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Oil production and fatty acid composition of *Chlorella vulgaris* cultured in nutrient-enriched solid-agar-based medium

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ABSTRACT

This current research demonstrated the potential of culturing microalgae by embedding the microalgae cells in nutrient-enriched solid-agar-based medium (SABM) which was casted as a thin agar-film in a specially design apparatus. Results showed that the cell number increased from 1×10^5 to 9.17×10^7 cells mL^{-1} at day 21. Upon culture maturation, the microalgal-agar films were easily harvested by peeling them out from the photobioreactor and dried in an oven. The system attained biomass production of 0.98 g 0.3-L^{-1} culture and total oil content of 25.31% at day 21. Fatty acid analysis revealed that C16:0 and C18:1 were the most dominant fatty acids which constituted 36.9 and 31.3% (of total oil content), respectively. The SABM culturing technique provide an alternative to conventional liquid-medium-based microalgae culturing technique, which has the advantage of by-passing the conventional thickening and dewatering processes during microalgal biomass harvesting.

1. Introduction

In the current scenario, microalgal culture is mainly focused on two major systems: open systems (open ponds) and closed systems (photobioreactor). Open pond microalgal culture system is the culture of microalgae directly bare under sunlight which is prone to be contaminated by invasive microorganisms, yields low microalgal biomass and is difficult to control in the environmental conditions (Narala et al., 2016; Show et al., 2017). Photo-bioreactor is a culturing system that can be installed indoors or outdoors in which the reactor walls are usually made of transparent materials and the photons need to pass through the walls before being impinged onto the culture cells (Matos et al., 2015). Photobioreactor system is higher in the operating cost, such as premium building materials required, a technician for maintenance, and a large area for construction (Chisti, 2008). According to Carvalho et al. (2006), there is no best culture system that possesses all the advantages of open ponds and photobioreactors to obtain the maximum productivity of microalgae. One apparent constraint of both culture systems is the use of a large amount of water during mass cultivation of microalgae (Novoveská et al., 2016).

Moreover, open pond and photobioreactors are facing the same problem in harvesting the microalgal biomass as it is expensive (Santos-Sanchez et al., 2016). The harvesting process of microalgal biomass requires at least one solid-liquid separation technique (Fig. 1a) to separate microalgal biomass from liquid culture suspension (Grima et al., 2003). For instance, biomass can be harvested by centrifugation, filtration, flocculation, and gravity sedimentation (Grima et al., 2003; Japar et al., 2017). Flocculation precedes the harvesting process in which it merges the microalgae in suspension to form a precipitate. However, extreme pH level created during flocculation could be toxic to microalgae (Japar et al., 2017). A major problem arises due to the small size of the algal cells which cause unstable colloidal microalgae in suspension (Santos-Sanchez et al., 2016). Another constraint of conventional harvesting method is that the oleaginous microalgae such as green microalgae suspended in water do not settle easily by gravity sedimentation due to negative surface charges (Milledge and Heaven, 2013). Filtration is applied following flocculation which provides high microalgal recovery efficiency; however, it is only suitable for harvesting large colonies and long length microalgae (Mallick et al., 2016) but not commonly applied in large-scale processes (Barros et al., 2015). Additionally, the biomass of microalgae can be recovered by centrifugation for its high-value products (Japar et al., 2017) with large volumes of microalgae can be harvested within time but require high energy and may damage microalgal cells due to high shear forces (Estime et al., 2017).

In recent years, microalgae biofilm culture technique has been

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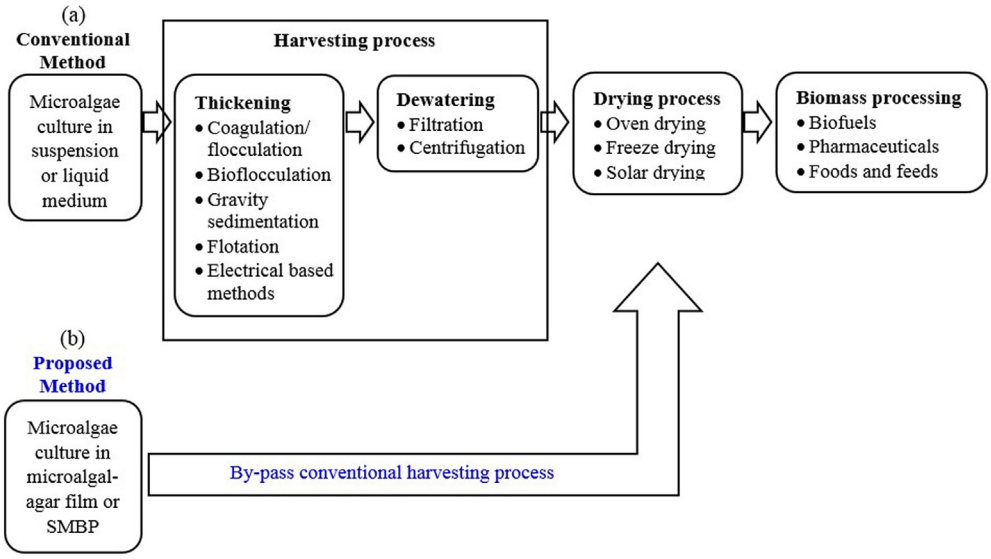

