Improved biological wastewater treatment and sludge characteristics by applying magnetic field to aerobic granules

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Abstract: Permanent magnets with non-uniform magnetic field and an electromagnet with 3–5 mT uniform magnetic field were applied to investigate their effects on both aerobic granulation and COD and ammonium removal in reactors with less than 7% coverage of magnetic field. It was found that both types of magnets had little influence on the granulation speed and the settling ability of granular sludge at the steady state. However, the maximum specific COD degradation rate and the maximum specific NH_4^+-N removal rate were increased by 45–54% and 30–50%, respectively, in the magnetic fields. Mean effluent COD with the electromagnet and the permanent magnet field, respectively, at the steady state, was 28 mg l^{-1} and 6 mg l^{-1}, respectively, lower than the control at a statistical significance level of alpha = 0.05. No statistically significant increase in NH_4^+-N removal was observed at the steady state probably due to almost complete NH_4^+-N removal before the end of the cycle. In addition, it was found that extracellular polymeric substances in granular sludge with electromagnet were 77% more while soluble microbial products were much less compared with the control, suggesting a positively changed metabolism of granular sludge at steady state. The results in this study indicated that low-intensity magnetic field has a great potential to be applied in granular sludge for an improved wastewater treatment.

Keywords: waste-water treatment; biodegradation; heterotrophs; aggregation; aerobic granules; magnetic field

1. Introduction

Biological effects of magnetic field have attracted great attention from the 20th century. Although the mechanism of how magnetic field changes enzyme activity, metabolism, bacteria growth, i.e. so-called magnetic bio-effects, is not clear, the effects of magnetic field on enzyme and bacteria have been observed in many cases. So far, biological effects of magnetic field have been used to improve the growth of plants [1], enhance the growth and activity of bacteria [2], increase the accumulation of fermentation products [3,4] and lipid production in biomass with municipal wastewater [34], and fasten substrate degradation rate [5]. Apart from the application of magnetic field to a taxonomic single species [6,7], magnetic field was also employed to mixed culture such as activated sludge to promote sludge sedimentation or improve wastewater treatment performance. Magnetic field could be exerted to biological wastewater treatment by either adding magnetic powders or applying an external magnetic field [8]. It has been reported that static magnetic field of 30 and 50 mT increased ammonium oxidation rate in municipal wastewater by up to 77% and stimulated the growth of ammonia oxidisers [9]. A dynamic magnetic field enhanced the chromium bioreduction by sludge significantly compared with the control [10]. By recirculating activated sludge through a static magnetic field of 80–130 mT with a rate of 0.33–1 L/min, both sludge volume index (SVI) and effluent chemical oxygen demand (COD) were always lower than the control without exposure to magnetic field [11]. When a lower magnetic strength such as 40 mT was used, no increased COD was observed by periodically exposing sludge to static magnetic field but nitrogen removal was improved compared with the control unit [12]. Given the enhanced role for sludge sedimentation [11], magnetic field was employed to fasten nitrifying graduation, which was shortened from 41 to 25 days [13].

So far, although there are quite few of reports about the enhancement of wastewater treatment by exposing sludge to magnetic field, most research focused on activated sludge. In addition, nitrogen removal or nitrifying sludge sedimentation enhancement by magnetic field was relatively confirmed [13], but reports on COD removal improvement were still limited. Given the rapid development of aerobic granule technology, it is necessary to investigate if magnetic field has similar influence on aerobic granulation and substrate degradation. In addition, it is still unclear if lower magnetic intensity such as down to 3–5 mT still could play a positive role to granular sludge. Therefore, this paper aims to study effects of external magnetic fields with low intensity and low reactor coverage on hybrid granulation with both nitrifying bacteria and heterotrophs for COD and nitrogen removal. Meanwhile, the types and intensity of external magnetic fields in a lower range were also investigated.

