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MORPHOLOGICAL CHARACTERIZATION OF PHOTOSYNTHETIC MICROBIAL GRANULE FROM PALM OIL MILL EFFLUENT (POME)

(Pencirian Morfologi Butir Mikrob Fotosintesis Daripada Effluen Kilang Minyak Sawit (POME))

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Abstract

Presently, global warming is the most highlighted subjects in the environmental issues which relates closely to greenhouse gases (GHG) emissions. In 2007, the Intergovernmental Panel on Climate Change (IPCC) assigns only methane (CH_4) emissions to wastewater treatment rather than GHG emissions specifically carbon dioxide (CO_2) gas from the aerobic treatment processes. Focusing on the palm oil industry in Malaysia, the most commonly used treatment of palm oil mill effluent (POME) which is the conventional ponding system, has caused excessive generation of GHG such as CH_4 and CO_2 gases. To develop a novel, innovative and environmental-friendly mitigation method, this study explores into the possibility of growing the photosynthetic bacteria in the form of granules via the aerobic granulation process with potential applications in reducing CO_2 gases. The cultivation of photosynthetic microbial granules was investigated using POME as the substrate in a sequencing batch reactor (SBR) system via the sequencing cycle of feeding, reacting, settling and decanting. Evidence of the formation of granule was based on microscopic examination of the morphological changes during the development of the granule in the SBR system over a period of 90 days. It shows changes from dispersed loose structure of the sludge merging into small flocs of irregular shapes and finally into dense and compact granular form. The granule was formed by applying an organic loading rate (OLR) at 2.75 kg COD/m^3.day, hydraulic retention time (HRT) at 4 h and superficial air velocity of 2.07 cm/s. The biomass concentration began to decreased first (initial sludge biomass = 16750 mg/L) and then increased steadily to a constant value of 32000 mg/L after 90 days. Besides, the results also demonstrated a good accumulation of biomass as the settleability between raw sludge and granule increased from 0.03 cm/s to 0.94 cm/s. The maximum settling velocity obtained in the reactor was approximately 2.0 cm/s.

Keywords: GHG, CO_2 gas, POME, granulation, photosynthetic bacteria, morphological change

Abstrak

Pada masa ini, pemanasan global adalah subjek yang paling menyerlah dalam isu alam sekitar yang berkait rapat dengan gas rumah hijau (GHG). Pada tahun 2007, Panel Antara Kerajaan mengenai Perubahan Iklim (IPCC) pendekatan menyerahkan hanya pelepasan metana (CH_4) untuk rawatan air sisa berbanding pelepasan GHG daripada proses aerobik khususnya gas CO_2. Memberi tumpuan kepada industri minyak sawit di Malaysia, rawatan yang paling lazim digunakan untuk rawatan POME adalah sistem kolam konvensional yang telah menghasilkan GHG yang berlebihan seperti gas CH_4 dan CO_2. Untuk membangunkan kaedah mitigasi baru yang lebih inovatif dan mesra alam, kajian ini melihat kepada kemungkinan menghidupkan bakteria fotosintesis dengan menggunakan penggranulan aerobik yang berpotensi untuk mengurangkan gas CO_2. Penanaman granul mikrob fotosintesis telah disiasat menggunakan POME dalam reaktor kelompok urutan (SBR) sistem. Dengan memerhatikan perubahan morfologi butir dalam reaktor, ia memaparkan berlakunya proses penggranulan yang integrasi - penggabungan semula - membesar. Ia menunjukkan perubahan daripada struktur longgar tersebar bergabung ke dalam floks kecil dengan bentuk berbutir berbentuk tidak teratur dan akhir sekali menjadi lebih padat dan padat. Butir itu akhirnya terbentuk dengan menggunakan kadar loading organik (OLR) pada 2.75 kg COD/m^3.day, masa tahanan hidraulik (HRT) pada 4 jam dan halaju udara cetek 2.07 cm/s. Kepekatan biomas pada mulanya menurun (biomas awal lumpur = 16750 mg/L) dan kemudian terus meningkat sehingga mencecah nilai 32000 mg/L selepas 90 hari. Selain itu, keputusan juga menunjukkan pengumpulan baik biomas sebagai kebolehan-mendakan antara kumbahan mentah dan butir meningkat daripada 0.03 cm/s hingga 0.94 cm/s. Halaju pemendakan maksimum yang diperoleh dalam reaktor telah mencapai sehingga kira-kira 2.0 cm/s.

