



# Effect of Static Magnetic Field on the Efficiency of Granular Sludge Development for Pharmaceutical Wastewater Treatment

Ahmad Hanis Omar<sup>1</sup>, Susilawati Toemen<sup>1,2</sup>, Khalida Muda<sup>1,\*</sup>, Siti Fadzianna Sulaiman<sup>1</sup>

<sup>1</sup>Department of Environmental Engineering, Faculty of Civil Engineering, Universiti Teknologi Malaysia, Johor, MALAYSIA

<sup>2</sup>Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, Johor, MALAYSIA

\*Corresponding Author

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**Abstract:** The effect of static magnetic field (SMF) on the development of granular sludge was evaluated in sequential batch reactor (SBR) system under alternating aerobic and anaerobic condition fed with pharmaceutical wastewater which is categorized as a high strength wastewater. The magnetic field intensity used in this research was 20 mT and compared with the control system without magnetic field exposure. The results showed that sludge granulation was enhanced significantly in the presence of magnetic field which made the reaction time for granulation process faster compared with that of the control. Matured, dense and compact granules with diameter of  $6.5 \pm 1.0$  mm with maximum size reaching up to 8.9 mm were developed with good settling velocity of 76.92 m/h. Magnetic field could stimulated EPS production by accumulating iron, calcium and magnesium compounds in the sludge. The contents of total EPS in R2 (with SMF) were 28.5 mg/gVSS, while in R1 (without SMF) were 21.7 mg/gVSS. The magnetic field system was efficiently treated COD, TP, orthophosphate, TN, nitrate and nitrite with over 90% removal from pharmaceutical wastewater. The application of magnetic field is then helpful and reliable for accelerating the granulation process and is capable in treating the pharmaceutical wastewater

**Keywords:** Magnetic field; Pharmaceutical wastewater, Granulation, Sequential batch reactor

## 1. Introduction

The technology of biogranular sludge is the most common and well known treatment process for domestic and industrial wastewater in recent year [1], [2]. It has an ability to withstand high-strength wastewater, shock loading and tolerance to toxicity due to the strong microbial structure [3]. In addition, granular sludge is a dense and compact microbial community with better settleability compared to floccular activated sludge [4].

However, the development of granular sludge in the treatment of this type of wastewater is slow especially for the treatment of high-strength wastewater such as pharmaceutical wastewater. Besides, there are exposed to a variety of disadvantages such as sludge expansion, low biomass and loose granular structure [5]. In order to overcome these problems, improving the parameter on the development of granular sludge is essential. A few studies have been done to explore the effect of magnetic technology on activated sludge process whether on microbes or on pollutant degradation.

With regards to the effects of magnetic field on microbes, previous study by Ji et al. [6] demonstrated that magnetic field induction of 20 mT had a positive effect on bacterial growth in activated sludge. Rao et al. [7] stated that the influence of magnetic field could accelerate the growth rate of microbes. In addition, Łebkowska et al. [8] found that magnetic field could stimulate the growth of sludge biomass, the number of bacteria and microfauna in the activated sludge. Wang et al. [2] proved that an intensity of 48 mT static magnetic field was beneficial to the growth the activity nitrite-oxidizing bacteria.

In terms of pollutants degradation using magnetic technology, Jung et al. [9] showed that the efficiency of phenol biodegradation rate increased to 30% by applying a magnetic field of 450 mT as compared to the control system. Łebkowska et al. [10] found that a static magnetic field of 7 mT could enhance formaldehyde biodegradation and decrease in COD concentration by greater 30% and 26% respectively, in comparison with the control. Zhu et al. [11] noted that the significant biodegradation rate of polyhydroxyalkanoates (PHA) was at 11 mT of magnetic field which exposed in the famine period. Meanwhile, Liu et al. [12] found a maximum 50% increase on nitrogen removal at the value of 75 mT. These investigations indicated that the application of magnetic field on activated sludge could enhance the growth of microbes and also improve the removal efficiencies of some pollutants. It is believed that magnetic field assisted the accumulation of iron compounds in the sludge. This is because iron compounds can be magnetized when exposed to magnetic field. This helped to enlarge the flocs size when the iron compounds were assembled together under the influence of magnetic field [13]. Thus, more microbes aggregated, and growth resulted more biogranules were formed and increased the efficiencies of the removal performance.

