International Journal of Environmental Science and Technology (2019) 16:3801–3808
https://doi.org/10.1007/s13762-018-1897-2

ORIGINAL PAPER

Effects of extra-cellular polymeric substances towards physical properties of biomass under magnetic field exposure

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Received: 7 September 2017 / Revised: 17 May 2018 / Accepted: 6 July 2018 / Published online: 11 July 2018

Abstract

Extra-cellular polymeric substances are responsible towards bioflocculation and settleability of sludge. This substances act as glue that holds the bioflocs to form larger aggregates by intervening both cells cohesion and adhesion. Different composition of this substances lead to different circumstances of sludge problem including bulking. Numerous studies have been conducted in optimizing the content of extra-cellular polymeric substances using various approaches. However, none of the approaches investigated on the use of magnetic field. Therefore, this study is aimed to investigate the effect of magnetic field on extra-cellular polymeric substances specifically its soluble-bound composition and its chemical constituents. Magnetic field of 88 mT was used in Reactor A, while Reactor B acted as control system. Results showed that Reactor A obtained mostly higher tightly bound extra-cellular polymeric substances compared to Reactor B in order of predominance: protein > polysaccharides > carbohydrates. These observations implied that magnetic field influenced the production of polymeric substances mainly its tightly bound constituent as well as protein and polysaccharides content. These productions were significant in improving bioflocculation and sludge settleability, thus minimizing potential occurrence of bulking.

Keywords Magnetic field · Sludge bulking · Tightly bound extra-cellular polymeric substances · Protein · Bioflocculation

Introduction

Extra-cellular polymeric substances (EPS) are metabolic products excreted by microorganisms in the form of sticky substances (Liu et al. 2004). EPS mainly consist of carbohydrate, polysaccharide, protein and humic acid, together with small quantities of deoxyribonucleic acid (DNA) and uronic acid (Higgins and Novak 1997; Liu and Fang 2002; Wang et al. 2005). EPS are present both outside of cells and in the interior of microbial aggregates. The forms of EPS that subsist outside of cells can be categorized into bound EPS and soluble EPS (Nielsen and Jahn 1999; Laspidou and Rittmann 2002; Sheng et al. 2010). The structure of bound EPS can then be divided into two layers (Nielsen and Jahn 1999). The inner layer consists of tightly bound EPS (TB-EPS), which is bound tightly and stably with the cell surface, while the outer layer, which consists of loosely bound EPS (LB-EPS), comprised a loose and dispersible slime layer.

Generally, EPS act as glue that holds the bioflocs to form larger aggregates by intervening both cells cohesion and adhesion (Higgins and Novak 1997). The aggregates combine through several binding interaction such as hydrogen bonding, hydrophobic and ionic interactions, and protein–polysaccharide interactions. The protein–polysaccharide interactions can either improve stability or lead to macroscopic destabilization. Aggregated structures can be formed as the polysaccharide molecules interact with proteins and adsorb to more than one colloidal particle. This interaction causes protein particles to become closer to one another. Associate interactions occur because of the electrostatic attraction between oppositely charged portions of proteins and polysaccharides. Additionally, hydrogen bonding

Editorial responsibility: M. Abbaspour.

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and hydrophobic interactions play part in the stabilization of the complexes formed (Corredig et al. 2011).

Content of EPS can be associated with settleability problem such as sludge bulking. Soluble EPS are the components that have weak capacity to bind the floc structure. This type of EPS always corresponds with high sludge volume index (SVI) and suspended solids in the wastewater treatment systems (Jin et al. 2004; Sheng et al. 2006; Yang and Li 2009). Consequently, highly soluble EPS may create a great chance for the sludge bulking to occur. In order to minimize the occurrence of sludge bulking, various approaches have been done, mostly by adding synthetic polymers, metal salts, chlorine, and others (Xie et al. 2007; De Gregorio et al. 2010; Wang et al. 2010; Li et al. 2011). Wang et al. (2010) reported by adding nickel ions to the bulked sludge, the SVI was greatly reduced. That nickel addition has led to low contents of EPS priory its soluble EPS, thus indicating that normal metabolism of bacteria has been inhibited. This had thus reduced the secretion of EPS and eventually minimized the occurrence of sludge bulking in the treatment system.