Kata kunci: GHG, gas CO_2, POME, *granulation*, fotosintesis bakteria, perubahan morfologi

Introduction

Currently, global warming is the most highlighted subjects in the environmental issues. This climate change is normally related to greenhouse gases (GHG) emissions from anthropogenic activities like the combustion of fossil fuel used for power generation, transportation, and industrial processes [1]. Inefficient wastewater treatment results in excessive production of carbon dioxide (CO_2) and methane (CH_4) continuously which will over time period contribute to global warming. The current Intergovernmental Panel on Climate Change [2] assigns only CH_4 emissions to wastewater treatment, but this approach may overestimate GHG emissions from the highly aerobic processes specifically CO_2. Hence, enhancement of photosynthetic activities in the microenvironment of wastewater is potentially promising for atmospheric CO_2 mitigation in the wastewater.

In Malaysia, one of the major sources of GHG is the palm oil mill wastewater treatment system. Earlier studies have indicated that the end products of the anaerobic digestion of palm oil mill effluent (POME) waste treatment produced biogas with 65% CH_4 [3] A research project prepared by SIRIM [4] discovered that approximately 335.27 kg of CO_2 gases was generated directly from POME treatment of the crude palm oil (CPO). Unfortunately, the biogas is released into the atmosphere from the open ponds and digesters of the waste treatment system. It is estimated that 50 million tonnes of POME are produced annually in Malaysia. In terms of biochemical oxygen demand (BOD) and chemical oxygen demand (COD), these generally exceed 25,000 mg/L and 50,000 mg/L, respectively.

The emissions of CO_2 and CH_4 is presently captured and stored in various anaerobic processes [5, 6, 7, 8]. There is a lot of interest in reducing emissions in anaerobic wastewater treatments especially in the CO_2 biofixation by algae and microalgae. However, the aerobic processes should also be emphasized in research to minimize greenhouse emission from the wastewater. Thus, reduction of emission from aerobically treated wastewater is needed. This can be achieved by utilizing photosynthetic bacteria in a more compact wastewater treatment system.

Aerobic granulation technology is a promising aerobic wastewater treatment system that can overcome the limitations in conventional activated sludge system. This technology provides an excellent characteristic of sludge in granular form with compact structure, good settling rate, high biomass concentration, and simultaneous removal of organic matter, nitrogen and phosphorus [9]. Aerobic granules cultivated via the aerobic granulation process is a form of biofilm in which various microorganisms including photosynthetic bacteria adhere to each other (self-immobilisation) to form a compact structure, which is good for solid-liquid separation in wastewater.

The role of photosynthetic bacteria in the aerobic granules is important for the CO_2 sequestration within the microsystem of wastewater. This will minimize the emission of CO_2 to the environment. Microbial communities in aerobic granules have been shown to be highly distinct from activated sludge, even within a single reactor system. Recent studies imply the importance of gaining an understanding of the functions of microbial communities, as population diversity alone may not be adequate in determining the microbial characteristics. This study focuses on the characterizations of photosynthetic microbial granules developed from POME. The presence of photosynthetic bacteria were determined, the change in morphologies, biomass concentration and settling properties were investigated during growth of the bacteria.

Experimental

Seed Sludge

The reactor was seeded with a 1:2:1 ratio of mixed sludge taken from a local sewage treatment oxidation pond, POME facultative pond treatment system and POME wastewater. Initially, the sludge was sieved (< 3 mm) to remove any debris and large particles. It was then mixed together and acclimatized up to at least 40 days in the reactor by applying anaerobic and aerobic condition similar to SBR operating parameters. After the acclimatisation period, a sludge volume of 0.5 L was filled inside the reactor, making up 50% of the working volume.