From above descriptions, it can be concluded that these investigations more focused on the effect of magnetic field towards activated sludge whether by external magnetic field or supplemented the activated sludge with magnetic powder to create magnetic activated sludge. In addition, previous researches have more concentrated on synthetic wastewater which was only conducted in the laboratory [2, 11, 14]. The main objective of this research was therefore to evaluate the utility of magnetic field towards the growth of microbial granular sludge for pharmaceutical wastewater treatment. This work could contribute to a better understanding of the ability of these granules to handle such high strength wastewater containing various chemicals which actually would inhibit the microbial growth.

## 2. Material and Method

### 2.1 Reactor and Operation

The development of granular sludge was done in two parallel SBR reactors (termed as R1 and R2) with the working volume of 3 L. Fig. 1 shows the schematic layout of the two column-type SBR in this study. The internal diameter and total height of the reactor are 8 cm and 125 cm, respectively. R1 was operated as control which is without magnetic field, while R2 was operated with magnetic field in which permanent magnets (cuboid: 50 x 50 x 5 mm) were attached to the exterior reactor body R2 with an intensity of 20 mT. The operation of the treatment was the same for R1 and R2. The cycle time of 6 h consisted of 5 min filling, 120 min aeration (aerobic period), 40 min without aeration (anaerobic period), 15 min settling, 5 min decanting and 5 min idling were used in this study. 1.5 L of pharmaceutical wastewater was pumped into the reactor from the bottom of column during each filling. Compressed air was supplied to the SBR by a fine air bubble diffuser with superficial air velocity of 2.5 cm/s during aerobic period. pH was not controlled during the operation, while DO was kept at 6-7 mg/L. The solid retention time (SRT) was set by the discharge of biomass with effluent.

### 2.2 Pharmaceutical Wastewater and Inoculated Sludge

Pharmaceutical wastewater sample was obtained from KPJ Johor Specialist Hospital, Malaysia. The location of the site is about 38 km from Universiti Teknologi Malaysia, Skudai. A few plastic containers with the volume of 20 L were used to collect the wastewater. The amount of samples taken from each trip was approximately 80 L which can cater for 4 days. The samples were then stored in cold storage room at 4°C to avoid biodegradation due to microbial action.

The development of granular sludge involved the sludge from sewage treatment plant since the sludge contains very high concentration of microbial populations that may be useful for granule development. Both reactors were inoculated respectively with 1.5 L of activated sludge taken from a sewage wastewater treatment plant in Taman Sutera, Indah Water Konsortium Treatment Plant System, Johor, Malaysia. The sludge inoculums were sieved with a mesh of 1.0 mm to remove large debris and inert impurities. The initial mass liquid suspended sludge (MLSS) of 7000 mg/L was used in this research and sludge volume index (SVI) of 60 mg/L.

### 2.3 Analytical Methods

The treatment performances of chemical oxygen demand (COD), total phosphorus (TP), Orthophosphate (OP), total nitrogen (TN), nitrate, nitrite, MLSS and MLVSS were monitored in each reactor every week. The parameters were carried out according to Standard Methods for the Examination of Water and Wastewater (APHA, 2005). The developed granules in the SBR column were analysed for their physical, chemical and biological characteristics.

Physical characteristics of settling velocity (SV) and sludge volume index (SVI) was explained by Beun et al. [15], Ghangrekar et al. [16] and Muda et al. [17].

The image observation of the granules was carried out using a stereo microscope equipped with digital image processing and analyzer (PAX-ITv6, ARC PAX-CAM). The morphological and microbial compositions within granules were observed with scanning electron microscopy (FESEM-Ziess Supra 35 VPFESEM). The granules were left to dry at room temperature prior to gold sputter coating (Bio Rad Polaron Division SEM Coating System).

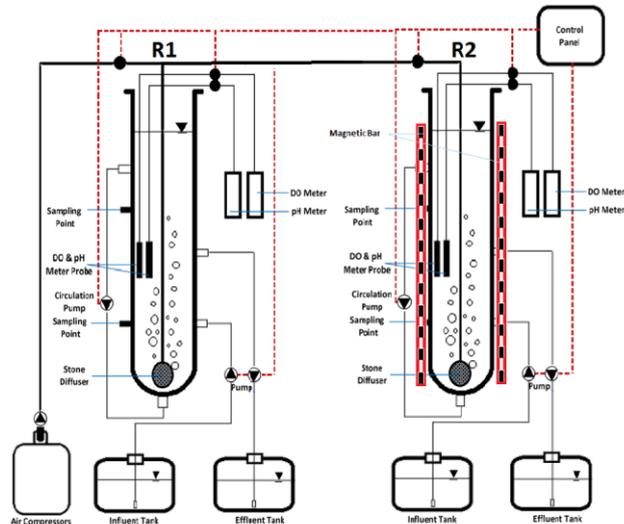


Fig. 1 - Schematic layout of the two column-type SBR in this study.

