Feature Article

Microalgae Cultivation In Palm Oil Mill Effluent (POME)

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ABSTRACT

Developing alternative fuels is an essential step towards solving fossil fuels issues such as fuel cost and pollution. Microalgae can be a promising feedstock for alternative fuel as it is fast growing and easily cultivated. Exploring wastewater such as palm oil mill effluent (POME) for feasible microalgae cultivation is essential as POME is abundantly available from palm oil milling activities. The high content of nutrients in POME makes it a potential microalgae growth medium. This study demonstrated that a microalgae species, i.e. Chlorella vulgaris UMACC 001 can grow at a specific growth rate of 0.39 day^{-1} and produce 0.14 mg biomass litre day^{-1} in POME outdoor conditions. The extracted algal oil showed 48.9% saturated fatty acids and 51.1% unsaturated fatty acids equivalent to palm oil as a biodiesel feedstock.

INTRODUCTION

Oil palm products such as palm oil, palm kernel oil, palm kernel cake, oleochemicals, finished products and biodiesel have contributed to RM 77.8 billion of export revenues in 2017. Approximately, 17.89 t ha^{-1} of fresh fruit bunch (FFB) was produced leading to 101.02 million tonnes of annual FFB processing from a total of 454 palm oil mills. Malaysia covers about 30% of the world's palm oil production and 34% of palm oil exports (Kushairi *et al.*, 2018). The high production of FFB yields 19.92 million tonnes of crude palm oil (CPO), and huge quantity of by-product, *i.e.* palm oil mill effluent (POME). POME is rich in organic matter but could be contaminated due to high content of biological oxygen demand (BOD), chemical oxygen demand (COD), oil and grease, total solids and suspended solids (Loh *et al.*, 2013). About 0.75 tonnes of POME is generated for every tonne of FFB processed (Vairappan and Yen, 2008). Technologies for POME treatment consist of conventional biological treatment methods, *i.e.* acidification, anaerobic, facultative and aerobic

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degradation (Loh *et al.*, 2013), open and closed digester tank with biogas recovery (Vairappan and Yen, 2008), and more recently an integrated biological treatment process is introduced consisting of pre-treatment, biological treatment and membrane separation (Loh *et al.*, 2013). Microalgae, a potential substrate for the production of biofuel, can be cultivated using POME as cultivation medium since it provides nitrogen and phosphorus to produce lipids and proteins (He *et al.*, 2013).

Biofuels can potentially be an alternative energy to replace fossil fuels. In particular, biofuels derived from microalgae are termed as the third generation biofuel. Microalgae are photosynthetic prokaryotic or eukaryotic microorganisms that grow rapidly when sunlight, water, carbon dioxide (CO_2) and nutrients are available and have the ability to live in different environments due to their unicellular or simple multicellular structure and simple growth needs (Chisti, 2007). This enormous growth potential contributes biomass for food, feed, fine chemicals and biofuels as well. They produce lipids, protein and carbohydrates that are common metabolite for bio conversion into both biofuels and valuable co-products. It is envisaged that microalgae have higher biomass productivity than plant crops based on land area required for cultivation and hold potential in greenhouse gas (GHG) emissions reduction.

Microalgae biomass can be produced either in closed or open systems. It is more economical for the latter but with less control of contamination from predators while the former has better control of nutrients and cultivation parameters such as temperature, dissolved CO_2, pH and lighting (Moreno-Garcia *et al.*, 2017). The ability of these microorganisms to produce and accumulate energy molecules (lipids) and their cells, plus the fact that their growth has no impact on agriculture have made them a premising feedstock for biofuels.

Different microalgal species accumulate varying quantities of lipids. The highest lipid yielding species reported so far are *Botrryococucus braunii, Desmodesmus sp., Nannochloropsis sp., Scenedesmus SDEC-8, Nannocloropsis sp.,* and *Sorokiniana FCG IITG* with 45%-64% lipid contents (dry weight) which are convertible into biofuel. *Chlorella vulgaris* stands up as one of the most attractive feedstock for biofuels owing to its fast growth and easy cultivation (Al-lwayzy *et al.*, 2014). As a suitable biofuel feedstock, species of microalgae must have high specific growth rate, high lipid content and robust, *i.e.* survive well in stressful environment caused by lack of heat, nutrient input and light intensity, and contamination from bacteria. The biomass harvesting process and lipid extraction method must be simple and economical too (Hannon *et al.*, 2010). This paper investigates and identifies the potential of POME as a low-cost cultivation medium for microalgae obtained from UM culture collection, *i.e. Chlorella vulgaris* UMACC 001 and the resulting algal oil as a biodiesel feedstock.

