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Poly aluminum chloride (PAC) enhanced formation of aerobic granules: Coupling process between physicochemical–biochemical effects

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HIGHLIGHTS

- The temporal effect of PAC during the whole aerobic granulation was investigated.
- PAC mainly performed during the start-up period (the first 15 days) of granulation.
- Major factors for flocculability and stability of enhanced granule were identified.
- Correlation between physicochemical and biochemical effects was revealed.

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ABSTRACT

Transforming conventional flocculent sludge to aerobic granular sludge is drawing increasing global interest in a quest for an efficient and innovative technology in wastewater treatment. However, long start-up time and low granule stability are the main challenging issues for its application. In this study, long-term and short-term PAC feeding strategies were applied in parallel to enhance the sludge granulation and with the aim to figure out the temporal effect of PAC during the whole process. Nevertheless, both of them identified to allow a rapid start-up formation of aerobic granules with better performances in physicochemical characteristics. More extracellular polymeric substances (EPS) were secreted in all PAC-fed reactors, especially for polysaccharides. By using enzymatic hydrolysis, factors which account for the flocculating ability and stability of granules were identified. Notably, the correlation between them was also revealed. Based on these findings, dosage of PAC played a positive role mainly during the start-up period (first 15 days) of the aerobic granules formation, extending its dosing time made no significant sense. The granules formed under this condition were the result of physicochemical–bio chemical effects coupling process.

1. Introduction

Aerobic granular sludge (AGS) has been extensively investigated using sequential batch reactors (SBRs). Compared to conventional floccular sludge, aerobic granular sludge become more attractive by dense microbial aggregates, faster settling velocities, high resilience against variations in pollutant loading and environmental condition [1,2]. Moreover, aerobic granular sludge process has been utilized in a wide range, both municipal and industrial wastewater [3,4]. However, reluctance to accept this technology is largely from perceptions of long start-up time and low granule stability [5,6].

As a complex process, granulation is dominated by various factors such as seed sludge, substrate composition, pH, temperature and reactor operations [5,7]. Additionally, extracellular polymeric substances (EPS) secreted by microorganisms can be regarded as another crucial role since it could alter the surface properties, such as cell surface charge and cell hydrophobicity [7]. Strong evidence has been also proved that EPS are closely related to aggregation, matrix structure formation, microbial physiology and long-term stability improvement of granules [8]. For the purpose to accelerate granulation, different strategies have been proposed. When mixed with the pure culture which had high self-aggregation and coaggregation ability, only 8 days was needed for activated sludge

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to transform to granules [9]. However, accumulation for the specified pure culture is complex and expensive in practice. It was also believed to be able to accelerate the start-up of aerobic granules by seeding sludge with anaerobic granules [10] or crushed granular sludge [6], while the granules form under these conditions might broken up and be washed out before a new granulation occurred [10]. In addition, aerobic granulation could be enhanced by metal ions, such as Ca^{2+} and Mg^{2+} [11,12]. Above all, although all of these abovementioned strategies could accelerate the formation of granules, more possibilities should be taken into consideration for the further enhancement of aerobic granulation.

Consist of monomeric Al, polymeric Al, and aluminum hydroxide ($Al(OH)_3$), poly aluminum chloride (PAC) is widely used for particulate matter destabilizing during coagulation in water treatment plants [13]. In contrast to Al salts, more attention was appealed by PAC, which was more effective and lower costing. Moreover, inherent difference in the coagulation behavior and particle removal mechanism between alum and PAC was also reported by Wu et al. [14]. Compared to the numerous studies regarding the efficiency of PAC addition to wastewater in the context of coagulation-flocculation, reports dealt with enhancement of granules formation is scarce. In our previous study, dosing PAC during the first 8 days can reduce the time for aerobic granulation from 17 to 7 days and the characteristics of PAC-fed granules were also improved [15]. These results suggested that PAC dosage also could be a good option to enhance the formation of aerobic granules. However, in order to optimize its application, more information is needed for the temporal role of PAC in the whole process, and maybe a more appropriate dosing strategy of PAC can be put forward. It can be very interesting to verify whether the strengthening effect can be further improved by prolonging the PAC feeding period or some inhibitory effect will be caused under the long time exposure to aluminum [16]. Furthermore, demonstrating the correlation between PAC and other possible factors is also necessary to understand the formation mechanism of PAC enhanced granules in depth.

Therefore, this study attempts to identify the temporal effect of PAC during formation of aerobic granular sludge by investigating the granulation with both long-term and short-term feeding of PAC. In order to make a comparison of the granules formed under different conditions, key parameters of physical characters were evaluated. Analysis of EPS was also involved. Additionally, the factors accounting for the PAC enhanced aerobic granulation were figured out and then the correlation among them was revealed. The results derived from the present study are expected to offer useful information for the optimization and further practical application of PAC enhanced aerobic granulation.

2. Materials and methods

2.1. Reactor and operation

Three identical sequential batch reactors (1.5 m in height and 0.05 m in diameter with a working volume of 2.4 L in each reactor) were used. STF_{SBR} and LTF_{SBR} stand for the two SBRs which fed with PAC: one for short-term feed (8 days, same duration as the adjustment of settling time which will be described later) and the other for long-term feeding (40 days, for the whole process of experiment), respectively. The rest was the control, C_{SBR} (no PAC feeding). A 6-h operation cycle was implemented in the running program of each reactor, comprising 5-min feeding, 330-min aeration, 5-min settling, 5-min decanting and 15-min idling. Moreover, a selective discharge operation was also included during the first 8 days: the settling time gradually reduced from the initial 15 min to 5 min at last. During the whole process, the pH of suspended liquor remained almost consistent at 7.0 by adding $NaHCO_3$ and the temperature was controlled at 25 ± 1 °C. Aeration was at an airflow rate of 2.0 L/min and a 50% volume exchange was also applied.