## 2.4 Extra Cellular Polymeric Substances (EPS)

### 2.4.1 Heat Extraction Method

8 mL of mixed granular sludge sample in R1 system was dewatered in a 50 mL centrifuge tube at 3000 rpm for 5 minutes. The water was drained and the sludge was dissolved with distilled water to the original volume of 8 mL. The steps were repeated five times or more until the clear water is obtained. After that, the sample was heated in 80°C of water bath for 10 minutes before it was centrifuged at 3000 rpm for 5 minutes. The supernatant obtained was filtered through 0.2 µm membrane filter and was considered as loosely bound EPS (LB-EPS).

The mixed sludge sample left in the centrifuge tube was dewatered again for at least twice and centrifuged at 3000 rpm for 5 minutes. Then, the sample was heated in 80°C of water bath for 60 minutes, and centrifuged at 5000 rpm for 5 minutes. The supernatant obtained was transferred into another centrifuge tube, and later centrifuged again at 10000 rpm at 4°C for 5 minutes. The supernatant then was filtered with 0.2 µm membrane filter and was considered as tightly bound EPS (TB-EPS).

Both samples (LB-EPS and TB-EPS) were freeze at -20°C and proceed with the lyophilisation process at -80°C for 24 hours. After the freeze drying process, the dry mass of the solid from LB-EPS and TB-EPS obtained were weighed, and the concentrations of both samples were calculated in mg/g VSS. The total EPS was calculated by summed up the LB-EPS and TB-EPS. The above procedure was repeated for granular sludge in R2 system.

### 2.4.2 Determination of Protein Content

For protein determination, different BSA standard protein solutions (0, 20, 40, 60, 80 and 100 mg/L) were prepared from 100 mg/L of stock solution. In the meantime, the samples from heat extraction method in Section 2.4.1 were taken out from -20°C freezer to thaw. When the samples were totally thawed, vortex the samples and dilute with distilled water if necessary. 0.5 mL of sample was transferred to 10 mL glass test tube in triplicates. 0.7 mL of Lowry solution was added into each of the glass test tube and vortex to mix well with the sample. The samples then were incubated in dark for 20 minutes under room temperature. After 20 minutes, 0.1 mL of diluted Folin Reagent was added into each of the tube, cap and vortex immediately to mix. The samples were incubated once more for 30 minutes or longer, in dark and under room temperature. Later, the absorption of the samples and standards were ready to be quantitatively measured by using the HACH machine at 750 nm wavelength.

### 2.4.3 Determination of Polysaccharide Content

For polysaccharide content determination, phenol-sulphuric acid method was applied. The glucose standard solutions (0, 20, 40, 60, 80 and 100 mg/L) was prepared from 100 mg/L of glucose stock solution. 0.4 mL of totally

thawed samples solution from heat extraction method (Section 2.4.1) was transferred into 10 mL of glass test tubes in triplicates. Then, 0.4 mL of 5% phenol solution and 2 mL of concentrated (95 – 97%) sulphuric acid were added into each of the test tubes. The samples were mixed well and incubated for 30 minutes. Lastly, the absorption of the samples and standards were measured at 490 nm wavelength using the HACH machine.