CULTIVATION OF MICROALGAE IN POME

The nutrients for cultivation (mainly nitrogen and phosphorus) can be obtained from POME, therefore besides providing a suitable growth environment for microalgae, POME is potentially degraded and polished to lower its organic contents. Since the palm oil industry generates around 65 million tonnes of POME a year, the wastewater discharged into waterways should be properly managed due to high BOD which causes depletion of dissolved oxygen. At the same time, the POME also contains high concentrations of protein, carbohydrate, nitrogenous compounds, lipids and minerals that can be utilised as food source for aquatic life (Habib *et al.*, 1997). *Table 1* shows the characteristic of raw POME and treated POME via anaerobic digestion. Raw POME has higher content of total solid (TS) (~60175 mg litre^{-1}) and COD (~74900 mg litre^{-1}), and these are much reduced in the treated POME, hence providing a neutral medium for microalgae cultivation with sufficient dissolved oxygen. Furthermore, the acidic characteristic and dark colour of raw POME may also reduce light availability and cause low growth

of microalgae. Besides, the treated POME still have sufficient content of nitrate and ortophosphate necessary for photosynthesis to support microalgae growth which then produces biomass such as lipid, protein and carbohydrate (Hadiyanto and Nur, 2012).

Chlorella vulgaris can be cultivated in either open pond system or closed photobioreactor (PBR) systems (*Figure 1*) under controlled environments and close monitoring has to be accomplished to detect and get rid of other microorganisms that can dominate during cultivation (Schenk *et al.*, 2008). Moreover, high BOD / COD of POME also contributes to contamination in terms of oxygen depletion. There are four microalgal metabolic pathways, namely autotrophic, mixotrophic, heterotrophic and photoheterotrophic (Lam and Lee, 2011). Dependent on the culture and growth conditions, varying biomass and lipid productivities are to be anticipated (Pratoomyot *et al.*, 2005).

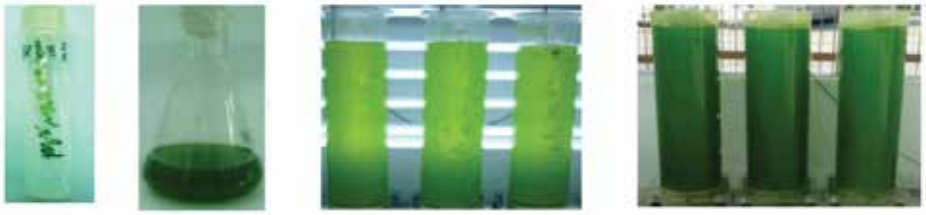
The accumulation of lipid in microalgae biomass is correlated with the growth stage of microalgae. Production of fatty acid is normally higher at the stationary phase than the exponential phase (Mata *et al.*, 2010). During stationary phase, microalgae utilise almost all the nutrients in the cultivation growth media. This creates a stressed environment, of which the rate of microalgae cell division starts to decrease, leading to accumulation of lipids (Mansour *et al.*, 2005). As POME is naturally colloidal, viscous and dark, it has to be diluted prior to media preparation for microalgae culture. Dilution also reduces the rather high concentration of ammoniacal nitrogen of POME that microalgae might not tolerate (Bello *et al.*, 2013). A 5% diluted POME has been found suitable for culturing marine microalgae species, *Isochrysis sp.* (Vairappan and Yen, 2008).

The optimisation of microalgae cultivation conditions is related to factors such as temperature, mixing, fluid dynamics and hydrodynamic stress, gas bubble size and distribution, gas exchange, mass transfer, light cycle and intensity, water quality, pH, salinity, mineral and cell density and growth inhibition (Schenk *et al.*, 2008). Optimal media formulation is important to ensure sufficient and stable supply of nutrients for microalgae to reach maximum growth acceleration and cell density, and to produce good quality biofuel feedstocks (Dayananda *et al.*, 2005). The majority of microalgae cultivated today are grown in open ponds due to economic advantages and ease of maintenance. The main disadvantages of open ponds are rapid water evaporation rate and susceptible to contamination by unwanted species as they are largely exposed to the atmosphere (Schenk *et al.*, 2008).

TABLE 1. POME CHARACTERISTICS

Parameter	Raw	Anaerobic digested
pH	4.5	7.26
Chemical oxygen demand (COD)	74 900	16 166
Total solid (TS)	60 175	22 133
Total suspended solid (TSS)	15 350	10 683
Total volatile solids (TVS)	49 004	12 191
Chlorophyll a	0.22	0.16
Carotenoid	0.12	0.10
Ammoniacal nitrogen (AN)	64.0	50.0
Nitrate	453.3	133.3
Nitrite	1.03	0.53
Orthophosphate	504.0	444.0

Note: Units in mg litre^{-1} except pH



Laboratory scale

Annular photobioreactor (PBR)

Air bubble PBR

Tubular PBR

Open raceway pond

Figure 1. Microalgae cultivation system (from laboratory to bioreactor scales).