Preparation of growth medium for photosynthetic paration

For cell growth of photosynthetic bacteria, the sludge sample was inoculated and grown in two different growth media [10]. The preparation of Succinate Medium and G5 Medium were based on the following compositions. (i) Succinate Medium consists of the following chemical reagents: 0.33 g K_2HPO_4, 0.33g $MgSO_4.7H_2O$, 0.33 g NaCl, 0.5 g NH_4Cl, 0.05 g $CaCl_2.2H_2O$, 1.0 g sodium and 0.02 g yeast extract; (ii) G5 Medium: 5.0 g peptone, 5.0 g yeast extract, 4.0 g L-glutamic acid, 3.5 g malic acid, 0.12 g KH_2PO_4, and 0.18 g K_2HPO_4. Each medium was adjusted to pH 7.0. Both of the medium were autoclaved at 121^{o}C, 15 lb/in^2 steam pressure for 15 min. After the temperature has cooled down, the growth media were poured into sterilised petri dishes and allowed to solidify at room temperature. The agar plates were incubated at 30^{o}C overnight to ensure the plates were not contaminated prior to use.

Culture Conditions

The bacteria used in this study were isolated from the sludge sample. The sludge was inoculated and cultured in both Succinate and G5 medium in stoppered bottles in separate experiments. Then, the cultures were incubated at 28-32 ^{o}C and illuminated with 3600 lux light source using a 12 h light/ 12 h dark regime for about 1 week.

Pigment Analysis

To detect the presence of photosynthetic bacteria, the colour change of the medium was visually examined during growth of the bacteria. Furthermore, pigment analysis was performed using a UV-Vis spectrophotometer (wavelength ranging between 300-1100 nm) to identify the type of bacteriochlorophyl pigment present in the microorganism according to the wavelength absorption characteristics.