2.2. Feeding

All the reactors were inoculated with 1.2 L seeding sludge taken from a local activated sludge sewage treatment plant (The Fourth Wastewater Treatment Plant, Xi'an, China). The detailed composition of synthetic wastewater was as the following (per liter): Glucose, 1000 mg (800 mg/l as chemical oxygen demand (COD) basis in actual); NH_4Cl, 700 mg (200 mg/l as ammonia nitrogen (NH_4^+—N) basis in actual); $MgSO_4{\cdot}7H_2O$, 25 mg; $FeSO_4{\cdot}7H_2O$, 20 mg; $NaHCO_3$, 600 mg; KH_2PO_4, 25 mg; $CaCl_2{\cdot}2H_2O$, 30 mg; $FeCl_3{\cdot}6H_2O$, 1.5 μg. The trace element solution contained (μg/L): H_3BO_3, 0.15; $CoCl_2{\cdot}6H_2O$, 0.15; $CuSO_4{\cdot}5H_2O$, 0.03; $MnCl_2{\cdot}H_2O$, 0.12; $Na_2Mo_7O_{24}{\cdot}2H_2O$, 0.06; $ZnSO_4{\cdot}7H_2O$, 0.12; KI, 0.03.

For PAC feeding strategy, the operation in our previous study was modified [15]: After each discharge, 50 mL poly aluminum chloride (PAC) with the concentration of 20 g/L was pumped from the bottom of the SBRs (except the C_{SBR}) in 1 min. In order to let the PAC and sludge flocs mixed completely, the aquarium air pump simultaneously worked. Meanwhile, rapid mixing with stirrers at a speed of 100 rpm was maintained for 5 min. After then, slow mixing at 50 rpm for another 5 min followed and the aeration idled. The same operation was also conducted in C_{SBR}, except for the PAC dosing. In the present study, commercial grade of PAC (Gongyi fuyuan water purification materials Co., purity: 30% w/w Al_2O_3, China) was utilized and solution freshly prepared before each feeding.

2.3. EPS content

EPS was extracted from the sludge by using formaldehyde-NaOH method [17]. The protein (PN) content in EPS was quantified using a modified Lowry colorimetric method with bovine albumin serum as standard [18]. The polysaccharides (PS) or carbohydrate content in EPS was determined using the phenol–sulfuric acid method with glucose as the standard [19]. The deoxyribose nucleic acid (DNA) content was measured by the diphenylamine colorimetric method using fish DNA as the standard [20]. Furthermore, the lipids, polar and neutral lipids were extracted from sludge by adding methanol/chloroform (1:2 v/v) and were determined as Adav et al. [20] described.

2.4. Granule staining

The granules were stained by using the following scheme proposed by Chen et al. [21]. The fluorescein isothiocyanate (FITC) is an amine reactive dye and used to stain proteins and amino sugars. Fluorescently labeled lectins Concanavalin A conjugated with tetramethyl rhodamine was utilized to bind to α-mannopyranosyl and α-glucopyranosyl sugar residues. Calcofluor white was applied to stain the β-D-glucopyranose polysaccharides. Nile red was utilized to stain lipids and extracellular polymeric substances were distinguished from cells by SYTO 63 stain. The detailed staining sequence and wavelengths of CLSM imaging are available in Chen et al. [21]. Confocal laser scanning microscopy (CLSM; Leica TCS SP8 Confocal Spectral Microscope Imaging System, Gmbh, Germany) was employed to probe the internal structure of granules. In addition, for each reactor, more than 100 CLSM images were sampled for a typical granule.

2.5. Enzymolysis treatment of granules

Proteinase K was utilized to degrade the protein by cleaving peptide bonds. Moreover, α- and β- amylases were used to cleave (1 → 4)-α- and β-glycosidic linkages, respectively. Lipase was also applied to enzymatically break down lipids and Dnase was utilized to degrade nucleic acids. For every reactor, 60 ml granules were sampled and separated into 6 test tubes, respectively. The suspension in each tube was centrifuged at 4000 rpm for 10 min and the supernatant was discharged. The sludge floc was washed with phosphate buffer solution (PBS) by three times and resuspended with Milli-Q water. As has been reported before [22], these tubes were severally treated with different specific enzymes: Proteinase K (2350 U/μL in PBS, pH 8.0), α-amylase (205 U/μL in PBS, pH 6.9), β-amylase (5.13 U/μL in PBS, pH 5.0), lipase (3.15 U/μL in PBS, pH 8.0) and dnase (2.47 U/μL in PBS, pH 5.8). The rest was the control (no enzyme added). Afterwards, the sample in each tube was completely mixed and incubated at 37 °C for 2 h. Following incubation, the supernatant collected for total Al content measurement and the granules transferred to other clean serum bottle for flocculating ability and mechanic strength analysis.

2.6. Analytic methods

COD, NH_4^+-N, mixed liquor suspended solid (MLSS), mixed liquor volatile suspended solid (MLVSS), sludge volume index (SVI) and specific oxygen uptake rate (SOUR) were measured according to standard methods [23]. Sludge retention time (SRT) was calculated from the biomass concentrations in both of reactor (dry weight) and effluent. Wet sieving separation method as described by Liu et al. [15] was applied to measure the size distribution of sludge particles. Zeta potential, a measure of the surface charge of a particle, was quantified by a Nanosizer ZS instrument (Malvern Instruments Co., UK). The relative hydrophobicity of sludge was measured as Wilen et al. [24] reported. After digestion by nitric acid, total aluminum concentration of all the samples was determined by ICP-MS (PerkinElmer Co., USA).