Fig. 1. Diagram of microalgal culture, harvesting and drying techniques for various downstream applications. (a) The conventional method of microalgae culture in suspension or liquid medium and (b) the potential of new proposed culturing method using solid-agar-based medium (SABM) which by-pass the conventional harvesting process. The diagram is modified from Barros et al. (2015).

introduced and employed to reduce nutrient level in wastewater. This culturing technique enables the concentration of microalgal biomass on specially constructed surface materials which reduce the labour in harvesting processes (Berner et al., 2015; Gao et al., 2015). Biofilm was mostly made up of certain surfaces such as fungi, bacteria or fiber for microalgae attachment and with a rotating reactor for mixing of microalgae (Gao et al., 2015; Rajendran and Hu, 2016). For example, the rotating algal biofilm reactors (RABR) cultivation method provides the advantage of bioremediation using microalgae when cultured in municipal wastewater and reduced water consumption (Estime et al., 2017). However, the major challenges for implementation of biofilm technology for large-scale cultivation are the construction cost and the complex construction of biofilm in which heterogeneity of surface material for microalgae adhesion and liquid velocity to replenish nutrients for microalgae to prevent drying on biofilm make it less applied commercially (Gao et al., 2015).

On the other hand, alginate beads are also used as alternatives to immobilize microalgae for easy and cost-effective harvesting (Pannier et al., 2014). However, they have drawbacks, which make them unable to retain the bead structure in the presence of high phosphate concentration in culturing microalgae and matching of alginate's physical properties to the microalgae encapsulation for cultivation (Lee and Mooney, 2012). To date, no economically feasible method to harvest microalgal biomass has yet to be invented (Barros et al., 2015). Following the harvesting of microalgal biomass, drying to obtain dried biomass is the next step (Fig. 1a) which may utilize oven-drying, freeze-drying or sun-drying (Bagchi et al., 2015) before further downstream processing for pharmaceuticals and biofuels use.

Agar is a gelling agent which consists of polysaccharides extracted from red marine algae such as *Gelidium* and *Gracilaria*. The two major polysaccharides are agarose (about 70%) and agaropectin. Agarose is a natural linear polymer of repeating units of agarobiose (a D-galactose and 3,6-anhydro-L-galactopyranose disaccharide) which is a neutral gelling fraction that is low in sulfate content. Meanwhile, agaropectin is a heterogeneous mixture formed by alternating units of highly charged D-galactose and L-galactose (Armisén and Galatas, 2009). Agar possesses several important properties such as hysteresis property, resistant to a breakdown by bacterial enzymes, resistant to shear forces, good clarity, good diffusion characteristics, the absence of toxic bacterial inhibitors, and relative absence of metabolically useful minerals and compounds. These properties have made agar widely used in food industries and for scientific applications. The uses of agar/agarose in scientific applications can be grouped into five broad categories: (a) immunodiffusion and diffusion techniques; (b) electrophoresis of particles carrying electrical charges (e.g. nucleic acids and proteins); (c) chromatographic techniques in gel chromatography, ion exchange chromatography, and affinity chromatography; (d) in bioengineering as a raw material for beads used in chromatographic columns for separations of proteins; and (e) as excellent base medium in microbiology and plant tissue cultures applications (Armisén and Galatas, 2009). Recently, its application has been extended into the development of new micro-extraction techniques such as agarose film liquid phase microextraction (AF-LPME) (Sanagi et al., 2012), multi-walled carbon nanotube-impregnated agarose film microextraction (MWCNT-AFME) (Loh et al., 2013a), solvent-impregnated agarose gel liquid phase microextraction (AG-LPME) (Loh et al., 2013b) and electro-mediated microextraction (EMM) (Chong et al., 2018).

In this present study, a new microalgae culturing technique by embedding the microalgae cells in nutrients-enriched solid-agar-based medium (SABM) is introduced. Microalgae cells are embedded in a low percentage of Bacto agar (0.5%) and cultured in a specially designed small-scale photobioreactor. This culturing technique is introduced to provide an alternative to the conventional liquid-medium-based microalgae culture techniques. In addition, this innovative culture technique could streamline the microalgal biomass harvesting processes (Fig. 1a). With this new culturing technique, the thickening and dewatering processes during harvesting are by-passed. The thin microalgae-agar sheets can be easily peeled off from the photobioreactor and dried in an oven or under the sun for further biomass processing (Fig. 1b).

2. Materials and methods

2.1. Solid-agar-based medium (SABM) culture system design

The SABM culture apparatus is a rectangular flat-panel glass cuboid culture system (Fig. 2). The dimensions of this SABM apparatus are 25 cm in length, 15 cm width, and 5 cm in height. There are four 0.6 mm holes, two at the sides for air inlet and two on top of the glass cover for air outlet. The air inlet was fitted with 0.22 μm air filter for air filtration whereas air outlet was fitted with 3 cm of 0.6 mm diameter polycarbonate (PC) tube.

2.2. Preparation of C. vulgaris

C. vulgaris (strain UMT-M1) inoculum was initiated from a single colony taken from a stock collection at the Institute of Marine Biotechnology, Universiti Malaysia Terengganu and cultured in BBM