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Developed microbial granules containing photosynthetic pigments for carbon dioxide reduction in palm oil mill effluent

M.Z.M. Najib [a, *], Salmiati [a, b, **], Z. Ujang [b], M.R. Salim [a, b], Z. Ibrahim [c]

[a] Department of Environmental Engineering, Faculty of Civil Engineering, Universiti Teknologi Malaysia (UTM), 81310, Skudai, Johor, Malaysia
[b] Centre for Environmental Sustainability and Water Security (IPASA), Research Institute for Sustainable Environment (RISE), Universiti Teknologi Malaysia (UTM), 81310, Skudai, Johor, Malaysia
[c] Department of Biosciences and Health Sciences, Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia (UTM), 81310, Skudai, Johor, Malaysia

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ABSTRACT

Since the photosynthetic process offers a natural way of sequestering carbon dioxide (CO_2) from industrial wastewater treatment such as the ponding system to treat palm oil mill effluent (POME), microbial granules possessing photosynthetic pigments were developed in a sequencing batch reactor (SBR) system using POME. POME from the last pond was used as source of nutrients for the biogranulation process. The developed microbial granules had shown the potential of retaining high accumulation of biomass concentration in the reactor (6.90–8.25 g L^{-1}), good settling properties (18.0–103.0 m h^{-1}) and improvement in size collected ranging from 0.3 to 2.36 mm as well as physical strength at integrity coefficient of 2% with most of the granules retained in the last day were 1.4 mm. The pigment analysis indicated the presence of the bacteriochlorophyll *a* implying the presence of anoxygenic photosynthetic bacteria. However, it is important to note that the molecular identification of the bacteria showed the presence of *Enterobacter cloacae*, *Bacillus cereus* and *Lysinibacillus* sp. which are typically known to be non-photosynthetic. These bacteria were found to possess photosynthetic pigments, mainly bacteriochlorophyll *a* and carotenoids.

1. Introduction

Among all greenhouse gases (GHG), nearly 64% of carbon dioxide (CO_2) gas contributes to climate change. From the year 1980–2011, the abundance of atmospheric CO_2 has increased globally averaging at 1.7 CO_2 ppm per year (Hartmann et al., 2013; Alshboul et al., 2016). Biological carbon sequestration using technologies such as controlled photosynthetic reactions may help to alleviate GHG problems, by carrying out reactions where CO_2 is transferred to the aqueous phase of the system (Jacob-Lopes et al., 2009). The photosynthetic bacteria signify as a promising tool for the development of various fields of biotechnology because of their capabilities to assimilate CO_2 gas, fix molecular nitrogen via photosynthetic metabolism and grow on different types of wastes (Paronyan and Gasparyan, 2009; Kim and Lee, 2016; Mohan et al., 2016).

In Malaysia, one of the primary sources of GHG is from industrial wastewater treatment such as the ponding system to treat palm oil mill effluent (POME). Malaysia's palm oil industry had generated approximately 80 million dry tonnes of solid biomass per annum as the volume is expected to increase to 85–110 million dry tonnes by 2020. (Agensi Inovasi Malaysia, 2013). Generally, 1 tonne of crude palm oil production needs 5.0–7.5 tonnes of water from which 50% is released in the form of POME. Besides, POME contained high organic content (COD = 50 g L^{-1}, BOD = 25 g L^{-1}) and substantial amounts of plant nutrients (Abdul Rahman and Azhari, 2013; MPOB, 2014; Norfadilah et al., 2016). If discharged, the untreated POME can cause considerable environmental problems.

The conventional ponding system, especially the anaerobic process produces harmful and odorous gases such as sulphur dioxide (SO_2), methane (CH_4) and CO_2 (Daelman et al., 2012; Kusrini

* Corresponding author. Department of Environmental Engineering, Faculty of Civil Engineering, Universiti Teknologi Malaysia (UTM), 81310, Skudai, Johor, Malaysia.
** Corresponding author. Centre for Environmental Sustainability and Water Security (IPASA), Research Institute for Sustainable Environment (RISE), Universiti Teknologi Malaysia (UTM), Skudai, Johor, Malaysia.
E-mail addresses: mzuhaili08@gmail.com (M.Z.M. Najib), salmiati@utm.my (Salmiati).

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et al., 2016). Basically, CH_4 formed during the treatment of wastewater in open ponds, reacts with water to form CO_2 and water; these gases are GHGs that contribute to the greenhouse effect causing global warming synergism (Bandara et al., 2011; Chen et al., 2013; WRI, 2014). There is a lot of interests in reducing these emissions in anaerobic wastewater treatments (Martinez et al., 2013; Sánchez-Hernández et al., 2013; Wiedemann et al., 2016), algae and microalgae were also investigated for CO_2 biofixation (Pankaj and Awasthi, 2013; Moreira and Paire, 2016; Yadav et al., 2016). Recently, photosynthetic bacteria play significant role in the CO_2 sequestration within the microsystem of wastewater as the bacteria utilize the CO_2 from the environment (Bently and Melis, 2012; Farrelly et al., 2013; Salmiati et al., 2015).