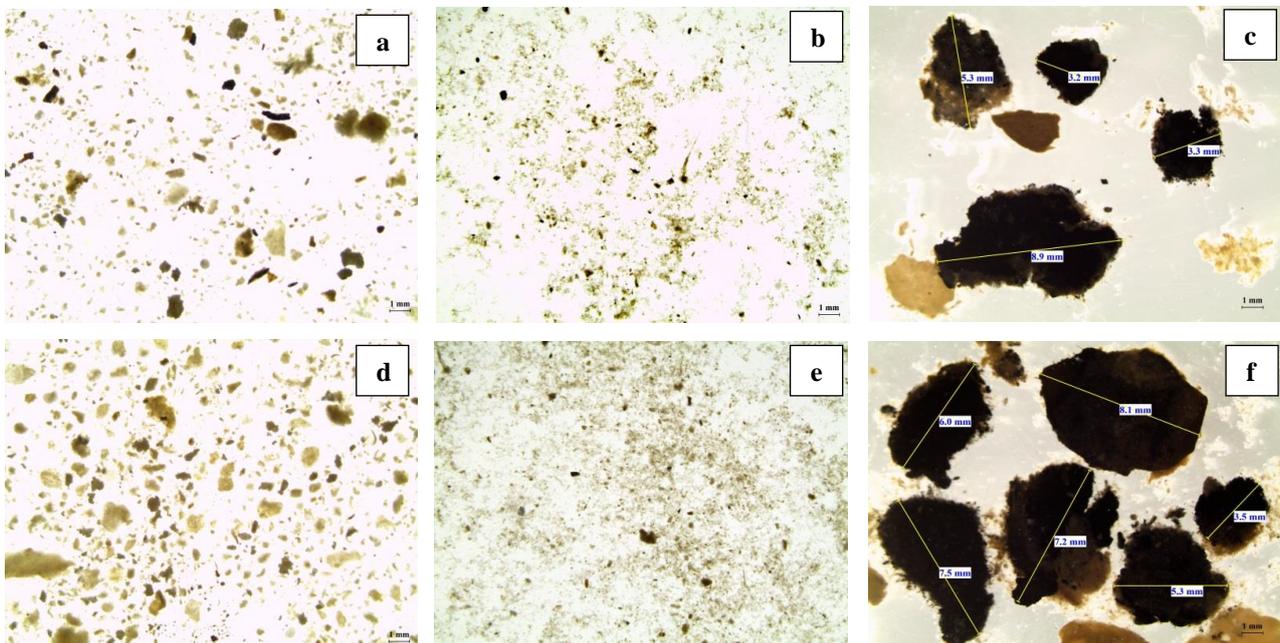
### 2.4.4 Determination of Carbohydrate Content

The different concentration of glucose standard solutions (0, 20, 40, 60, 80 and 100 mg/L) was prepared from 100 mg/L of glucose stock solution. The samples from heat extraction method were taken out from -20°C freezer to thaw. In the meantime, the freshly prepared anthron reagent and the 75% sulphuric acid solutions were placed on the ice to chill. 1 mL of sample was transferred into the glass test tube in triplicates. The samples then were placed on ice to chill. 2 mL of already chilled 75% sulphuric acid solution was added into each of the glass test tube. Briefly cap and vortex the samples to mix. Then, 4 mL of already chilled anthron solution was added into the test tubes and mixed well. The tubes were later placed on the heating block and boil at 100°C for 15 minutes. After 15 minutes, the tubes were cool down to room temperature before ready to be measured. The absorption of the samples and standards was determined quantitatively at 578 nm wavelength using the HACH machine.

## 3. Results and Discussion

### 3.1 Formation and Stability of Granular Sludge

The visual and microscopic observations of granule formation were made a week after inoculating both reactors (Fig. 2(a) and Fig. 2(d)). At this stage, fluffy and irregular structures of sludge particles with an average size of  $0.02 \pm 0.01$  mm were observed. On day 20th, some small granules, spherical in shape and black in colour with average diameter of 1 mm were firstly observed in R1 (Fig. 2(b)), whereas R2 granules are yellow in colour with the same average size (Fig. 2(e)). The developed granules for both reactors composed mainly of loosely clumped sludge which could easily break up into pieces if placed under vigorous shaking [17].



**Fig. 2 - Structural development of granular sludge taken by stereo microscope with magnification of 6.7 x, scale bar of 1 mm**

After 90 days of operation, the formation of granules becoming more dense and compact. The outer layer of matured granules was yellow in colour representing the dominant of aerobic bacteria, while the darker spot within the granules indicating the presence of anaerobic bacteria. The granules that consisted with the combination of aerobic and anaerobic portions are classified as Type B granules as suggested by Linlin et al. [18]. The average particles size of granules in R1 were  $3.2 \pm 0.1$  mm (Fig. 2(c)), whereas  $6.5 \pm 1.0$  mm for R2 (Fig. 2(f)) with maximum size reaching up to 8.9 mm. It can be concluded that sludge cultivated in magnetic field had a positive effect on the sludge granulation especially on the size of the granules.

The microstructure of the microbial granular sludge was further examined by SEM and shown in Fig. 3. The granular sludge morphologies for both reactors were different significantly. The SEM observation on 90th day showed that

bacteria of granules in R2 are dense and compact with non-filamentous cocci bacteria spread on all surface of the granules. Meanwhile, the granules in R1 were dominant by filamentous bacillus bacteria and the structure of granules was loose. This indicated that magnetic field could successfully influence the dominant of the bacteria on the granules as suggested in the research of Wang et al. [2].

As the magnet could attract metals such as iron, the compositions and contents of elements in granules were analyzed by SEM/EDX. As can be seen in Fig. 4, the content of Fe over granule cultivated in magnetic field was 41.8%, compared with only 6.6% of those the control reactor. It was probably due to the presence of Fe compounds in pharmaceutical wastewater which would attach to activate sludge in the reactor. Iron compounds in activated sludge could be magnetized in the presence of magnetic field which then resulted in the enlargement of the floc size by assembling the iron compounds together by the magnetic forces between them [2, 13].