Application of magnetic field is also seemed to be potential in minimizing the sludge bulking. To date, there is lack of information on the effect of magnetic field in inhibiting sludge bulking by affecting the composition of EPS in the sludge biomass. According to Barnothy (2013), implementation of magnetic field had possibly initiated a triggering process which releases or redirects ordinary metabolic energy to produce certain biological effects. The effects are inclusive of the protein–polysaccharide formation. For the protein–polysaccharide interactions to be produced, the binding site of the ion-binding protein is unoccupied initially. A polysaccharide ion, therefore, occupies the binding site, causing the protein to undergo a conformational change and form active protein–polysaccharide interactions (Male 1992; Belyavskaya 2004). Therefore, this study is conducted in order to investigate the possibility effect of magnetic field towards EPS specifically in terms of its composition (soluble/bound) and its chemical constituents such as protein, polysaccharides and carbohydrates. This study is conducted at Environmental Laboratory, Faculty of Civil Engineering, Universiti Teknologi Malaysia (UTM) and completed on January 2017.

Materials and methods

Experimental set-up and operational conditions

Two laboratory-scale sequencing batch reactors—Reactor A (SBR_A) and Reactor B (SBR_B)—were designed with a working volume of 6 L. SBR_A was equipped with the magnetic device, while SBR_B acted as a control system. Magnetic device attached to SBR_A comprised series of permanent magnets that arranged in an alternate order. Each permanent magnet was a square prism with two faces of 100 mm × 50 mm and a thickness of 5 mm. The stationary magnetic field was exhibited by neodymium–iron–boron (NdFeB–N42) permanent magnets manufactured by Ningbo Newland International Trade Co., Ltd, China. The applied magnetic field was at intensity of about 88.0 mT.

Both SBR_A and SBR_B were operated in parallel with hydraulic retention time (HRT) of 8h in three successive cycles. Each cycle comprised of 10-min filling, 380-min reaction, 80-min settling and 10-min decanting. An average organic loading rate (OLR) throughout the operation was 27.7 kg COD/m^3/day. The volumetric exchange rate (VER) was fixed at 50%. Both reactors were inoculated with activated sludge from a municipal wastewater treatment plant and fed with raw domestic wastewater from the same treatment plant. The compositions of the influent feed are shown in Table 1. In order to study the effect of magnetic field on the production of EPS under sludge bulking condition, both reactors were set at dissolved oxygen (DO) concentration of below than 2 mg/L.

Analytical methods

Extraction of extra-cellular polymeric substances (EPS)

A modified heat extraction method was used to extract the loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) from the sludge (Chen et al. 2013). A 8-mL sample of mixed sludge, each drawn from SBR_A and SBR_B was filled in a 50-mL centrifuge tube for the dewatering process. The process involved centrifugation at 3000 rpm for 5 min. The water was drained, and the sludge was dissolved with distilled water to the original volume of 8 mL. The sample was then heated in a water bath at 80 °C for 10 min, and further centrifuged at 3000 rpm for 5 min. The supernatant obtained was considered to be the LB-EPS. Meanwhile, the sludge left in the centrifuge tube was redissolved with distilled water to original volume of 8 L and then centrifuged at 3000 rpm for 5 min. The water was drained and the sludge

Table 1 Characteristics of influent/feed wastewater during the experimental study

Parameters	Values
Temperature (°C)	22–25
pH	7.0–7.9
Suspended solids (mg/L)	120–250
Chemical oxygen demand—COD (mg/L)	140–500
Ammonia (mg/L)	17–30
Total nitrogen (mg/L)	41.0–70.0
Total phosphorus (mg/L)	12.0–39.0

was dissolved with distilled water to the original volume of 8 mL. The sample was then heated in a water bath at 80 °C for 60 min and then centrifuged at 5000 rpm for 5 min. The supernatant was centrifuged at 10,000 rpm, 4 °C, for 5 min. After centrifugation, the supernatant in the tube was regarded as the TB-EPS.

Both obtained supernatants (LB-EPS and TB-EPS) were filtered through a 0.2-μm membrane filter (nylon or cellulose acetate) to remove impurities. The supernatants were frozen at − 80 °C/− 20 °C and proceed by freeze-drying/lyophilization at − 80 °C for 24 h. After lyophilization, the LB-EPS and TB-EPS were changed into solid form. The dry mass of the EPS can be weighed and the concentration of LB-EPS and TB-EPS can be calculated in mg/g VSS. The total EPS is the addition of LB-EPS and TB-EPS.