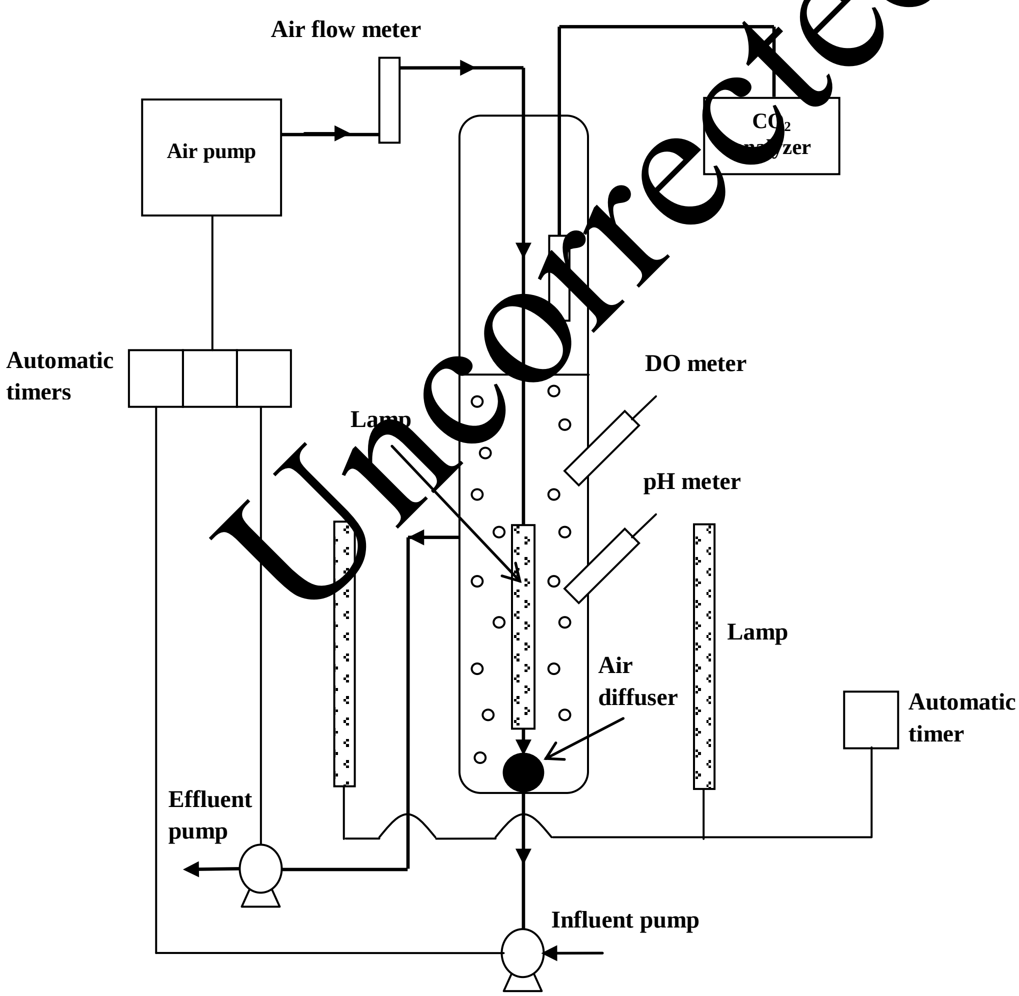

Figure 1. Schematic diagram of SBR system

Characterisation of Granules

During the development period, the morphologies of the photosynthetic microbial granules were examined visually and using the light microscope using PAX-IT™ image analyzing system. All analytical measurements were conducted according to Standard Methods for the Examination of Water and Wastewater [11].

Operation of the Sequencing Batch Reactor (SBR)

The experiment was operated using a 3L double-jacketed cylindrical column type-sequencing batch reactor (SBR) system (diameter = 6.4 cm) with working volume of 1.2 L at an organic loading rate (OLR) of 2.75 kg COD/m^3.day (Figure 1). The SBR was run in a cycle of 4 h per day and consisted of 5 min for filling up of influent, 80 min of anaerobic condition, 130 min for aeration, 15 min for settling of sludge, and 5 min for pumping out of the effluent. Aeration was supplied using an air diffuser by controlling the air flow rate at ± 4 L/min along with maintaining the temperature in the reactor between 20-27^{o}C. Besides, the reactor was equipped with two light sources providing illumination of 3600 lux each (12 h light/ 12 h dark regime) for creating photosynthetic condition. Table 1 summarizes the important operational details of the SBR system.

Table 1. Operating parameters of SBR system

SBR parameter	Unit	Value
HRT	hour	4
Cycle per day	-	6
Working volume	m^3	1200
pH	-	7
Inlet volume	m^3	600
Organic loading rate	kg COD/m^3.day	2.75
Superficial air velocity	cm/s	2.07
Light intensity	lux	3600
Each step of cycle:		
a) Fill	min	5
b) React:		
Anaerobic	min	80
Aerobic	min	130
c) Settle	min	15
d) Decant	min	5
e) Idle	min	5

Results and Discussion

Presence of Photosynthetic Bacteria

To determine the presence of photosynthetic pigment analysis for bacteriochlorophyll α, β and carotenoid pigments were carried out for the cell culture using the UV-Vis spectrophotometer within a range of 380-1100 nm. After 1 week of incubation, the succinate medium showed the appearance of red 'bloom' (Figure 2(a)) while no growth in the G5 medium (Figure 2(b)). This characteristic reddish bloom had implied the presence of photosynthetic bacteria that of purple non-sulfur photosynthetic bacteria and the presence of only bacterichlotophyll b [12]. This was because the average wavelength absorption gained mostly within the range of 976 to 1050 nm as shown in Figure 3.

Bacteria were further isolated and single cultures of bacteria were obtained using the spread plate and dilution streak technique.

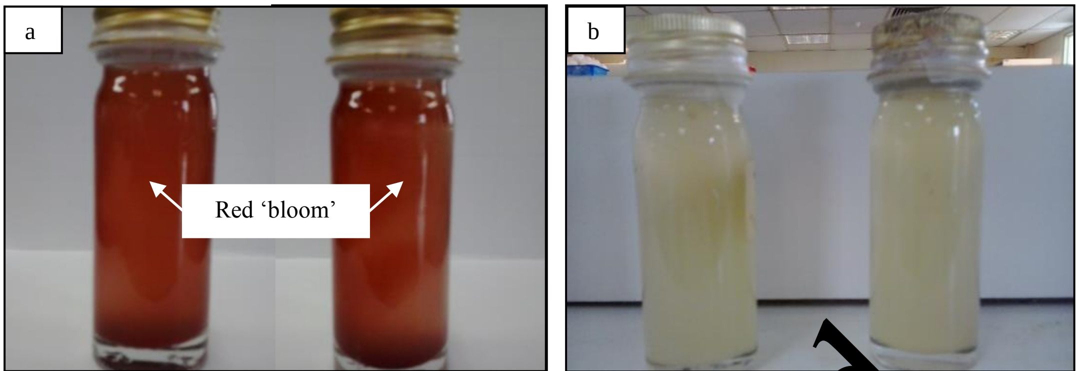

Figure 2. Final results on isolation of bacteria using (a) Succinate and (b) G5 medium.