2. Materials and Methods

2.1. Reactor operation

Three bubble columns were used to cultivate aerobic granules with an internal reactor diameter of 6.5 cm and working volume of 2.5 L. Fine air bubbles for aeration were supplied through an air sparger at the reactor bottom with an upflow air velocity of 2.5 cm s^{-1} to all reactors. The reactors were operated at ambient temperature under the sequential batch mode with 5-min feeding, 200–225min aeration, 5–30min settling and 5min discharging within 4-hour cycle. The settling time was set at 30 min initially to start up the reactors, and then gradually decreased to 5 min with the improvement of biomass settling ability, and maintained at 5 min at the steady state as reported by Kong et al [14].

12 pairs of circular disc magnets with size of 10mm × 8mm were applied to reactor 2 (R2), which were scattered at the bottom of reactor with three layers of arrangement as shown in Figure 1. The intensity of magnetic field at each layer was around 30 mT but the intensity between layers was reduced dramatically, which created a discontinuous and uneven distribution of magnetic field. 1mm copper wire connecting to DC power supply was wrapped to the bottom part of reactor 3 (R3) to

generate a uniform magnetic field with the intensity of 3–5mT. In this way, the reactor volume coverage by magnetic field in R3 was 7% while it was less than 7% in R2 due to the discontinuity. Reactor 1 (R1) without magnetic field was used as the control. The specific information of magnets was listed in Table 1.

Activated sludge collected from a local municipal wastewater treatment plant without nitrification capability was evenly inoculated into three reactors after 1-week of acclimatization with the synthetic wastewater. The initial seed sludge in three reactors had a biomass concentration of 2.41 g l^{-1} and a SVI value of 236 ml g^{-1}.

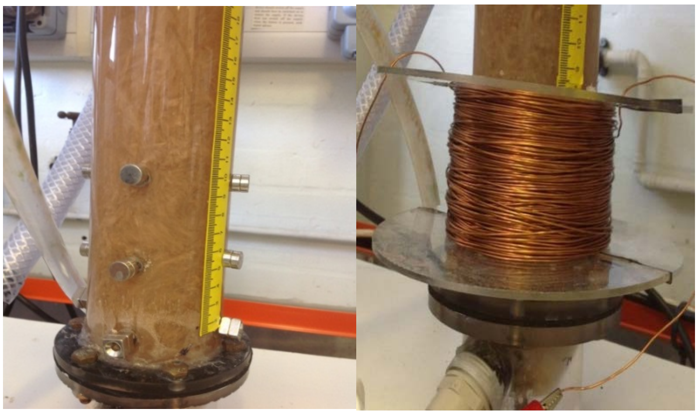

Figure 1. The installation of permanent magnet to reactor R2 and electromagnet to reactor R3.

Table 1. Magnets applied to reactors and their parameters.

	Magnet type	**Intensity of magnetic field (mT)**	**Coverage volume (%)**
Reactor (R1)	None	0	0
Reactor (R2)	Permanent magnets (12 pairs of circular disc magnets with size of 10mm × 8mm)	30 on layer (Non-uniform magnetic field)	Discontinuous Less than 7%
Reactor (R3)	Electromagnet	3-5 (Uniform magnetic field)	7%

2.2. Medium

The synthetic wastewater, fed into the reactor by peristaltic pumps, consisted of: COD (sodium acetate) 700–1000 mg l^{-1}, NH_4Cl 80–200 mg l^{-1}, K_2HPO_4 22.5 mg l^{-1}, $CaCl_2 \cdot 2H_2O$ 15 mg l^{-1}, $MgSO_4 \cdot 7H_2O$ 12.5 mg l^{-1}, $FeSO_4 \cdot 7H_2O$ 10 mg l^{-1} and trace elements with the same concentrations as reported by Chen et al. [15]. The organic loading rate of three reactors was controlled at around 2.1–3 g COD l^{-1} d^{-1}.