Closed PBR is increasingly used to grow microalgae for biofuel production due to water, energy and chemical savings as well as cost reduction. Most closed PBRs are designed as tubular reactors, plate reactors or bubble column reactors (*Figure 1*). Mixing is necessary in all these PBRs to prevent sedimentation of cells and support distribution of CO_2 and O_2.

PERFORMANCE OF MICROALGAE CULTIVATION IN POME

Upscaling microalgae cultivation using closed PBR is dependent on lipid content, growth rate and cell size. The lipid productivity is critical for microalgae to be used as a biodiesel feedstock. A high lipid content may improve the processing efficiency of biomass into biofuels (Rodolfi *et al.*, 2009). *Chlorella vulgaris* UMACC 001 was cultivated in 5% POME in 250-ml flasks for 12 days. The flasks were incubated under controlled environment at 25±1°C, illuminated with cool white fluorescent lamp (40 μmol photon m^{-2} s^{-1}) on 12:12-h light-dark cycle and supplied with 100 ml filtered ambient air and then transferred to an enclosed PBR at a laboratory for another six days. It was then transferred to an outdoor enclosed PBR for 12 days using Bold's Basal Medium (BBM) as a control.

Table 2 shows the growth of *Chlorella vulgaris* UMACC 001 in different culture system (Idris *et al.*, 2017). The maximum optical density (OD_{620}) / cell size in BBM culture using annular PBR was in the range of 1.57-1.64 while the culture in 5% POME showed maximum OD_{620} of 1.45 in an annular PBR and 1.81 in a flat panel PBR. Flat panel PBR yielded better OD_{620} at outdoor condition in medium enriched with POME due to exposure of more sunlight leading to more biomass accumulation at an earlier stage compared to annular PBR. This was evidenced by higher biomass content (1.00±0.00 g $litre^{-1}$), specific growth rate (0.39 day^{-1}) and biomass productivity (0.14±0.00 g $litre^{-1}$ day^{-1}).

BIODIESEL PRODUCTION FROM MICROALGAE

Microalgal oil can be converted into biodiesel via transesterification. The fuel quality (*i.e.* cetane number, exhaust emission, heat of combustion, cold flow, oxidative stability, viscosity and lubricity) of the biodiesel derived depends very much on the fatty acid composition (chain length and number of double bonds or unsaturation, and chain branching) of the microalgal oil used (Schenk *et al.*, 2008). The biodiesel quality can be further improved by adequate mixing of different microalgae feedstocks with oils having desirable fatty acids, or with genetically modified microalgal oil.

TABLE 2. CHARACTERISTICS OF *CHLORELLA VULGARIS* UMACC 001 CULTURED IN PHOTOBIOREACTORS (PBRS) IN LABORATORY AND OUTDOOR CONDITIONS IN BOLD'S BASAL MEDIUM (BBM) AND 5% PALM OIL MILL EFFLUENT (POME)

System	Condition	OD_{620}	Biomass content (g litre^{-1}) (dwb)	Specific growth rate (day^{-1})	Biomass productivity g litre day^{-1}
Annular PBR	Laboratory (BBM)	1.64±0.02	0.70±0.08	0.29	0.12±0.02
Annular PBR	Outdoor (BBM)	1.57±0.03	0.65±0.05	0.19	0.05±0.02
Annular PBR	Outdoor (5% POME)	1.45±0.00	0.80 ±0.00	0.19	0.11±0.00
Flat panel PBR	Outdoor (5% POME)	1.81 ±0.00	1.00±0.00	0.39	0.14±0.00

Ideally, a good biodiesel feedstock helps balancing the oxidative stability and the cold flow characteristics of the resulting biofuels (Schenk *et al.*, 2008). *Chlorella vulgaris* UMACC 001 showed predominantly C16:0 and C18:1 fatty acids quite similar to palm oil. The balanced saturation (48%) versus unsaturation (51.1%) level, although not able to provide an ideal solution, shows biodiesel fuel properties comparable with studies by Velasquez-Orta *et al,* (2012), Johnson and Wen (2009) and Ehimen *et al,* (2010).

BENEFITS

An integrated POME treatment and microalgae cultivation may provide a sustainable way to conserve the environment. Utilisation of POME will increase the cultivation viability of microalgae as shown by other studies where a positive energy balance is possible in an integrated energy system consisting of POME anaerobic digestion, biomass power generation and microalgae cultivation (Abdullah *et al.*, 2007; Abdullah *et al.*, 2017). Microalgae-based biofuel is more promising compared to other bioenergy feedstock in terms of land area used. Moreover, advances in PBR engineering for microalgae cultivation will further lower the cost of biofuel production.

CONCLUSION

Chlorella vulgaris UMACC 001 is most productive when cultured in 5% POME using PBR at outdoor conditions. The algal oil assembles that of palm oil with an almost equal amount of saturated and unsaturated fatty acids dominated by C16 and C18. Thus, *Chlorella vulgaris* can be a potential biodiesel feedstock.

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