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Characteristics of aerobic granulation at different organic and ammonium compositions

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ABSTRACT

Aerobic granulation was characterized using three different wastewaters consisting of a sole organic substrate, a sole ammonium substrate, and combination of both organic and ammonium substrates. The formation of aerobic granules in each wastewater presented different physical, chemical, morphological, and microbial characteristics. The faster formation of larger and loose granules occurred at the high organic concentration of 1,000 mg COD/L; while the compact small size (0.6 mm) aerobic granules could be developed when nitrification was involved in aerobic granulation. The chemical changes and biomass growth were mainly dominated by heterotrophs and autotrophic nitrifiers. The simulation results of biofilm model demonstrated that the biofilm thickness depended on the substrate concentration at the outer surface and the attachment surface of granule's film. The formed granules can turn into media at a low substrate concentration for developing biofilms on the outer layer.

Keywords: Aerobic granulation; Biofilm; Characteristics; Chemical composition; Simulation

1. Introduction

The technology for forming aerobic granules has been intensively studied in the last decade because it promises a novel effective approach in the field of biological wastewater treatment [1,2]. Aerobic granulation can be regarded as self cell-to-cell immobilized microbial consortium which is usually accomplished in a column-type airlift sequencing batch reactor (SBR) [3]. The unique structure of aerobic granules can lead to a good sludge settleability, a long sludge retention time (SRT), and a high biomass concentration in the reactor as well as the capability of the treatment of various wastewaters [4].

The formation of aerobic granules is mostly studied by applying organic substrates and affected strongly by operating conditions. The aerobic granules can be formed in a wide range of organic loading rates (OLRs) [5]. The granules developed at different loading rates could present different morphology, structural properties, and microbial communities [6]. Furthermore, in order to investigate the simultaneous organic and nitrogen removal, the substrate nitrogen (N)/chemical oxygen demand (COD) ratio effect on the formation of aerobic granules had attracted research interests [7,8]. It was reported that

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the N/COD ratio could affect the microbial and physicochemical characteristics of aerobic granules [9,10].

Nitrifying bacterial have a low growth rate and tend to be washed out from reactors, while aerobic granulation can be a method of immobilizing a large population of nitrifying bacteria within a reactor [11,12]. In comparison with heterotrophic granulation, the autotrophic nitrifying granulation is undesired due to fewer production of extracellular polysaccharide substances (EPS), which hinder the metabolism of organic compounds [13,14]. Therefore, the cultivation of nitrifying granules from matured aerobic granular sludge has been suggested as an effective strategy for rapid start-up [15].

The aerobic granule is a spherical biofilm that consists of different microbial compositions in accordance with the space distribution of substrates and dissolved oxygen [16]. Different organic and ammonium compositions in the wastewater can decide the growth competition between autotrophs and heterotrophs, and hence influence the characteristics of granule's film [17]. The substrate transport is driven by the concentration differences and the cell's location within the granules, thus substrate utilization and cell growth are not uniform [18].

Removal of organic and ammonium pollutants is the main purpose of biological wastewater treatment. Both organic matters and ammonium compounds are always appearing simultaneously in various domestic and industrial wastewaters [19]. Aerobic granulation promises a great potential for the simultaneous removal of both pollutants in a cost-effective way [20]. Therefore, the understanding of physical, chemical, and microbial characteristics of aerobic granulation at different organic and ammonium compositions can be helpful for the development of the application potential of aerobic granules. However, these characteristics have not been studied comprehensively.

Accordingly, in order to investigate the characteristics of the formation of aerobic granules at different organic and ammonium compositions, the wastewaters consisting of a sole organic substrate, a sole ammonium substrate, and both organic and ammonium substrates were applied to the operation of column-type SBRs. In addition, the start-up of nitrifying granulation was investigated by inoculating aerobic granular sludge. The aerobic granulation was comparatively characterized based on physical, chemical, and microbial properties. The biofilm phenomena on the granules were simulated using a steady-state biofilm model.

