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THE MONITORING OF AEROBIC FLOC-LIKE SLUDGE INFLUENCED BY CALCIUM IONS

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Abstract : Aerobic floc-like sludge was formed in a batch reactor and the effect of cations on the formation of aerobic floc-like sludge was studied. In order to enhance the formation (rate) of aerobic floc-like sludge, cations such as K^+, Na^+, Ca^{2+}, and Mg^{2+} were added to the seed sludge. It was found that Ca^{2+} had positive effect on the formation of floc-like sludge, as measured by sludge volume index (SVI) for settle ability. The formation of floc-like sludge was confirmed by the microscopic observation after DAPI staining. The scattered forms of sludge samples at the initial stage became aggregated to form flocs after Ca^{2+} addition. To ensure the functions of sludge flocs in a treatment plant, the gradient of ionic species around the surfaces of floc-like sludge was monitored by ion selective microelectrodes for NH_4^+, NO_3^-, and pH. The effective concentration of Ca^{2+} ion to form floc-like sludge was determined to be 750 mg/L (0.15 mg Ca^{2+}/mg MLSS). Under the effective Ca^{2+} condition, the SVI value was the lowest and large distribution of nitrifying bacteria at the outer surface was observed in the aerobic floc-like sludge. From the results, it was found that the calcium ion functioned as an agent for the formation of aerobic floc-like sludge, resulting in the enhanced nitrification.

Key Words : Aerobic floc-like sludge, Calcium ion, Ion-selective microelectrodes (ISEs), Sludge volume index(SVI).

INTRODUCTION

In biological wastewater treatment processes, maintaining the high biomass concentration is one of most important conditions to ensure high enough treatment performance under fluctuating organic loading rates, disposal of wasted sludge, and to diminish a large space requirement for reactors and settlers.1) Regarding the biomass concentration, various studies have been conducted and finally developed the biofilm process to resolve the above problems. While conventional biological processes require several unit processes such as aerobic tank, nitrifying and denitrifying reactors for each biological reaction, biofilm process allows compact space requirement, at the same time performs effective removal of contaminants.2)

Many papers reported the biofilm process with granulation, but most of the studies were conducted under anaerobic condition since it is known that the granulation is better achieved under anaerobic conditions than aerobic condition. Lettinga, *et al.* (1993) studied extensively the anaerobic granulation process in the biological system for wastewater treatment.3) Their study resulted in the UASB reactor build-up as a compact, high performance reactor. In many places, successful operation of UASB reactor

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and the modified forms have revealed the roles of granules in biological treatment system. Recently, more interest is given to the aerobic granulation process and studies conducted to evaluate the process feasibility. Granular sludges have characteristics of high settling velocities leading to a good solid-liquid separation, high biomass retention, high activity, and an ability to withstand high loading rates. Studies for the aerobic granulation applied in SBR systems[4-7] revealed that the solid-liquid separation was very efficient during the treatment, so that high accumulation of the active biomass could be achieved. The results also showed a potential to save the operational cost, as there was no carrier material required in the SBR process.

In spite of the advantages, the term and mechanism of granulation has not been defined well. It is likely that the granulation starts up with suspended cells or non-settling flocs that may aggregate with the time passage. The aggregated floc-like sludges in the SBR are then forced to form granules, otherwise they would be washed out due to the upward liquid velocity. The compactness and high settling velocity of the granules allow the settling time required to be reduced as well. In this study, the formation of aerobic floc-like sludge was initially studied in a lab scale, as a pre-test for the evaluation of optimum conditions for aerobic granulation.

Since the activated sludge has flocs composed of diverse microorganisms, biological polymers, and inorganic cations,[8-11] many researchers have suggested that cations interact with the negatively charged biopolymers in activated sludge so as to cause structural change into the floc. It has been studied that divalent cations tend to improve settling and dewatering properties of sludge.[10-12] Van der Hoek[13] hypothesized that calcium might create a matrix for the formation of floc-like sludges. In addition, the rate of mass transport on the surface of floc-like sludges can be described in terms of a mass transport coefficient by defining a mass boundary layer over which the concentration of a chemical change gradually form the bulk concentration to the surface concentration. The thickness of the mass boundary layer is controlled not only from the flow and viscosity but also from the diffusion coefficient itself. As the bulk fluid moves, for example, the depth of the boundary layer decreases and convective transport dominates in the bulk phase. Only in a small area above the biofilm surface and inside the biofilm diffusion then occurs the main transport mechanism.[14] It can be assumed that due to the boundary layer, the concentration at the surface of the biofilm may differ considerably from the bulk concentration. Therefore, transport through the mass boundary layer must be taken into account if it significantly contributes to the total diffusional resistance.[15]