Chemical analysis

The dry weight of sludge and volatile suspended solids (VSS) in samples were measured based on Standard Methods (APHA 2005). The carbohydrate content in EPS was measured using Anthrone method (Frølund et al. 1996) with glucose as the standard. The protein content in EPS was measured by the modified Lowry method (Lowry et al. 1951) using bovine serum albumin (BSA) as the standard. As for polysaccharides content in EPS, method used was based on phenol–sulphuric method by Dubois et al. (1956). In each test, blanks with respective extracting reagents but with no sample were performed and treated as control.

Sludge volume index (SVI)

Sludge volume index (SVI) was determined following the Standard Methods—2710D (APHA 2005). It is measured as the volume occupied by settled sludge biomass after 30 min of settling divided by the dry weight of biomass in this volume. In total, 1000 mL of sludge was poured into 1 L of graduated cylinder. The volume of the settled sludge solids was recorded after 30 min.

Relative hydrophobicity

Relative hydrophobicity of the activated sludge is measured as adherence to hydrocarbons. The method for this parameter is referred from Chang and Lee (1998). Two of 30-mL sludge samples were taken; one was washed by Tris buffer solution (pH = 7.1, 0.05 mmol/L) and was shaken for 2 min. Then, the uniform suspended sludge was transferred to a separating funnel together with 15 mL *n*-hexadecane and uniformly agitated for 5 min. After 30 min, when the two phases had separated completely, the aqueous phase was transferred into glassware, and the concentration of the remaining solid phase was measured as $MLSS_e$. Another sludge sample was untreated, and its concentration was marked as $MLSS_i$. The relative hydrophobicity is expressed as in Eq. (1).

$$\text{Relative hydrophobicity}\,(\%) = \left\{1 - \frac{MLSS_e}{MLSS_i}\right\} \times 100, \tag{1}$$

where $MLSS_i$ = MLSS concentration in the aqueous phase before emulsification (mg/L), $MLSS_e$ = MLSS concentration in the aqueous phase after emulsification (mg/L).

Surface charge

The surface charge of activated sludge can be determined by colloidal titration (Morgan et al. 1990). One (1) mL sample of activated sludge was added to 200 mL deionized water. Five (5) mL of Polybrene (0.002 N) was then added to the mixture. The mixture was pipette with 200 μL of Toluidine Blue O. Then, 50 mL of polyvinyl sulphuric acid potassium salt (PVSK) (0.001 N) was finally titrated in the mixture. As for blank, the same volume of PVSK was titrated to the mixture without addition of sludge sample. The titration was stopped as the colour of mixture turn from blue to pink/purple. The volume of titrated PVSK in the mixture was calculated and used in the determination of surface charge shown in Eq. (2).

$$\text{Surface charge (meq/g MLSS)} = \frac{(A-B) \times N(1000)}{(V \times \text{MLSS})}, \tag{2}$$

where A = volume of PVSK titrated to the sample (mL), B = volume of PVSK titrated to blank sample (mL), N = normality of PVSK (eq/L), V = sludge sample volume (mL), MLSS = biomass concentration (g/L).

Results and discussion

Effect of magnetic field on content of LB-EPS and TB-EPS

Table 2 shows the total content of EPS in both reactors—SBR_A (with magnetic field exposure) and SBR_B (without magnetic field exposure) under the induced sludge bulking condition. Based on the table, mean total EPS (EPS_T) at end of experiment was 162.2 mg/g VSS for SBR_A and 147.7 mg/g VSS for SBR_B. Slightly high EPS_T in SBR_A compared to SBR_B could be due to excess secretion of EPS by bacteria as its response towards magnetic field exposure. According to Sheng et al. (2010), bacteria tend to excrete more EPS materials under abnormal condition. These EPS_T values in both reactors were also relatively higher compared to one that have been reported such as by Wang et al. (2010)

Table 2 Content of EPS during sludge bulking in SBR_A and SBR_B

Time (d)	EPS_T (mg/g VSS)		LB-EPS (mg/g VSS)		TB-EPS (mg/g VSS)	
	SBR_A	SBR_B	SBR_A	SBR_B	SBR_A	SBR_B
0	155.9	177.9	64.5	107.8	91.4	70.1
29	177.7	141.5	72.4	74.0	105.3	67.5
54	284.2	285.6	86.4	186.8	197.8	98.8
67	215.9	251.6	101.6	150.0	114.3	101.6
89	161.7	199.6	56.4	124.4	105.3	75.2
95	154.8	185.5	67.5	108.6	87.3	76.9
117	125.5	91.3	48.6	62.5	76.9	28.8
138	145.8	136.4	67.7	74.4	78.1	62.0
144	178.6	158.9	65.5	97.4	113.1	61.5

whom also reported on the content of total EPS under sludge bulking condition.