2.3. Analytical procedures

COD, SVI, and biomass dry weight (MLSS and MLVSS) were analyzed in accordance to the standard methods [16]. Morphology of sludge was observed and photos were taken with Olympus SZX9 microscope (Olympus SZX9, Tokyo, Japan). Capillary suction time (CST) of sludge was measured using a Triton-WRPL type 130 single CST, a Triton type 319 Multi CST apparatus and paper (Triton Electronics Ltd, UK).

For extraction of extracellular polymeric substances (EPS) of granules, 10 ml of granule sample from the reactor was centrifuged at 4000 g in a 15 ml centrifuge tube and washed with distilled water twice. The remaining pellet was grinded in a ceramic mortar carefully to a state with an average particle size from 100 to 200 µm, which was then transferred to a clean centrifuge tube and topped up to 10 ml. This sample was kept at 4 °C for 1 hour after adding 12 µl of formaldehyde and another 3 hours after adding 800 µl of 1 M NaOH. After that, the sample was centrifuged at 13, 200 g for 20 min. Protein (PN) of the extracted EPS supernatent was measured with Bradford regent at a wavelength of 595 nm according to Bradford Method [17], and polysaccharide (PS) of supernatant was assayed with 80% phenol and 98% sulphuric acid at a wavelength 490 nm suggested by Dubois [18].

3. Results and Discussion

3.1. Granulation of suspended sludge in magnetic fields

SVI is usually used to indicate the settling ability of sludge. As shown in Figure 2, during the first week of the operation, SVI decreased sharply from 236 ml g^{-1} to around 100 ml g^{-1} in R1 without magnet and R2 with the permanent magnet and then gradually dropped further until around 50 ml g^{-1} on day 35. However, SVI in R3 with the electromagnet fluctuated from 100 ml g^{-1} to 300 ml g^{-1} during the first 3 weeks and then reduced to 50 ml g^{-1} on day 35. Based on SVI and microscopy observation shown in Figure 3, the reactor operation was separated into two phases, i.e. granulation period before day 35 during which activated sludge was converted into granular sludge and steady state after day 35 during which mature granules were dominant. Obviously, the permanent magnet in R2 had no obvious influence on sludge settling ability while the electromagnet in R3 played a negative role during granulation period. However, the sludge morphology observed under microscope as shown in Figure 3 did not show much difference among three reactors. Tiny granules appeared in all three reactors within the first week although the settling velocity of sludge in R3 was quite poor. By measuring SVI and observing sludge morphology during the granulation period, no direct link could be found between the initial granulation and sludge settling ability although the settling ability of sludge usually improves with granulation. After 35–day operation, magnetic field did not show any influence on sludge settling ability, which is mainly the function of SVI, mean size of granules and biomass concentration of granules [31]. MLVSS/MLSS of sludge in three reactors were at around 70% at steady state indicating that magnetic field applied in this study had no effects on the precipitation of metal compounds or accumulation of inorganic compounds in granular sludge. Magnetic field could enlarge floc size [11], settling ability of sludge [19] or enhance aggregation of nitrifying bacteria for better sludge sedimentation [13]. It was reported that the surface zeta potentials of the sludge were not changed in the magnetic field while that addition of

