Study on the effect of a static magnetic field in enhancing initial state of biogranulation

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ABSTRACT

Successful aggregation and surface hydrophobicity play a significant role towards effective initial state development of the biogranulation process. Considering previous studies, there is sparse research available for the study of the static magnetic field effect on aggregation and surface hydrophobicity of microbial granules. Therefore, this work aimed at exploring the feasibility of enhancing both aggregation and surface hydrophobicity using a static magnetic field. The influence of the static magnetic field on the removal of chemical oxygen demand (COD) was also monitored. The results showed that magnetically exposed activated sludge of 15 mT has better performance than other investigated intensity levels of static magnetic field. At 15 mT intensity, a maximum of 54% surface hydrophobicity was retained in 48 hours of starting the experiment and 90.4% aggregation was achieved in 10 hours. The removal efficiency of COD was also increased under similar static magnetic field intensity compared to the case without static magnetic field exposure. With this initial finding, it can be concluded that a static magnetic field of moderate field intensity stands a good chance of positively influencing the initial state of biogranulation.

Key words | aggregation, biogranulation, sludge, static magnetic field, surface hydrophobicity

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INTRODUCTION

Biogranulation involves cell-to-cell interaction, which includes biological, physical and chemical phenomena. Development of biogranules requires aggregation of microorganisms (Liu & Tay 2004; Liu *et al.* 2004a; Muda *et al.* 2010). There are various triggering forces that can bring bacteria together and further make them aggregate. Previous research showed that cell hydrophobicity could serve as a triggering force for biogranulation to begin (Liu *et al.* 2003). When bacteria became more hydrophobic, cell-to-cell adhesion, which is observed as cell surface hydrophobicity, increases. This condition allows cells to aggregate, thereby increasing the granulation rate (Kos *et al.* 2003; Liu *et al.* 2004b). There are many advantages associated with the use of biogranulation technologies in wastewater treatment, including high biomass retention, strong microbial structures with good settleability, ability to withstand high-strength wastewater and shock loadings, and also high tolerance to toxicity (Wang *et al.* 2005; Sheng *et al.* 2010; Seviour *et al.* 2012). Nevertheless, biogranulation technology has some drawbacks. These include the need for a long start-up period, instability caused by granule disintegration, a relatively high operation temperature and its unsuitability for low strength organic wastewater (Liu & Tay 2004; Kocaturk & Erguder 2016). In order to overcome these drawbacks, various research works have been completed to improve the development of biogranulation. A major focus for improvement is investigating the key parameters that positively influence the cell aggregation and surface hydrophobicity. These include the type and concentration of substrate, nature of the seed sludge, availability of essential nutrients, composition of the media, pH, temperature, the operational set-up of the reactor system and many others (Wang *et al.* 2009; Zhu

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et al. 2012; Muda et al. 2014). Alias et al. (2017) reported that addition of cation Ca^{2+} increased the aggregation of biogranules by up to 62% and at the same time, it positively affected the cell's surface hydrophobicity. When the aggregation and surface hydrophobicity of biogranules are well enhanced, the settleability of biomass can be improved thus further enhancing the wastewater treatment.

Application of a static magnetic field can also enhance the biomass settleability in wastewater treatment. The magnetic field plays its role by manipulating the positive and negative charges of the bacterial cells. The charges are well separated and easily aligned in the direction of the magnetic field (Vick 1991). This strengthens the electrostatic force and polymeric interaction that can further enhance the cell's adhesion. Consequently, this results in an increase in the cell's surface hydrophobicity and increases the aggregation and settleability properties (Zaidi 2016). Since application of a magnetic field is able to improve the biomass settleability, it may improve the initial stage of biogranulation.

MATERIALS AND METHODS

Synthetic wastewater and inoculated sludge

A synthetic wastewater was used in the study. Its composition was in accordance with Muda et al. (2010): NH_4Cl (0.16 g/L), KH_2PO_4 (0.23 g/L), K_2HPO_4 (0.58 g/L), $CaCl_2.2H_20$ (0.07 g/L), $MgSO_4.7H_2O$ (0.09 g/L), EDTA (Ethylenediaminetetraacetic Acid) (0.02 g/L) and trace solution (1 mL/L). Additionally, glucose (0.5 g/L), ethanol (0.125 g/L) and sodium acetate (0.5 g/L) were used as mixed carbon sources. However, the trace elements used were based on the composition recommended by Smolders et al. (1995). The initial chemical oxygen demand (COD) for this study was 2,388 mg/L. As for the granular sludge, it was developed from the common sludge obtained from Indah Water Konsortium Sewage Treatment Plant System which is located at Taman Sutera, Johor, Malaysia. This sludge contains a very high concentration of microbial populations that are useful for granule development. The collected sludge inocula were sieved using a mesh of 1.0 mm in order to remove large debris and inert impurities. The mixed liquor suspended solids of the sludge that was used throughout the experiment was 1,000 mg/L.