Similarly, the content of divalent metal ions such as  $Ca^{2+}$  and  $Mg^{2+}$  in R2 was higher than in the control reactor (R1). Previous research have indicated that the metal cations such as  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Fe^{2+}$ ,  $Fe^{3+}$  and  $Al^{3+}$  could enhance granulation process by bind to the sludge due to negative charge (zeta potential) of the sludge [13]. Granules with more cations content would have greater densities, which can then explain why granules in R2 hold better settling properties in the whole process (Fig. 5). A short settling time is believe to be beneficial for granulation process as stated in previous studies of Adav et al. [19]. The high settling velocity enabled the granules to be retained in the system without being flushed out during the decanting phase. Therefore, settling time was decreased from 15 min to 5 min on 14<sup>th</sup> day of experiment, and then sludge with poor settling properties in the reactor was washed out.

It can be seen that the average settling velocity of the granular sludge developed in R2 system increased from 8.78 m/h to 92.54 m/h at the end of experiment. The settling velocity in R2 was much higher than the settling velocity of R1 system (76.92 m/h). From these observations, it can be concluded that the use of magnetic field would be a potential alternative as it can accelerate the process of granulation as well as producing more dense and compact granular sludge. Faster development of the granules would improve the treatment system by speeding up the biodegradation or removal process.

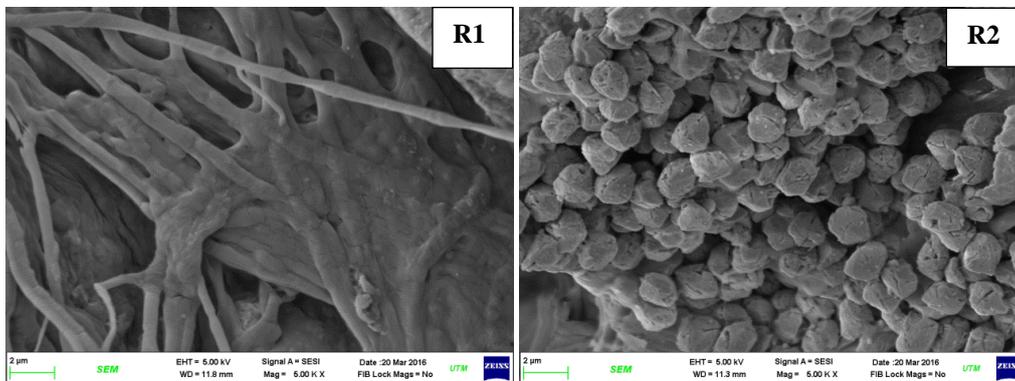


Fig. 3 - Morphology image of mature microbial granular sludge in R1 and R2 at a magnification of 5 K

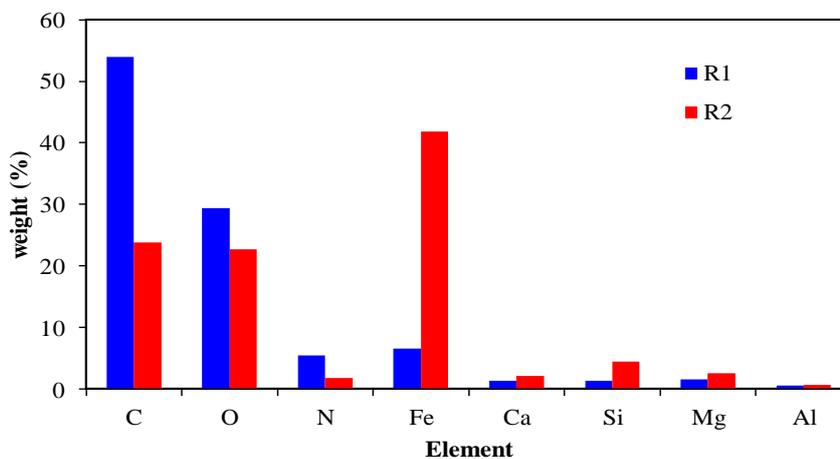


Fig. 4 - Element analysis of granular sludge collected on 90th day in R1 and R2 by SEM/EDX

