Theme Number: V Sub-topic: Biomaterials & biological approach

Photosynthetic Microbial Granules Developed From Palm Oil Mill Effluent (POME)

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Abstract

In the 1980s, carbon dioxide (CO_2) gas was proven to be one of the most hazardous greenhouse gases (GHG) as it increases from 57 to 80% of the current GHG contribution to global warming. In Malaysia, one of the major sources of GHG is from industrial wastewater treatment such as the ponding system to treat palm oil mill effluent (POME). Alternatively, this study looks into the possibility of applying biogranulation technology by growing photosynthetic bacteria to consume CO_2 gas within a treatment system. The photosynthetic microbial granule was cultivated for 100 days in a sequencing batch reactor (SBR) system by applying an organic loading rate (OLR) of 1.53 kg COD/m^3. day, hydraulic retention time (HRT) at 8 hours and superficial air velocity of 0.58 cm/s. The results showed the morphological structure of sludge evolved from dispersed loose shaped into a more stable and smoother compact granular form with good settling properties. The settleability of the sludge improved from 18.0 to 103 m/h periodically due to the increased of biomass concentrations (6.90 - 8.25 g/L) as well as achieving maximum granule size of 2.8 mm. The granules also displayed great physical characteristics in strength and stability, which attained low integrity coefficient (2.22%). Based on the pigment analysis using the UV-Vis spectrophotometer (380-1100 nm), the presence of purple non-sulfur photosynthetic bacteria were detected in the sludge. The average wavelength adsorption obtained was within 976 to 1050 nm which implied the existence of bacteriochlorophyll b.

Keywords: CO_2 gas, POME, photosynthetic microbial granule, SBR system, bacteriochlorophyll b

Introduction

In the 1980s, carbon dioxide (CO_2) gas was proven to be one of the most hazardous greenhouse gases (GHG) as it increases from 57 to 80% of the current GHG contribution to global warming (Lahoff and Lahuja, 1990). In Malaysia, one of the major 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, 1.0 tons of crude palm oil production need 5.0-7.5 ton of water which 50.0% of them come out as POME. Besides, POME contained high organic content (COD 50.0 g/l, BOD 25.0 g/l) and substantial amounts of plant nutrient (Singh *et al*.,1999; Ahmad *et al.*, 2005; Abdul Rahman *et al*., 2013; MPOB, 2014). If discharged, the untreated POME can cause considerable environmental problems**.** Oil palm cultivation per se, constituting 16% of the 32.86 million hectares of the total land mass of Malaysia, is generally of little threat to the environment. The oil palm plantation, a perennial with a closed canopy, also stores carbon, both above and below the ground. Playing no small role in carbon dioxide (CO_2) balance and carbon fixation, a plantation may accumulate 100-120 tonnes of biomass per hectare by maturity (Su *et al*., 2009)

The conventional ponding system, especially the anaerobic process produces harmful and odor gases such as sulfur dioxide (SO_2), methane (CH_4) and CO_2 gases (Alimahmoodi and Mulligan, 2008;Olah *et al*., 2009; Daelman *et al.,* 2012). Methane is formed during wastewater treated in open ponds. Methane off the air will increase the threat of global warming, because methane in the air will react with water to form carbon dioxide and water. Reactions that occur in the air cause the accumulation of methane gas and carbon dioxide as well. Methane and carbon dioxide gases are gases that contribute to the greenhouse effect causing global warming synergism (Bandara *et al*., 2011; WRI, 2014). There is a lot of interest in reducing these emissions in anaerobic wastewater treatments (Chotwattanasak and Puetpaiboon, 2011; Martinez *et al*., 2013) with the latest study on the CO_2 biofixation by algae and microalgae (Nayak *et al*., 2013; Pankaj and Awasthi, 2013). 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 granular sludge is a promising new technology in aerobic wastewater treatment system and has increasingly attracted interest in recent years for its ability to overcome the limitations in conventional activated sludge system. This technology improves the characteristics of sludge whereas its granular form provides/consists of a compact structure with good settling capability, high biomass concentration, and simultaneous removal of organic matter, nitrogen and phosphorus (Bassin *et al*., 2012; Wei *et al.,* 2012).

The role of photosynthetic bacteria is important for the CO_2 sequestration within the microsystem of wastewater as it utilizes the CO_2 in the environment (Bently and Melis, 2013; Farrelly *et al*., 2013). 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 (Zak *et al.,* 2011; Rastogi and Sani, 2011; Egan *et al*., 2013) as population diversity alone may not be adequate in determining the microbial characteristics. Thus, this study intended to investigate the characteristics of photosynthetic microbial granules developed from POME. The main parameters included were the change in term of morphological structure, biomass concentration and settling properties. Also, during the cultivation of photosynthetic bacteria, the presences of the bacteria were identified.

Materials and Method

Experimental set-up and SBR operation

The experiment was operated using a 3L double-jacketed cylindrical column (6.4 cm in diameter and 90 cm in height) was used as sequencing batch reactor (SBR) system with a 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 for 4 h cycle per day consisted of 5 min for filling up 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%. An air diffuser for the aeration was controlled at an air flow rate of ± 4 L/min (superficial air velocity = 2.07 cm/s) while keeping the reactor at room temperature (between 20-27 ± 2 ^{o}C). In addition, for creating photosynthetic condition, 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). Table 1 summarizes the important operational details of the SBR system.

