Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2013.780768

51 (2013) 6201–6209
September

Taylor & Francis
Taylor & Francis Group

Settling and dewatering characteristics of mixed microorganisms according to changes in the SRT

Kwang Ho Ahn[a,b], Kwang Baik Ko[b], Baek Woo Sung[b], June Seok Choi[a], Bong-Soo Kim[c], Kwang Soo Kim[a,*]

[a]*Water Resources & Environment Research Division, Korea Institute of Construction Technology, 283, Goyangdae-Ro, Ilsanseo-Gu, Goyang-Si, Gyeonggi-Do 411-712, Korea*
Tel. +82 31 910 0279; Fax: +82 31 910 0291; email: kskim@kict.re.kr
[b]*School of Civil and Environmental Engineering, Yonsei University, Sinchondong 134, Sudaemungu, Seoul 120-749, Korea*
[c]*Chunlab Inc., Seoul National University, Gwanak-ro, Gwanak-Gu, Seoul 151-742, Korea*

Received 14 November 2012; Accepted 5 March 2013

ABSTRACT

This study was carried out to evaluate the settleability and dewaterability of mixed microorganisms according to the changes in sludge retention time (SRT) after mixing granulated methane-oxidizing bacteria and activated sludge (AS). The results of increasing SRT of microorganisms from 15 to 20 days showed that the settling rate of microorganisms increased, while specific resistance to filtration decreased. From the results of analyzing extracellular polymeric substances (EPS), no significant change was found in the bound EPS, but total soluble EPS showed a tendency to decrease as the SRT increased from 15 to 20 days. The average particle size of the mixed microorganisms was two to three times larger than that of the general AS, and as the SRT increased, the average particle size also increased, indicating that there is a close correlation between SRT and granule formation. The results of analyzing microbial community in SRT 15 and 20 days showed the dominance of *Proteobacteria* at long SRT.

Keywords: Aerobic granule; Methane-oxidizing bacteria; Settleability; Specific resistance to filtration; Extracellular polymeric substances

1. Introduction

In general, biological sewage treatment systems, a large amount of sludge occurs, and spacious grounds for various facilities, such as aeration tank and secondary clarifier, are required [1]. One of the most important operating factors of a biological treatment system is the solid-liquid separation of sludge. Settling and dewatering characteristics of sludge are considered as important in adjusting the treatment process appropriately. The settling and dewatering characteristics of activated sludge (AS) can vary depending on the operating conditions and characteristics of the sludge. Studies on the granules have been conducted as an effective method for improving settleability and dewaterability of AS [2,3].

*Corresponding author.

Presented at The Fifth Desalination Workshop (IDW 2012), October 28–31, 2012, Jeju, Korea

Studies on the granular sludge began with the development of the upflow anaerobic sludge blanket reactor in the late 1970s, and the formation and application of aerobic granules were studied until the late 1990s. Meanwhile, since anaerobic granular sludge has its shortcomings, such as long-term operation, relatively higher operating temperature, difficulty in long-term persistence in high concentrations of organic wastewater, and low efficiency in eliminating nutrients of nitrogen and phosphorus from the sewage, an interest in aerobic granules has increased [4,5]. The first experiment on aerobic granules was done by using pure oxygen in the continuous aerobic upflow sludge blanket reactor by Mishima and Nakamura [6].

The aerobic granular sludge has physical, chemical, and biological characteristics, and each characteristic shows the difference in typical sewage treatment microorganisms, such as AS. The physical characteristics of aerobic granular sludge include less than sludge volume index (SVI) 80 mL/g of sludge settleability and maintenance of physical granule stability caused by the higher specific gravity compared with flocculent sludge due to the high density of microorganisms and compact structure [7]. The chemical characteristics of aerobic granules can be explained by the increase in the hydrophobicity of cells leading to the increase in Gibbs energy, when self-aggregates between cells are formed to create granules [7,8]. In addition, extracellular polymeric substances (EPS), which formed due to the metabolism of microorganisms, contain protein, polysaccharide, humic acids, nucleic acids, and lipids as their major components, whose concentration appears higher in aerobic granules than flocculent sludge [9,10]. As biological characteristics, aerobic granules form a microbial community with a variety of substrates (glucose, acetate, ethanol, phenol, etc.), while granule characteristics and forms vary [11,12].

As a Gram-negative aerobic bacteria using methane as a carbon source, methane-oxidizing bacteria (MOB) can be seen in multiple environments in which methane is generated, such as swamps, rivers, mud, fallen leavens, sewage sludge, etc. [13,14]. The previous researches on MOB have focused on the removal of nutrients, contaminants, and nonbiodegradable pollutants such as trichloroethylene [15,16], and there was no studies on the settling and dewatering using the MOB.

This study was designed to identify the characteristics of settling and dewaterability according to the changes in sludge retention time (SRT) of mixed microorganisms by mixing granulated MOB with AS in terms of their physicochemical and biological properties.

2. Materials and methods

2.1. Cultivation and granule formation of methane-oxidizing bacteria

For the cultivation of MOB, the top soil from landfill whose adulterants were removed through a series of sieves 50 was mixed with a 200 mL modified nitrate-minimal salt (NMS) medium prepared according to Best and Higgins [17] and then put in a 350 mL Erlenmeyer flask. The opening of the flask was covered with a silicon stopper, and then methane gas was injected into the flask with 20% of the head space using a syringe, which was agitated in the shaking incubator after sealing. A supernatant (100 mL) was then injected into a new NMS medium after incubation for one day under the culture conditions of 30˚C incubation temperature and 150 rpm agitation speed, which was repeated several times for the cultivate MOB. The MOBs were transferred to the mixed reactor shown in Fig. 1, and the reactor was operated for 30 days to facilitate formation of granules. After granule formation, granular methane-oxidizing bacteria (GMOB) was maintained at the SRT 20 days.