The objectives of this research are to investigate the effect of calcium ion on the granulation of activated sludge through the settling properties of biosolids, to monitor the gradient of ion concentration within the biofilm of aerobic flocs with ISEs (Ion selective microelectrodes) and DAPI (4,6-diamidine-2-phenylindole) staining method, and to determine the boundary layer of the floc-like sludge.

MATERIALS AND METHODS

Batch Test

In order to investigate more effective cations for aerobic granulation, cations including Na^+, K^+, Mg^{2+}, and Ca^{2+} were selected and added to each chamber of the Jar-tester (PHIPPS & BIRD, Richmond, VA 23228). Before the addition of cations to each chamber, the Jar-tester containing the pure seed sludge was operated for 12 hours at 60 rpm. The rotation speed was then reduced down to 30 rpm after addition of the cations at room-temperature. The jar-tester was equipped with six jars (beaker) and rotating controller to regulate the speed. The seed sludge was the activated sludge taken from the municipal wastewater treatment plant in Gwangju, Korea. Initial characteristics of the seed sludge are described as follows; 5,000 MLSS mg/L pH 7.6, SVI 210 (ml/g). Four levels of cations (0, 250, 750, and 1250 mg/L as chloride salts were added to the seed sludge. The ranges of cation concentrations were based on the previous studies.[16] After the effective cation species and the appropriate concentration ranges were determined, only calcium ion was used as an additive within narrow concentration ranges, such as 550, 750, and 950 mg/l. For the sludge samples with the calcium dose, sludge volume index (SVI) was monitored every 24 hours for seven days of experimental periods to observe the granulation process. Biomass concentrations were measured as MLSS levels. All the experimentalmethods were followed as described by method 2710D in Standard Method.[17]

Microelectrode Measurements

After the batch test, the aerobic floc-like sludge samples were immediately attached on the thin nylon thread sieve (size: w 2.5 cm × h 2 cm; cellular area of sieve: 0.04 mm^2). The thread sieve was then transferred to a small open chamber designed to be suitable for microelectrode measurement (Figure 1). The microelectrode measurement was conducted in a Faraday cage in order to prevent external electric force. The small chamber was continuouslysupplied with the medium containing 7 mg/L of NH_4^+ and the pH level was 7.8. Due to the mucus' properties of floc-like sludges, in general, the sludge sample was easily attached on the thread sieve and the floc-like forms were not broken during the microelectrode measurement.

The ISEs (i.e., pH, NO_3^-, and NH_4^+ microelectrodes) with a tip size of about 10 m were fabricated as described previously by Yoon (2003).[2] After the fabrication of ISEs in the laboratory, the ISEs were calibrated with dilution series of pH, NO_3^-, and NH_4^+ medium to test the accuracy and the performance of ISEs. Only the microelectrodes which satisfied the calibration test(the error rage of standard curve$\leq$5%) were used in the experiment. As nitrification occurred through the sludge sample, the ion concentrations in the floc-like sludge were measured in every 100 m depth. As shown in Figure 1, the measurement system of microelectrode consisted of the working electrode (i.e., ISEs), the reference electrode, a micromanipulator, a dual light source, a stereomicroscope, a microelectrode chamber, and the data acquisition apparatus.