The mechanical strength of the granules was determined according to the method developed by Ghangrekar et al. [25]. The integrity coefficient (%), which is defined as the ratio of residual granules to the total weight of the granular sludge after 5 min of shaking at 200 rpm on a platform shaker, had been utilized to present the mechanical strength.

To estimate the flocculating ability of sludge flocs after enzymatic hydrolysis, the method described by Jorand et al. [26] was modified: 50 mL milli-Q water was added into each bottle and fully mixed. Ten mililiter suspensions was taken and centrifuged at 1200 rpm for 2 min, and absorbance of the supernatant was measured at 600 nm (Abs_1). The rest was stirred on a magnetic stirrer kept at a constant low speed to facilitate the flocculation of the sludge in suspension. This operation was kept at ambient temperature for 15 min. After then, another 10 mL suspension was sampled and centrifuged at 1200 rpm for 2 min. The absorbance of the supernatant at 600 nm was also recorded (Abs_2). The flocculating ability of the sludge flocs was given as

$$Flocculability = (1 - Abs_2/Abs_1) \times 100\%$$

3. Results

3.1. AGS formation

During the experiment, aerobic sludge granulation was successfully achieved in all the three reactors. Visible granules firstly appeared in LTF_{SBR} on the 7th day and became dominant in the sludge after about 15 days. A similar phenomenon was also observed in STF_{SBR} (8th day) and the granulation was nearly completed after 14 days, 2 days later than that in LTF_{SBR}. In contrast, the proportion of granules in C_{SBR} did not increase significantly until the 21th day and the granulation fully achieved on day 29. Comparison among the three SBRs suggests that PAC regulation has a positive effect on the formation and growth of aerobic granules, especially during the start-up period.

3.2. Physical characters of AGS

3.2.1. MLVSS

Fig. 1a demonstrates the overall profile of MLVSS changing. As a commonly used approach to enhance granulation, progressively reduction of the settling time was also applied in this study [6], and significant decrease of MLVSS occurred in all reactors during the initial days. Then, more biomass was reserved in both LTF_{SBR} and STF_{SBR} since the flocculation of PAC. Following the PAC dosing ceased in STF_{SBR} on the 8th day, difference between the two PAC-fed reactors tended to be significant, but only lasted for approximately 12 days. Compared with C_{SBR}, the reactors fed with PAC performed better in biomass accumulation, even after the fully granulation. In this case, the sludge flocs would get more chance to touch with others and aggregate together. Additionally, the pollutants removal efficiency of reactors could be guaranteed.

3.2.2. Settling property

The settling property of the sludge was compared and a constant decrease of SVI was observed in all SBRs during the granules formation (Fig. 1b). The SVI decreased from the initial 165 mL/g to levels lower than 40 mL/g when all the granulation completed. Compared with STF_{SBR}, lower SVI could be easily observed in LTF_{SBR} during the 10th to 17th day. This could be attributed to the extra dosage of PAC. Nevertheless, the difference in SVI between these two reactors tended to decrease along with the complete formation of granules and finally disappeared. For the C_{SBR}, more time was needed for the sludge to obtain the stable SVI value, which was a little bit higher than that of other reactors. Throughout the whole process, PAC-fed sludge had a better settling property.

3.2.3. Surface characters

The cell surface charge and hydrophobicity of sludge in different systems were also evaluated (Fig. 1c). During the process of granules formation, the cell surface characters of sludge were substantially improved in all the reactors. In LTF_{SBR}, obvious granules achieved when the cell surface charge of sludge, expressed as zeta potential, increased from initial −34.5 to −14.8 mV. Moreover, this value maintained at 5.4 ± 1.2 mV after the fully granulation achieved. In STF_{SBR}, disturbed by the cease of the PAC feeding, a slight retard of the cell surface charge rose from the 10th day. Nevertheless, no significant difference exists between PAC-fed reactors afterwards.

In terms of cell hydrophobicity, similar results were obtained. During the first week, a drastic increase of cell hydrophobicity occurred in both LTF_{SBR} and STF_{SBR} since the regulation of PAC. In PAC-fed reactors, the cell hydrophobicity of sludge rose into a narrow range of 80–85% coped with dominance of granular sludge, 8.3 ± 2.4% higher than the sludge cultivated in the C_{SBR}. Above all, regulation of PAC played a positive role on the surface properties of sludge.

3.2.4. Size distribution

Granules size was evaluated to present the growth and aging process of sludge during the granulation. The results are illustrated in Fig. 1d. The visible granules were firstly discovered in the LTF_{SBR} on the 7th day and approximately 3% of sludge larger than 2.5 mm