Various contradictions were found in terms of high or low EPS_T that may correspond towards effective bioflocculation and sludge settleability. According to Meng et al. (2006) and Yang and Li (2009), observation by analysing only at EPS_T was not sufficient to conclude the effectiveness of bioflocculation. The quantification should also be analysed upon the composition of EPS which are LB- and TB-EPS. If high EPS_T production is contributed by high LB-EPS compared to TB-EPS, thus settleability problem indicates by sludge bulking can occurs. Nevertheless, if high EPS_T production is contributed by high TB-EPS rather than LB-EPS, bioflocculation can be enhanced.

Based on Table 2, it can also be seen that the content of EPS_T for both reactors was not able to achieve the steady-state condition. In fact, contents for both reactors were fluctuated from early to the end of experiment. Such fluctuation that occurred particularly in SBR_A could be due to the shock effect exhibited by the magnetic field. The shock effect caused microorganisms in the reactor to be under stressed condition, thus allowing them to produce more EPS materials (Aquino and Stuckey 2004). Throughout the experiment, the shock effect was represented by the amplified intensity of magnetic field which gets stronger towards the end of the experiment. The amplification, which occurred randomly, was due to the long exposure time. Randomness of the amplification caused variation in EPS production. The fluctuation of the EPS_T content in SBR_A could also be due to the susceptibility of the microorganisms towards the magnetic field. It seemed reasonable that different species of filamentous microorganisms may have different microbial functions and that their contributions to the microbiologically produced substances (i.e. EPS) under different conditions (such as under magnetic field exposure) may be different (Jenkins et al. 1993; Li et al. 2008; Zaidi et al. 2014).

Table 2 also showed that contents of LB-EPS (mean value of 66.6 mg/g VSS) were lower than that of TB-EPS (mean value of 95.6 mg/g VSS) for SBR_A, while vice versa in SBR_B. Such observations of LB- and TB-EPS in SBR_A indicated better performance of settleability compared to SBR_B. This is because LB-EPS are the component that has weak capacity to bind flocs structure. Such observation is corresponding to high SVI and total effluent suspended solids in the systems (Jin et al. 2004; Sheng et al. 2006; Yang and Li 2009). Meanwhile, TB-EPS contributes to the stability of the flocs because the component is more hydrophobic and hard to be extracted (Laspidou and Rittmann 2002; Yu et al. 2009; Liang et al. 2010). Hence, having high content of TB-EPS and low content of LB-EPS as been observed in SBR_A confirmed that the settleability of the sludge was increased as the sludge flocs were at strong density and low porosity (Meng et al. 2006; Yang and Li 2009). This in turn could inhibit the sludge bulking occurrences. Eventually, these observations have also been supported by the results of SVI that were positively improved in SBR_A compared to SBR_B as shown in Fig. 1. Figure 1 showed that the sludge biomass in SBR_A obtained consistently lower SVI with mean value of less than 20 ± 2 mL/g compared to SBR_B which obtained rather high and fluctuated SVI (32 ± 4 mL/g). Consequently, this observations also showed that the magnetic field which facilitated SBR_A had positively affected the production of TB-EPS composition, thus helping in enhancing the settleability of sludge biomass.

Effect of magnetic field on chemical components of EPS

In this study, three major components in EPS were extracted which are carbohydrates, polysaccharides, and protein. The compositions of carbohydrates, polysaccharides, and protein in both LB- and TB-EPS for SBR_A and SBR_B are shown in Fig. 2. Based on Fig. 2, the mean contents of LB-EPS in SBR_A were 15.9 ± 0.9 mg carbohydrates/g VSS, 15.0 ± 0.4 mg polysaccharides/g VSS, and 24.6 ± 0.4 mg protein/g VSS with the order of predominance: protein > carbohydrates > polysaccharides. Meanwhile in SBR_B, the mean contents of LB-EPS were 20.8 ± 0.8 mg carbohydrates/g VSS, 21.3 ± 0.6 mg polysaccharides/g VSS