An autoclaved palm oil mill effluent (POME) wastewater was used as feeding. This was implied to lower the oil and grease/ fatty acid content that could affect aerobic granulation process (González *et al*., 2009) and also limit the rate of photosynthesis due to low light penetration (Strickland *et al*., 1958). Besides, 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. Preliminary, 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 anoxic and aerobic condition similar to SBR operating parameters. After the acclimatization period, a sludge volume of 0.5 L was filled inside the reactor, making up half of the working volume.

Table 1: Timing of SBR cycles

Phases	**Duration (min)**
1) Fill	5
2) React: (i) Anoxic	80
(ii) Aerobic	130
3) Settle	15
4) Decant	5
5) Idle	5
Total cycle length	240

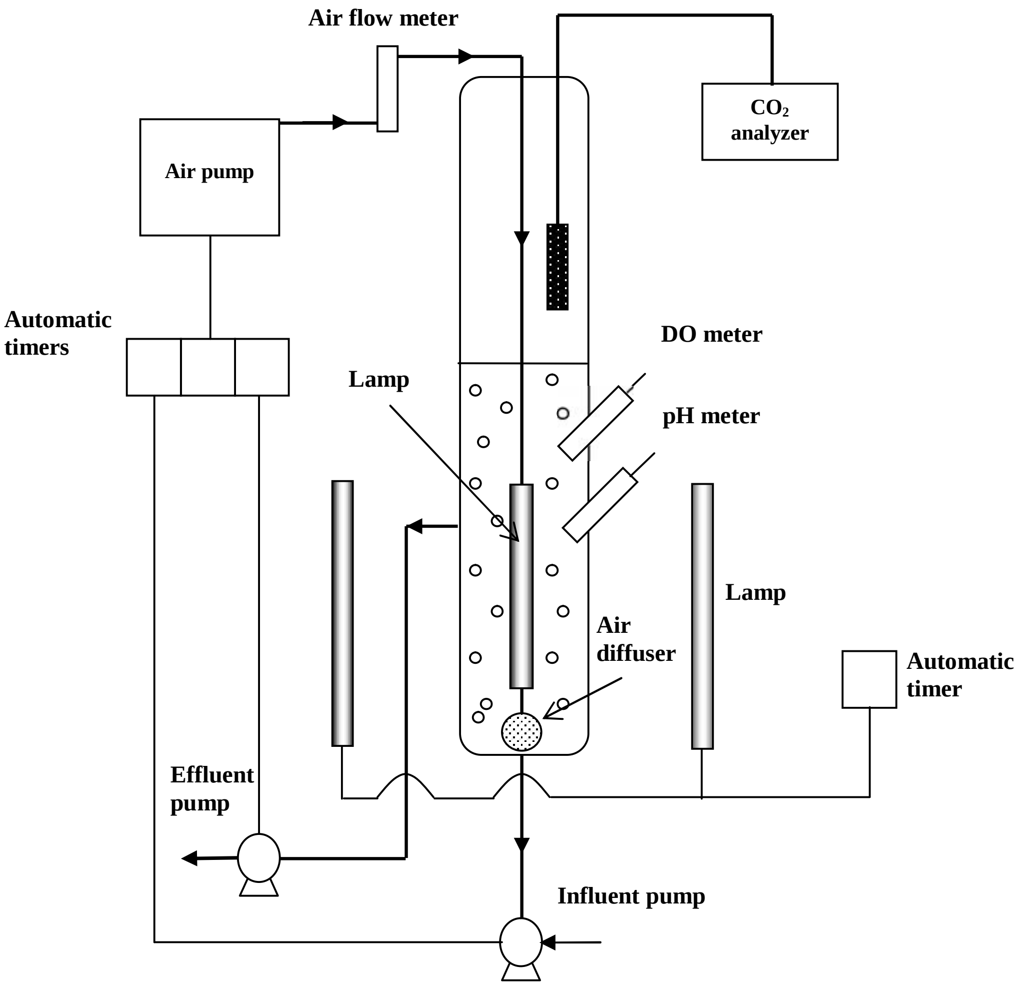


Figure 1: Schematic diagram of SBR system

Analytical procedures

Characterization of Granules

During the development period, the morphological observations of the photosynthetic microbial granules were examined using the light microscope with PAX-IT™ image analyzing system and photos were also taken. The biomass concentration and the sludge volume index (SVI) were analyzed according to the Standard Methods for the Examination of Water and Wastewater (U.S.EPA, 1999; APHA, 1998). Also, the other parameters for granules were conducted based on previous study such as the settling velocity (Linlin *et al*., 2005) and the granular strength (Ghangrekar *et al.,* 1996)

Cultivation and identification of photosynthetic bacteria

Preparation of growth medium for photosynthetic bacteria

For cell growth of photosynthetic bacteria, the sludge sample was inoculated and grown in two different growth media (Prasertsan *et al*., 1993) 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 sterilized 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 color 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.

Results and discussion

Physical and morphological structure

The morphology of granules varies substantially, depending on substrate types and cultivation conditions (De Kreuk *et al*., 2005a). Based on the physical observation, the seeding of raw sludge used was dispersed and loose structure shaped. Initially, the sludge had an average diameter around 0.04 mm during the start-up of the experiment and some of them also consisted of flocs (Figure 2A). After two weeks, the small particles of the sludge and flocs disintegrated under aerobic condition and started to merge together slowly forming flocculated sludge (Figure 2B). This recombined was similar as the flocculation process. It continued to grow and developed into small aggregates (Figure 2C) on the next 30 days with an average diameter of 1.8 mm. Shortly, two months later, an abundant of flocs obtained and started to form bigger aggregates as shown in Figure 2D. Moving on to Figure 2E, the bacteria tend to colonize into particles, which produced extra cellular polymer generally bind the flocs properties together and contributed to the formation of dense aggregates (Snidaro *et al*., 1997).