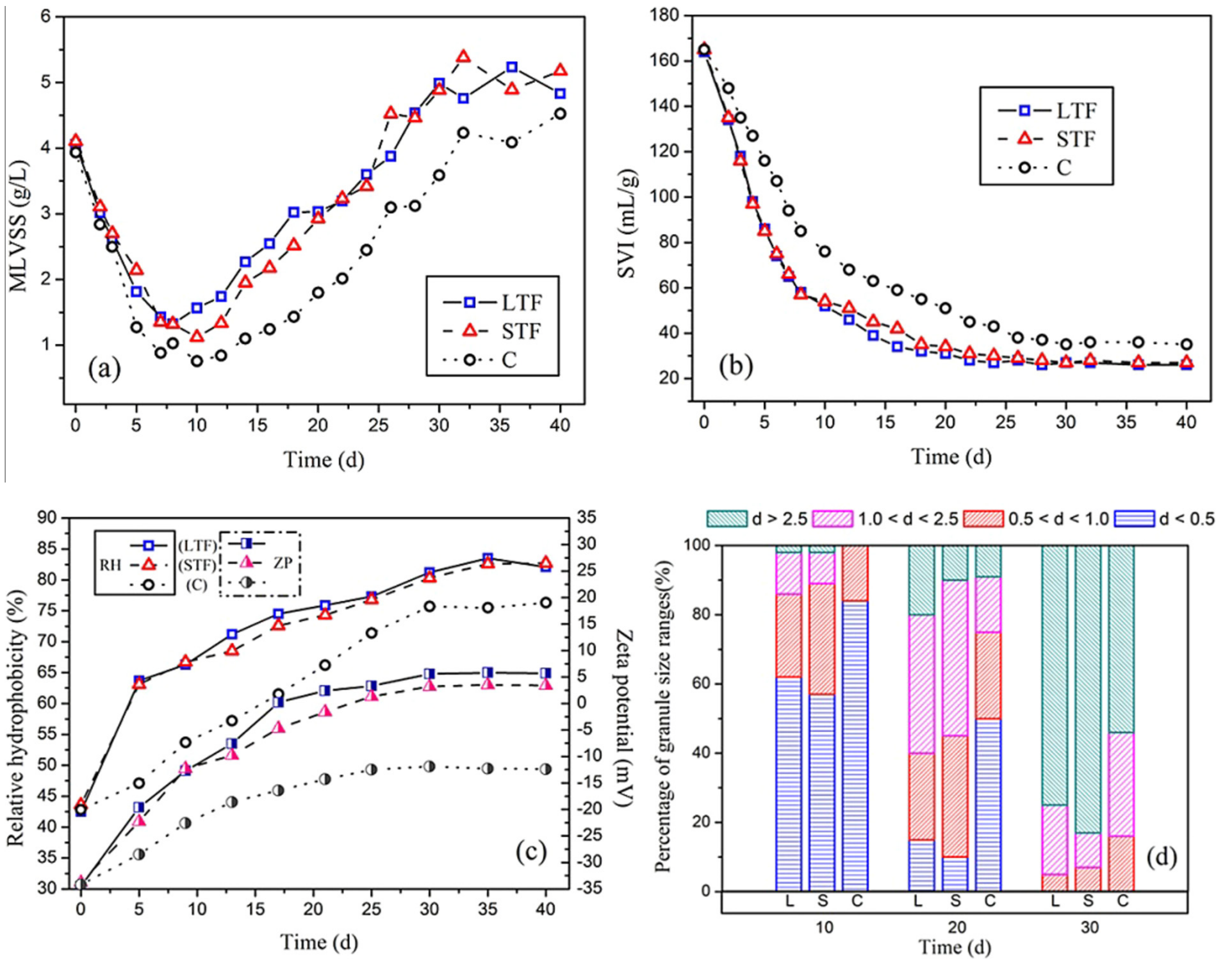

Fig. 1. Physical characters variation of sludge during operation (a) MLVSS, (b) SVI, (c) relative hydrophobicity and zeta potential, and (d) sludge size distribution (L: LTF_{SBR}; S: STF_{SBR}; C: C_{SBR}).

after 3 days. On the 20th day, this proportion increased to 20% and the granules which had a size over 1.0 mm dominated. As particle size of most granules (75%) was above 2.5 mm, the granulation fully achieved. An analogous variation also appeared in STF_{SBR}, but the distribution of granules diameter was more symmetrical. In comparison, sludge cultivated without PAC-feeding displayed a worse performance in this filed and the proportion of granular sludge larger than 2.5 mm only accounted for 54% on the 30th day.

3.3. Extracellular polymeric substances (EPS)

3.3.1. Components of EPS

The sludge EPS content at different stages was quantified and compared (Fig. 2). During the start-up period, owing to the various selective pressures, significant increases of EPS production were observed in all the SBRs, especially in PAC-fed systems. However, following the maturation of granular sludge, EPS content tended to be stable, 74.1 ± 2.3 mg/g·SS in PAC-fed systems and 64.3 ± 1.8 mg/g·SS in C_{SBR}, respectively.

Generally, as being defined as the biopolymers which are presented around the cells and in the interior of various microbial aggregates, EPS contains different classes of macromolecules such as proteins, polysaccharides, lipids, nucleic acids and other polymeric compounds. In this study, EPS components of granules cultivated in the three reactors were also investigated (Table 1 and Fig. 2). Proteins and carbohydrates accounted for the majority of EPS content and the PN/PS ratio varied during the granules

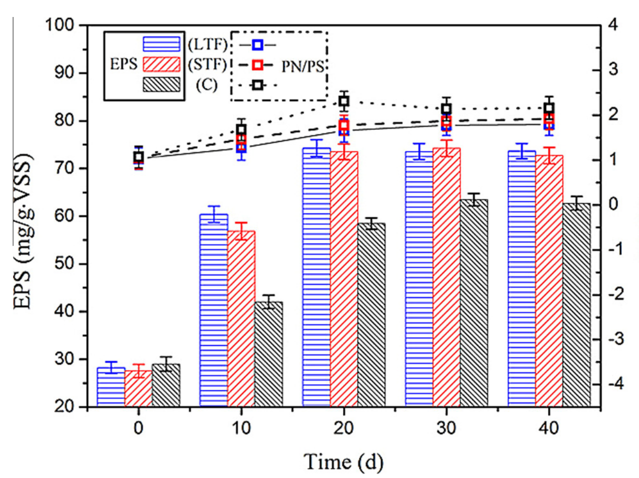

Fig. 2. Profiles of the EPS content variation of sludge in the different SBRs during operation.

formation. During the initial 20 days, PN/PS ratio of the granules constantly increased in all SBRs, especially in the C_{SBR}. In C_{SBR}, the rate of PN/PS improved from 1.07 to 2.31 and maintained at the range of 2.14 ± 0.2 after the mature granules achieved. In comparison, more polysaccharides were produced both in LTF_{SBR} and STF_{SBR}. This may imply that the EPS composition of biogranules is fairly related to the operating conditions.