$FeCl_3$ to the sludge enhanced the effects of the external magnetic field of 80 mT [11]. It was thus believed that activated sludge containing iron was magnetized and coagulated in the magnetic field leading to bigger floc size and better sedimentation. The similar strategy was adopted to enhance nitrifying granulation by Wang et al. [13], who reported that the aggregation of iron decreased the full granulation time from 41 to 25 days by enhancing the setting properties of granules in a static magnetic field with an intensity of 48.0 mT. Therefore, the effects of magnetic field on better sedimentation of sludge are based on the paramagnetic characteristics of iron in water although this explanation is not very convincing. The same phenomenon had been expected in this study, however, the granulation of sludge was not increased by magnetic field. In addition, the settling ability of granules in R3 with electromagnetic field was even worse during the granulation period as shown in Figure 2. By comparison, it could be known that Fe^{2+} was added in this study with a concentration of only 2 mg l^{-1} while 15 mg l^{-1} Fe^{2+} was employed by Wang et al. [13]. In addition, the coverage volume of reactor by magnetic field in this study was less than 7% while the reactor was 100% covered by permanent magnetic field by Wang et al.[13] and 4 g l^{-1} magnetic particles were added in the reactor [19]. The magnetic field strength used by Wong et al. [13] and Hatorri et a. [11] were 48 mT and 80–300 mT, respectively, which was much higher than 30 mT and 3–5 mT employed in this study. Therefore, the contradictory results of this study with Wong et al. [13] and Hatorri et al. [11] indicate that the effects of magnetic field on sedimentation may be closely related with wastewater quality with different iron concentrations, magnetic field strength, magnet types as well as reactor operation conditions. Therefore, the careful optimization has to be carried out to the specific reactor setup fed with a specific wastewater to get the positive effects of magnetic field on the enhancement of settling ability of sludge or sludge granulation. This causes a great uncertainty to apply magnetic field to enhance sludge settling ability. Recently, however, a report about aerobic granulation within 24 hours [20] provides a novel strategy to enhance formation of aerobic granules without any external assistance such as magnetic field. Thus, magnetic field does not have special benefit to enhance aerobic granulation in terms of simplicity and capital cost compared with other strategies.

3.2. Reactor performance in magnetic fields

The performance in three reactors was shown in Figure 4. During the granulation period, effluent COD was almost same in three reactors. The distinct difference, however, appeared after the complete granulation. The effluent COD in R3 was statistically significantly lower than that in R1 and R2 after 35 days. For example, mean effluent COD in R1 was stable at 57 mg l^{-1} in R1 and 51 mg l^{-1} in R2 at steady state but it was only 28 mg l^{-1} in R3 from day 35 to 50, resulting in 97–98% COD removal efficiency in R3 while 95% in R1 and R2. This difference in effluent COD concentration between control (R1) and R2 with permanent magnet, and control (R1) and R3 with

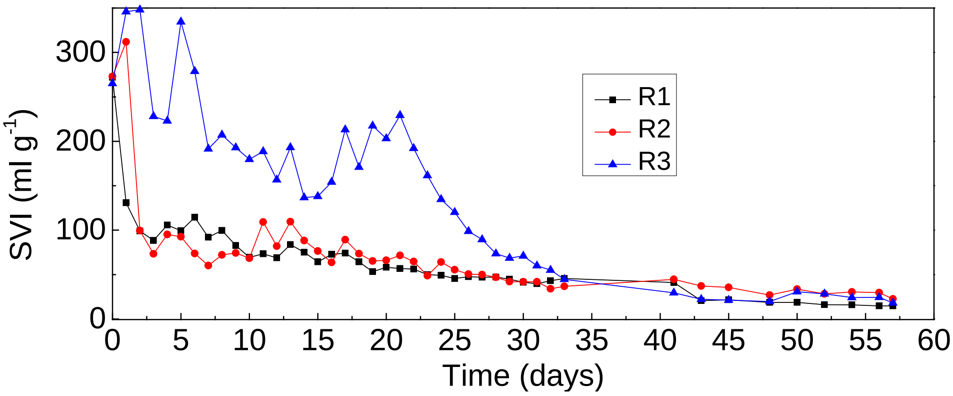

Figure 2. SVI over the operation time in three reactors.