2. Materials and methods

2.1. Wastewaters

The substrate in synthetic wastewaters mainly consisted of organic carbon ($C_6H_{12}O_6$), inorganic carbon ($NaHCO_3$), ammonium nitrogen ($(NH_4)_2SO_4$), and other necessary nutrients for bacteria growth. The chemicals added to the synthetic wastewater included KH_2PO_4 (70–120 mg/L), $MgSO_4$ (20 mg/L), $CaCl_2$ (100 mg/L), trace solution 1 (1 mg/L), and trace solution 2 (1 mg/L). Trace solution 1 contained (per liter deionized water) ethylenediaminetetraacetic acid (EDTA) (5 g) and $FeSO_4{\cdot}7H_2O$ (9.144 g). Trace solution 2 contained (per liter deionized water) EDTA (15 g), $ZnSO_4{\cdot}7H_2O$ (0.43 g), $CoCl_2{\cdot}6H_2O$ (0.24 g), $MnCl_2{\cdot}H_2O$ (0.66 g), $CuSO_4{\cdot}5H_2O$ (0.25 g), $NaMoO_4{\cdot}2H_2O$ (0.22 g), $NiCl_2{\cdot}6H_2O$ (0.19 g), $Na_2SeO_4{\cdot}H_2O$ (0.21 g), and H_3BO_4 (0.014 g) [21].

2.2. Reactor operation

Three columns (120 cm in height and 6 cm in diameter) with an effective volume of 2.85 L were used as SBRs for aerobic granulation (Fig. 1). The reactors were equipped with the controller system for adjusting pH, DO, and temperature (water jacket). A fine bubble aerator was provided in the bottom of the reactor to supply air. The hydraulic retention time (HRT) was determined by the ratio of working volume to daily flow rate. The SRT was determined based on the effluent suspended sludge that was washed out during steady-state phase. The OLR, the nitrogen loading rate (NLR), and the details of operating conditions for each reactor (*R*1, *R*2 and *R*3) are shown in Table 1. Activated sludge from an aeration tank of the wastewater treatment plant in Ansan City, Korea, was used as inoculums for *R*1 and *R*2. The aerobic granular sludge developed from *R*1 was inoculated to *R*3 for starting up nitrifying granulation. Each reactor was operated over 60 d without excess sludge discharge.

2.3. Analytical procedures

Alkalinity, Mixed liquor volatile suspended solids (MLVSS), and sludge volume index (SVI) were determined according to standard methods [22]. COD and NH_4–N were measured using a spectrophotometer (DR/2500, Method 8038, Hach Co., USA). NO_2–N and NO_3–N were measured using an ion chromatography (790 Personal IC, Metrohm Ltd, Switzerland). The samples of effluent water were filtered by a 0.45 μm nylon syringe filter (Whatman, USA) for the analysis of COD, NH_4–N, NO_2–N, and NO_3–N. The concentration of free

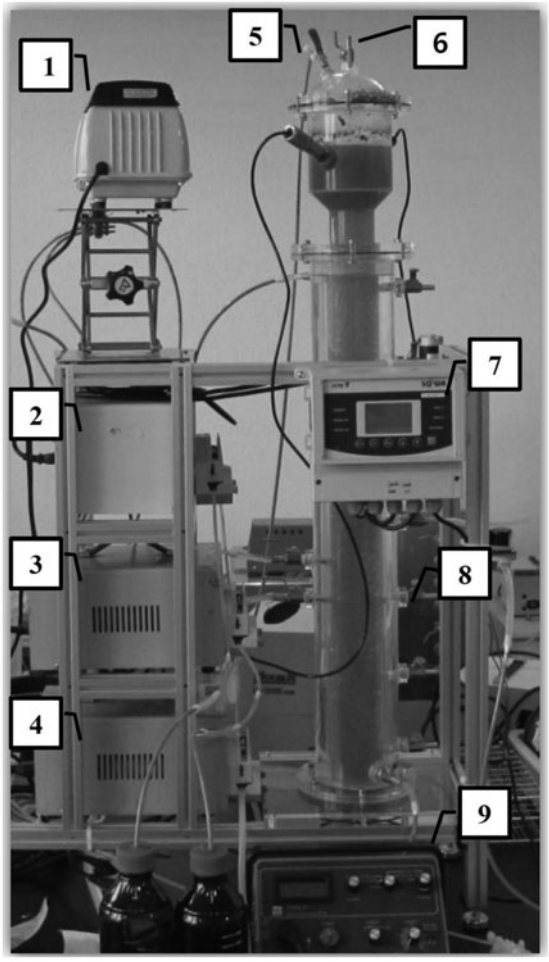

Fig. 1. Photographic illustration of SBR for aerobic granulation (1) air pump, (2) influent pump, (3) acid controller, (4) base controller, (5) influent valve, (6) gas valve, (7) pH controller, (8) effluent valve, and (9) temperatures and DO monitor.