### 3.2 Reactor Performance and Removal Efficiencies

Fig. 6 displays the performance of two reactors system based on the removal of COD, TP and orthophosphate during the whole operation period. Overall, the removal performance in R2 treatment system was much better than the system without magnetic field. At the beginning of experiment, the percentage removal for COD (Fig. 6(a)) over R1 and R2 was 56% and 64% respectively. The removal efficiency increased to over 90% at the end of the experiment for both reactors. This trend certainly indicates the occurrence of high biological activity in the reactor system. During the first 50<sup>th</sup> day of operation, the removal of COD was fluctuated for both reactors but then became stable for the remaining period. From Fig. 6(b), the R2 treatment system has better removal of total phosphorus in the first 50<sup>th</sup> day operation compared to R1 system. As the flocculent sludge grew and developed into granular sludge (after 50<sup>th</sup> day operation), the total phosphorus removal efficiency improved to between 92% and 99% and this value was approximately similar for both reactors. Meanwhile, the percentage removal of orthophosphate for both reactors showed some fluctuation almost throughout the study period (Fig. 6(c)). The removal was about 45% and 9% for R1 and R2 treatment system during the start-up and increased to approximately 85% and 93% respectively.

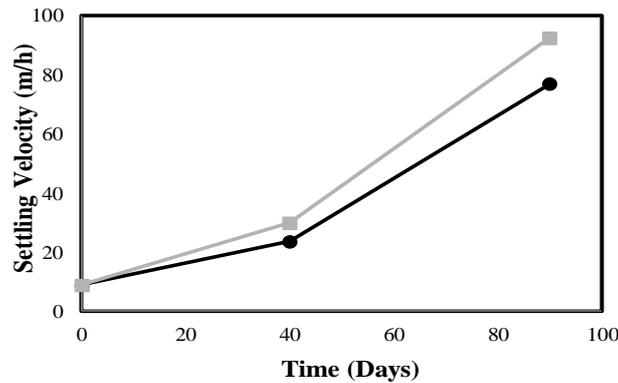


Fig. 5 - Effect of magnetic field on settling velocity. (●) R1 and (■) R2

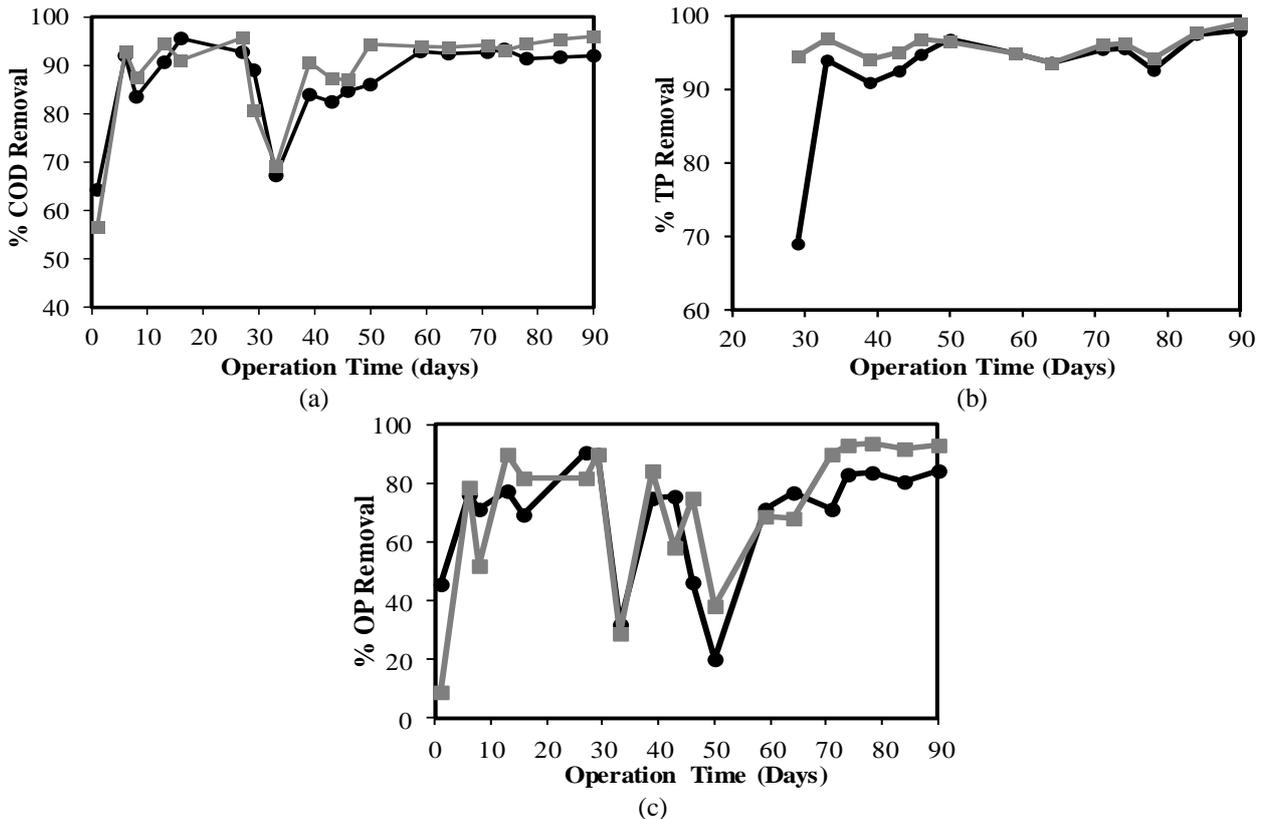


Fig. 6 - Percentage removals of (a) COD, (b) Total phosphorus and (c) Orthophosphate in SBR system. (●) R1 and (■) R2

Fig. 7 depicts the trend of TN, nitrate and nitrite removal performances of the SBR throughout the granulation process. The result of TN removal over R1 and R2 systems in Fig. 7(a) shows some fluctuated between 60% and 99%, with the average removal of 84.16% and 78.77% respectively. During start-up, the percentage removal of nitrite and nitrate were below 60% for both R1 and R2 systems (Fig. 7(b) and Fig. 7(c)). The low percentage could be due to the adaptation of activated sludge with the reactor’s environment as well as experiment conditions. The removal performance however, starts to improve by showing gradual increased to above 80% towards the end of the experiment resulting in good nitrate and nitrite removal efficiencies.