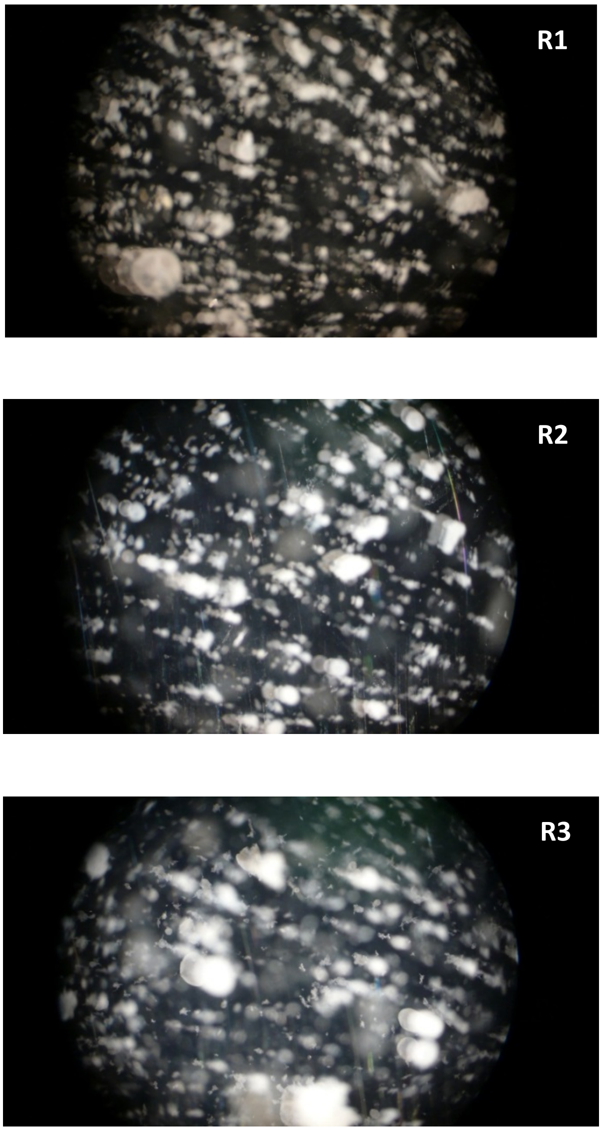

Figure 3. The morphology of granular sludge in three reactors on day 35.

electromagnet is statistically significant at confidence level of 95%. From day 55 to 60, the reactors were fed with lower COD concentration followed by higher COD concentration, which resulted in a fluctuation of effluent COD in three reactors. But effluent COD in R3 was always statistically lower than that in R1 and R2.

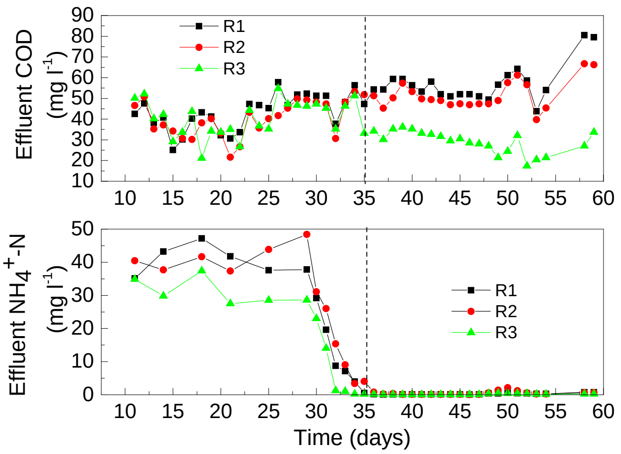

Figure 4 Reactor performances over the operational period in three reactors.

Effluent NH_4^+-N was measured only from operation day 10. During the first 30 days, sludge in three reactors did not have good ammonium oxidation capability as nitrifying bacteria were not enriched well. But mean effluent NH_4^+-N concentration in R3 was 31 mg l^{-1} while it was 40–41 mg l^{-1} in R1 and R2. The effluent NH_4^+-N concentration in R3 was statistically significantly lower than R1 and R2 at a significance level of alpha = 0.05. It seems that electromagnet played a weak but positive role for ammonium oxidation during the enrichment period of nitrifying bacteria. From day 30 to 35, effluent NH_4^+-N concentration dropped dramatically from 30–40 mg l^{-1} to below 0.1 mg l^{-1} in all reactors. This phenomenon was highly in agreement with the result reported by Liu et al. [21] that NH_4^+-N removal was improved significantly after the granulation. Therefore, the effective retention of sludge by an improved settling ability is crucial for the enrichment of nitrifying bacteria, suggesting granulation is important for nitrification in hybrid granular sludge reactor. Since effluent NH_4^+-N concentration in all reactors were lower than 1 mg l^{-1} , no statistically significant difference in effluent NH_4^+-N concentration could be observed after day 35.