After 100 days of development, the final form of granules observed were compact structure, smoother, non-uniform spherical shaped with an average diameter of 2.8 mm which in the range of 2.0 to 4.0 mm (Abdullah *et al*., 2011; Othman *et al*., 2013). Furthermore, the morphological changes of the granules during the development period had displayed integration – recombination – growing up process as studied by (Linlin *et al*., 2003).

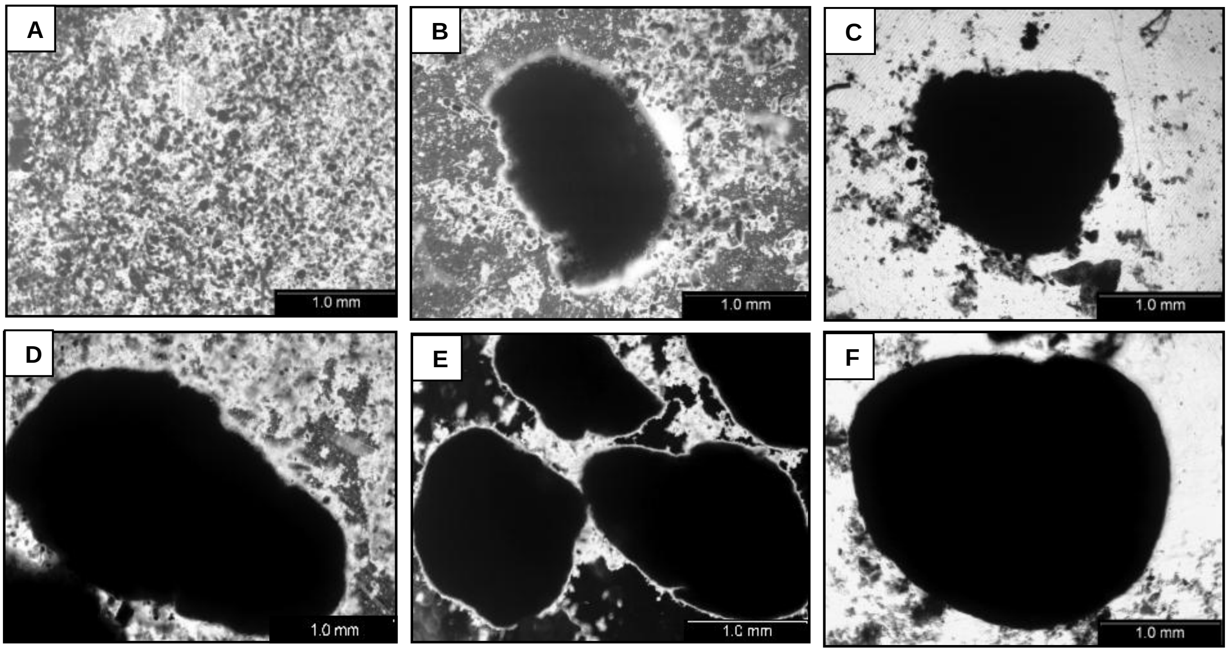


Figure 2: Morphological observation of the granules in the reactor using light microscope PAX-IT (magnification = 40x): (A) Initial sludge, (B) After two weeks, (C) 30 days, (D) two months, (E) 90 days, and (F) 100 days

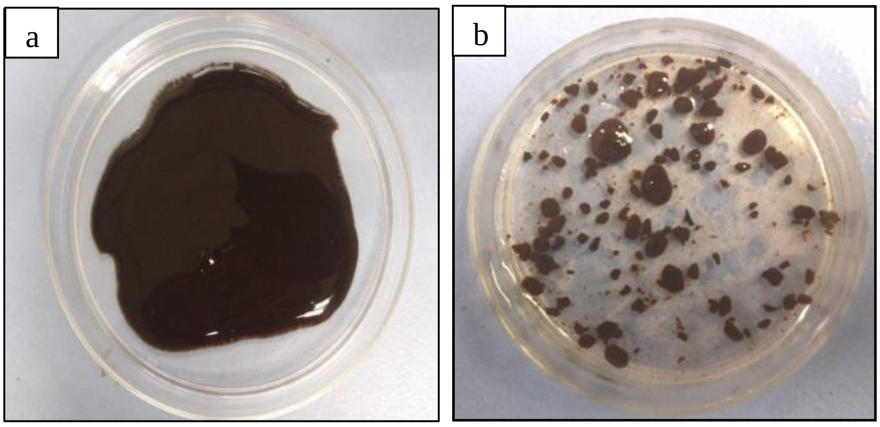


Figure 3: Photos taken to compare between (a) raw sludge and (b) microbial granules