Table 1
EPS components of granules cultivated in different reactors on the 30th day.

Reactors	Proteins (mg/g·VSS)	Carbohydrates (mg/g·VSS)	Humic substances (mg/g·VSS)	Lipids (mg/g·VSS)	DNA (mg/g·VSS)
LTF	36.8 ± 2.4	20.4 ± 2.7	15.5 ± 1.7	11.4 ± 0.7	0.53 ± 0.16
STF	35.7 ± 1.8	19.7 ± 2.3	17.3 ± 0.8	8.9 ± 1.1	0.34 ± 0.07
C	34.6 ± 3.1	16.6 ± 1.2	13.8 ± 1.5	7.3 ± 0.4	0.42 ± 0.02

3.3.2. Fractions distribution of EPS

Fig. 3 shows the CLSM images of granules cultivated in both LTF_{SBR} and C_{SBR} (on day 30), which were achieved by using the multicolor fluorescent technique. Distributions of proteins, lipids, nucleic acids, α- and β-D-glucopyranose were probed. According to the graphic information which was shown in the figure, the outer layer of granules cultivated in LTF_{SBR} was mainly occupied by the cells and α-D-glucopyranose, consistent with those of McSwain et al. [27]. In contrast, proteins and lipids were spread throughout the interior of granule. Moreover, just as Chen et al. [28] mentioned, the distribution of β-D-glucopyranose was not only concentrated in the outer layer but also penetrated within the whole granules. For STF_{SBR}, the results were almost same with LTF_{SBR}, data not shown.

In comparison, for the granule which appeared in C_{SBR}, the cells were mainly accumulated in the core. Absence of mass transfer limitation could account for this phenomenon, since more obvious interstices existed in the no PAC-fed granules (Fig. 3). Furthermore, as embedded in a large cross-network of β-D-glucopyranose, proteins and α-D-glucopyranose spread over the granule. Meanwhile, the lipids were also involved in the core of granules. Although there were some disparities of EPS composition and components distribution existed between the systems which ran with PAC or not, the major components distribution of EPS such as proteins and β-D-glucopyranose remained the same.

3.4. Enzymatic hydrolysis of AGS

Table S1 demonstrates the extracted EPS content of hydrolyzed granules which were sampled from the LTF_{SBR} on the 30th day. Obviously, individual EPS compounds of granules were generally hydrolysed by the specific enzymes.

Fig. 4a, b and c illustrate the sludge flocculability variation of different reactors in response to the enzymolysis treatment. In addition, for each sample, the initial flocculability measured before enzymolysis were also shown as control. Due to surface characters regulation by PAC, the flocculating ability of sludge in LTF_{SBR} substantially increased from 54.3% of raw sludge to 83.2% during the first 10 days. After the granules fully achieved, the flocculability tended to be stable at 80.4 ± 0.6%. Similar situation was also presented in STF_{SBR}, except the slight less flocculating ability on the 20th day which may be caused by the cease of PAC feeding. In contrast, on the 10th day, the flocculating ability of sludge cultivated in C_{SBR} was only 73.7% and the gap in this parameter compared with PAC-fed reactors should not be ignored until 10 days later. In terms of specific enzymolysis treatment, the most dramatic decrease in flocculating ability was observed in the presence of protease K and this decrease became more obvious as the time went on. This is in accordance with an earlier study in which it has been observed that proteins had the biggest influence on the flocculating ability of sludge flocs, and had relatively strong positive correlations with flocculating performance [24]. Furthermore, the effect of α-D-glucopyranose was also indispensible, especially in C_{SBR} .

Variation of granular sludge stability before and after enzymatic hydrolysis is also shown in Fig. 4d, e and f. The mechanical strength of granules cultivated in all SBRs gradually increased and the granules appeared in PAC-fed systems were more closely knitted. In this respect, the β-D-glucopyranose played an important role. The drop in mechanical strength for all the β-amylase-treated samples was greater than others, especially on the 10th day. This may be due to the fact that β-D-glucopyranose formed the backbone of the network-like structure of granules (Fig. 3c and h), and this finding was also confirmed by Adav et al. [22]. Meanwhile, after the hydrolysis of protease K, noteworthy reduction in stability of granule was also observed.

4. Discussion

4.1. Effect of PAC in the aerobic granule formation

Due to the regulation of PAC, the physical characteristics of sludge floc substantially improved, especially on the start-up duration. Compared with C_{SBR}, both zeta potential and cell hydrophobicity of sludge which was cultivated in PAC-fed systems were significantly increased (Fig. 1). Moreover, visible granules appeared in both LTF_{SBR} and STF_{SBR} even the cell surface charge still low. These results can be likely explained by the neutralization and electrostatic patch coagulation of PAC [14]. In general, after dosing of PAC, the preformed Al species adsorbed to surface of flocs quickly, and the negative charge can be neutralized. Moreover, the adsorbed species could aggregate, rearrange and finally form 'electrostatic patches' over portions of the surface, after then, the collision frequency of oppositely charged patches and surfaces of other floc would be considerably improved [14]. Meanwhile, bridge-connection might be also included since the SVI in PAC-fed system continuously dropped even the zeta potential turned to positive (Fig. 1), and this effect usually caused by the formation of aggregated Al_{13} and other polymeric species during pre-hydrolyzation [29]. Due to most of the sludge was washes out by the progressive reduced settling time, in all SBRs, both COD and ammonia have a high concentration in the effluent during the initial days. As the reactors running, more microorganisms generated and the COD removal ability in all the SBRs rapidly improved. However, in terms of NH_4^+—N removal, significant lagging was observed in C_{SBR} (Fig. 5). As it has been recognized as a slow grower, nitrifiers usually need more time to recover after shocking. This is also a common problem when researchers try to develop the granules with different types of wastewater [6]. In this study, excellent performance of NH_4^+—N removal firstly appear in PAC-fed systems since more biomass was saved and thus the SRT increased by the coagulation effect of PAC during the start-up period (Fig. 1a and S1). Nonetheless, in this respect, there was no significant difference between the three reactors at the end since the low concentration of ammonia in influent and the decreased nitrogen load which was along with the increase of biomass.