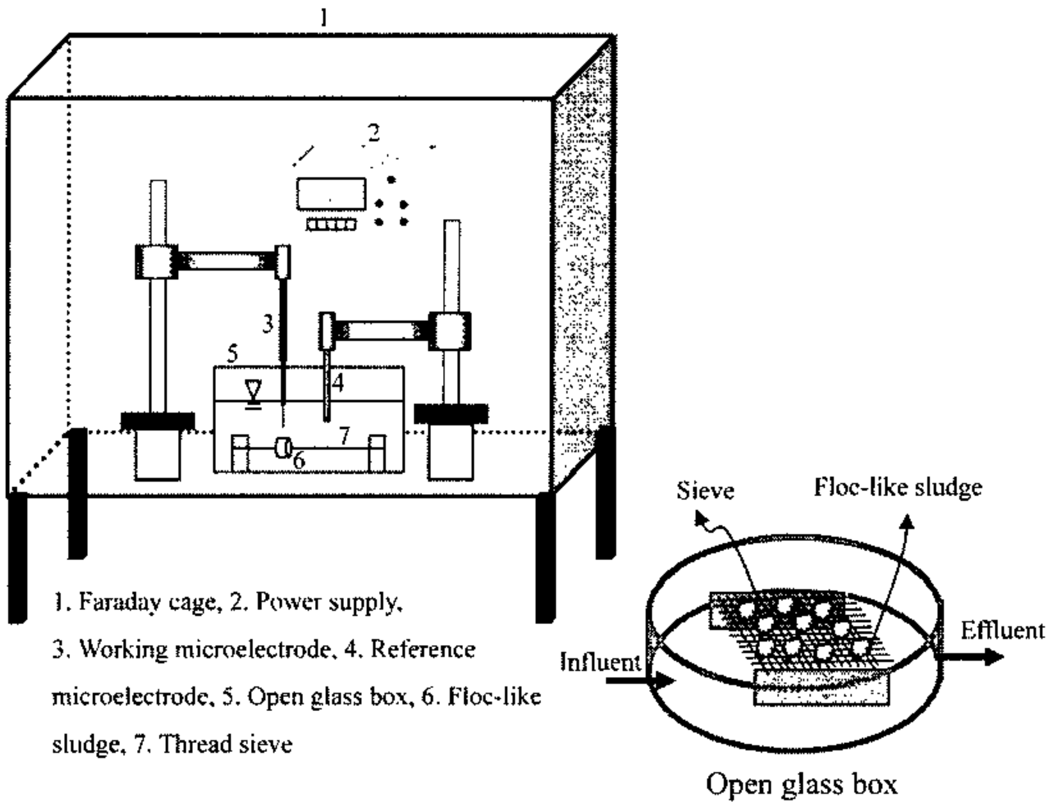

Figure 1. Micro-sensor settings in Faraday cage to measure EMF (electromotive force).

DAPI Staining

The DAPI (4,6-diamidine-2-phenylindole, 0.33 g/ml) staining method was applied during the granulation process through the observation of microbial aggregation. Samples of floc-like sludge were taken from the jar-tester, then they were fixed in one third ratio with 4% paraformaldehyde solution for 1 hour at 4℃. After fixation, the floc-like sludges were rinsed with PBS (Phosphate Buffered Saline). Each sample was placed on a gelatin coated slide glass. The sample on the slide glasses were dehydrated by successive soak in the different concentrations of ethanol solutions and then air-dried before used for DAPI staining for 5 min in the dark. After then, DAPI stained samples were observed through the epifluorescence microscopy (Carl ZEISS, Ser.Nr.995179) or scanning electron microscopy (SEM, Hitachi High-Technology Corporation, S-4700) in a dark room at 25℃.

Kinetics of Floc-Like Sludge

The flux (J_s) through the interface of floc-like sludges with radius (r) should be equal to the flux across the diffusion layer. It is assumed that the flux across the boundary layer occurs only by diffusion, so that it can be described by Fick's law (Eq. 1).

$$J_{s,x} = -D_{sw}\left(\frac{dc_{sw}}{dr}\right)_{r\downarrow R} \qquad (1)$$

where D_{sw} is the molecular diffusion coefficient of compound S in the liquid phase, r is the distance from the center, R is the radius, and $(dc_{sw}/dr)_{r\downarrow R}$ is the concentration gradient in the boundary layer at the biofilm-liquid interface. Since the concentration profile in the fluid is linear, equation (1) can be simplified for a stagnant fluid film thickness of h, as;

$$\delta_h = \frac{D_{sw}}{J_{s,x}} (C_{sw,\infty} - C_{sb,0}) \qquad (2)$$

where $C_{sw,\infty}$ is the bulk concentration at the interface between the well-mixed fluid and stagnantfluid interface, and $C_{sb,0}$ is the concentration at the interface between the biofilm and the stagnant fluid. With microelectrodes, the ionic concentrations around the biofilm surface can be measured directly, at 100 m interval. The diffusion coefficients of ammonium and nitrate at 30℃ were taken at 1.8×10^{-9} and 1.6×10^{-9} m^2/s, respectively.[15]