Biomass concentration, settleability, and size change

By the time, the biomass of the sludge in the reactor inconsistently going up and down, possibly cause by the dominant number of small filamentous granules and flocs present (Liu and Liu, 2006) in the first two months (Figure 4). The settleability during that period was inconsistent with initial settling velocity of 18.0 m/h, it suddenly rises at day 31 up to 57.8 m/h at the same time it dropped after 10 days till day 70. This unstable condition occurred during the aggregation process which is considered a balanced between the floc formation and floc breakage (Wan *et al*., 2011). Eventually, advanced granulation took place between 70 to 110 days as the settling velocity began to display steady improvement (from 43.5 to 103.0 m/h). This situation was due to the shearing effects of intense aeration which made the granule surface become smoother and spherical shaped. In addition, this granule formation was also due to the microorganism production of their own extracellular polymers tending to encapsulate the cells and cause them to flocculate or build their colonies (Li and Ganczarczyk, 1990). In conclusion, compared to the conventional raw sludge, the granular form displays exceptional potential with high biomass retention and excellent settleability (Kusmierczak *et al*., 2012). These characteristics are important for easier separation of biomass from treated effluent which led to more efficient and economic technology revolution (Venugopalan *et al*., 2012)

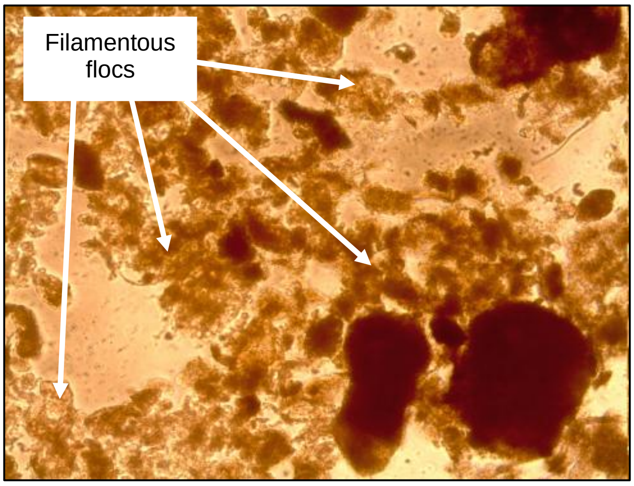


Figure 4: Filamentous floc-forming bacteria presented during the granulation process (magnification = 100x)

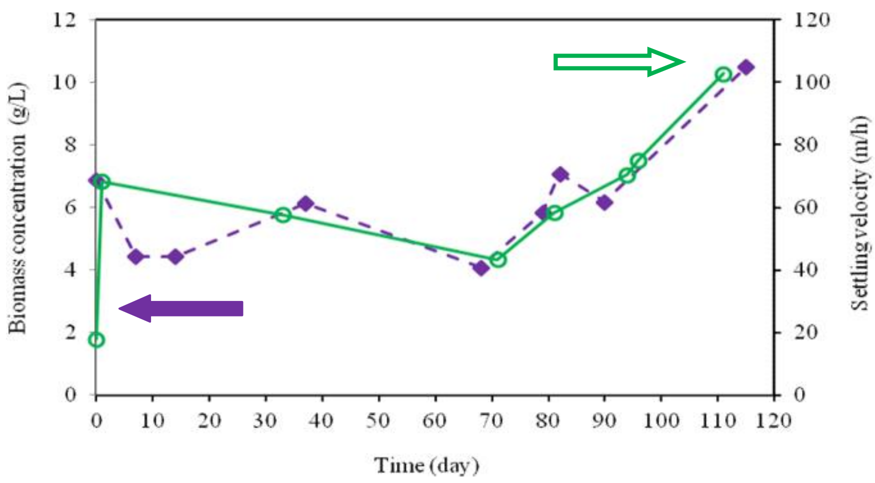


Figure 5: The changing trend of biomass concentration and settling velocity with operation time. (-◆-) Biomass concentration, (—ο—) Settling velocity.

Moving on, Figure 6 displayed there was increased in settling velocity of initial raw sludge throughout the granulation period. This excellent improvement was due to the increase of sludge size as mentioned in the Stoke's Law (Muda *et al*., 2010). The formation of granules had changed the actual raw sludge physically which the size seems to increase from majorly 0.04 mm initial size up to a maximum size of 2.8 mm in the form of granule. Besides having a good settling property, it also leads a good solid-liquid separation compared to initial sludge condition (Kosaric, and Blasczyzk, 1990). The settleability of the sludge was seen to be unstable in the first two months, however, it began to increase gradually from day 71 (settling velocity = 43.5 m/h) then achieved consistent velocities of 70.6-75.2 m/h at day 94-96 until it reaches maximum velocity of 103 m/h at the end of the experiment. These values, varied from 18 m/h to 103 m/h, were similar to earlier report, which situated in the range of 18 to 90 m/h (Xiao *et al*., 2008; Shi *et al*., 2010). This also implies that the physical characteristics of microbial aggregation produce a significant effect on the settling properties (Clifford *et al.*, 1996). An increasing of aggregate diameter had resulted in a higher settling velocity.