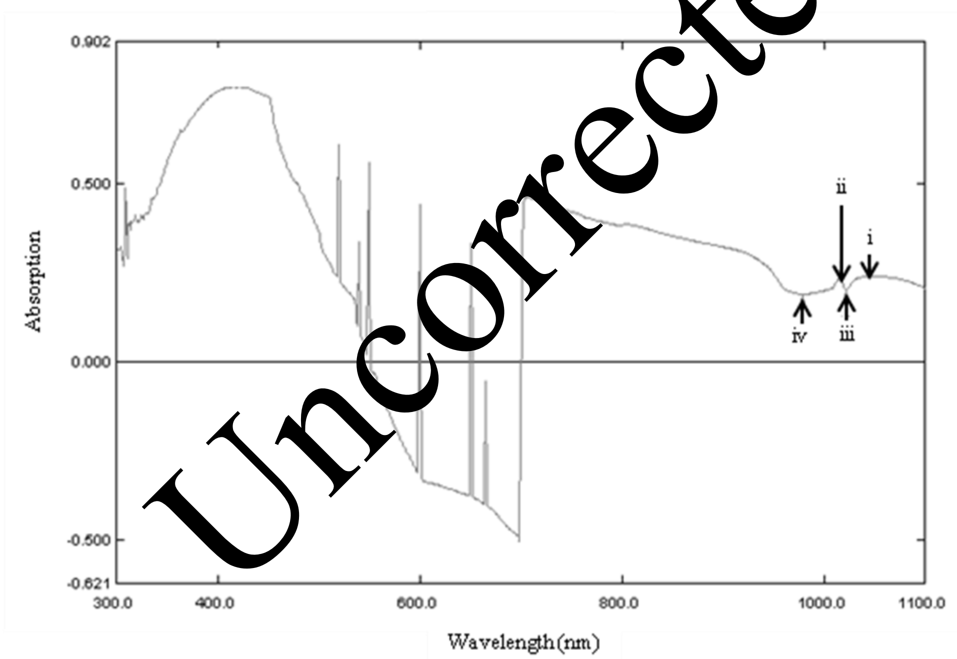

Figure 3. In vivo absorption spectrum for G5 medium. (i) 1046 nm; (ii) 1016 nm; (iii) 1022 nm and (iv) 979 nm.

Morphological Change

Focusing on the morphological characteristics during the development of granules, the initial sludge consisted of mainly dispersed loose structure with an average diameter of 0.07 mm. These were further developed into small flocs after two weeks of cultivation (Figure 4(A)). After 28 days, the flocs began to merge together forming small irregular aggregates with diameter size ranging between 0.02-0.11mm (Figure 4(B)). The number of aggregates was found to increase with time during the experiment. However, after 75 days of operation, the small aggregates had generated into bigger floc size with fluffy shape granular form (Figure 4(C)). Later on, the appearance of smoother non-uniform spherical granule (day 90) was observed as shown in Figure 4(D). During this phase, the maximum size of mature granule achieved was 1.11 mm with total area of 0.96 mm^2. Based on the morphological variation of granules developed in the reactor, the phenomenon displayed during the granulation process was that of integration – recombination – growing up as described by Linlin *et al.* [13].