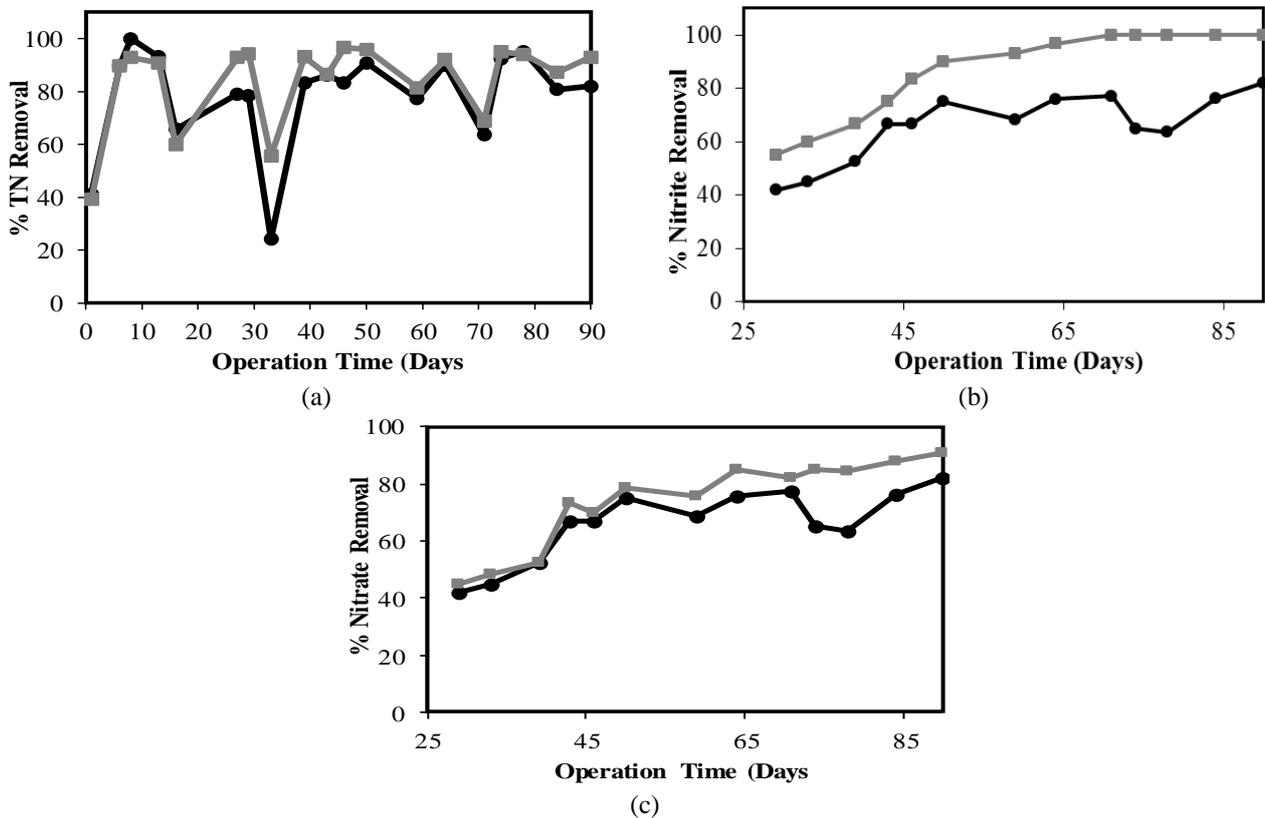


Fig. 7 - Percentage removals of (a) Total nitrogen, (b) Nitrate and (c) Nitrite in SBR system. (●) R1 and (■) R2

As can be seen from Fig. 7, the removal efficiency of magnetized sludge was much better than non-magnetized sludge. This can be explained in terms of the changes of water characteristics that occurred due to the magnetic field which may further influence the metabolic activities of bacteria such as their enzyme activity [20]. This may be the reason for the higher nitrite oxidizing bacteria (NOB) activities which resulting the higher nitrite oxidation rate under magnetic field application. Similar results were also found by Tomaska and Janosz-Rajczyk [21] and Tomaska and Wolny [22]. They reported that the nitrification rate of the magnetized activated sludge was higher than that of the control, and the rate of oxygen uptake by NOB was intensified more significantly.

### 3.3 EPS of Matured Granules

Table 1 summarizes the quantities of EPS extracted from the granule samples in R1 and R2 systems. The quantities of total EPS are the sum of the loosely bound EPS (LB-EPS) and tightly bound EPS. The mean quantities of total EPS for R1 system was 21.7 mg/g VSS, whereas 28.5 mg/g VSS for R2 system. High content of EPS would contribute greatly to the strength and stability of granular sludge [11, 23] .

Table 1 – EPS contents from granular sludge over R1 and R2 systems

	R1 (mg/g VSS)		R2 (mg/g VSS)	
	LB-EPS	TB-EPS	LB-EPS	TB-EPS
Proteins	73.6	75.1	104.5	110.3
Carbohydrates	22.7	34.5	39.3	61.2
Polysaccharides	7.5	13.8	10.5	14.0
Total EPS	21.7		28.5	

The EPS of granular sludge in R1 system was consisted with 73.6-75.1 mg proteins/g VSS, 7.5-13.8 mg polysaccharides /g VSS and 22.7-34.5 mg carbohydrates /g VSS. Granular sludge extracted from R2 system had greater quantity of EPS than R1 system: 104.5-110.3 mg proteins/g VSS, 10.5-14.0 mg polysaccharides /g VSS and 39.3-61.2 mg carbohydrates /g VSS. From the results obtained for both reactors, it can be concluded that the induction of magnetic field could influence the content of EPS by stimulating bacteria to produce more protein, carbohydrate and polysaccharide in granular sludge.