2.2. Mixing of GMOB and AS

The AS process used in the experiments as an anaerobic oxic (A/O) process, it was composed of an anaerobic tank, an aeration tank, and a clarifier, and the effective capacity was 3.5, 6, and 2.5 L, respectively, while hydraulic retention time was 8 h based on the aeration tank. An experiment was conducted by seeding the aeration tank sludge of the sewage treatment plant, and the influent was operated at COD_{cr} 150–200 mg/L, NH_4^+–N 30–50 mg/L, PO_4^{3-}–P 10 mg/L, and alkalinity 300 mg/L (as $CaCO_3$). The AS process was operated at the SRT 20 days, and the experiment was done at 20˚C.

Fig. 1 shows a reactor in which the aeration tank AS of the A/O process is mixed in GMOB of equal proportions of 1:1. In the mixed (GMOB and AS) reactor, methane and oxygen were injected into the gas dissolution tank at 10 mL/min, respectively, along with the influent, and the dissolved methane and oxygen were introduced into the mixed reactor through the bottom of the reactor, which was then stirred up by the agitator for granulation of the mixed AS and methane-oxidizing bacteria. The dissolved oxygen concentration in the gas dissolution tank was maintained at 1.3–1.7 mg/L and that of granulation tank at 0.2–0.7 mg/L. The capacity of the gas dissolution tank and the mixed reactor was 1.2 and 4.5 L, respectively, while residence time was 1.6 and 6 h, respectively. The pH of the influent was 6.9–7.4, and the temperature of the reactor was

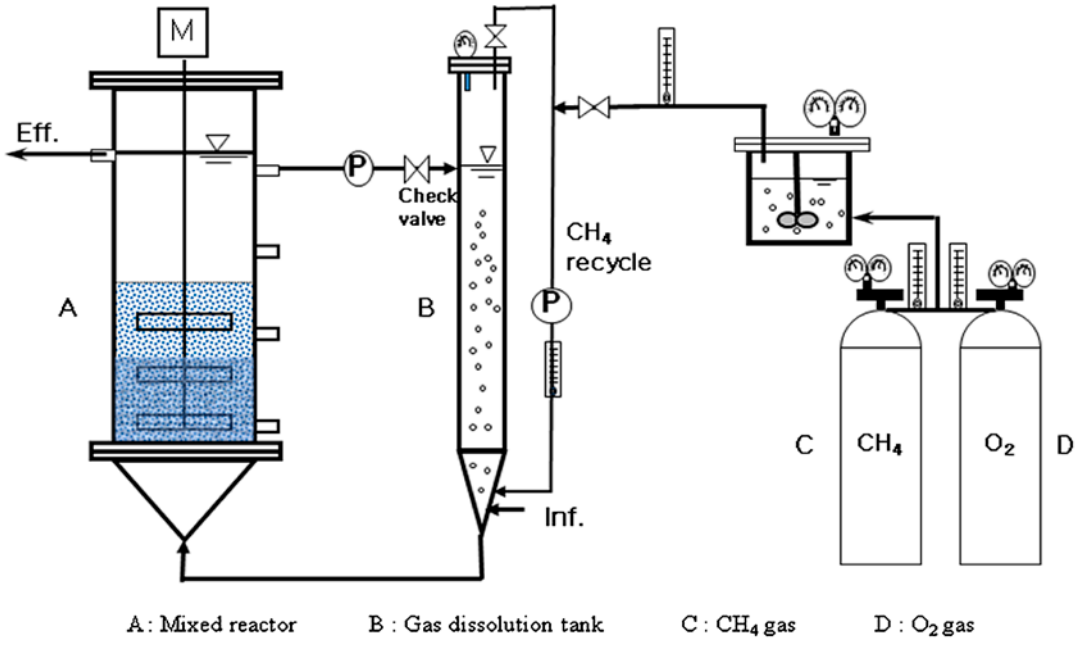

Fig. 1. Schematic diagram of mixed microorganisms reactor.

Table 1
Major constituents in the influent of the mixed reactor

Constituents	Typical range	Chemicals used
NH_4^+–N (mg/L)	50–100	NH_4Cl
NO_3^-–N (mg/L)	10–20	KNO_3
PO_4^{3-}–P (mg/L)	10	KH_2PO_4, K_2HPO_4
Trace elements (mg/L)	2	$MgSO_4{\cdot}7H_2O$
	5	$CaCl_2{\cdot}2H_2O$
	0.6	$FeSO_4{\cdot}7H_2O$
	1	$MnSO_4{\cdot}5H_2O$
	1	$CuSO_4{\cdot}5H_2O$

maintained at 20°C. The influent characteristics of the mixed reactor are shown in Table 1.

2.3. Experimental methods

2.3.1. Filterability and specific resistance to filtration test

Filterability and Specific resistance to filtration (SRF) experiments were conducted to evaluate dewaterablity, in which microorganisms were taken at SRT 15 day, 17 day, and 20 day from the mixed reactor, and the amount of filtrate dehydrated depending on the elapsed time was measured at 2,000 and 4,000 mg/L. The SRF was calculated by using Whatman No. 1 at 51 kPa [18].