RESULTS AND DISCUSSION

The Effect of Cation on the Formation of Floc-Like Sludge

After the Jar-tester was operated for 7 days to form the aerobic floc-like sludge, the sludge formation of the each sample was examined by SVI measurement. Compared to the initial SVI values, all the SVI values were consistently decreased indicating the aggregation of the activated sludges under the conditions of no chemical addition. However, when the SVI was measured after the addition of various cations (Na^+, K^+, Mg^{2+}, and Ca^{2+}), the range of SVI changes between the different chemicals was significantly different from each other. Figure 2. shows the result of SVI values of floc-like sludge with the four different doses of cation species. With the addition of monovalent cation, no significant change in the SVI values was observed between the initial and final states of the floc-like sludge. However, the SVI values of the floc-like sludge added with divalent cations such as Mg^{2+} and Ca^{2+} decreased more conspicuously than those with the monovalent cations. It was reported that increasing concentrations of monovalent cation resulted in the deterioration of floc-formation and lowered sludge performance (poor sludge settling and dewatering) through the possible ion exchange mechanisms.[9,11] On the other hand, when the concentrations of divalent cation (Mg^{2+} and Ca^{2+}) increased, improved sludge performance was observed due to the increased divalent cation bridging.[18]

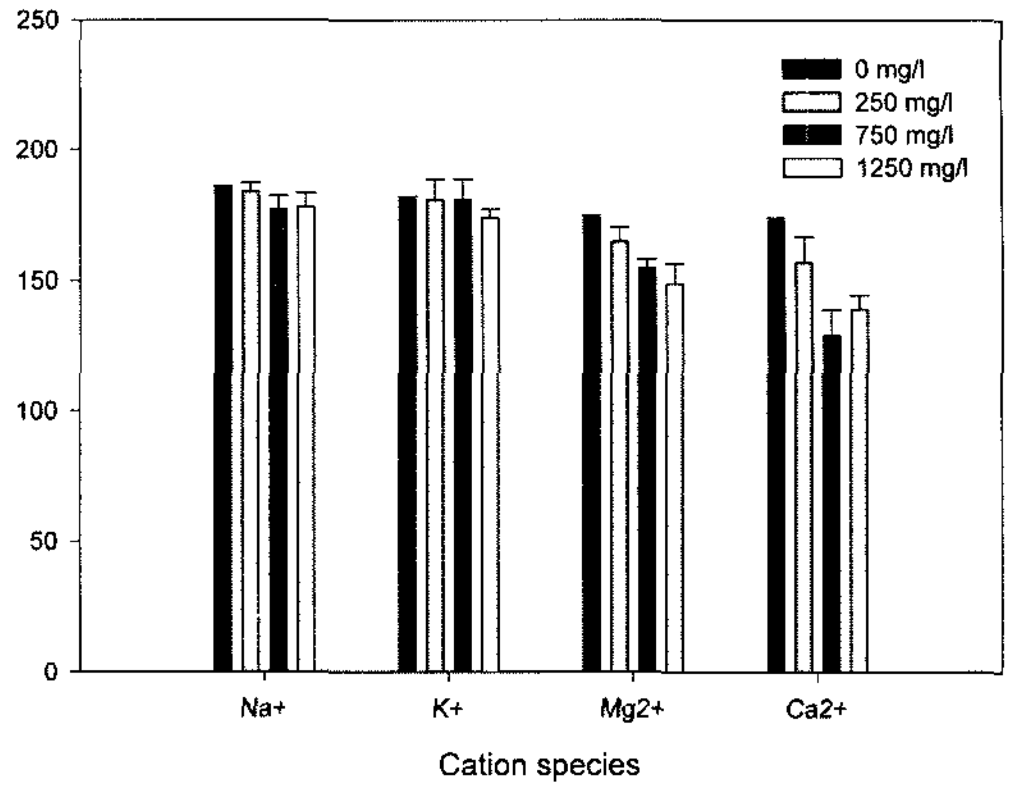

Figure 2. The final SVI of seed sludge influenced by several cations.