Since the reactors were operated in SBR mode, COD and NH_4^+-N were degraded to the minimum levels within 1 and 3 hours for a 4-hour cycle, respectively, at steady state, the total removal efficiency is mainly decided by the effluent COD and NH_4^+-N concentrations. To compare the degradation rates of COD and NH_4^+-N in batch operation, cycle analysis of COD and NH_4^+-N removal in three reactors at steady state were conducted and shown in Figure 5. The maximum specific COD degradation rate and the maximum specific $NH4^+$-N removal rate in magnetic fields were increased by 45–54% and 30–50%, respectively, indicating an significant improvement of both COD and NH_4^+-N degradation rates by exposing granular sludge to either permanent magnet field or electromagnet field . This result was in highly agreement with those reported for formaldehyde degradation with a magnetic field strength of 7 mT [22], ammonium oxidation by activated sludge at

30–50 mT [9], glucose degradation with 17.8 mT [23]. It can be seen that degradation rates of different types of substrates could be improved by magnetic field.

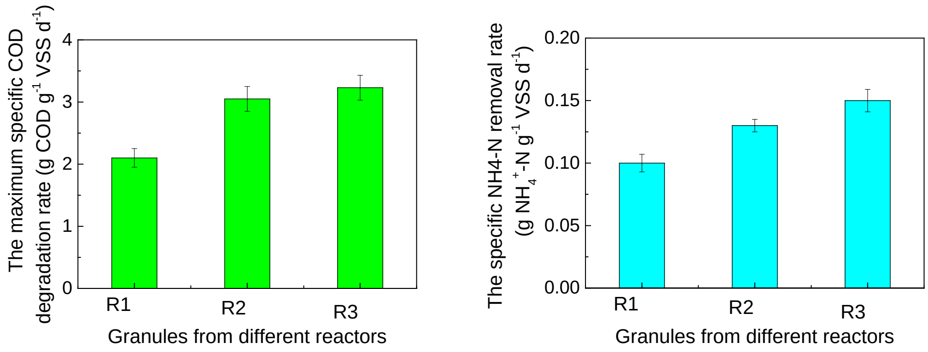

Figure 5. The maximum specific COD and NH_4^+-N degradation rates of granular sludge from three reactors at the steady state.

Increased substrate degradation rate by suspended sludge was observed by Yavuz and Celebi [23] in an electromagnetic field of 17.8 mT, 30-minute induction in a 10.0 T magnetic field by Hu et al. [24], by Lebkowska et al. [5] in a 7 mT permanent magnet and by Kriklavova et al. in a 370 mT static magnetic field [6]. Tomska and Wolny [12] found that periodic exposure of activated sludge to a 40 mT permanent magnetic field did not affect COD removal but improve nitrifying bacteria activity. Based on literature and the results in this study, bacteria activity in both suspended and granular sludge could be enhanced by magnetic field, which were not highly dependent on the types of magnet fields, strength of magnetic field, reactor volume covered and sludge exposure time to magnetic field although a certain optimal conditions still exist to get the maximum improvement. This allows a more practical and flexible application of magnetic field to wastewater treatment as sludge could pass through a magnetic field mounted on pipe for sludge recirculation instead of installing magnet on the entire reactor or tank, which could reduce the cost incurred by magnets significantly. Deeper understanding of positive effects of magnetic field on sludge bacteria activity was rarely investigated due to the nature of mixed sludge culture with different species. A bit of evidence shows that enzyme activity was enhanced by magnetic field leading to improved substrate degradation rate [25,26]. Magnets could thus be expected to be a quite useful tool to enhance bacteria activity and substrate degradation rates especially when the bacteria activity is reduced significantly under some circumstances such as low temperature or presence of toxic compounds.

