microalgal growth and its lipid production which needs to be optimized for achieving best results. In addition, using wastewater Palm Oil Mill Effluent (POME) as a rich carbon and nutrient source is a promising approach either as natural environment treatment or as high-lipid-content raw material for production of biofuel.

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