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Lipid for biodiesel production from attached growth *Chlorella vulgaris* biomass cultivating in fluidized bed bioreactor packed with polyurethane foam material

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HIGHLIGHTS

- Thermodynamic favorability in selecting fluidized beds' support material.
- Polyurethane foam for growing attached microalgae *Chlorella vulgaris* biomass.
- Determination of suitable harvesting period of attached microalgae biomass.
- Polyurethane foam of 1.00-mL cubes achieved the highest biomass and lipid weights.
- FAME composition of harvested microalgae biomass complied with biodiesel standards.

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ABSTRACT

The potential to grow attached microalgae *Chlorella vulgaris* in fluidized bed bioreactor was materialized in this study, targeting to ease the harvesting process prior to biodiesel production. The proposed thermodynamic mechanism and physical property assessment of various support materials verified polyurethane to be suitable material favouring the spontaneous adhesion by microalgae cells. The 1-L bioreactor packed with only 2.4% (v/v) of 1.00-mL polyurethane foam cubes could achieve the highest attached growth microalgae biomass and lipid weights of 812 ± 122 and 376 ± 37 mg, respectively, in comparison with other cube sizes. The maturity of attached growth microalgae biomass for harvesting could also be determined from the growth trend of suspended microalgae biomass. Analysis of FAME composition revealed that the harvested microalgae biomass was dominated by C16-C18 (>60%) and mixture of saturated and mono-unsaturated fatty acids (>65%), satiating the biodiesel standard with adequate cold flow property and oxidative stability.

1. Introduction

Since the beginning of industrial revolution, global energy consumptions have increased exponentially rising from the rapid population growth and economic developments. The most consumed energy source was fossil fuel which accounted for 82.68% in 2013, encompassing of crude oil (30.92%), coal (28.95%) and natural gas (22.81%) (Wan Ghazali et al., 2015). Excessive consumption of fossil fuel has resulted in serious environmental issues preponderantly air pollution. The indiscriminate spews of greenhouse gases have blanketed the Earth, spurring global warming that insidiously afflicts the natural environments of all living organisms. The fossil fuel sources are also non-renewable since those are derived from pre-historic fossils and will not be instantly available once they are depleted. Thus, the fossil fuel sources are finite in supply and depleting at a rapid rate in recent decades. Accordingly, the needs for renewable energy sources have become the key challenge of late, besides admonishing a more sustainable energy development for the future.

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The International Energy Agency (2010) had vindicated that only energy generated from combustible renewables and wastes has the highest compatibility potential with fossil fuel energy. As such, Lam and Lee (2012) envisaged the renewable energy from combustible sources, namely, biodiesel would play a crucial role as an alternative renewable energy in the near future. Indeed, biodiesel has gained worldwide attention in recent years as it contains several favourable properties such as high degradability, no toxicity and low emissions of carbon dioxide, particulate matters and unburned hydrocarbons upon the combustion (Veljković et al., 2015). Among the feedstock used for biodiesel production, microalgae biomass is anticipated to guarantee remarkable benefits. The cultivation of microalgae generally requires lower demands for water and footprint than commercial oil crops, in addition to serving as an effective carbon dioxide sink (D'Alessandro and Filho, 2015). The capability of microalgae in loading its cells with lipid that can be eventually extracted for biodiesel production has intrigued global researchers to delve further in enhancing the lipid content in microalgae biomass (Kumaran et al., 2016; Abomohra et al., 2017; Kim et al., 2017; Lam et al., 2017). Better yet, Chisti (2007) also confirmed that microalgae possessed higher growth rate and productivity than the conventional forestry, agricultural crops and other aquatic plants, hastening the generation of microalgae biomass in a short period of time. On another note, in addition to the lipid accumulation potential, the photosynthetic microorganisms of microalgae are as well able to synthesize a wide range of valuable bioproducts such as proteins, omega-3 fatty acids and powerful antioxidants (Haruna et al., 2010). In this regard, virtually, microalgae are perceived to be the only possible feedstock that can significantly sate the growing population necessities inclusive of replacing the petroleum-based fuels (Liu et al., 2013). Among the microalgae species, *Chlorella vulgaris* had been attested to possess high growth and lipid accumulation abilities while producing short-chain fatty acids (C16 to C18 of carbon length) which are suitable for biodiesel production (Johnson and Wen, 2010; Song et al., 2013).