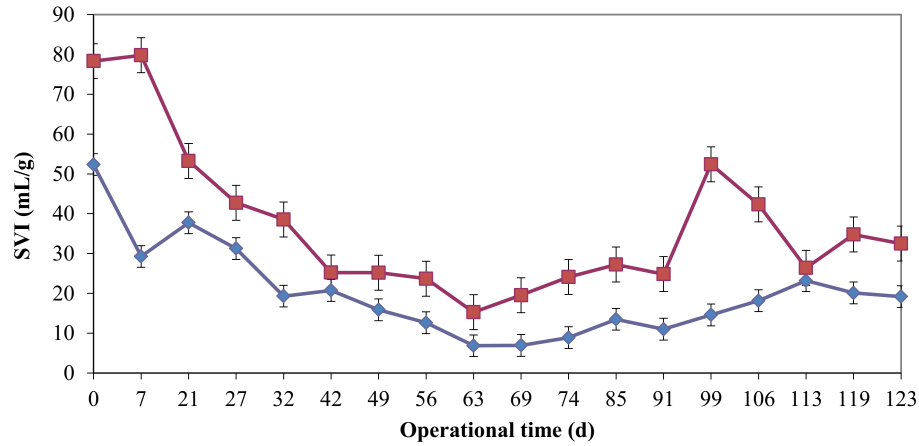

Fig. 1 Sludge volume index (SVI) profiles of activated sludge for SBR_A and SBR_B (blue diamond SVI_A; brown square SVI_B)

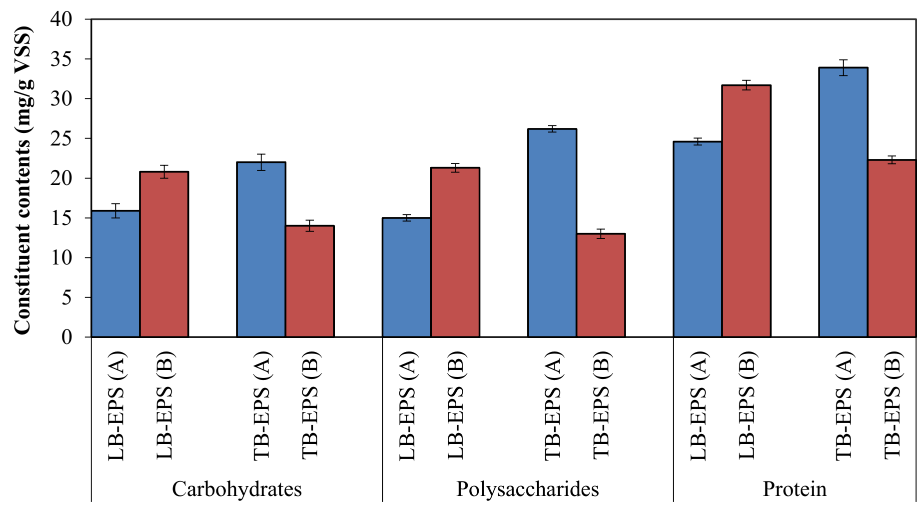

Fig. 2 Composition of chemical components (carbohydrate, polysaccharides and protein) in each LB-EPS and TB-EPS for SBR_A and SBR_B

and 31.7 ± 0.6 mg protein/g VSS with the order of predominance: protein > polysaccharides > carbohydrates. It is noted that the content of every component in LB-EPS was higher in SBR_B rather than in SBR_A by 23.6% (carbohydrates), 29.6% (polysaccharides), and 22.4% (protein).

For the mean contents of TB-EPS in SBR_A, the results recorded were 22.0 ± 1.0 mg carbohydrates/g VSS, 26.2 ± 0.4 mg polysaccharides/g VSS, and 33.9 ± 1.0 mg protein/g VSS with the order of predominance: protein > polysaccharides > carbohydrates. As for SBR_B, the mean contents of TB-EPS were 14.0 ± 0.7 mg carbohydrates/g VSS, 13.0 ± 0.6 mg polysaccharides/g VSS and 22.3 ± 0.5 mg protein/g VSS with the order of predominance: protein > carbohydrates > polysaccharides. It can be acknowledged that the content of TB-EPS were higher in SBR_A rather than in SBR_B. High content of TB-EPS and low content of LB-EPS for all the chemical constituents in SBR_A were in accordance with the theory conveyed by Yang and Li (2009) which indicated enhancement of bioflocculation and settleability of the biomass.