Experimental set-up

The experiment was carried out using Schott bottles with inner diameter of 101 mm and height of 230 mm. A working volume of 500 mL of the synthetic textile wastewater was used. Aeration was produced by an air pump at a flow rate of 6.5 L/min and at standard room temperature of 24 °C. The dissolved oxygen (DO) concentration was maintained at more than 5 mg/L and the pH was between 7 and 8. Four identical sets of Schott bottles were prepared. One was set as a control experiment without magnetic field exposure while the other three were attached to permanent magnets so as to induce required magnetic fields. The strength of the magnetic field was varied (9, 15 and 30 mT) in the respective Schott bottles. The permanent magnets were in the shape of cuboids of size $50 \times 50 \times 5$ cm. These magnets were attached at exterior sides of the Schott bottles. The strength of the magnetic field was detected using a TM-701 Tesla Meter, Kanetec. Throughout the entire aeration period, samples were taken every two hours and were analysed for aggregation and surface hydrophobicity assays as well as for COD measurement. The entire experiment was allowed to run for only 2 days.

Aggregation assay

The aggregation assay (%Ag) was determined by turbidity. A sample of 15 mL was taken from each Schott bottle and used for aggregation assay. The turbidity of the sample was read instantaneously and the collected data was recorded as initial turbidity (T_i). The sample was then centrifuged at a speed of 200 rpm for 2 min. Thereafter, the turbidity data of the sample was measured again and the data was set as final turbidity (T_f). The reading of turbidity was taken using a turbidity meter (H 2100Q Portable Turbidimeter). The percentage of aggregation can be calculated using Equation (1).

$$\%\,\mathrm{Ag} = [(T_i - T_f)/T_i] \times 100 \tag{1}$$

Surface hydrophobicity assay

The cell surface hydrophobicity was conducted based on microbial adhesion to the hydrocarbon assay. This method was originally reported by Rosenberg et al. (1980) and Canzi et al. (2005). However, a few steps were modified to suit the experimental conditions of this study. About 15 mL of the sample from each Schott bottle was used for the surface hydrophobicity assay. This setup was operated by centrifugation at 9,000 rpm for 5 min in order to harvest the bacterial cells from the sample. The supernatant was discarded, while the pellet was washed twice with 50 mM K_2HPO_4 (pH 7.0). After that, the pellet was suspended again in the same buffer to obtain an absorbance of about 0.5 at 660 nm (A_i). 5 mL of the bacterial suspension was mixed with 1 mL of xylene ($C_6H_4(CH_3)_2$) by vortex for 120 sec and then allowed to be idle for another 1 h at room temperature (A_f). The absorbance was measured at 660 nm using a HACH DR 6000 spectrophotometer. The surface hydrophobicity can be expressed as the percentage surface hydrophobicity (% SHb) and can be calculated using Equation (2).

$$\% \text{SHb} = [(A_i - A_f)/A_i] \times 100 \quad (2)$$

RESULTS AND DISCUSSION

Aggregation and surface hydrophobicity

Figure 1 shows the trend of aggregation under different intensities of the magnetic field exposure. Based on the figure, the percentage of sludge aggregation increased abruptly to about 87% within 4 hours of the reaction process either with or without the magnetic field. Ten hours into the experiment, the aggregation of the magnetized sludge having an intensity of 15 mT reached the highest aggregation with a percentage aggregation of 90.4% compared to activated sludge without the magnetic field exposure (86.9%). After 30 hours of operation, the aggregation of the activated sludge under the magnetic field exposure achieved more than 95% for all the activated sludge samples. With the increase in aggregation, the surface hydrophobicity correspondingly increased.

Figure 2 illustrates the percentage of surface hydrophobicity with and without the exposure to a magnetic field. The cell surface hydrophobicity plays a key role in the self-immobilization and attachment of cells to a surface. Based on the experimental conditions used in this study, induction of the magnetic field on the sludge may have a significant effect on the surface hydrophobicity. However, based on Figure 2, the percentage of surface hydrophobicity after reaching 48 hours of operation was not high, being approximately 54% for the sludge that had been exposed under 15 mT magnetic field intensity. A slight increase in the surface hydrophobicity (about 55%) was observed when magnetic field intensity of 30 mT was applied to the sludge. Although the intensity of the magnetic field was double, the increment of surface hydrophobicity was small. This may be due to the limitation of microbes to sustain the intensity of magnetic field. Based on previous research, every microbe has a unique susceptibility level towards the magnetic effect. If the level is exceeded, the microbe itself can be self-degraded and consequently can cause an adverse effect on the physical, chemical, biological and removal performances of a system (Yavuz & Çelebi 2000; Zaidi *et al.* 2014).