In the settleability and sludge settling velocity experiments, mixed microorganisms of GMOB and AS were injected into a mass cylinder with a 300 mL capacity. Settling velocity were obtained by measuring sludge blanket, depending on the time elapsed. For the SVI, mixed microorganisms were injected into a 100-mL mass cylinder at SRT 15 day, 17 day, and 20 day, respectively, and the settled sludge volume was measured in 30 min. The SRF was calculated as follows: the modulus of volume change in sludge filtration is

$$\frac{dV}{dt} = \frac{PA^2}{\mu(rCV + R_m A)} \quad (1)$$

where P is the pressure of filtration (N/m^2), A is the area of the filter paper (m^2), μ is the viscosity of the filtrate ($N\text{-}s/m^2$), r is the SRF (m/kg), C is the weight of the dry solids per volume of filtrate (kg/m^3), V is the volume of the filtrate (m^3), and R_m is the resistance on the medium (1/m). (R_m was ignored as it had a very small value compared with the resistance on the sludge cake.)

$$\frac{dt}{dV} = \frac{\mu r C}{PA^2}V + \frac{\mu R_m}{PA} \quad (2)$$

where dt/dV is a linear equation on the Y-axis, and V on the X-axis. If r is calculated from a slope, b, on the plot of dt/dV versus V, Eq. (3) can be used for calculating r, and C can be also calculated as shown in Eq. (4).

$$r = \frac{2A^2P}{\mu C}b \quad (3)$$

$$C = \frac{C_c C_s}{100(C_c - C_s)} \quad (4)$$

where C_c is the cake suspended solid concentration and C_s is the slurry suspended solid concentration.

2.3.2. EPS analysis

In general, EPS is divided into bound EPS and soluble EPS [19]. In the case of soluble EPS, effluent of mixed reactor was centrifuged at 4,000 g at 4℃ for 20 min to analyze EPS, while the EPS of mixed organisms according to SRT was extracted, respectively, in bound EPS. To extract EPS, a supernatant was removed through centrifugation at 4,000 g at 4℃ for 20 min, and residual microorganisms were extracted by using the Comte et al. method [20]. A total of four components of EPS, such as protein, polysaccharide, humic substance, and uronic acid, were analyzed. In the case of protein, absorbance was measured at 750 nm using the Lowry method that uses bovin serum albumin as a standard protein [21]. Polysaccharide was analyzed by means of the phenol/sulfuric acid method used by Dubois et al. [22] that uses glucose as a standard, which was measured at 490 nm [22,23]. Humic substance was measured at 630 nm by using the modified Lowry method in which humic acid is used as a standard [24]. Uronic acid was measured at 520 nm by using the *m*-hydroxydiphenyl sulfuric acid method of Blumenkrantz and Asboe-Hansen et al. [25]. Total EPS was obtained from the sum of the four components, and protein/polysaccharide (PN/PS) ratio was calculated by dividing polysaccharide into protein.

2.3.3. Particle size analysis

The particle size analysis of mixed microorganisms taken from SRT 15 day, 17 day, and 20 day was performed by using Malvern Mastersizer 2000® (Malvern Instruments Ltd. UK), and all samples were measured by repeating them 10 times to indicate the mean value.

2.3.4. Comparison of microbial communities

To observe microbial community in SRT 15 day and 20 day of mixed microorganisms, genomic DNA was extracted from collected samples using the FastDNA SPIN kit for Soil (MP Biomedicals, CA, USA). Bacterial 16S rRNA genes (ranged from V1 to V3) were amplified from genomic DNA of samples using fusion primers followed by previous descriptions [26] with a 454 GS FLX Junior Sequencing system (Roche, Branford, CT, USA). Any reads containing two or more ambiguous nucleotides, average quality score <25, or short reads (<300 bp) were filtered from raw pyrosequencing reads. Chimera check was conducted after filtering process, and taxonomic assignments of reads were performed using the extended EzTaxon database (http://eztaxon-e.ezbiocloud.net/) [27]. To compare samples with different reads, the numbers of reads were normalized by random subtraction. After normalized reads, the statistical analyses of microbial communities were conducted using the Mothur program [28].

3. Results and discussion

3.1. Filterability

Fig. 2 shows the filtration volume of mixed microorganisms in specific times (mixed liquor suspended solids [MLSS] 2,000 mg/L, 4,000 mg/L) of SRT 15 day, 17 day, and 20 day. The filtration volume was small at SRT 15 days, but it increased at SRT 20 days.

The filtration volume filtrated at MLSS 2,000 mg/L for 1 min was 13.8, 25, and 59 mL at mixed microorganism of SRT 15, 17, and 20 days, respectively, and that one filtered at MLSS 4,000 mg/L was 7, 25, and 64 mL at 15, 17, and 20 days, respectively, which shows that, as SRT increases, the filtration volume also increases for 1 min. In the case of mixing GMOB with AS, dehydration volume decreased in the initial stage, but it decreased by degrees.

3.2. Settling velocity and SVI

Fig. 3 shows the settling velocity of mixed microorganisms according to the SRT changes. The settling velocity showed a tendency to increase after 5 min, but it decreased sharply after 5 min. The initial settling velocity at 15-day SRT was lower compared with the 20-day SRT, and as SRT increased, settling velocity gradually became faster. As SRT increased, settleability improved gradually, when SVI turned out to be 122, 75, and 62 mL/g at SRT 15, 17, and 20 days, respectively, which indicates that as SRT increases, SVI decreases.