ammonia (FA) was calculated according to the method which was given by Anthonisen et al. [23]. The granular sludge size was determined using a computer-based image analysis system (Image-Pro Plus 6.0, MediaCybernetics, USA). The microstructure of mature granules was examined with a scanning electron microscope (SEM) (MIRA3, TESCAN Inc., USA). The chemical compositions of sludge were analyzed by an energy dispersive spectrometry system for the SEM (TEAM^{TM} EDS, EDAX Inc., USA).

2.4. Biofilm modeling

The substrate mass balance for a steady-state concentration profile in the biofilm is described as follows:

$$D_f \frac{d^2 S_f}{dz^2} = \frac{q X_f S_f}{K + S_f} \quad (1)$$

where S_f is the substrate concentration at that point in the film (mg/cm^3) and z is the depth dimension normal to biofilm surface (cm).

Eq. (1) has two boundary conditions, the first is no flux into the attachment surface and the second is substrate transport at the biofilm/water interface [24]. By specifying the concentrations at both boundaries of the biofilm, Eq. (1) can be analytically solved as follows:

$$J = \sqrt{2qX_f D_f \left(S_o - S_a + K \ln\left(\frac{K + S_a}{K + S_o}\right)\right)} \quad (2)$$

where J is the substrate flux into the biofilm (mg/cm^2-L), S_o is the substrate concentration at the outer surface (mg/cm^3), and S_a is the substrate concentration at attachment surface (mg/cm^3).

Biofilm thickness (L_f, cm) is obtained by following equation:

$$L_f = \frac{JY}{X_f b'} \quad (3)$$

The description of the parameters for biofilm modeling is shown in Table 2. The details for the estimation of parameter values can be found in published papers [18,25]. The calculations were carried out using MATLAB (MATLAB R2012a, MathWorks, USA).

3. Results and discussion

3.1. Formation of aerobic granules

The operating results for aerobic granulation in each reactor are shown in Fig. 2. The formation of aerobic granules presented different processes in *R*1, *R*2, and *R*3. The aerobic granules could be observed in *R*1 after 7 d of operation, whereas the sludge was comprised of a few aerobic granules and a large mass of flocs. The granules became dominant in *R*1 after 21 d when the settling time was 5 min. Throughout the operation of *R*1, the effluent contained the suspended solids that were unable to settle in the zone below the discharge point. The complete formation of aerobic granules in *R*2 was observed with the significant disappearance of suspended flocs in the reactor after 21 d. At day 56, the high biomass concentration of 6,645 mg MLVSS/L was determined in *R*2. The inoculated

Table 1
Operating conditions of SBRs for aerobic granulation

Inoculation	Unit	*R*1 Activated sludge	*R*2 Activated sludge	*R*3 Aerobic granular sludge
Initial MLSS	mg/L	2,500	2,500	3,000
Influent COD	mg/L	1,000	400–500	
Influent NH_4–N	mg/L	50	100–150	200–600
OLR	kg COD/m^3/d	4.20	1.12–1.40	–
NLR	kg N/m^3/d	–	0.28–0.42	0.52–1.37
Alkalinity	mg $CaCO_3$/L	500	3,000	3,500–4,000
Influent pH		7.0–7.5	7.5–8.0	8.0–8.5
Temperatures	℃	20–25	20–25	35
Aeration rate	L/min	3.0	3.0	3.0
Aeration time	min	220–230	340–350	350
Settling time	min	5–15	5–15	5
Total cycle time	min	240	360	360
HRT	d	0.24	0.36	0.48
SRT	d	20	60	40