The higher protein content in granular sludge over R2 system is probably due to higher  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentrations (Fig. 4) as reported by previous research of Higgins and Navok [24] and Sheng et al. [25]. They found that the protein content in the sludge EPS increased at higher  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  concentrations and that higher  $\text{Na}^{2+}$  concentration led to a lower protein content. With the increasing concentration of Fe in the reactor with static magnetic field, the EPS characteristics and compositions could also be altered by this metal concentration.

According to Liao et al. [26] and Wilén et al. [27], the protein has the biggest influence on the surface properties and flocculating ability of the sludge flocs and had relatively strong positive correlations with negative surface charge and hydrophobicity of microbial flocs. High carbohydrates and polysaccharides content could also provide better bioflocculation which is attributed to the polymeric interactions that similar to bacterial adhesion to the cell surfaces (Badireddy et al. [28]; Pei et al. [29]). Thus, it showed that magnetic field influent the microbial activity during development of biogranules which is high EPS was produced in R2 system and enhances the development of biogranules.

#### 4. Conclusion

The induction of magnetic field was more effective in granulation process than that of control system without magnetic field. Matured, compact and dense granular sludge with diameter  $6.5 \pm 1.0$  mm with maximum size reaching up to 8.9 mm was successfully developed after an operation period of 90 days in SBR system under aerobic and anaerobic conditions fed with pharmaceutical wastewater. It was observed that the use of static magnetic field could improve settleability of granular sludge to 76.92 m/h and stimulated EPS production by accumulating iron, calcium and magnesium compounds in the sludge. The removal efficiencies of COD, total phosphorus, orthophosphate, total nitrogen, nitrate and nitrite in treating wastewater were at over 90%.

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