The observations also evidenced that protein was the predominance content in both LB- and TB-EPS. This could be due to the presence of exoenzymes in large quantities which mainly exhibited due to the bacterial excretions, such as lysis and extra-cellular products in the flocs (Sponza 2002; Meng et al. 2006). These excretions depended very much on the types of microorganisms, substrate properties, and experimental conditions as well. Exposure by magnetic field in SBR_A seemed to provide suitable condition for these microorganisms to excrete more exoenzymes. This could be the reason of high protein content in TB-EPS which dominated most by SBR_A. Besides that, high protein content particularly in TB-EPS of SBR_A is also believed in resulting positive enhancement of the settling property (i.e., in form of surface charge, relative hydrophobicity). According to Liao et al. (2001) and Wilén et al. (2003), 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. This justification is evidenced in the results obtained by the parameter of relative hydrophobicity and surface charge as shown in Fig. 3. Based on Fig. 3, SBR_A showed high hydrophobicity with average of 57% compared to SBR_B with almost half of the SBR_A's average percentage. Additionally, SBR_A also showed lower negative surface charge compared to SBR_B. The mean surface charge for SBR_A was − 0.9 meq/g MLSS, while for SBR_B was − 2.0 meq/g MLSS. A low value of negative surface charge obtained in SBR_A indicated that there can be high electrostatic binding of cations on the surfaces of the sludge flocs, thus increasing the potential of flocculating ability (Jin et al. 2003). These results can also be supported as the mechanism of magnetic field is in terms of enhancing magnetization of particles' or cells' charges. By having the exposure of magnetic field, charges of the particles' or cells' can be easily aligned which consequently favouring the bioflocculation process.

In terms of carbohydrates and polysaccharides content, its composition was less compared to the protein content. This is because both the carbohydrates and polysaccharides have been found to be particularly strong bound, thus making it hard to extract (Frølund et al. 1996; Wilén et al. 2008). High concentration of carbohydrates as been indicated in SBR_A could positively affect the solid–liquid separation properties of the sludge flocs (Wilén et al. 2008). In addition, high carbohydrates and polysaccharides content could also provide better bioflocculation which is attributed to the polymeric interactions that can helps bacterial adhesion to the cell surfaces (Tsuneda et al. 2003; Badireddy et al. 2010; Pei et al. 2010). Indirectly, these above observations conveyed that the magnetic field in SBR_A was potential in influencing the production of EPS mainly its predominance content as well as other important compositions. Thus, able to improve the settleability through efficient bioflocculation and eventually control the sludge bulking occurrences.

Role of protein in improving bioflocculation of sludge flocs

In order to further determine the role of protein in reducing the sludge bulking occurrences in SBR_A and SBR_B, the protein–polysaccharides ratio (PN/PS ratio) in EPS_T were assessed. The data obtained from EPS analysis is shown in Fig. 4. Overall, severe fluctuations in PN/PS ratio were observed for both reactors. SBR_B showed slightly high PN/

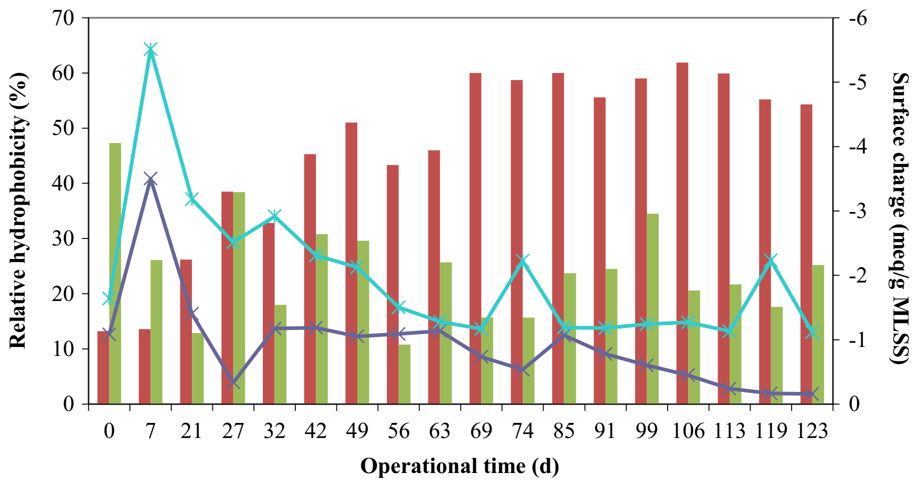

Fig. 3 Relative hydrophobicity and surface charge profiles of sludge biomass in SBR_A and SBR_B (brown rectangle RH_A; green rectangle RH_B; violet asterisk SC_A; blue asterisk SC_B)