Table 2
Estimated parameter values for the simulation of granule's film

Parameter	Unit	Definition	Reactor		
			*R*1	*R*2	*R*3
Y	g VSS/g COD or NH_4–N	Biomass yield coefficient	0.56	0.62	0.24
q	mg COD or NH_4–N/mg VSS-d	Maximum specific substrate utilization rate	18	5	6
K	mg/cm^3	Substrate half-saturation coefficient	0.02	0.04	0.002
b	1/d	Endogenous decay coefficient	0.15	0.25	0.10
b'	1/d	Overall biofilm loss coefficient	0.20	0.30	0.15
X_f	mg/cm^3	Biomass density of aerobic granules	30	50	40
D_f	cm^2/d	Molecular diffusion coefficient		0.8	

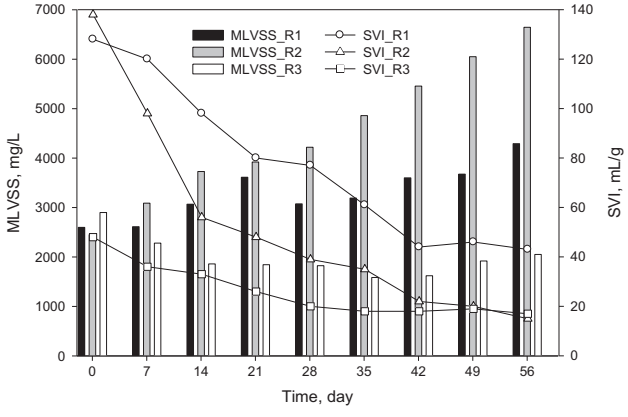

Fig. 2. Biomass (MLVSS) and settleability (SVI) variations during the operation of SBRs for aerobic granulation.

aerobic granular sludge could be maintained in *R*3 by forming smaller size granules. During the operation of *R*3, the biomass in the reactor was decreased by the sludge wash out and it began to increase after 42 d. The SVI in *R*1, *R*2, and *R*3 on day 56 was 43, 15, and 17 mL/g, respectively, which demonstrates that the settleability had been dramatically improved comparing with activated sludge.

The operations of *R*1, *R*2, and *R*3 developed the aerobic granules with different characteristics. The aerobic granules in each reactor had a clear and regular round shape. The average diameter of dominated granules in *R*1, *R*2, and *R*3 (after 50 d) was 3.0, 6.0, and 1.0 mm, respectively. Fig. 3 shows different shapes of granules under SEM observation. The granules in *R*1 and *R*2 had smooth and compact surfaces which indicated no significant filamentous cells. The size of granules in *R*2 was much smaller than the granules in *R*1 but had a

more stable structure that the outer biomass was not easy to detach. This may be because the nitrifying bacteria had a higher hydrophobic interaction than that of heterotrophic bacteria [9]. The growth of filament flocs was observed in *R*1, whereas most of them were washed out due to poor settleability. The inoculated aerobic granules significantly decreased in size under the operating conditions of *R*3. After the use of organic substrate was stopped, the heterotrophic biomass could rapidly decay and detach from the granules which might cause the appearance of smaller granules and the biomass loss in the reactor. The surface of granules in *R*3 showed a rough structure with spherical and rod-shaped micro-organisms. It was estimated that the