Being exposed to various environmental stresses such as desiccation, heavy metals or others, many microorganisms can instinctively secrete EPS to protect the bacterial cells [30]. In the present study, more EPS were also produced in both LTF_{SBR} and STF_{SBR}. Furthermore, production of extracellular carbohydrates acted as the major difference between the SBRs fed with PAC or not (Table 1). Analogous result was reported that nearly 82% increase of extracellular carbohydrates appeared in the sulfate-reducing bacterial biofilms after exposure to trivalent Cr [30]. Further supports were

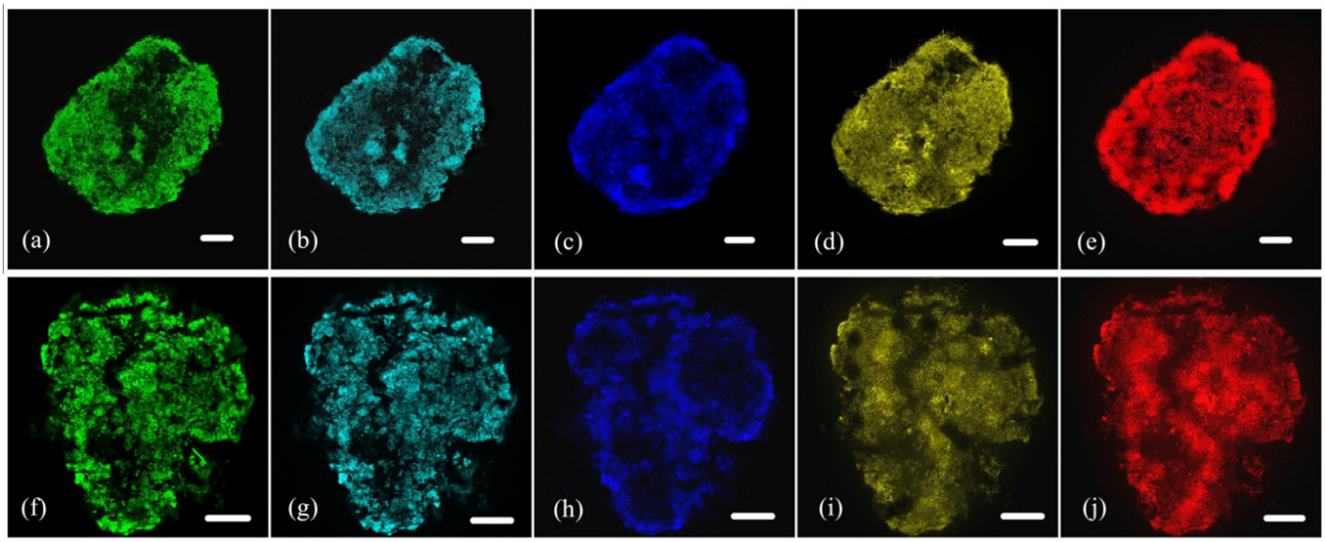

Fig. 3. CLSM images of granules cultivated in LTF_{SBR} (a, b, c, d, e) and C_{SBR} (f, g, h, i, j), bars = 500 μm. (a), (f) Green (FITC): proteins; (b), (g) cyan blue (ConA): α-D-glucopyranose polysaccharides; (c), (h) blue (calcofluor white): β-D-glucopyranose polysaccharides; (d), (i) yellow (Nile red): lipids; (e), (j) red (SYTO 63): nucleic acids. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