Figure 3. describes the changes of SVI values in the aerobic floc-like sludge samples influenced by various amount of calcium ion with the passage of time. The SVI of seed sludge (avg. 200 SVI) decreases to 185 (R1, blank), 158 (R2, 550 mg Ca^{2+}/L), 135 (R3, 750 mg Ca^{2+}/L), and 139 (R4, 950 mg Ca^{2+}/L). The low SVI value indicates the high floc-like biomass concentration, as the biomass tends to make more compact form of flocs with the time passage. From the results shown in Figure 3, it can be assumed that the aggregation of biomass can be stimulated by calcium ion addition as it functions as a bridging agent for the microorganisms to combine each other.

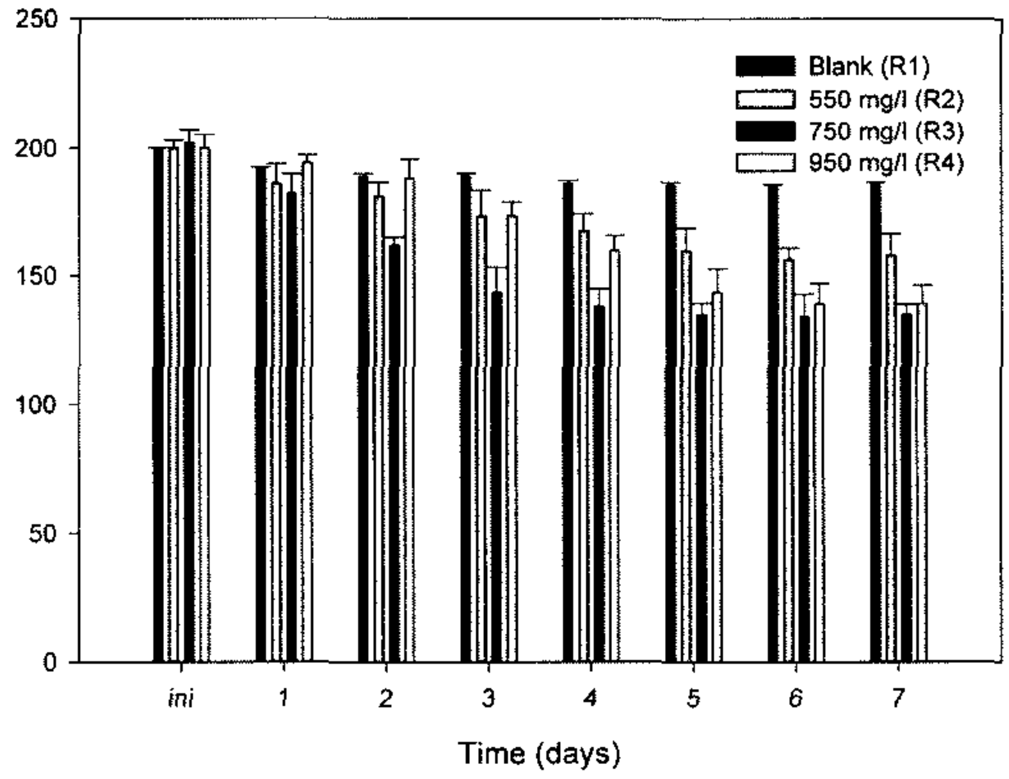

Figure 3. The result of SVI trends through the various concentrations of calcium ion with time passages.

Formation of the Floc-Like Sludge

Microscopic examination of the initial seed sludge showed a typical morphology of general activated sludge, in which filamentous microorganisms were dominant. The average SVI value of the seed sludge used in this test was 210 ml/g, which implied the existence of filamentous microorganisms. It is known that SVI value above 150 ml/g in activated sludge is related with filamentous growth. The seed sludge had many scattered microorganisms and did not make aggregate or flocs. However, the floc-like sludge formed during the experimental period barely contained any filamentous microorganisms. The formation of floc-like form of sludge was observed after 5 days of operation. From the result of DAPI staining shown in Figure 4. (a) and (b), it was clearly shown that the microorganisms could easily build a floc-like sludge by the aid of calcium ion. The DAPI staining method is for living cell counting using the fluorescent dye combined with rRNA or DNA. The detailed microstructure of the floc-like sludge in Figure 4. (c) was obtained using SEM. It shows a compact bacterial structure in which cells were tightly combined with each other, and a rod-shaped species was found to be dominant. The distribution of microorganisms was similar to the result of Tay et al (2001), which showed the cells tightly linked together and they have rod-like species predominant in outer surface.