3.3. Specific resistance to filtration

Fig. 4 shows the results of the comparison of the SRF of the mixed microorganisms. The SRF turned out to be 8.87×10^{12}–5.20×10^{13} at MLSS 2,000 mg/L,

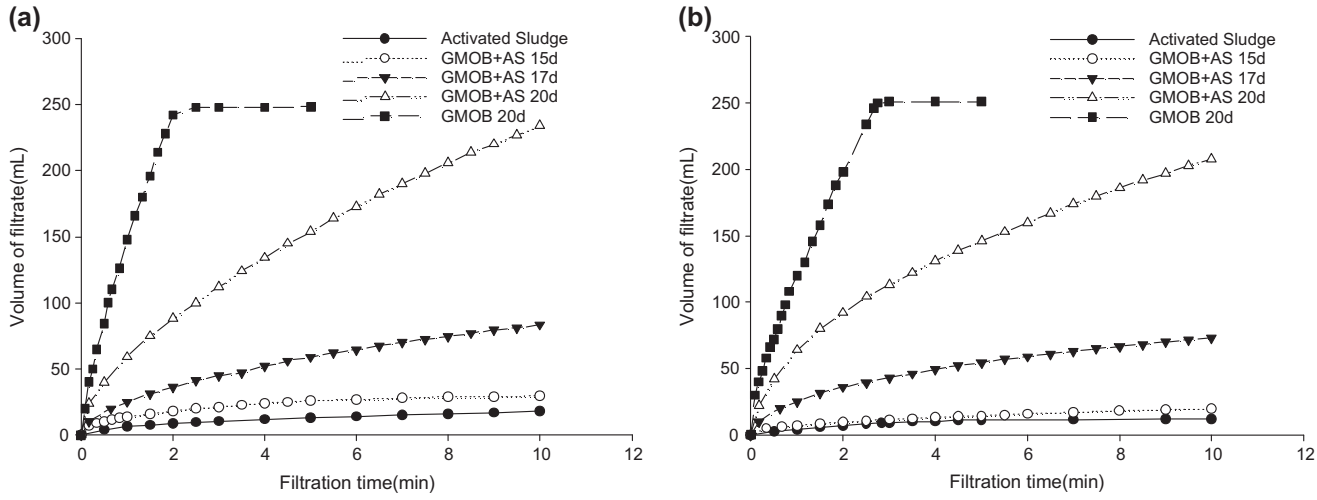

Fig. 2. Filtrate volume of the mixed microorganisms with respect to time elapsed at the MLSS concentrations of (a) 2,000 mg/L and (b) 4,000 mg/L.

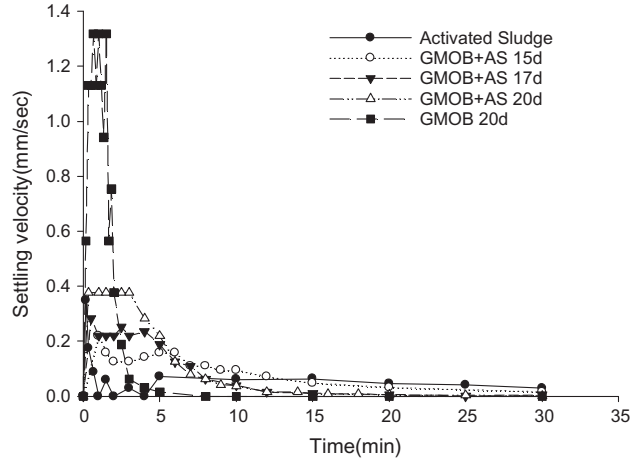

Fig. 3. Settling velocity of the mixed microorganisms with respect to time elapsed.

and the SRF at MLSS 4,000 mg/L was 5.03×10^{12}–4.42×10^{13}, which indicates that as SRT increases, SRF decreases. This phenomenon led to more difficulty in the dehydration of mixed microorganisms at SRT 15 days compared with that of the GMOB. In addition, the increase in SRT resulted in improved flocculation and sedimentation of sludge, which indicates that there is a correlation between SRT and dewaterability in GMOB as well [29].

The SRF used in evaluating the dewaterability of sludge is closely related to flocculation and sedimentation characteristics of microorganisms, and it varies depending on operating conditions and the analytical method of the AS. In this study, the SRF of mixed microorganisms was 5.0×10^{12}–5.2×10^{13} m/kg, and it showed a somewhat lower range of SRF 2.5×10^{13}–210×10^{13} m/kg [30] in wastewater treatment plants.

In the measurement of specific resistance coefficient, as sludge concentration increases from 2,000 to 4,000 mg/L, the specific resistance coefficient tends to decrease generally. In dewaterability and settleability test, the C_c of Eq. (4) represents the remaining amount of sludge cake after filtration, and it shows similar values in MLSS 2,000 and 4,000 mg/L in this study. In addition, in the concentration of C_s, the initial slurry volume increases from 2,000 to 4,000 mg/L, C increases, and r, the SRF, decreases.

3.4. EPS analyses

The amount and characteristics of EPS due to changes in the SRT of mixed microorganisms of

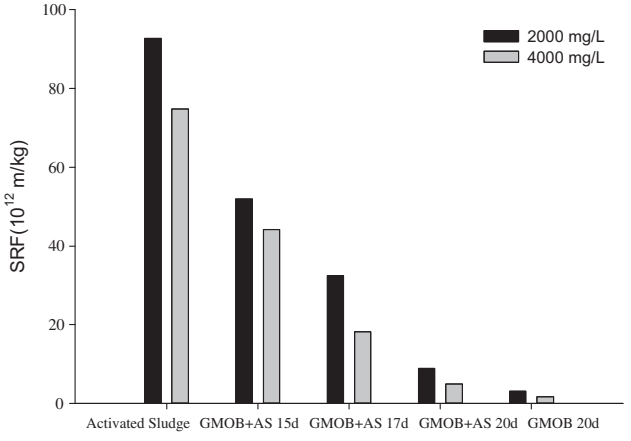

Fig. 4. The comparison of the resistance to filtration (SRF) of the mixed microorganisms.