3.3 Characteristic of granular sludge in magnetic field

Granular sludge on operation day 50 was sampled and analysed for its extracellular polymeric substances (EPS). Figure 6a shows that extracellular protein extracted from sludge in R1 and R2 was around 5 mg g^{-1} VSS while it was around 9 mg g^{-1} VSS from granular sludge in R3. Extracellular polysaccharide of sludge from three reactors followed the similar trend, which was much higher in sludge from R3 compared with R1 and R2. This leads to a 77% higher total EPS content in granular sludge from R3 than that from R1 and R2 with EPS of 53.4 mg g^{-1} VSS in R3 and 30.7 mg g^{-1} VSS and 29.8 mg g^{-1} VSS in R1 and R2, respectively. This result is very interesting. Although the physical properties of granular sludge in three reactors at the steady state seem similar in terms of sludge settling velocity and granule morphology, the chemical properties with regard to EPS have shown distinct difference. In addition, it was noticed from Figure 6b that protein/polysaccharide (PN/PS) of granular sludge at steady state in different reactors were not significantly different after considering error propagation from PN and PS, but it is higher than that in activated sludge or sludge with magnetic particles inside [19]. Recently, it has been reported that aerobic granular sludge could be used to produce sizing agents by extracting extracellular polymers from aerobic granular sludge [32]. Recovering resources from sludge is a critical step towards to circular economy. In such situation, magnetic field could be a potential assistant tool to further improve production of extracellular polymers in aerobic granules based on the enhanced EPS production results shown in Figure 6 by weak electromagnet.

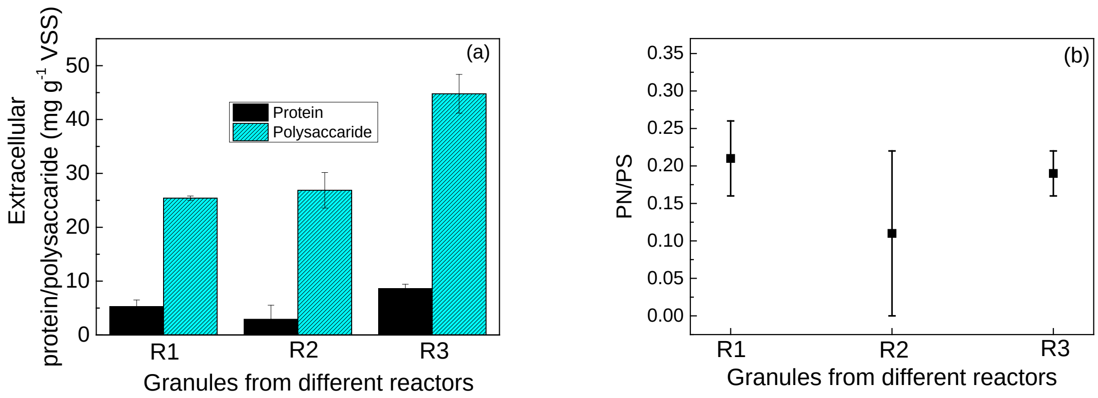

Figure 6. Contents of polysaccharide and protein, and PN/PS in granular sludge from three reactors on operation day 50.