By introducing the photosynthetic characteristics within the developed granules, this study will be a significant endeavour in promoting an alternative approach to current CO_2 mitigation strategies in minimizing the CO_2 emission from POME. Furthermore, the sludge production in the palm oil industry could be improved by developing into granules consisting of compact, denser structure, and higher settleability lead to a better solid-liquid separation in the wastewater. Thus, the main aim of the present study was to investigate the granulation process and the advancements in the characterization of microbial granule under photosynthetic growth condition. In this study, the microbial granules changes in morphology, biomass concentration, settling properties, diameter, size distribution, sludge volume index (SVI) and strength of the granules were discussed. The bacteria making up the granule were isolated and cultivated in succinate broth prior to analysis of the photosynthetic pigments. Microbial analyses for the identification of microorganisms were conducted and the phylogenetic tree was constructed to reveal the evolutionary relationship of the bacterial group. This research will help intensify the knowledge of cultivation procedure and the application of granulation technology in wastewater treatment especially for future reduction of CO_2 emission.

2. Methodology

2.1. Operation of the sequencing batch reactor (SBR) system

The experiment was carried out using a sequencing batch reactor (SBR) system consisting of a 3 L double-jacketed cylindrical column (6.4 cm in diameter and 90 cm in height) with a working volume of 1.2 L at an organic loading rate (OLR) of 2.75 kg COD m^{-3} day^{-1}. The SBR was run for 4 h cycle per day consisting of 5 min fill up time for the influent, 80 min of anoxic condition, 130 min of aeration, 15 min for settling of sludge, and 5 min of effluent withdrawal with a volume exchange ratio (VER) of 50% (Fig. 1). An air diffuser for the aeration was controlled at an air flow rate of ±4 L min^{-1} (superficial air velocity = 2.07 cm s^{-1}) while keeping the reactor at room temperature (28 ± 2 °C). In addition, the reactor was equipped with two light sources providing illumination of 3600 lux each (12 h light/12 h dark regime controlled using automatic timers) to provide condition conducive for photosynthesis environment. Fig. 2 illustrates the overall system of the operated SBR.

The POME from the last pond used as nutrient sources was initially autoclaved to reduce the oil and grease that could affect aerobic granulation process (González et al., 2009) and also limit the rate of photosynthesis due to low light penetration (Naing et al., 2016). Besides, the reactor was seeded with 1:2:1 ratio of mixed sludge taken from a local sewage treatment oxidation pond, POME facultative pond treatment system and POME wastewater. Prior to use, the sludge was sieved (<3 mm) to remove any debris and large particles. It was then mixed and acclimatized up to at least 40 days in the reactor by applying anoxic and aerobic condition similar to SBR operating parameters. After the acclimatization period, sludge volume of 0.6 L was filled inside the reactor, making up half of the working volume.

2.2Characterization of microbial granules

During the development period, the morphological observations of the microbial granules were examined using the light microscope with PAX-it™ image analyzing system. The biomass concentration was analyzed according to the Standard Methods for the Examination of Water and Wastewater (U.S.EPA, 1999; APHA, 2005). Other parameters for granules were also conducted such as the settling velocity (Linlin et al., 2005) and the granular strength (Ghangrekar et al., 1996). The SVI value was determined by dividing the bed volume of sludge biomass with the dry weight of biomass retained in the reactor.

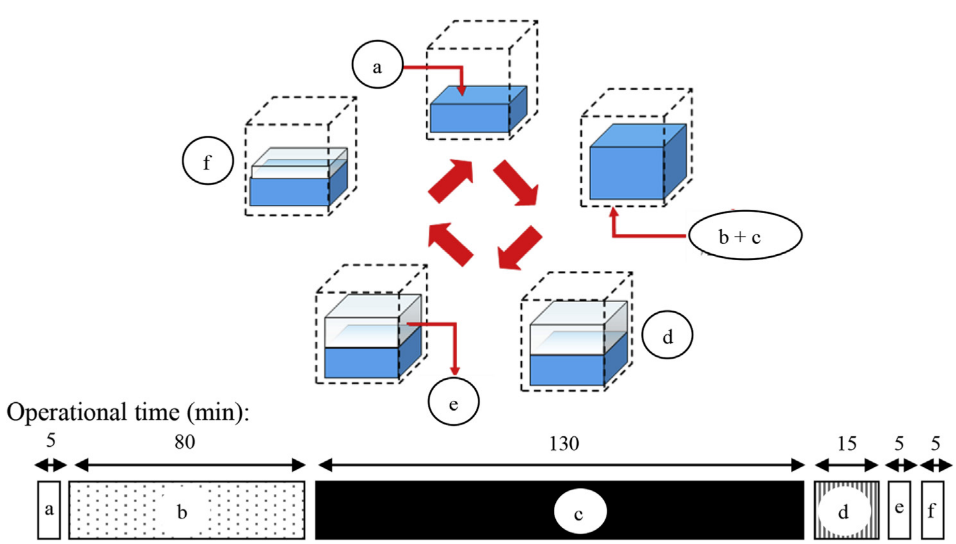

Fig. 1. Timing of each phase for one complete SBR cycle: (a) Fill, (b) anoxic, (c) aerobic, (d) settle, (e) decant, and (f) idle. (All the above are illustrative only).