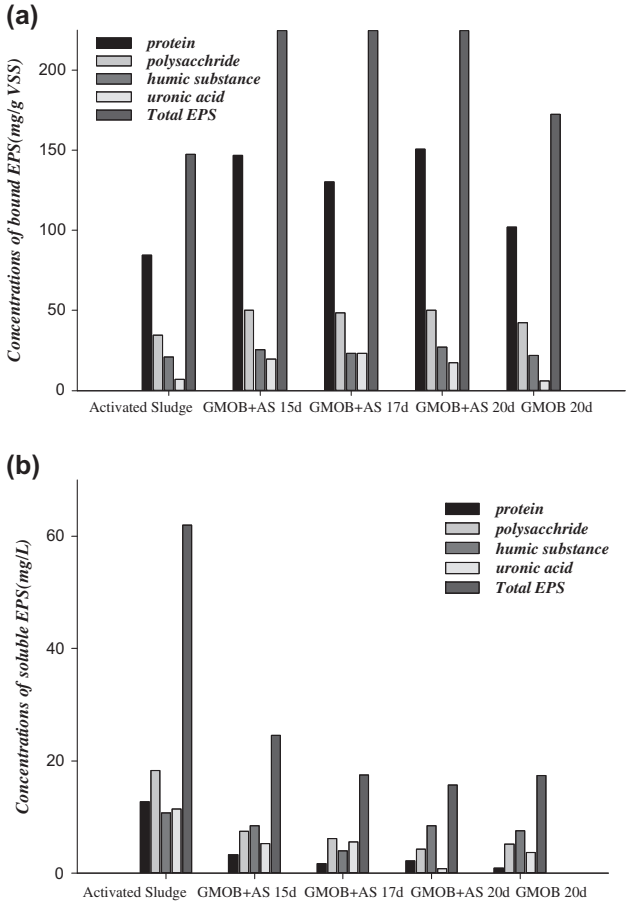

Fig. 5. Major constituents of the EPS for the mixed microorganisms: (a) bound EPS of microorganisms; (b) soluble EPS of microorganisms.

GMOB and AS are shown in Fig. 5. No significant change was found in the total bound EPS (225.3–245.8 mg/g-VSS) despite SRT changes from 15 to 20 days. The total soluble EPS of mixed microorganism was 15.7–24.5 mg/L, which indicates that as SRT increases, the concentration of soluble EPS decreases.

The concentration of soluble EPS turned out to be high at short SRT after GMOB is mixed with AS, and as SRT became longer, the concentration of soluble EPS decreased, thereby improving dewaterability. It is

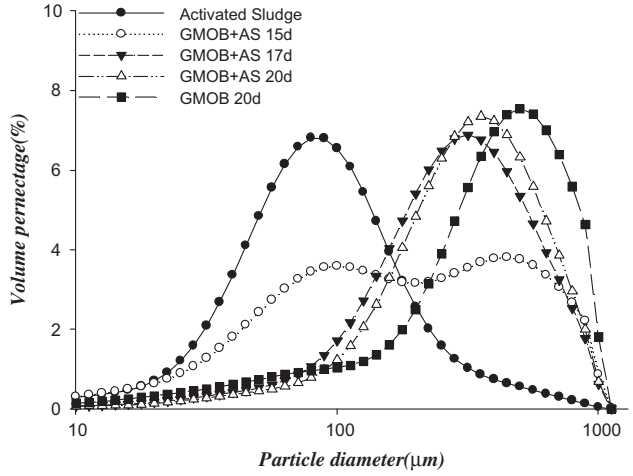

Fig. 6. Particle size distributions mixed microorganisms according to SRT changes.

known that the dewaterability of the microbial treatment process is closely related to soluble EPS, and it is the cause of degradation in filtration performance [31].

The composition ratio of EPS in general AS varies, depending on extraction methods, analytical methods, and sludge operating conditions, but the proportion of protein and polysaccharide is higher compared with other components in general [20,32]. In this study, the proportion of protein and polysaccharide turned out to be higher compared with the humic substance and uronic acid in bound EPS, and a consistent tendency was not found in soluble EPS. However, it was found that the concentration of the total EPS tends to decrease as SRT increases.

PN/PS ratios appeared similar both in bound EPS and in soluble EPS, since they were 2.7–3.0 in bound EPS of mixed microorganisms and 0.1–0.4 in soluble EPS. The PN/PS ratios of AS varied depending on operating conditions, and they were found to have a wide range of 0.5–2.12 [20,33]. In comparison with the results from previous studies, PN/PS ratios showed similar ranges in bound EPS, and lower ranges of 1.6–1.9 were found in previous studies in the case of soluble EPS [20].

Table 2
Particle size distribution of the mixed microorganisms: 10, 50, and 90 percentiles

Particle diameter	AS	GMOB + AS 15 day	GMOB + AS 17 day	GMOB + AS 20 day	GMOB 20 day
Mean particle size (μm)	107	249	314	340	402
D_{10} (μm)*	31	36	100	116	81
D_{50} (μm)*	79	162	274	305	385
D_{90} (μm)*	202	605	598	623	738

*D_{10}, D_{50} and D_{90} values indicate the 10, 50, 90% of the particles measured were less than or equal to the size stated.