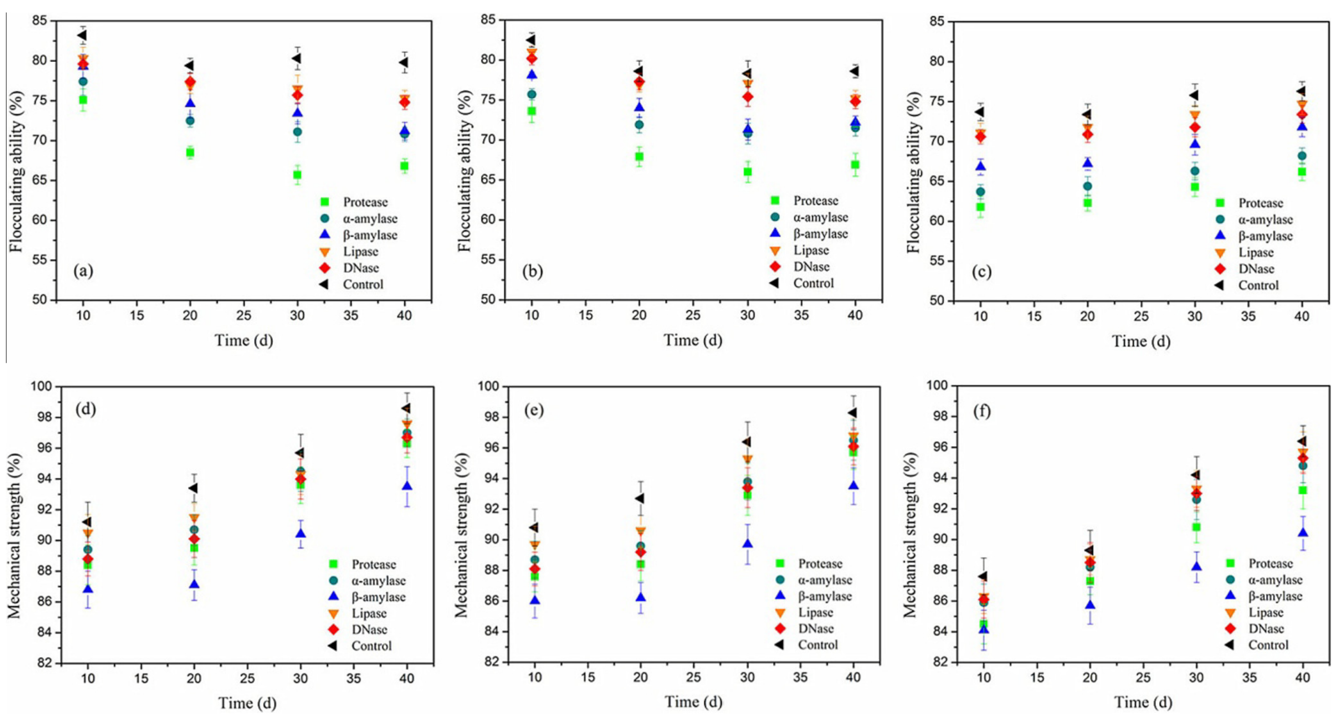

Fig. 4. Flocculating ability and stability response for aerobic granules with specific enzyme at different stages: (a), (d) sludge sampled from LTF_{SBR}; (b), (e) sludge sampled from STF_{SBR}; (c), (f) sludge sampled from C_{SBR}.

also obtained that the carbon consumption in other systems could shift towards polysaccharide production in the presence of excess divalent ions such as Mg^{2+} and Ca^{2+}, and thus high polysaccharide levels could achieve [31,32]. In brief, the production of extracellular carbohydrates can be stimulated by adverse exogenous shocking. As generally acting as a bioglue, extracellular polysaccharides could facilitate cell-to-cell interaction and further strengthen microbial structure through forming a polymeric matrix [8]. In this case, the formation of aerobic granular sludge can be enhanced. Additionally, as previous studies suggested, the ratio of PN to PS also could be used to indicate the performance of microbial aggregates since the protein content positively correlated with the flocculating ability of sludge [24,27]. In the present study, the highest ratio of PN to PS was observed in C_{SBR} while the flocculating ability of the sludge in this reactor was weaker than that of other SBRs (Figs. 2 and 4). This was likely inferred from the less protein (Table 1). For the fractions distribution of EPS in the granules, PAC played a minor role inside. Similar arrangements of proteins, α-D-glucopyranose and β-D-glucopyranose were observed in both PAC-fed system and the C_{SBR}. In general, these three groups of organic matters account for major effect on the stability of granules, especially for proteins and β-D-glucopyranose.

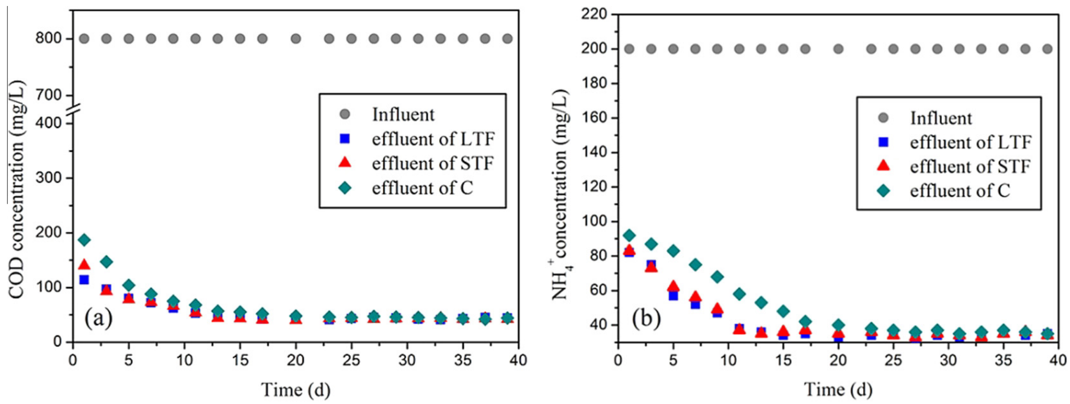

Fig. 5. Performances of SBR reactors stressed by different PAC feeding strategy. (a) COD removal. (b) Ammonia removal.

The protein core always apparently stabilizes granule integrity, and the possible role of β-polysaccharides on granule stability should be also included, as it presents a network over the entire granule [20].

As some Al had been detected after the enzymatic treatment of granules (Table S2), interactions between aluminum and EPS should be also taken into consideration. Two types of mechanisms could be involved to explain the binding reactions between EPS and metals: (1) metals integrated with EPS by electrostatic interactions since abundant negatively charged groups displayed on the EPS surface [33]; (2) complexation potential existed between EPS and metals by the complexing bonds [34]. In the present study, notable release of Al occurred after the hydrolysis of β-amylase and it may imply that Al mainly interacted with β-polysaccharides (Table S2). This result can be also supported by the previous report that polysaccharide found in natural water was selectively removed by aluminum polychlorosulfate during the drinking water treatment [35].

In this study, successful granulation was also achieved in the C_{SBR}, which with no PAC dosing. This result suggested that the selection pressure also should be taken into consideration for the aerobic granulation. As previous studies reported, a short settling time could significantly improve the surface characteristics of sludge cell and these selection pressure-induced microbial changes were in favor of the aerobic granulation [36,37]. For the detail information about the correlation between PAC regulation and selection pressure, and their respective roles in the granulation enhancement, further research is required.