It has been generally believed that EPS in sludge might play a negative role for sludge dewaterability, which means that higher EPS in sludge could lead to poorer dewaterability due to the highly hydrated nature of EPS [27]. However, CST values of sludge at the steady state from three reactors ranged within 9–10s, suggesting little difference of sludge dewaterability although they contained different EPS contents. Obviously, total EPS content is not the decisive factor to affect the dewaterability of aerobic granules. For example, EPS content in granular sludge is usually much higher than activated sludge as EPS provides matrixes to allow bacteria to aggregate compactly to from dense structure of granules [28]. However, dewaterability of aerobic granules is much better than activated sludge as reported by Zhou et al [29], who suggested that the structural characteristics, distribution features of extracellular polymeric substances and PN/PS values of aerobic granular sludge were important factors for excellent dewaterability of aerobic granules.

The most striking effect of electromagnetic field in this study is reactor's performance at steady state. Effluent COD concentration in the electromagnetic field is distinctly lower than both control and permanent magnetic field. Meanwhile, it was observed that EPS in granular sludge from R3 is 77% higher than that in R1 and R2. Much higher EPS secretion by nitrifying granules in a 48 mT magnetic field was observed too by Wang et al. and Lan et al. [13,19]. Since only acetate, 100% biodegradable substrate, was used as carbon source in this study, it was quite reasonable to speculate that effluent COD mainly comprised soluble microbial products (SMP), which are currently regarded as ‘the pool of organic compounds that are released into solution from substrate metabolism and biomass decay’ [33]. SMP have been found to comprise the majority of soluble organic material in the effluents from biological treatment processes and their presence is, therefore, of particular interest in terms of achieving discharge consent levels for BOD and COD [33]. Although SMP production is related to reactor type, substrate type, and operational conditions [33], the only difference for three reactors in this study is the application of magnetic field. The lower effluent COD (i.e. lower SMP production in this study) and the higher EPS in granular sludge in R3 suggested reasonably that metabolisms of bacteria in sludge were affected in the electromagnetic field. The substrate was more directed to synthesize EPS located in sludge instead of SMP released into the solution. This result is quite important. On one hand, the quality of treated water is much improved due to reduced SMP. On the other hand, it might reduce some operational problems such as membrane fouling in membrane bioreactors when membrane technology is combined as SMP is the mostly reported factor causing membrane fouling [30].

Although both permanent magnets and electromagnet used in this study are static magnets, the permanent magnets in this study create non-uniform and discrete magnetic field with a higher magnetic field strength at the magnet layer while electromagnet has a very uniform magnetic field at the bottom of R3. In addition, the direction of magnetic field is vertical in R3 and it is horizontal in R2. But the intensive and chaotic turbulence of fluid at the reactor bottom might weaken the effect from magnetic field direction. Therefore, it is believed that the main difference with regard to effects of two types of magnets on effluent COD and EPS in granular sludge in this study is mainly because of the magnetic field intensity, magnetic field coverage to reactor volume and uniformity of magnetic field.

4. Conclusions

Formation of aerobic granules was not enhanced by employing magnetic field with intensity up to 30 mT and a magnetic field coverage less than 7% of the reactor working volume. However, both COD and ammonium nitrogen degradation rates at the steady state were increased significantly by applying magnetic field intensity down to 3–5 mT. Even with batch cycle operation including starvation period, COD removal efficiency was statistically significantly improved, which was likely due to the metabolic change of sludge in magnetic field by producing more EPS in granular sludge instead of SMP. Therefore, the results shown in this study indicated that the magnetic field with low intensity and small reactor coverage has great potential to be used to aerobic granular sludge at steady state for an improved wastewater treatment. EPS and SMP production in magnetic field by aerobic granular sludge needs further investigation to understand the mechanism behind as well as their potential impact on maintaining the long-term physical stability of aerobic granules and sludge dewaterability.

Acknowledgement

This research was financially supported by Civil, Maritime, Environmental Engineering and Science Academic Unit, Faculty of Engineering and Science, University of Southampton, as primepumping project. In addition, Dr. Dominic Mann and Mr. Simone Cinquepalmi were appreciated for the fabrication of reactors, timer design and reactor set-up.

Conflict of Interest

The authors declare no conflict of interest.

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