Table 3
Summary of statistical analysis was obtained from pyrosequencing reads using Mothur program

Sample	Analyzed reads[a]	Normalized reads[b]	Observed OTUs[c]	Estimated OTUs (Chao1)	Shannon diversity	Good's coverage
SRT 15 day	5,667	5,600	987	5,459	4.22	0.86
SRT 20 day	11,668	5,600	352	1,617	1.64	0.95

[a]Analyzed reads were obtained through quality filter process.
[b]Normalized reads were obtained using random subsampling from analyzed reads to compare between samples that have different read numbers.
[c]Operational taxonomic units (OTUs) were obtained by clustering sequence reads using 97% similarity cutoff value.

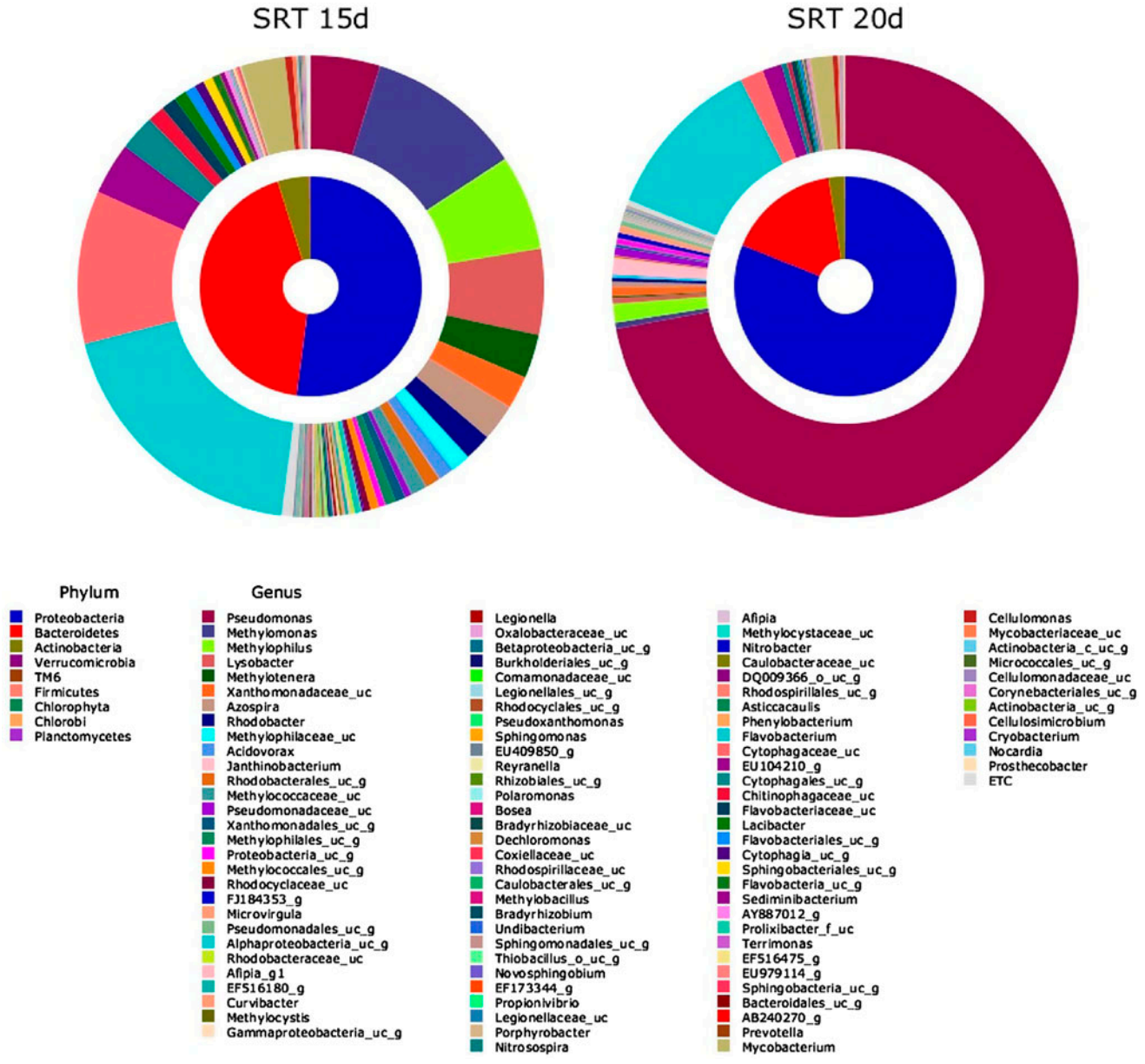

Fig. 7. Double pie charts of bacterial communities in SRT 15 and 20 days were compared. Inner circle indicates phylum composition of community and outer circle indicates genus composition of samples. The differences of communities in SRT 15 day and 20 day were observed.

3.5. Particle size distribution

Fig. 6 and Table 2 show the particle size distribution of mixed microorganisms according to SRT changes. The average particle size distribution was 249, 314, and 340 μm at SRT 15 day, 17 day, and 20 day, respectively, which indicates that as SRT increases, the average particle size also increases due to the improvement of the granules. Based on the results of the comparison between the AS and the mixed microorganisms, the average particle size of the mixed microorganisms was found to be 2 to 3 times larger than that of the AS, and the settleability and the dewaterability of the particles appeared to be high, as the granulation status of the microorganisms improved due to the increase in SRT. The average particle size of aerobic granules varies from 0.2 to 16 mm, depending on the kinds of substrate and operating conditions [34,35], and aerobic granules of less than 0.5 mm were suggested as the optimum [34,36].