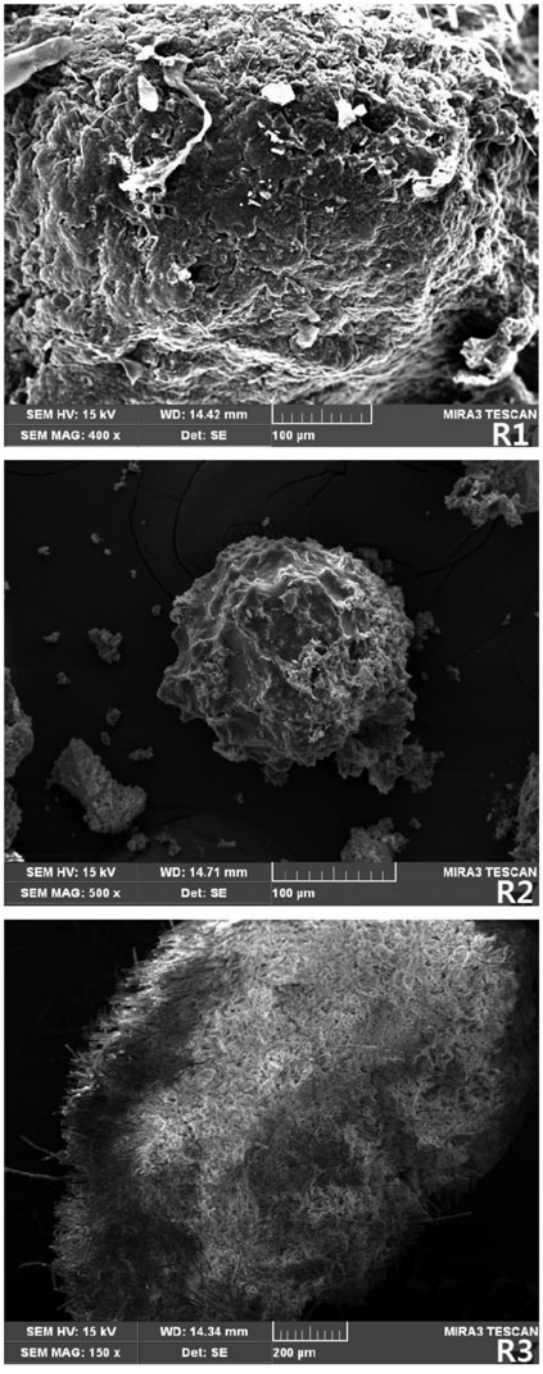

Fig. 3. Morphology of different aerobic granules under the observation of SEM image (after 50 d).

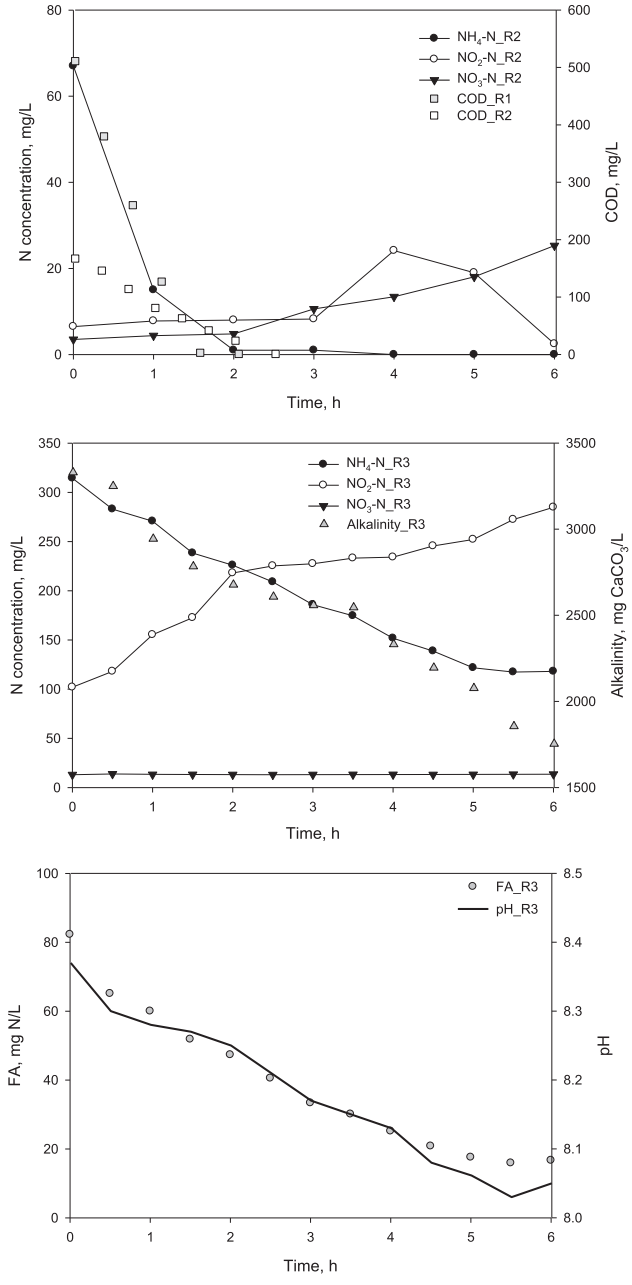

Fig. 4. Organic removal, nitrification and FA and pH profiles in the SBRs on day 50.

nitrification process developed different microbial diversity and changed the structure of granules. Nevertheless, the granules could be maintained in the reactor and the formation and the structure of granules were stabilized eventually. In conclusion, the faster formation of larger and loose granules occurred at a higher OLR and the formation of smaller and more compact granules occurred at a higher NLR.