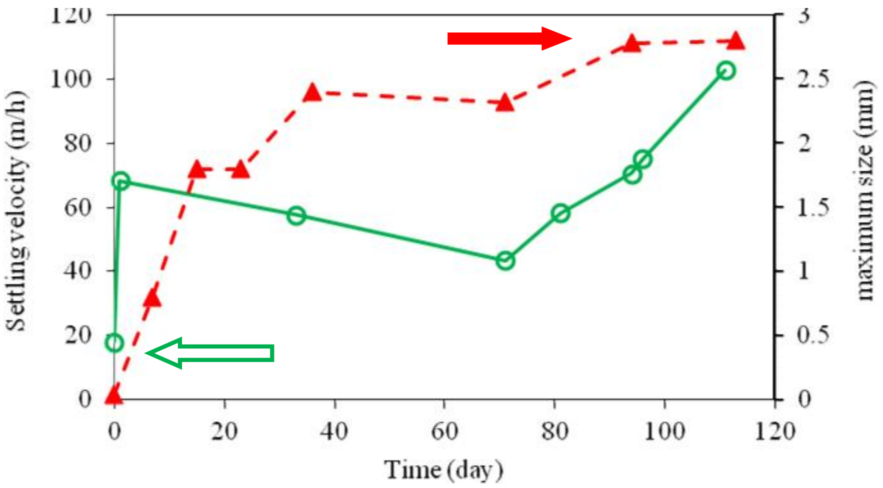


Figure 6: The relationship between settling velocity and the size of developed microbial granules. (–⊖–) Settling velocity, (-▲-) Size in diameter.

Granular strength

The granulation process had successfully finished after 120 days. During the process, the granules displayed a good strength and stability when the final strength of mature granules achieved less than two percent (2.22%) expressed in terms of integrity coefficient. The higher ratio or the integrity coefficient indicates the lower the strength of the granules and via versa. The graph showed the improvement in strength throughout operation time compared to the initial raw sludge (integrity coefficient = 46%). From the graph, after one month, the granules obtained were weak while the granular strength becomes stronger with time from 71.05% to 40% on day 35. Later, the granules maintained at a certain range approximately 35% till day 95 and finally reached at coefficient of 2.22% (day 120). This result suggested that the granules had reached a stability form with further granule development lead to denser granules plus higher settling velocity (Ibrahim *et al*., 2012). Furthermore, the granules with a integrity coefficient of more than 20 were categorized as poor strength granules consisted of loose structure (Park *et al*., 2012). The strength of the granules is essential for their stability and for solid-liquid separation of effluent from the reactors (Kolekar *et al.,* 2012)

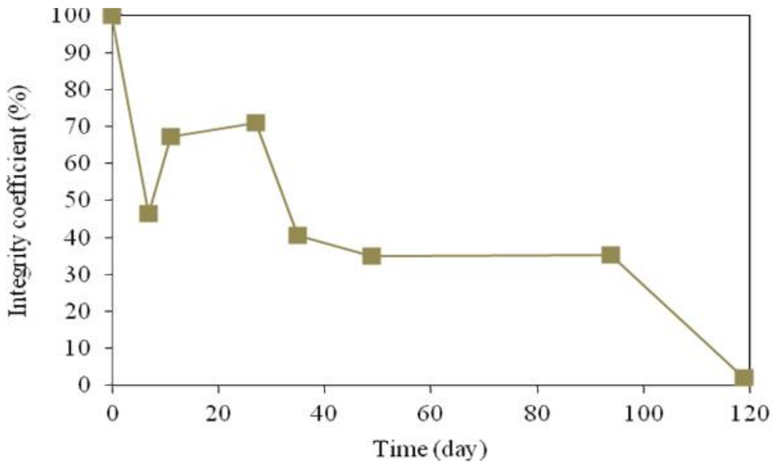
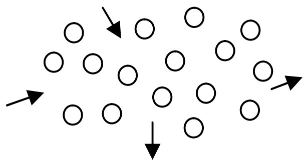
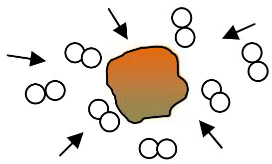
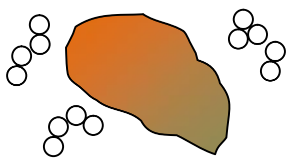


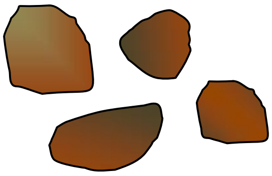
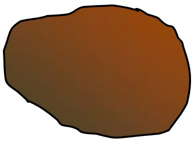
Figure 7: The profile of integrity coefficient representing the granular strength of microbial granules

Characteristics of microbial photosynthetic granules

Below are the summary of the results showing the characterization of the granules obtained after 100 day development period.

Table 2: Overall details of microbial granules throughout development process

Time (day)	Characteristics				
	morphology illustrated	**Size range (mm)**	**Biomass (g/L)**	**Settling velocity (m/h)**	**Granular strength (%)**
1-7	 Small and disperse loose shaped sludge flocs	0.04 – 0.10	6.90	18.0	46.42
30	 Sludge flocs merged each other to form small bioflocs	1.50– 2.10	6.15	57.8	40.68
60-80	 Filamentous outgrowths and bigger flocculates formed	2.19 – 2.45	7.10	58.4	35.10

90	 Breakage of flocculates & small microbial aggregates presence	2.33 – 2.45	8.25	75.2	35.33
100	 Appearance of dense and smooth microbial granules	2.36 – 3.20	8.45	103.0	2.22

Presence of Photosynthetic Bacteria

To determine the presence of photosynthetic pigment analysis for bacteriochlorophyll a, ß 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 8A) while no growth in the G5 medium (Figure 8B). This characteristic reddish bloom had implied the presence of photosynthetic bacteria that of purple non-sulfur photosynthetic bacteria (Hiraish *et al*., 1995) and the presence of only bacteriochlorophyll b. This was because the average wavelength absorption gained mostly within the range of 976 to 1050 nm (Blankenship, 2013) as shown in Figure 9. Bacteria were further isolated and single cultures of bacteria were obtained using the spread plate and the dilution streak technique.