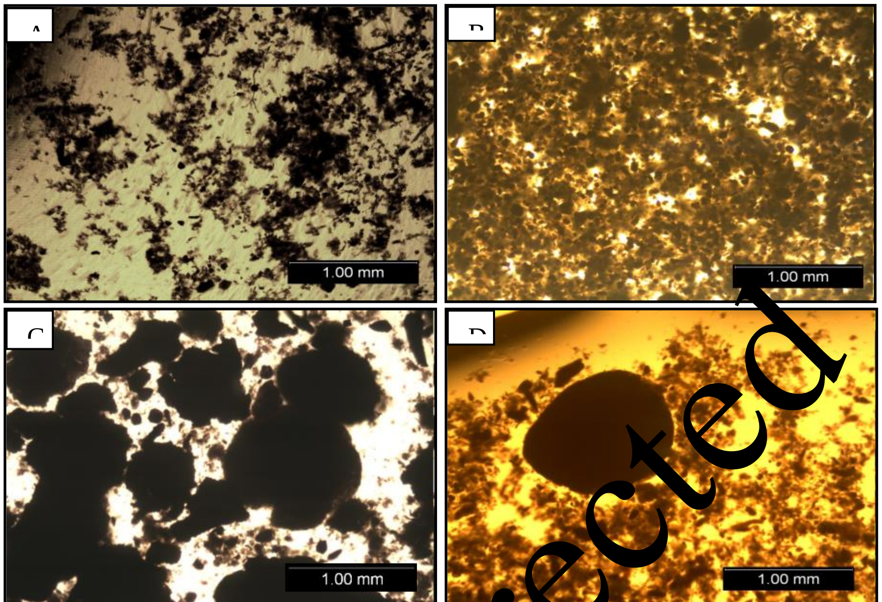

Figure 4. The morphological variation of granules captured using the PAX-IT™ during the experimental period (magnification = 40x). (A) Raw sludge; (B) after 28 days; (C) after [illegible]5 days and (D) after 90 days.

After 90 days, however, most of the granules underwent disintegration, forming smaller granules of regular shape and yellow-brownish in colour (Figure 5). This might be probably due to the formation of biogases such as hydrogen [14] and nitrogen gas [15] as secondary metabolites by the photosynthetic bacteria and other bacteria that makes up the granules.

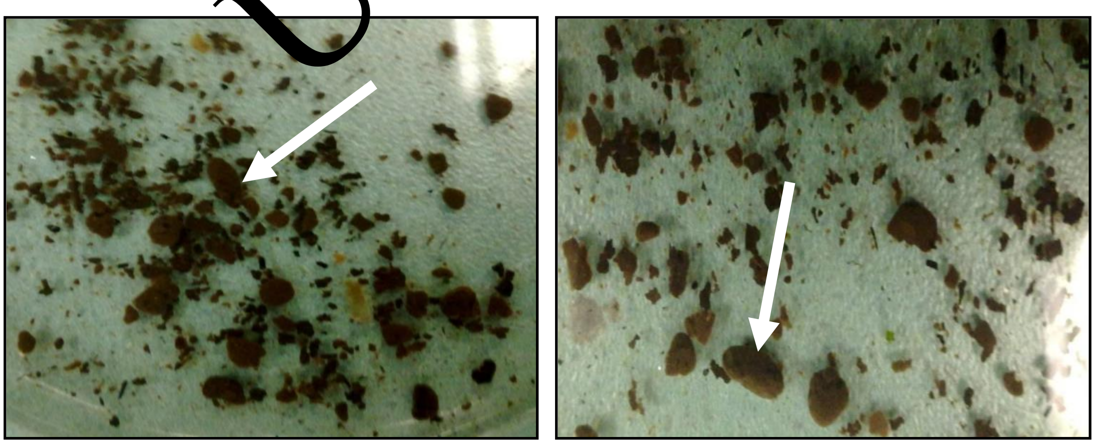

Figure 5. Granules developed at the end of experiment (arrow)

Biomass Properties

During the start-up period, the initial sludge with biomass concentration of mixed liquor suspended solid (MLSS) was 16750 mg/L and physically dispersed form. In the following days, the biomass increased to 17600 mg L^{-1} and continued to do so reaching approximately 32000 mg/L at the end of the experiment. Nevertheless, about 30 percent of the total sludge volume experienced loss of biomass after 14 days of the granule development possibly due to presence of filamentous microorganisms which made it difficult for some of the flocs to settle quickly [16, 17]. The average biomass was attained at about 23700 mg/L between 30 to 80 days with biomass loss up to 12100 mg/L while the highest biomass concentration of developed granule was 32000 mg/L as shown in Figure 6.