3.2. Organic removal and nitrification of aerobic granules

The SBRs for aerobic granulation were operated at different substrate compositions, (Table 1) the sole high organic substrate (*R*1), the high NH_4–N/COD ratios (*R*2), and the sole ammonium substrate (*R*3). Fig. 4 shows the profiles of COD and nitrogen concentrations during the one-cycle operation of SBRs. Almost all influent COD could be removed in 3 h in the operations of *R*1 and *R*2. The ammonium completely decreased, and both nitrite and nitrate were produced in *R*2. After finishing the one-cycle operation of *R*3, the ammonium concentration higher than 100 mg N/L was determined in the reactor. The decrease of alkalinity in *R*3 may demonstrate that a significant nitrifier developed in the reactor might utilize inorganic carbon (HCO_3) for their growth. Most of the nitrite was not further oxidized to nitrate because the high ammonium concentration at a high pH 8.0–8.5 could produce significant FA that mainly inhibits the activity of nitrite oxidizers [26]. During the operation of *R*3, the FA change was in the range of 17–82 mg N/L that could be considered as inhibitory concentration of nitrite oxidation [23]. The substrate profiles indicated that the *R*1 mainly developed heterotrophic biomass, the *R*2 simultaneously developed both heterotrophic and autotrophic biomass, and the biomass in *R*3 mainly comprised autotrophic ammonia oxidizers.

3.3. Elemental composition on aerobic granules

The characteristic differences of aerobic granules under different operating conditions can be related to the changes in chemical compositions. For the different formation of aerobic granules, the chemical composition may reflect the characteristics of microbial community and growth conditions. Fig. 5 shows the elemental compositions of activated sludge and the aerobic granules in *R*1, *R*2, and *R*3. The data indicated typical compositions of main elements which are C, O, N, S, and P in micro-organisms for the activated sludge and the granules in *R*1 (the main element H was not displayed here due to unavailable detection using EDX). The accumulation of calcium was determined in *R*2 and *R*3. The Ca/C ratios in *R*2 and *R*3 were much higher than the activated sludge and the aerobic granules in *R*1. The nitrification process could result in the increase of negative ions, such as nitrite and nitrate on the surface of granule's bacteria which may enhance the attraction of positive calcium ion. Calcium is an ionic cross-bridging molecule for the polysaccharides of bacteria that are negatively charged

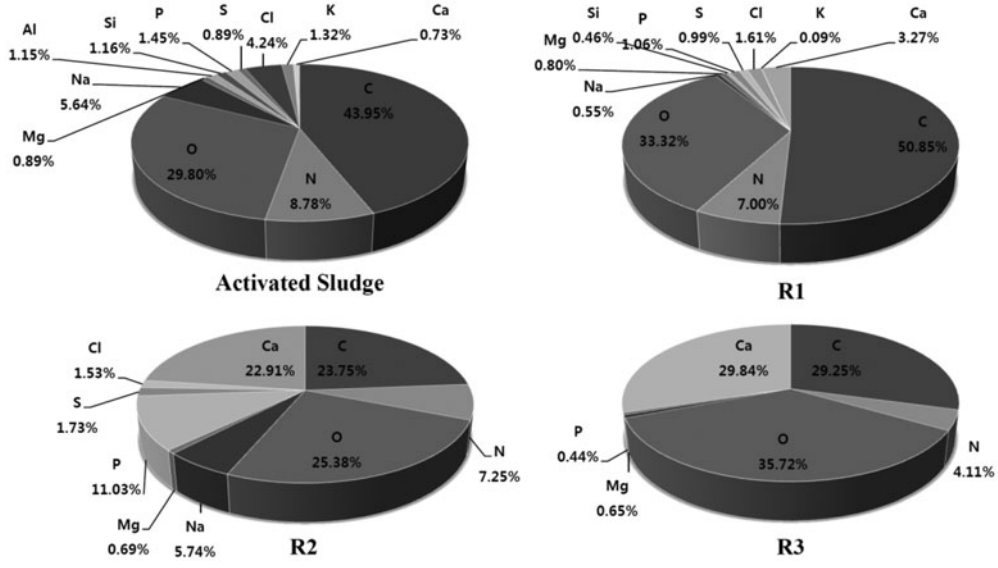

Fig. 5. Elemental compositions of activated sludge and aerobic granules after 50 d.