3.6. Comparison of microbial community

A total of 17,335 reads were obtained from two samples and their reads sizes were normalized as 5,600 reads for the comparison of statistical values. The necessity of normalized reads for the comparison of samples were described previous studies [37,38]. The microbial communities originate from SRT 15 days was more diverse than those from SRT 20 days sample (Table 3). Estimated values of SRT 15 days were higher than those of SRT 20 days samples.

The compositions of bacterial communities were compared between two samples (Fig. 7). Phylum of *Proteobacteria* was the most abundant group (81.1% of total reads) in SRT 20 days and followed by *Bacteroidetes* (16.4%), while *Proteobacteria* (52%) and *Bacteroidetes* (43.1%) were abundant groups in SRT 15 days. *Actinobacteria* was more abundant in SRT 15 days sample (4.6%) than SRT 20 days (2.4%). *Pseudomonas* was the most dominant genus (72% of total reads) within *Proteobacteria* phylum of SRT 20 day, while the proportion of *Pseudomonas* was decreased in SRT 15 days (4.8%). The aerobic granules by the GMOB showed the dominance of Proteobacteria at long SRT. Three kinds of microbial species were mostly found: *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria*. *Proteobacteria* significantly increased with the increase in SRT. *Proteobacteria* are known as a dominant type of microorganism at the time of the formation of aerobic granules [5,39].

4. Conclusions

Experiments were carried out by mixing GMOB and AS and increasing SRT. Based on the results, the following conclusions were obtained.

(1) The results of the filterability and settleabilty tests found that filterability and settleability improved according to changes in the SRT of the mixed microorganisms, and dewaterability also improved as SRT decreased.
(2) From the results of analyzing EPS according to SRT changes, no significant change was found in the bound EPS, but total soluble EPS showed a tendency to decrease due to changes in the SRT of mixed microorganisms, showing low concentration of the soluble EPS at long SRT.
(3) The particle size distribution results showed that the average particle size of the mixed microorganisms was 2 to 3 times larger than that of the general AS, and as SRT increased, the average particle size also increased, indicating that there is a close correlation between SRT and granule formation.
(4) The results of analyzing microbial community showed that three kinds of microbial species were mostly found: *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria*. *Proteobacteria* significantly increased with the increase in SRT.

References

[1] C.Di. Iaconi, R. Ramadori, A. Lopez, R. Passino, Aerobic granular sludge systems: The new generation of wastewater treatment technologies, Ind. Eng. Chem. Res. 46 (2007) 6661–6665.
[2] M.A. Alam, A. Fakhru'l-Razi, Enhanced settleability and dewaterability of fungal treated domestic wastewater sludge by liquid state bioconversion process, Water Res. 37 (2003) 1118–1124.
[3] H.P. Yuan, X.F. Yan, C.F. Y, N.W. Zhu, Enhancement of waste activated sludge dewaterability by electro-chemical pretreatment, J. Hazard. Mater. 187 (2011) 82–88.
[4] D. Gao, L. Liu, H. Liang, W. Wu, Comparison of four enhancement strategies for aerobic granulation in sequencing batch reactors, J. Hazard. Mater. 186 (2011) 320–327.
[5] S. Adav, D. Lee, K. Show, J. Tay, Aerobic granular sludge: Recent advances, Biotechnol. Adv. 26 (2008) 411–423.
[6] K. Mishima, M. Nakamura, Self-immobilization of aerobic activated sludge a pilot study of the aerobic upflow sludge blanket process in municipal sewage treatment, Water Sci. Technol. 23 (1991) 981–991.
[7] D. Gao, L. Liu, H. Liang, W.M. Wu, Aerobic granular sludge: Characterization, mechanism of granulation and application to wastewater treatment, Crit. Rev. Biotechnol. 31(2) (2011) 137–152.
[8] Y. Li, Y. Liu, L.X. Hai, Is sludge retention time a decisive factor for aerobic granulation in SBR? Bioresour. Technol. 99 (2008) 7672–7677.
[9] B.S. McSwain, R.L. Irvine, M. Hausner, Composition and distribution of extracellular polymeric substances in aerobic flocs and granular sludge, Appl. Environ. Microbiol. 71 (2005) 1051–1057.