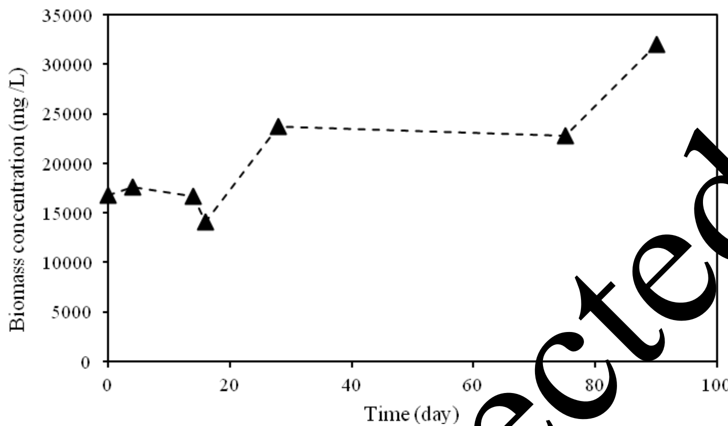

Figure 6. The change of biomass concentration in the reactor

As the relationship between biomass concentrations is proportional to the granule diameter, the settling velocity had also increased gradually (Figure 7). At this point, the maximum settling velocity remained in the reactor had reached approximately 2.0 cm/s. The average settling velocity of the granules were also found to increase linearly with the MLSS values, showing good accumulation of the biomass [18]. Furthermore, the settleability between the initial sludge and developed granule were found to increase (0.03 cm/s to 2.00 cm/s) within the range of 0.5 to 2.5 cm/s as reported by Gao *et al*. [19].

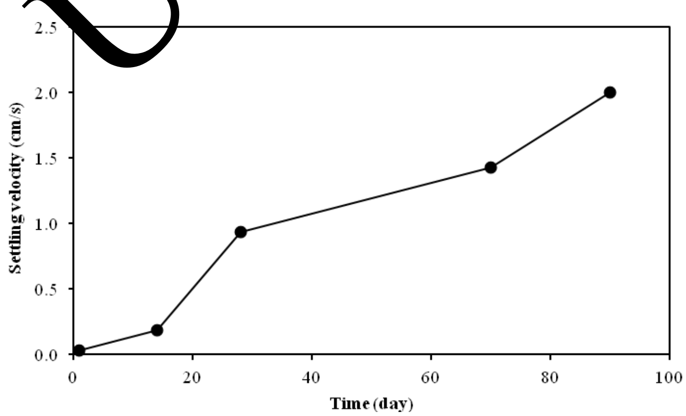

Figure 7. Settling properties of granule from the start-up period till the end of the experiment

Conclusion

In conclusion, the granules containing photosynthetic bacteria were formed after 90 days of cultivation by applying OLR at 2.75 kg COD/m^3.day, HRT of 4 hours and superficial air velocity of 2.07 cm/s. Based on the results, the characteristics of the developed photosynthetic microbial granule were proven better than the conventional sludge with a more compact (MLSS = 3200 mg/L; diameter = 0.40 - 1.11 mm), and higher settling properties of 2.0 cm/s. In addition, from the morphological change of the granules in the reactor, the occurrence of granulation process was that of integration – recombination – growing up. It showed changes from dispersed loose structure merging into small flocs of irregular shapes and finally forming a denser and compact granular form. In general, the granules demonstrated a good accumulation of biomass as well as increased in settleability compared to the conventional sludge (from 0.03 cm/s to 0.94 cm/s). The maximum settling velocity obtained achieved was approximately 2.0 cm/s. Future studies will include optimisation of the operational parameters such as the HRT and organic loading rate to produce granules that are compact, of higher settling abilities and durability. It is also noteworthy to include immediate applications of the granules in wastewater treatment to minimise the emission of GHG.