and involved in bacterial adhesion to a surface when binding with proteins [27,28]. Therefore, the calcium could have an important function for cell-to-cell aggregation with the involvement of nitrifying bacteria in aerobic granulation. In addition, the high phosphorous accumulation of 11.03% was determined in *R*2. A high-biomass concentration with the infinity retention time may have resulted in the accumulated phosphorous higher than conventional sludge. The potential of phosphorous removal could be expected under the operating conditions of *R*2.

3.4. Biofilms of aerobic granules

The aerobic granules can be considered as self cell-to-cell immobilized biofilms. Biofilm process is very complex, both physically and microbiologically. Experimental approach has limitations to fully understand and monitor the biofilm phenomena, such as mass transfer, substrate utilization, and biofilm growth. Therefore, the model simulation based on estimated kinetics could be a desirable approach for the insight of granule's film. Fig. 6 is the simulation results of the biofilm growth based on the changes of substrate concentrations. The simulation focuses on steady-state biofilms. The biofilm growth in *R*1 and *R*2 mainly depends on organic substrate. Since the growth rate of heterotrophs was much faster than the autotrophs, the growth of autotrophic biofilms in *R*2 can be negligible; while the autotrophic nitrifiers are the dominant biofilms on the granules for the operation of *R*3 which uses ammonium as main substrate. Two types of substrate phenomena lead to the development of bioflim thickness at different concentrations. For a deep biofilm, the substrate concentration approaches zero at some point in granule's film. Otherwise, the substrate penetrates the granule's film in a decreased concentration. Therefore, the development of biofilm thickness depends on the substrate at outer surface (S_o) and at the attachment surface (S_a). The biofilm thickness decreases with the increase of S_a. The microbial characteristics of the granules in *R*1, *R*2, and *R*3 result in different profiles of biofilm thickness. The simulation results show that the developed thickness in *R*1 is higher than the other reactors at the same concentration conditions. The autotrophic nitrifying granule's film (*R*2) has a potential to develop the thickness like heterotrophic biofilm. However, the slower growth and the lower EPS production can significantly slow down the rate of biofilm growth. Furthermore, the size of nitrifying granules can be much smaller than the heterotrophic granules due to the detachment of biofilms in a long development period of granule's film. The production of EPS at high shear force could lead to the formation of more compact and denser granule's film [29]. In the bubble