The typical cultivation approach of microalgae is via suspended growth method which gives rise to the microalgae biomass concentrations in the culture medium of lesser than 0.1% solids during the stationary growth phase (Barros et al., 2015; Gerardo et al., 2015). To top it off, the microalgae are also small size microorganisms, generally ranging from 1 to 20 μm, suspending in liquid culture medium with biomass densities close to that of water (Lam and Lee, 2012). Hence, extensive time and energy are required to handle large feedstock volumes prior to and amidst harvesting stage. To date, although tremendous researches on the microalgae-derived biofuels had been made, the commercial production system is still unable to achieve economic viability due to the expensive harvesting methods applied (Show et al., 2015). Among the microalgae biomass harvesting methods are flocculation, ultrasonic aggregation, flotation, centrifugation, filtration, etc. have largely accounted for approximately 20–30% of total production cost (Singh et al., 2013; Tan et al., 2015). So, alternative methods must be explored to overcome the cost restraint while making the microalgae-derived biodiesel a much reliable and economical energy source being used broadly.

Accordingly, Katarzyna et al. (2015) accentuated that the attached growth method in cultivating microalgae was more commercially feasible than the traditional method, i.e., suspended growth method. Using this method, the high demand for water, about 3800 kg of water to produce 1 kg of biodiesel (Yang et al., 2011), can be avoided. The microalgae cells are initially forming attachment with the surfaces of support materials introduced into the culture medium and growing thereafter in the form of biofilm. At the maturity period, the densely attached growth microalgae biomass can be facilely separated from the culture medium by simply collecting the support materials. The use of fixed support materials for growing microalgae biomass had been reported lately in scientific literature (Schnurr and Allen, 2015; Li et al., 2016). Nevertheless, the inherent drawbacks of fixed support materials such as clogging, nutrients diffusion limitation, inefficient light exposure, domination of non-photoautotrophic materials within the thick photosynthetically inactive layers, etc. (Schnurr and Allen, 2015) have pressingly called for improvement. To that end, fluidized support material is proposed to replace fixed support material in growing microalgae biomass in this study. The introduction of fluidized beds into the bioreactor is anticipated to offset the drawbacks experienced by fixed support materials as it can freely and sporadically mobile within the culture medium while microalgae cells attaching and growing on its surfaces.

The thermodynamic model has been exploited to predict various microbial adhesion mechanisms, for instance, the attachment of bacteria to substrate is related to the surface free energies in which the relationship can be positive or negative depending on microbial species and suspension medium (Cui and Yuan, 2013). Liu et al. (2003) on the other hand had use the thermodynamic model to study the mechanism of anaerobic granulation in order to ameliorate the start-up period of upflow anaerobic sludge blanket reactor used for treating wastewater. Other studies on thermodynamic mechanisms of microbial adhesion had also been executed to develop an anti-biofouling for marine vessel and design a new coating to prevent the attachment of microbial (Magin et al., 2010; Callow and Callow, 2011). According to Absolom et al. (1983), microbial adhesion will be favoured if the process itself causes the thermodynamic function to decrease and vice versa. From this viewpoint, a suitable type of support materials used as fluidized beds for growing attached growth microalgae biomass was initially selected in this study based on the physico-chemistry favourability of proposed thermodynamic mechanism. In enhancing the microalgae biomass attachment, the sizes of selected support material were subsequently optimized considering as well of the highest lipid content and best biodiesel quality achievability aspects. The outputs manifest from this study are anticipated to pave a smoother way for the mass microalgae biomass harvesting process, offering economical, practical and efficient alternative for biodiesel production.

2. Materials and methods

2.1. Stock culture of microalgae

The *Chlorella vulgaris* (USMAC 24) was used as the model of microalgae species in this study and its seed was acquired from the culture collections belong to Centre for Biofuel and Biochemical Research, Universiti Teknologi PETRONAS. The stock culture of microalgae was initially setup in a 5 L flask containing 4.5 L of Bold's Basal Medium (BBM), prepared by mixing: (1) 10 mL per liter of the following chemicals: $NaNO_3$ (25 g/L), $CaCl_2{\cdot}2H_2O$ (2.5 g/L), $MgSO_4{\cdot}7H_2O$ (7.5 g/L), K_2HPO_4 (7.5 g/L), KH_2PO_4 (17.5 g/L), NaCl (2.5 g/L) and (2) 1 mL per liter of the following chemicals: EDTA anhydrous (50 g/L), KOH (31 g/L), $FeSO_4{\cdot}7H_2O$ (4.98 g/L), H_2SO_4 (1 mL), H_3BO_3 (11.4 g/L), $ZnSO_4{\cdot}7H_2O$ (8.82 g/L), $MnCl_2{\cdot}4H_2O$ (1.44 g/L), MoO_3 (0.71 g/L), $CuSO_4{\cdot}5H_2O$ (1.57 g/L), $Co(NO_3)_2{\cdot}6H_2O$ (0.49 g/L) (Lam et al., 2017). The medium was then filled with microalgae seed culture to 5 L with pH being maintained at 3 throughout the cultivation period. The aeration using compressed air and illumination using cool-white fluorescent light (Philip TL-D 36W/865, light intensity of 60–70 μmol/m^{2}s) with surrounding temperature fluctuating between 25 °C and 28 °C were applied continuously until the stationary growth phase of microalgae was reached.