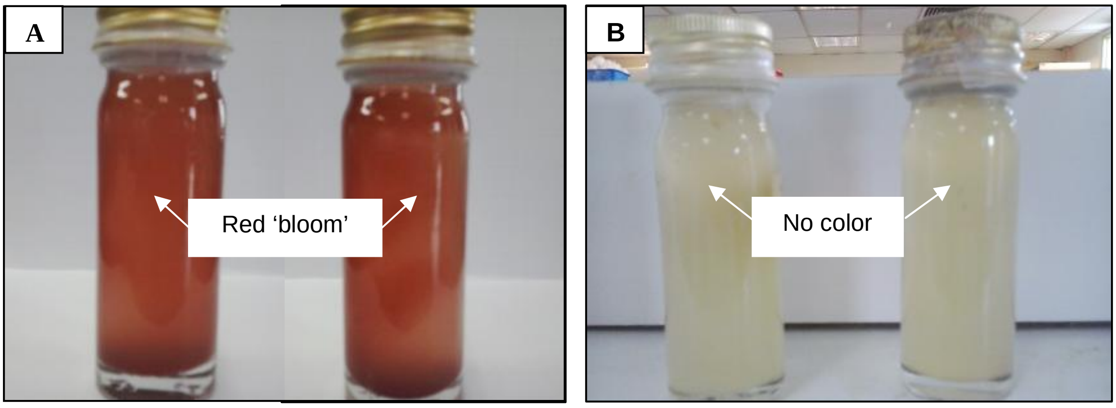


Figure 8: Results for isolation of bacteria using two different broths culture after one week of incubation. (A) Succinate and (B) G5 medium.

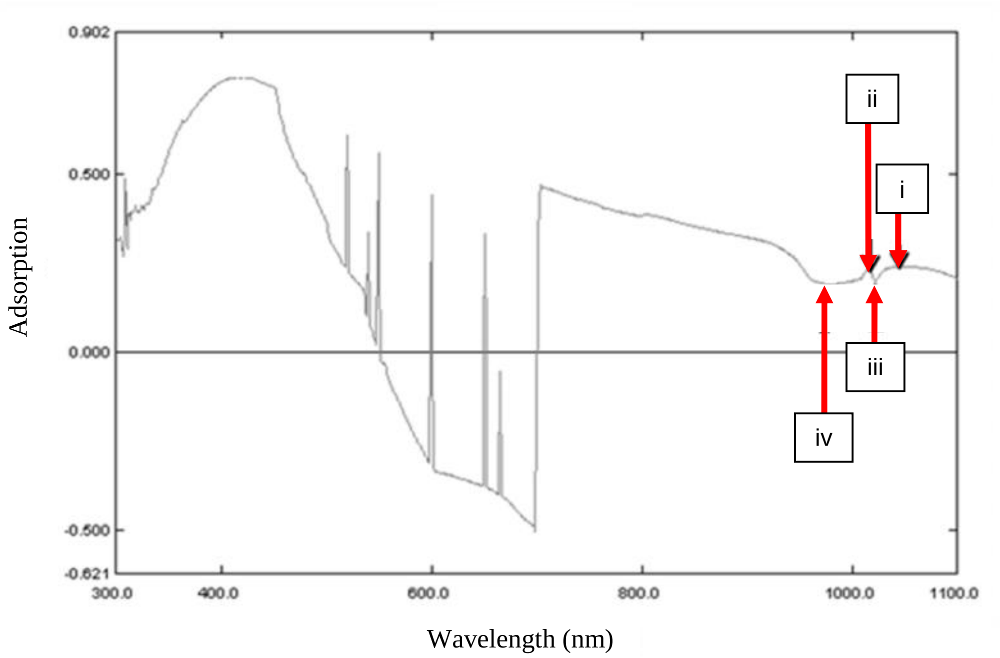


Figure 9: In vivo absorption spectrum for G5 medium. (i) 1046 nm; (ii) 1016 nm; (iii) 1022 nm and (iv) 979 nm.

Conclusion

At the end of this study, the granules containing photosynthetic bacteria were successfully formed after 100 days of cultivation by applying OLR at 1.53 kg COD/m^3.day, HRT of 8 hours and superficial air velocity of 0.58 cm/s. In conclusion, the photosynthetic microbial granules developed had enhanced the characteristics of the conventional sludge. From the morphological change of the granules in the reactor, the initial structure of sludge evolved from dispersed loose shaped into a more stable and smoother compact granular form with good settling properties. The settleability of the sludge improved from 18.0 to 103.0 m/h periodically due to the good accumulation of biomass concentrations (6.90 - 8.25 g/L) as well as achieving maximum granule size of 3.2 mm. The granules also displayed great physical characteristics in strength and stability, which attained low integrity coefficient (2.22%). Based on the pigment analysis using the UV-Vis spectrophotometer (380-1100 nm), the presence of purple non-sulfur photosynthetic bacteria were detected in the sludge. The average wavelength adsorption obtained was within 976 to 1050 nm which implied the existence of bacteriochlorophyll b. Future studies will include optimization of the operational parameters such as the HRT and organic loading rate to produce granules that are more compact, of higher settling abilities and durability. It is also noteworthy to include immediate applications of the granules in wastewater treatment to minimize the emission of GHG.

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References

Lashof, D.A. and Ahuja, D.R. (1990). Relative contributions of greenhouse gas emissions to global warming. *Nature 344: 529−531.*

Agensi Inovasi Malaysia (2013). National Biomass Strategy 2020: New wealth creation for Malaysia's palm oil industry, Version 2.0.