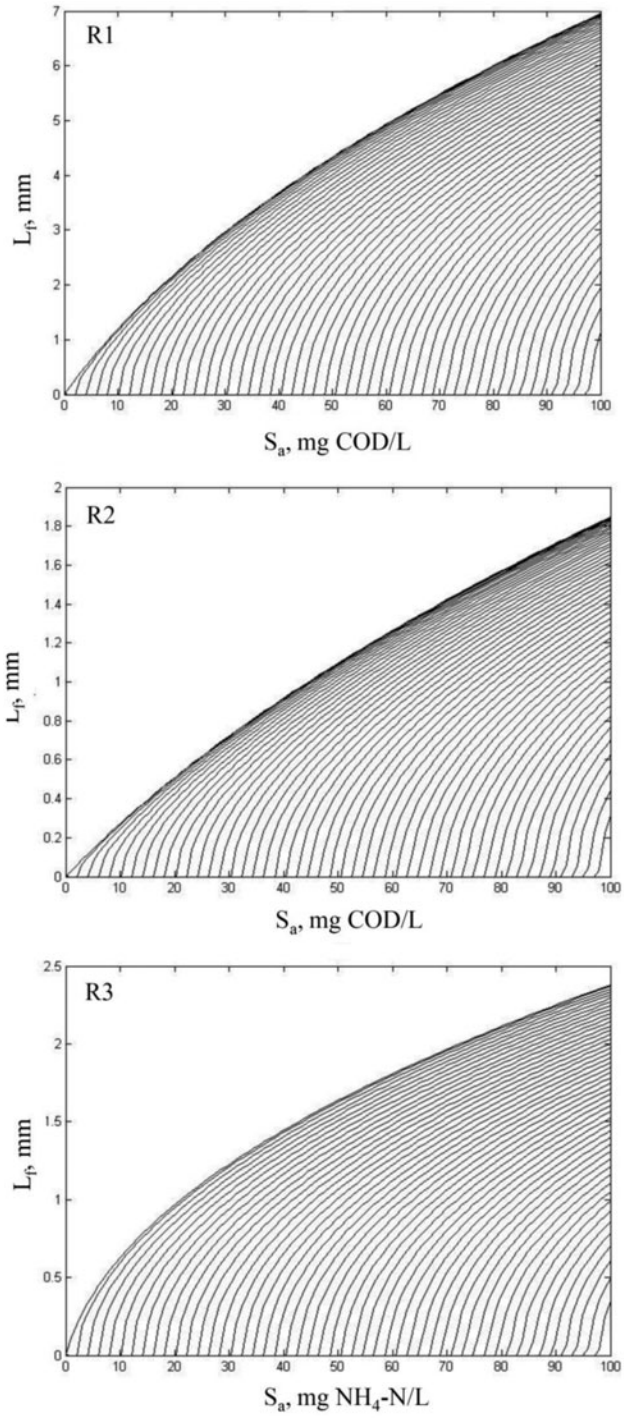

Fig. 6. Granule's film thickness (L_f) changes based on the substrate concentrations at the outer surface (S_o) and the attachment surface (S_a) (when S_a = 0, 2, 4, 6, … , 98, 100 mg COD or NH_4–N/L, total 50 lines in each figure).

column SBR, the up-flow velocity creates hydrodynamic shear force. Thus, the formation of a biofilm on the granule could be affected by the different levels of hydrodynamic shear forces.

Under different operating conditions, the aerobic granules in each reactor can create internal unique microenvironments (e.g. pH, O_2, and microbial community) depending on the compositions of organics and ammonium in substrate. As a result, the formation of aerobic granules is driven to have different characteristics by the developed biofilm. In addition, the formed granules turn into the carriers which can develop biofilm on the surface.

4. Conclusion

The purpose of aerobic granulation technology is to benefit the application in wastewater treatment. The understanding for the characteristics of aerobic granulation can be importantly involved with the operating strategy for system optimization. The characteristics of the mature aerobic granules could be different under different operating conditions. The desirable start-up for aerobic granulation could be achieved by operating the column-type SBR reactor at a high organic loading which generated loose and round-shape aerobic granules in two weeks. The compact small size (0.6 mm) aerobic granules could be developed when the nitrification process was involved. The formation of nitrifying granules could be derived from the inoculated aerobic granular sludge by reducing the start-up time. The development of both heterotrophic and autotrophic biomass in a simultaneous process was available by applying aerobic granulation. The chemical compositions may reflect the characteristics of microbial community and growth conditions when developing aerobic granules under different organic and ammonium compositions. The calcium composition of 22–30% in aerobic granules was determined when nitrification was involved in aerobic granulation. The substrate concentration influences the characteristics of granule's biofilm. The biofilm thickness depends on the substrate concentration at the outer surface and the attachment surface.

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List of symbols

- b — endogenous decay coefficient (1/d)
- b' — overall biofilm loss coefficient (1/d)
- D_f — molecular diffusion coefficient (cm^2/d)
- J — substrate flux into the biofilm ($mg/cm^2 L$)
- K — substrate half-saturation coefficient (mg/cm^3)
- Q — maximum specific substrate utilization rate (mg COD or NH_4–N/mg VSS-d)
- S_f — substrate concentration at that point in the film (mg/cm^3)
- S_o — substrate concentration at the outer surface (mg/cm^3)
- S_a — substrate concentration at attachment surface (mg/cm^3)
- X_f — biomass density of aerobic granules (mg/cm^3)
- Y — biomass yield coefficient (g VSS/g COD or NH_4–N)
- Z — depth dimension normal to biofilm surface (cm)

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