4.2. Coupling process between physicochemical–biochemical effects during the PAC enhanced granulation

Two different feed-strategies were applied in this study to clarify the work time of PAC during the granules formation and it was fixed at the start-up period, speculatively for the first 15 days, after when the Al consumption in LTF_{SBR} tended to be eliminated (Fig. S2). Extension of PAC dosing period will not further improve its reinforcement for aerobic granulation, although no specific inhibitory effect caused by long term exposure to PAC (Fig. S3). This finding was in accordance with the physical characters variation of granules which had been presented above. No matter for MLVSS or SVI, significant differences between LTF_{SBR} and STF_{SBR} were observed after the dosage of PAC in STF_{SBR} stop and tended to decrease only a few days later. For the residual disparity, it could be explained by the more produced EPS in LTF_{SBR} during the previous days (Fig. 2). EPS can improve surface characters of sludge through increasing the cell surface softness and decreasing the negative surface charge density surrounding the cell surface [8]. Furthermore, contributions of EPS to activated sludge aggregation were also explained by extended DLVO (Derjaguin, Landau, Verwey and Overbeek) theory that loosely bound EPS always displayed a positive effect on the sludge aggregation since the interaction energy of it was negative [38].

Despite the fact that difficulties exist in distinguishing the explicit effect of PAC and EPS throughout the granules formation process, some insight can be expected in examining the flocculating ability and stability of granules after the specific enzymolysis treatment (Fig. 4). In terms of flocculating ability, the main factor gradually changed from PAC to proteins along with the formation of aerobic granules. Meanwhile, the PAC induced influence in mechanical strength of granules tended to be negligible while the effect of β-D-glucopyranose on the maintaining of granules stability increased.

As mentioned above, the coupling process between physico chemical–biochemical effects during the PAC enhanced aerobic granulation was revealed (Fig. 6). Initially, physicochemical effect played a major role and the negatively charged sludge flocs mainly aggregated by the neutralization and electrostatic patches of PAC. Bridge connection should be also taken into consideration. Meanwhile, EPS production during this period was stimulated in the presence of various selective pressures such as progressive decrease of settling time, hydraulic shear and slight inhibition of PAC. After then, the whole surface of bacteria turned to be completely surrounded by EPS-layer when abundant EPS accumulated. In this case, different species of aluminum mainly bonded with EPS and the cells in bacteria could be protected against inhibitory of metals. With the SBRs running, adsorption between EPS and PAC saturated and thus the PAC intake of sludge ceased. During the following granules formation, the contribution of EPS dominated. This process demonstrated the formation of PAC enhanced aerobic granulation. Moreover, the reason why PAC-induced enhancement mainly focused on the initial period of granulation process was also adequately explained.

In conclusion, the role of PAC for the aerobic granulation enhancement was identified and the mechanism of this process was well revealed by the systematic investigation. Dosing PAC during the initial period is suggested to improve the application of aerobic granular sludge. Generally, the formation of aerobic granules in pilot-scale took much longer time than in lab-scale reactor [3,39]. In this case, enhancement of PAC may be more attractive for the upscaling development of aerobic granulation although some extra cost will be caused. Furthermore, for the full-scale application of aerobic granules, maintaining treatment performance during the transition from a floc to a granule biomass is also a challenge and high biomass washout during the first days of startup has been identified as the main reason [6,40]. In this study,

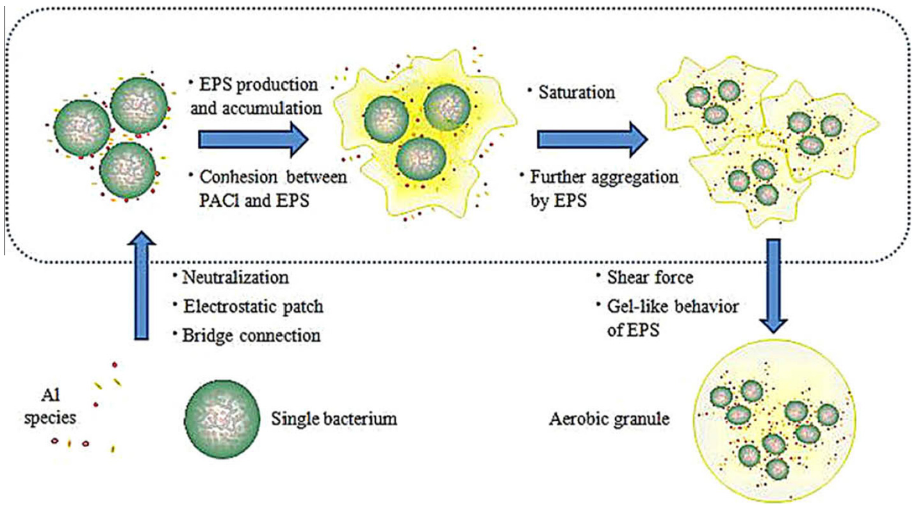

Fig. 6. Coupling process between physicochemical–biochemical effects during the PAC enhanced formation of aerobic granules.

more biomass was saved in PAC-fed reactors than in C_{SBR}, and the reactors performances in nutrient removal were guaranteed. Although the hypothetical working time of PAC has been proposed, a balance between the reactor performances and operating cost should be taken into consideration during the further application of PAC enhanced aerobic granulation.

5. Conclusion

In comparison, aerobic granules cultivated in PAC-fed SBRs preferably performed in settling property, biomass retention and nutrient removal. Moreover, EPS production was also improved, especially for polysaccharides. Enzymatic hydrolysis treatment results figure out the factors which were involved in the formation of aerobic granules. PAC, proteins, α-D-glucopyranose and β-D-glucopyranose were indentified to account for the flocculating ability and stability of granules. In addition, the coupling process between physicochemical and biochemical effects was also revealed. Overall, during the whole enhanced granulation process, the work time of PAC was mainly concentrated in the start-up stage (the first 15 days). Dosing PAC during this period can be an effective and resource-saving approach to enhance the aerobic granulation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cej.2015.09.061.

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