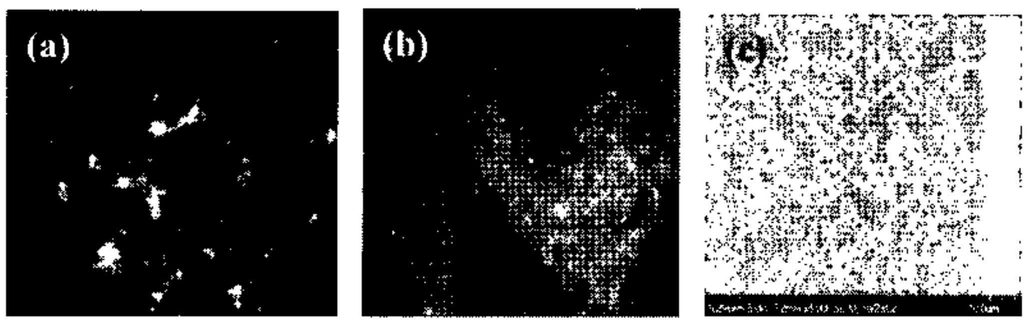

Figure 4. The aerobic floc-like sludge influenced by calcium through the DAPI staining (X200); (1) no addition of calcium ions, (b) addition of 750 mg Ca^{2+}/L. The figure (c) observed by SEM shows the surface of floc-like sludge.

Microelectrode Analysis

The final samples of floc-like sludge formed during the experimental period were transferred to the microelectrode chamber and the ion concentrations (pH, nitrate, and ammonium) were measured by the ISEs. The biological reactions resulted in the ammonium and nitrate concentration change with the depth of the floc-like sludge. As shown in the microprofiles in Figure 5. (a) and (b), the total amount of ammonium ion consumed was not balanced with the amount of the nitrate ion produced. The production of nitrite might cause the difference in the floc-like sludges.