Ahmad, A.L., Ismail, S. and Bhatia, S. (2005). Membrane Treatment for Palm Oil Mill Effluent Effect of Transmembrane Pressure Crossflow Velocity. *Desalination,179: 245-255*

Abdulrahman, N.H. and Azhari, N.H. (2013). Effect of Organic Loading Rate on the Performance of Ultrasonic-Assisted Membrane Anaerobic System (UAMAS) in Treating Palm Oil Mill Effluent (POME). *Journal of American Science, 9(9s): 23-31.*

Malaysian Palm Oil Board (MPOB) (2014). Oil Palm and the Environment (updated March 2014).

Su, F., Lu, C., Chen, W., Bai, H., and Hwang, J. (2009). Capture of CO_2 from Flue Gas via Multiwalled Carbon Nanotubes. *Science Total Environment (In press).*

Alimahmoodi, M., and 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.*

Olah, G., Goeppert, A., and 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.*

Daelman, M.R.J., van Voorthuizen, E.M., van Dongen, U.D.J.M., Volke, E.I.P. and van Loosdrecth, M.C.M. (2012).Methane Emission During Municipal Wastewater Treatment. *Water Research 46: 3657-3670*.

Bandara, W.H., Sato, H., Sasakawa, M., Nakahara, Y., Takahashi, M. and Okabe, S. (2011). Removal of Residual Dissolved Methane Gas in an Up flow Anaerobic Sludge Blanket reactor Treating Low-strength Wastewater at Low Temperature with Degassing Membrane. *Water Research 45(11): 3533-40.*

World Resources Institute (WRI) (2014). Climate Analysis Indicators Tools (CAIT) 2.0: WRI's climate data explorer. Accessed May 2014.

Chotwattanasak, J. and Puetpaiboon, U. (2011). Full scaled Anaerobic Digester for Treating Palm Oil Mill Wastewater. *Journal of Sustainable Energy and Environment 2: 133-136*.

Martinez, L., Otero, M., Moran, A. and Garcia, Al. (2013). Selection of Native Freshwater Microalgae and Cynobacteria for CO_2 Biofixation. *Env. Technol. 34(21-24): 3137-43*

Nayak M., Rath, S.S., Thirunavoukkarasu, M., Panda, P.K., Mishra, B.K. and Mohanty, R.C. (2013). Maximizing Biomass Productivity and CO2 Biofixation of Microalga, Scenedesmus Sp. By Using Sodium Hydroxide. *J. Microbial Biotechnol. 23(9): 1260-8.*

Pankaj, V.P. and Awasthi, M. (2013). Use of Algae through Different Approaches: A Review. *Special Issue of International Journal of Sustainable Development and Green Economics (IJSDGE) ISSN No.: 2315-4721, V-2,I-1,2.*

Bassin, J.P., Kleerebezem, R., Deezotti, M. and van Loosdrecht, M.C.M. (2012). Simultaneous Nitrogen And Phosphate Removal In Aerobic Granular Sludge Reactors Operated At Different Temperatures. *Water Research 46: 3805-3816.*

Wei, Y., Ji, M., Li, R. and Qin, F. (2012). Organic and Nitrogen Removal from Landfill Leachate in Aerobic Granular Sludge Sequencing Batch Reactor. *Waste Management 32:448-455.*

Bently, F.K. and Melis,A. (2012). Diffusion-Based Process for Carbon Dioxide Uptake and Isoprene Emission in Gaseous/Aqueous Two-Phase Photobioreactors by Photosynthetic Microorganisms. Biotechnology and Bioengineering 109(1): 100-109.

Farrelly, D.J., Everard, C.D., Fagan, C.C., and McDonnell, K.P. (2013). Carbon Sequestration and the Role of Biological Carbon Mitigation: A Review. *Renewal and Sustainable Energy Reviews 21:712-727.*

Zak,' D.R., Pregitzer,' K.S., Burton, A.J., Edwards, I.P. and Kellner, H. (2011). Microbial Responses to a Changing Environment: Implications for the Future Functioning Of Terrestrial Ecosystems. *Fungal Ecology 4(6): 386-395.*

Rastogi, G., and Sani, R. K. (2011). Molecular techniques to assess microbial community structure, function, and dynamics in the environment. In *Microbes and Microbial Technology* (pp. 29-57). Springer New York.

Egan, S., Harder, T., Burke, C., Steinberg, P., Kjelleberg, S. and Thomas, T. (2013). The Seaweed Holobiont: Understanding Seaweed–Bacteria Interactions. *FEMS Microbiology Reviews 37(3): 462-476.*

González, J., Figueiras, F. G., Aranguren-Gassis, M., Crespo, B. G., Fernández, E., Morán, X. A. G., and Nieto-Cid, M. (2009). Effect of a simulated oil spill on natural assemblages of marine phytoplankton enclosed in microcosms. *Estuarine, Coastal and Shelf Science*, *83*(3), *265-276*.

Strickland, J. D. H. (1958). Solar radiation penetrating the ocean. A review of requirements, data and methods of measurement, with particular reference to photosynthetic productivity. *Journal of the Fisheries Board of Canada*, *15*(3), *453-493*.

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.

APHA (1998). Standard Method for the Examination of Water and Wastewater. 19th Edition, American Public Health Association, Washington, D.C., U.S.A.