Based on Figure 2, the percentage of cell surface hydrophobicity is also increased in parallel with the exposure time of the activated sludge. The average increment is about 46% which was obtained when the exposure time reached 48 hours. This increment could be due to the decreasing of

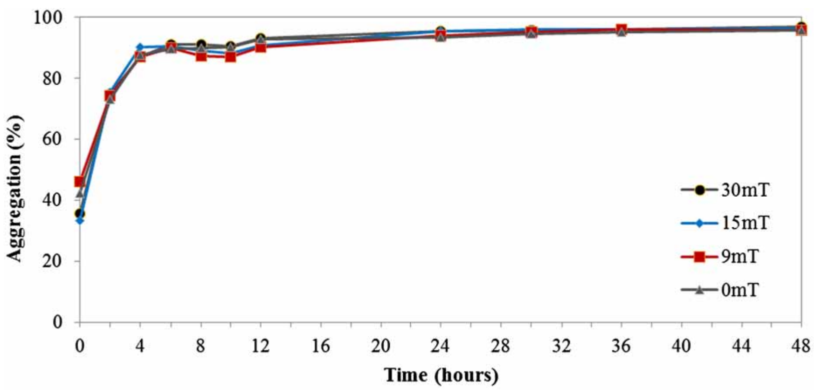

Figure 1 | Aggregation of sludge samples with and without magnetic field exposure.

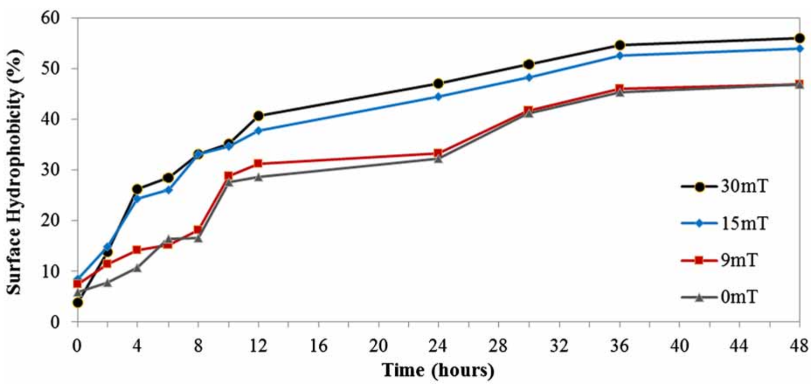

Figure 2 | Surface hydrophobicity for sludge with and without magnetic field exposure.

nutrients or less available organic compounds which would cause starvation to the microbial community. This starvation may induce the increment of cell surface hydrophobicity. This is in order to provide a protective shell for cells that are exposed to the unfavourable environments thus, further strengthening cell-to-cell interactions, leading to a stronger than usual microbial self-attachment (Liu *et al.* 2009).

Concentration of initial turbidity

Throughout the experiment, various concentrations of initial turbidity were observed with regard to the slight differences of aggregation percentage under the presence and absence of the magnetic field. All relevant curves in Figure 3 indicate that the exposure to the magnetic field resulted in an increase in initial turbidity of the activated sludge. It is obvious that higher turbidity may increase the collision between cells, which later could facilitate the adhesion and aggregation process. Liu & Tay (2002) pointed out that the collision between particles plays an important factor in inducing the formation and stabilization of biofilm, and anaerobic as well as aerobic granules.

Based on Figure 3, the higher magnetic field intensity of 30 mT corresponded to the higher average concentration of initial turbidity which was approximately 476 NTU. According to Zaidi (2016), the activated sludge particles are located randomly under normal conditions (absence of magnetic field), which makes it more difficult for them to attach to each other although collisions between the particles may occur. However, when the sludge is exposed to a magnetic field, the particles will be aligned according to their positive and negative charges, which would favour collisions between particles. This would, hence, lead to higher initial turbidity, eventually enhancing the coagulation and aggregation process.