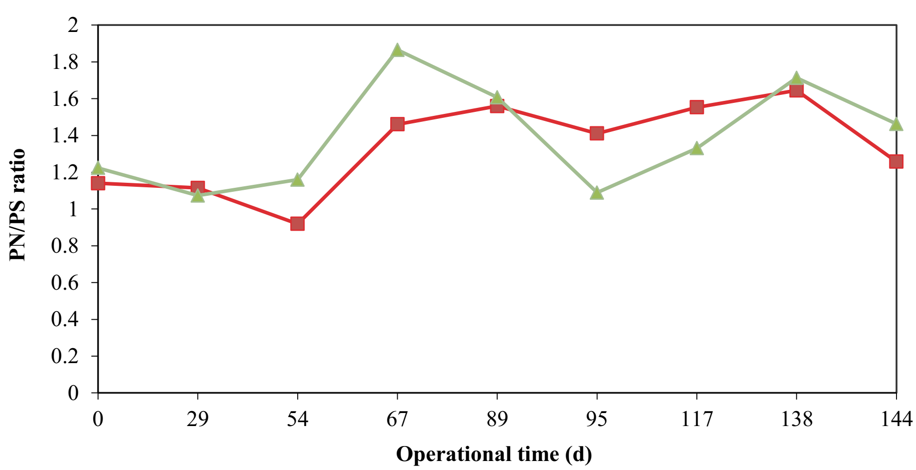

Fig. 4 Changes of PN/PS ratio in EPS for SBR_A and SBR_B throughout the experimental period (brown square PN/PS_A; green triangle PN/PS_B)

PS ratio with its mean value of 1.39 compared to SBR_A with its mean value of 1.34.

Lower PN/PS ratio indicated by SBR_A could be due to slightly high content of polysaccharides in EPS. That may be due to the sudden increase of influent organic loading sourced by the raw wastewater. These increments disabled the utilization of the carbon sources by the microorganisms that needed for the growth. As a result, the excess carbon sources which have not completely utilized were transferred as intra-cellular substances either for storage or for extra-cellular biopolymers. These extra-cellular biopolymers might be expected to account for the high content of polysaccharides in EPS (Zhang et al. 2007). In comparison between low and high PN/PS ratio, high PN/PS ratio might correlate with the less negative surface charges. This seemed to imply that high ratio of PN/PS could decrease the negative surface charges of bacterial cells. This condition can results in an increase in van der Waals forces of the cells that can strengthen the cells' adhesion. Consequently, this can reduces the electrostatic repulsion between cells, thus further favouring bioflocculation of sludge biomass (Zhang et al. 2007).

Although SBR_A showed slightly lower PN/PS ratio compared to SBR_B (Fig. 4), the overall performances in SBR_A which under the magnetic field facilitation were better than the SBR_B. Depending on the results achieved throughout the study, SBR_A was greatly evidenced in controlling the settleability problem which corresponding to the occurrence of sludge bulking by improving the settling properties of the sludge biomass. The lower PN/PS ratio in SBR_A may due to the fluctuated influent organic loading of the raw wastewater that cannot be controlled. That loading was the general cause to the high polysaccharides content, thus led to the low PN/PS ratio.

Conclusion

Overall, this work highlights that the magnetic field of 88 mT is able to minimize the occurrence of induced sludge bulking. This means that the magnetic field application stands a great chance to minimize the sludge settleability problem. Under the effect of magnetic field, it has been evidenced that the bacteria in biomass tend to secrete more EPS priory the TB-EPS and protein contents which in turn corresponded to the strong density flocs, thus further helping the bioflocculation process. High TB-EPS content specifically has been well reflected towards enhancement in negative surface charge and SVI which mainly indicated that the sludge settleability were improved in SBR_A (with magnetic field exposure) compared to SBR_B (without magnetic field exposure).

Acknowledgements The authors would like to express thanks to Universiti Teknologi Malaysia (UTM) for financially support this research (Grant Project No. 12H26 and 14J41).

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflict of interests.

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