[10] Y.Q. Liu, Y. Liu, J.H. Tay, The effects of extracellular polymeric substances on the formation and stability of biogranules, Appl. Microbiol. Biotechnol. 65 (2004) 143–148.
[11] S.D. Weber, W. Ludwig, K.H. Schleifer, J. Fried, Microbial composition and structure of aerobic granular municipal wastewater biofilms, Appl. Environ. Microbiol. 73 (2007) 6233–6240.
[12] Y. Liu, J.H. Tay, State of the art of biogranulation technology for wastewater treatment, Biotechnol. Adv. 22 (2004) 533–563.
[13] R.S. Handson, Methanotrophic Bacteria, Microbiol. Rev. (1996) 439–471.
[14] W.A. Corpe, A method for detecting methylotrophic bacteria on solid surfaces, J. Microbiol. Methods. 3 (1985) 315–323.
[15] M.M. Fogel, R. Taddeo, S. Fogel, Biodegradation of chlorinated ethenes by a methane-utilizing mixed culture, Appl. Environ. Microbiol. 51 (1986) 720–724.
[16] P.L. McCarty, Bioengineering issues related to in-situ remediation of contaminated soils and groundwater, In: G.S. Omenn (Ed), Environmental Biotechnology: Reducing Risks From Environmental Chemicals Through Biotechnology, Plenum Press, New York, NY, pp. 143–162, 1988.
[17] D.F. Best, I.F. Higgins, Methane-oxidizing activity and membrane morphology in a methanol-grown obligate methanotroph *Methylosinus trichosporium* OB3b, J. Gen. Microbiol. 125 (1981) 73–84.
[18] M.Z. Alam, A. Fakhru'l-Razi, A.H. Molla, Biosolids accumulation and biodegradation of domestic wastewater treatment plant sludge by developed liquid state bioconversion process using batch fermenter, Water Res. 37 (2003) 3569–3578.
[19] F.P. Sheng, H.Q. Yu, Z.Y. Li, Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review, Biotechnol. Adv. 28 (2010) 882–894.
[20] S. Comte, G. Guibaud, M. Baudu, Biosorption properities of extracellular polymeric substances (EPS) resulting from activated sludge according to their type: Soluble or bound, Process Biochem. 41 (2006) 815–823.
[21] K. Raunkjær, T. Hvitved-Jacobsen, P.H. Nielsen, Measurement of pools of protein carbohydrate and lipid in domestic wastewater, Water Res. 28 (1994) 251–262.
[22] M. Dubois, K.A. Gilles, F.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem. 28 (1956) 350–356.
[23] Z. Wu, Z. Wang, Z. Zhou, G. Yu, G. Gu, Sludge rheological and physiological characteristics in a pilot-scale submerged membrane bioreactor, Desalination 212 (2007) 152–164.
[24] G.P. Sheng, M.L. Zhang, H.Q. Yu, A rapid quantitative method for humic substances determination in natural waters, Anal. Chim. Acta. 592 (2007) 162–167.
[25] K. Mojica, D. Elsey, M.J. Cooney, Quantitative analysis of biofilm EPS uronic acid content, J. Microbiol. Methods. 71 (2007) 61–65.
[26] B.-S. Kim, J.N. Kim, S.H. Yoon, J. Chun, C.E. Cerniglia, Impact of enrofloxacin on the human intestinal microbiota revealed by comparative molecular analysis, Anaerobe 18(3) (2012) 310–320.
[27] O.S. Kim, Y.J. Cho, K. Lee, S.H. Yoon, M. Kim, H. Na, S.C. Park, Y.S Jeon, J.H. Lee, H. Yi, S. Won, J. Chun, H. Yi, S. Won, J. Chun, Introducing EzTaxon-e A prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species, Int. J. Syst. Evol. Microbiol. 62(3) (2012) 716–721.
[28] P.D. Schloss, S.L. Westcott, T. Ryabin, J.F. Hall, M. Hartmann, E.B. Hollister, R.A. Lesniewski, B.B. Oakley, D.H. Parks, C.J. Robinson, J.W. Sahl, B. Stres, G.G. Thallinger, D.J. Van Horn, C.F. Weber, Introducing mother: Open-Source, platform-independent, community-supported software for describing and comparing microbial communities, Appl. Environ. Microbiol. 75(23) (2009) 7537–7541.
[29] X.Y. Li, S.F. Yang, Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge, Water Res. 41 (2007) 1022–1030.
[30] G.H. Yu, P.J. He, L.M. Shao, Novel insight into sludge dewaterability by fluorescence excitation-emission matrix combined with parallel factor analysis, Water Res. 11 (2010) 797–806.
[31] M.E.H. Rojas, R. Van Kaam, S. Schetrite, C. Albasi, Role and variations of supernatant compounds in submerged membrane bioreactor fouling, Desalination 179 (2005) 95–107.
[32] S. Comte, G. Guibaud, M. Baudu, Effect of extraction method on EPS from activated sludge: An HPSEC investigation, J. Hazard. Mater. 140 (2007) 129–137.
[33] G. Guibaud, S. Comte, F. Bordas, S. Dupuy, M. Baudu, Comparison of the complexation potential of extracellular polymeric substances (EPS), extracted from activated sludges and produced by pure bacteria strains for cadmium lead and nickel, Chemosphere 59 (2005) 629–638.
[34] D. Gao, L. Liu, H. Liang, W. Wu, Aerobic granular sludge: Characterization, mechanism of granulation and application to wastewater treatment, Crit. Rev. Biotechnol. 31(2) (2011) 137–152.
[35] S.K. Toh, J.H. Tay, B.Y.P. Moy, S.T.L. Tay, Size-effect on the physical characteristics of the aerobic granule in a SBR, Appl. Microbiol. Biotechnol. 60 (2003) 687–695.
[36] L.H. Lin, L.W. Jian, H.W. Xiang, Q. Yi, The formation and characteristics of aerobic granules in sequencing batch reactor (SBR) by seeding anaerobic granules, Process Biochem. 40 (2005) 1–7.
[37] T.M. Gihring, S.J. Green, C.W. Schadt, Massively parallel rRNA gene sequencing exacerbates the potential for biased community diversity comparisons due to variable library sizes, Environ. Microbiol. 14(2) (2012) 285–290.
[38] L.B. Price, C.M. Liu, J.H. Melendez, Y.M. Frankel, D. Engelthaler, M. Aziz, J. Bowers, R. Rattray, J. Ravel, C. Kingsley, P. S. Keim, G.S. Lazarus, J.M. Zenilman, Community analysis of chronic wound bacteria using 16S rRNA gene-based pyrosequencing: Impact of diabetes and antibiotics on chronic wound microbiota, PLoS ONE 4(7) (2009), e6462.
[39] H.L. Jiang, J.H. Tay, AM. Maszenan, STL. Tay, Bacterial diversity and function of aerobic granules engineered in a sequencing batch reactor for phenol degradation, Appl. Environ. Microbiol. 70 (2004) 6767–6775.