Removal efficiency of COD

Figure 4 shows the profile of COD removal performance of the batch test throughout the operational period. Overall,

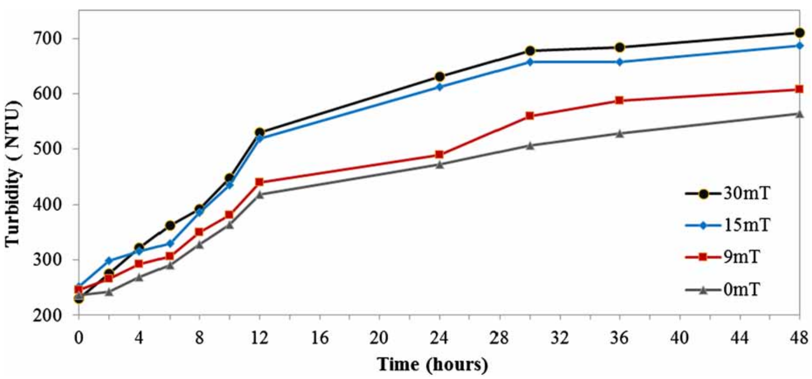

Figure 3 | Concentration of initial turbidity with and without magnetic field exposure.

high removal efficiency of COD was achieved in all of the batch tests. It was clear that the difference in COD removal was affected by the magnetic field within the initial 24 hours of the experiment. Over the next 24 hours of the experiment, the COD degradation rate reached more than 80% both with and without the magnetic field exposure. Greater or longer exposure time would ensure the effect of the magnetic field sustained by the activated sludge particles to last much longer, thereby enabling a continuously improved COD removal (Colic & Morse 1999; Zaidi 2016).

High removal efficiency of the COD as was indicated can also be explained in terms of aggregation ability and surface hydrophobicity. Previous studies suggested that the magnetic field could increase the organism biodegradation ability of aerobic bacteria from the activated sludge. This was due to the increase in the concentration of extracellular enzymes distributed on the bacterial surface under a magnetic field (Ji *et al.* 2010; Wang *et al.* 2012). This would in turn reduce the negative surface charges of the cells, thus resulting in cells of high hydrophobicity. When bacteria become more hydrophobic, cell-to-cell adhesion increases. This condition allows cells to aggregate, thereby increasing the granulation rate (Kos *et al.* 2003; Liu *et al.* 2004b). As the granulation rate increases, the removal efficiency of the COD is also increased.

Based on Figure 4, the curve of 9 mT intensity caused no effect on the COD removal throughout the experiment. This indicates that low magnetic field intensity can be ignored and would not cause any changes in the removal of COD or degradation of the wastewater. A similar result that supports this claim was reported by Tomska & Wolny (2008). The study stated that no removal of organic matters occurred when a weak magnetic field was applied. However, the change in COD removal as a result of the magnetic field of 15 mT intensity was notable. The percentage removal increased gradually to about 50% after 12 hours of exposure. This removal by the 15 mT intensity was higher compared to the removal for the experiment without magnetic field exposure which was about 43%. The removal of 50% under 15 mT field intensity was similar even after a further increase in the magnetic field intensity up to 30 mT. This indicated that the respective microbial granules involved in degradation of COD have a limitation around a magnetic intensity of about 15 mT. This is consistent with a previous study by Yavuz & Çelebi (2000).

CONCLUSIONS

The induction of certain intensities of static magnetic field appears more effective in enhancing the aggregation process and cell surface hydrophobicity of activated sludge. The experimental results in this study showed that magnetically-exposed activated sludge of 15 mT has a better performance than other intensity levels of magnetic field. At 15 mT intensity, a maximum of 54% of surface hydrophobicity was retained in 48 hours of the experiment, while 90.4% aggregation was achieved in 10 hours of experimentation. The COD removal efficiency was also increased under the magnetic field intensity in comparison to that without exposure to a magnetic field. With a preliminary study like this, it can be concluded that a static magnetic field of moderate field intensity could positively influence the initial state of biogranulation to improve wastewater treatment. Further study would be welcome in this research area.

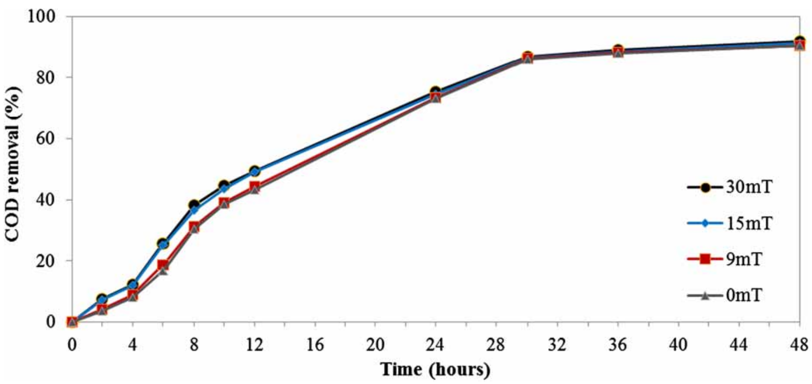

Figure 4 | Profile of COD removal performance throughout the operational period with and without magnetic field exposure.

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