Linlin, H., Jianlong, W., Xianghua, W., and Yi, Q. (2005). The formation and characteristics of aerobic granules in sequencing batch reactor (SBR) by seeding anaerobic granules. *Process Biochemistry*, *40(1), 5-11.*

Ghangrekar, M. M., Asolekar, S. R., Ranganathan, K. R., and Joshi, S. G. (1996). Experience with UASB reactor start-up under different operating conditions. *Water Science and Technology*, *34(5), 421-428.*

Prasertsan, P., Choorit, W. and Suwanno, S. (1993). Isolation, Identification And Growth Conditions of Photosynthetic Found in Seafood Processing Wastewater. *World Journal of Microbiology and Biotechnology. 9, 590-592.*

De Kreuk M.K., Heijnen J.J., and van Loosdrecht M.C.M. (2005a). Simultaneous COD, nitrogen and phosphate removal by aerobic granular sludge. *Biotechnol Bioeng 90(6): 761–769*.

Snidaro, D., Zartarian, F., Jorand, F., Bottero, J. Y., Block, J. C., and Manem, J. (1997). Characterization of activated sludge flocs structure. *Water science and technology*, *36*(4), 313-320.

Abdullah, N., Ujang, Z., and Yahya, A. (2011). Aerobic Granular Sludge Formation for High Strength Agro-Based Wastewater Treatment. *Bioresource Technology 102, 6778 – 6781.*

Othman, I., Anuar, A. N., Ujang, Z., Rosman, N. H., Harun, H., & Chelliapan, S. (2013). Livestock wastewater treatment using aerobic granular sludge.*Bioresource technology*, *133*, 630-634.

Linlin, H., Jianlong, W., Xianghua, W. and Yi, Q. (2003). The Formation and Characteristics of Aerobic Granules in Sequencing Batch Reactor (SBR) by Seeding Anaerobic Granules. *Process Biochemistry 40, 5–11.*

Liu, Y., and Liu, Q. S. (2006). Causes and Control of Filamentous Growth in Aerobic Granular Sludge Sequencing Batch Reactors. *Biotechnology Advances*, *24(1): 115-127.*

Wan, J., Mozo, I., Filali, A., Liné, A., Bessière, Y., & Spérandio, M. (2011). Evolution of bioaggregate strength during aerobic granular sludge formation.*Biochemical Engineering Journal*, *58*, 69-78.

Li, D. H., and Ganczarczyk, J. J. (1990). Structure of activated sludge floes.*Biotechnology and bioengineering*, *35*(1), 57-65.

Kusmierczak, J., Anielak, P. and Rajski L. (2012). Long-Term Cultivation of an Aerobic Granular Activated Sludge, Ejpau, 15(1), #06. Retrieved from http://www.ejpau.media.pl

Venugopalan, V.P., Nancharaiah, Y.V., Mohan, T.V.K. and Narasimhan, S.V. (2005, March). Biogranulation: Self – Immobilised Microbial Consortia for High Performance Liquid Waste Remediation. BARC Newsletter. Retrieved from www.barc.gov.in/publications/nl/2005/200503-0.htm

Muda, K., Aris, A., Salim, M. R., Ibrahim, Z., Yahya, A., van Loosdrecht, M. and Nawahwi, M. Z. (2010). Development of Granular Sludge for Textile Wastewater Treatment. *Water Research*, *44(15), 4341-4350*.

Kosaric, N. and Blasczyzk, R. (1990). Microbial Aggregates in Anaerobic WWT. *Advances in Biochem. Eng. and Biotech., 42: 28–55*

Xiao, F., Yang, S. F., and Li, X. Y. (2008). Physical and Hydrodynamic Properties of Aerobic Granules Produced in Sequencing Batch Reactors. *Separation and Purification Technology*, *63*(3*): 634-641.*

Shi, X. Y., Sheng, G. P., Li, X. Y., and Yu, H. Q. (2010). Operation of a Sequencing Batch Reactor for Cultivating Autotrophic Nitrifying Granules. *Bioresource technology*, *101*(9), *2960-2964***.**

Clifford P. Johnson,Xiaoyan Li, and Bruce E. Logan (1996). Settling Velocities of Fractal Aggregates. *Environmental Science & Technology 30* (6): 1911-1918

Ibrahim, Z., Amin, M.F.M., Yahya, A., Aris, A., and Muda, K. (2010). Characteristics of Developed Granules Containing Selected Decolourising Bacteria for the Degradation of Textile Wastewater. *Water Science and Technology61 (5): 1279 – 1288.*

Park, Y. J., Lee, J. W., and Song, I. H. (2012). The Optimization of Additive Contents and Presintering

Conditions for Si-Additive Mixture Granules. *Journal of the Ceramic Society of Japan, 120(1398), 77-81.*

Kolekar, Y. M., Nemade, H. N., Markad, V. L., Adav, S. S., Patole, M. S., & Kodam, K. M. (2012). Decolorization and Biodegradation of Azo Dye, Reactive Blue 59 by Aerobic Granules. *Bioresource technology*, *104, 818-822*.

Hiraishi, A., and Ueda, Y. (1995). Isolation and Characterization of *Rhodovulum Strictum Sp. Nov*. And Some Other Purple Nonsulfur Bacteria from Colored Blooms in Tidal and Seawater Pools. *Journal of systematic bacteriology*, *45*(2): *319-326.*

Blankenship, R.E. (2013). *Molecular Mechanisms of Photosynthesis*. Retrieved from books.google.com.my/books?isbn=1118685369