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References

1. Chen, S.S. (2008). The LCA Approach to Illustrate Palm Oil's Sustainability Advantage. *International Palm Oil Sustainability Conference 2008.*
2. Intergovernmental Panel on Climate Change (IPCC) (2007). *Climate Change 2007*: The Physical Science Basis. *Contribution Of Working Group I To The Fourth Assessment Report*. Intergovernmental Panel on Climate Change.
3. Hassan, M.A., Yacob, S. & Shirai, Y. (2004). Treatment of Palm Oil Wastewaters. *Handbook of Industrial and Hazardous Waste Treatment (2_{nd} edition), Marcel Dekker Inc., N.Y., pp. 719-735.*
4. SIRIM (2008). Interim National LCI Database, National LCA Project, SIRIM, Shah Alam.
5. Su, F., Lu, C., Chen, W., Bai, H., & Hwang, J. (2009). Capture of CO_2 from Flue Gas via Multiwalled Carbon Nanotubes. *Science Total Environment 407 (8): 3017 - 3023* .
6. Olah, G., Goeppert, A., & Prakash, G. (2009). Chemical Recycling of Carbon Dioxide To Methanol And Dimethyl Ether: From Greenhouse Gas To Renewable, Environmentally Carbon Neutral Fuels And Synthetic Hydrocarbons. *Journal of Organic Chemistry, 74 (2): 487-98.*
7. Huber-Humer, M., Gebert, J., & Hilger, H. (2008). Biotic Systems to Mitigate Landfill Methane Emissions. *Waste Management Resources, 26 (1): 33-46*.
8. Alimahmoodi, M., & Mulligan, C. (2008). Anaerobic Bioconversion of Carbon Dioxide to Biogas in an Upflow Anaerobic Sludge Blanket Reactor. *Journal of Air and Waste Management Association. 58(1): 95-103.*
9. Su, B., Cui, X., & Zua, J. (2012). Optimal Cultivation and Characteristics of Aerobic Granules with Typical Domestic Sewage in an Alternating Anaerobic/Aerobic Sequencing Batch Reactor. *Bioresource Technology Volume 110: 125–129.*
10. Prasertsan, P., Choorit, W. & Suwanno, S. (1993). Isolation, Identification And Growth Conditions of Photosynthetic Found in Seafood Processing Wastewater. *World Journal of Microbiology and Biotechnology*. 9, 590-592.
11. U.S.EPA (1999). Wastewater, Technology Fact Sheet: Sequencing Batch Reactors, U.S Environmental Protection Agency, Office of Water, Washington, D.C., EPA 932-F-99-037.
12. Bhise., A.,D. (2005). Biomimetic Approach for Synthesizing Artificial Light- Harvesting System Using Self-Sssembled. *Institut of Nanotechnology. 5-7*
13. Linlin, H., Jianlong, W., Xianghua, W. & Yi, Q. (2003). The Formation and Characteristics of Aerobic Granules in Sequencing Batch Reactor (SBR) by Seeding Anaerobic Granules. *Process Biochemistry 40, 5–11.*
14. Kaewsuk, J., Thorasampan, Thanuttamavong, M. & Tae, G. (2010). Kinetic Development And Evaluation of Membrane Sequencing Batch Reactor (MSBR) With Mixed Cultures Photosynthetic Bacteria For Dairy Wastewater Treatment. *Journal of Environmental Management 91, 1161-1168*.

15. Dapena, A., Arrojo, B., Campos, J.L., Mosquera, A. & Mendez, R. (2004). Improvement of the Settling Properties of Anammox Sludge in a SBR. *Journal of Chemical Technology and Biotechnology 79, 1417-1420.*
16. Mangrum, R.L. (1998). The effect of anoxic selectors on the control of activated sludge bulking and foaming. Master Thesis. Virginia Polytechnic Institute and State University.
17. Liao, B.Q., Allen, D.G., Droppo, I.G., Leppard, G.G. & Liss, S.N. (2001). Surface Properties of Sludge and Their Role in Bioflocculation and Setteleability. *Water Research Volume 35(2), 339 – 350.*
18. Abdullah, N., Ujang, Z., & Yahya, A. (2011). Aerobic Granular Sludge Formation for High Strength Agro-Based Wastewater Treatment. *Bioresource Technology 102, 6778 – 6781.*
19. Gao, D.W., Lin, L., Liang H., & Wu, W.M. (2011). Aerobic Granular Sludge: Characterization, Mechanism of Granulation and Application to Wastewater Treatment. Critical *Reviews in Biotechnology 31 (2), 137–152.*