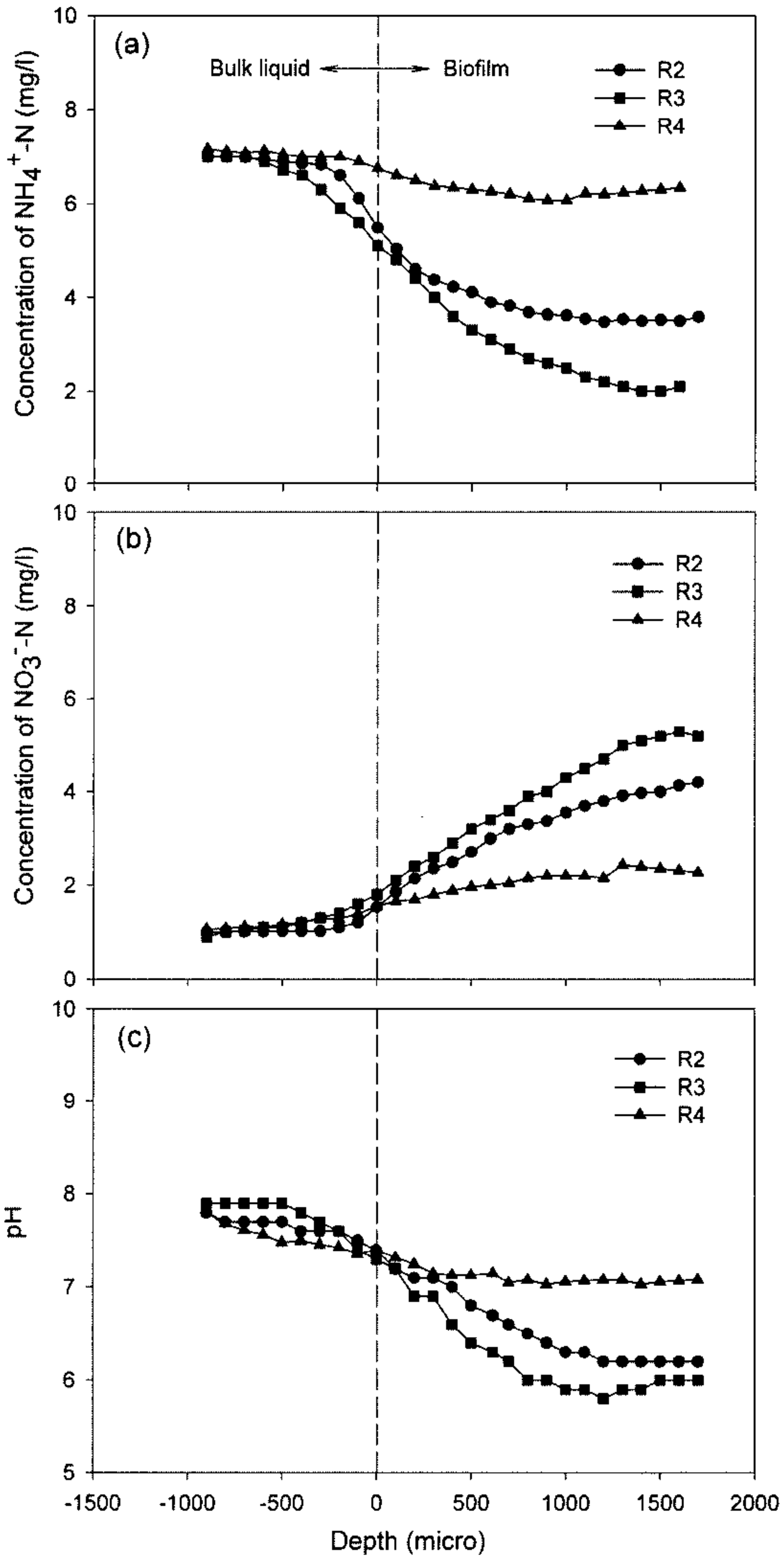

Figure 5. Microprofiles of several ion concentrations measured by ISEs; (a) NH^{4+}-N, (b) NO^{3-}-N, and (c) pH; R2 (550 mg Ca^{2+}/L), R3 (750 mg Ca^{2+}/L), R4 (950 mg Ca^{2+}/L).

The profile of pH through the surface of floc-like sludge (Figure 5. (c)) indicated the decrease of pH levels with the depth of flocs. Since the hydrogen ion is produced as a result of nitrification, it could be assumed that the nitrifying bacteria distributed in the outer surface on floc-like sludge. The amounts of hydrogen ion produced are proportional to the activity of nitrifying bacteria. As it was expected, the highest reduction in the pH level was shown in R3 where 750 mg Ca^{2+}/L was added. Therefore, the 750 mg Ca^{2+}/L seems to be the most effective amount in the formation of aerobic floc-like sludge.

From the profiles of ion concentration through the floc-like sludge sample measured by the several microelectrodes, the surface of floc-like sludge could be located. Table 1 lists the calculated values of the NH_4^+ and NO_3^- fluxes and thickness of boundary layer at the floc-like sludge surface using Eq. (1) and (2), respectively, based on the measured ionic parameters with ISEs.

CONCLUSION

The aerobic floc-like sludge with the addition of calcium ion was investigated through the SVI measurement, DAPI staining, and the microelectrode technique. From the SVI result, the most effective concentration of calcium dose for aerobic granulation was found to be 750 mg/L (32.2% decrease in SVI), compared to the addition of 550 mg/L (22.3% decrease) and 950 mg/L (30.2% decrease). The most active nitrification occurred at 750 mg/L dose, as found by ISEs measurements, and the nitrification at 550 mg/L addition was the least active. The nitrification with 950 mg/L calcium ion addition was poor. It is likely that high concentration of calcium ion functions as a good bridging agent for the granulation, but may have adverse effect on the nitrification as the interaction between ammonium ion and the nitrifying bacteria can be physically inhibited. The formation of thick granules with calcium ion may also have impact on the ammonia transfer through the inside of granule.

Overall, the addition of calcium ion to activated sludge was found to have a positive effect on the granulation, and the nitrification efficiency can be also improved with the proper calcium ion dose at concentrations not to inhibit the ion transfer in the granular sludge.

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Table 1. Thickness of mass boundary layers around the floc-like sludge for ammonium and nitrate and fluxes of ammonium and nitrate through the floc-like sludge and bulk liquid interface.

Amount of Ca^{2+} (mg/L)	h (NH^{4+}) (μm)	JS (NH^{4+}) (10^{-6}g/m^2/s)	h (NO^{3-}) (μm)	JS (NO^{3-}) (10^{-6}g/m^2/s)
550	241	11.3	161	5.28
750	380	9.0	450	4.8
950	280	2.7	320	2.4

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