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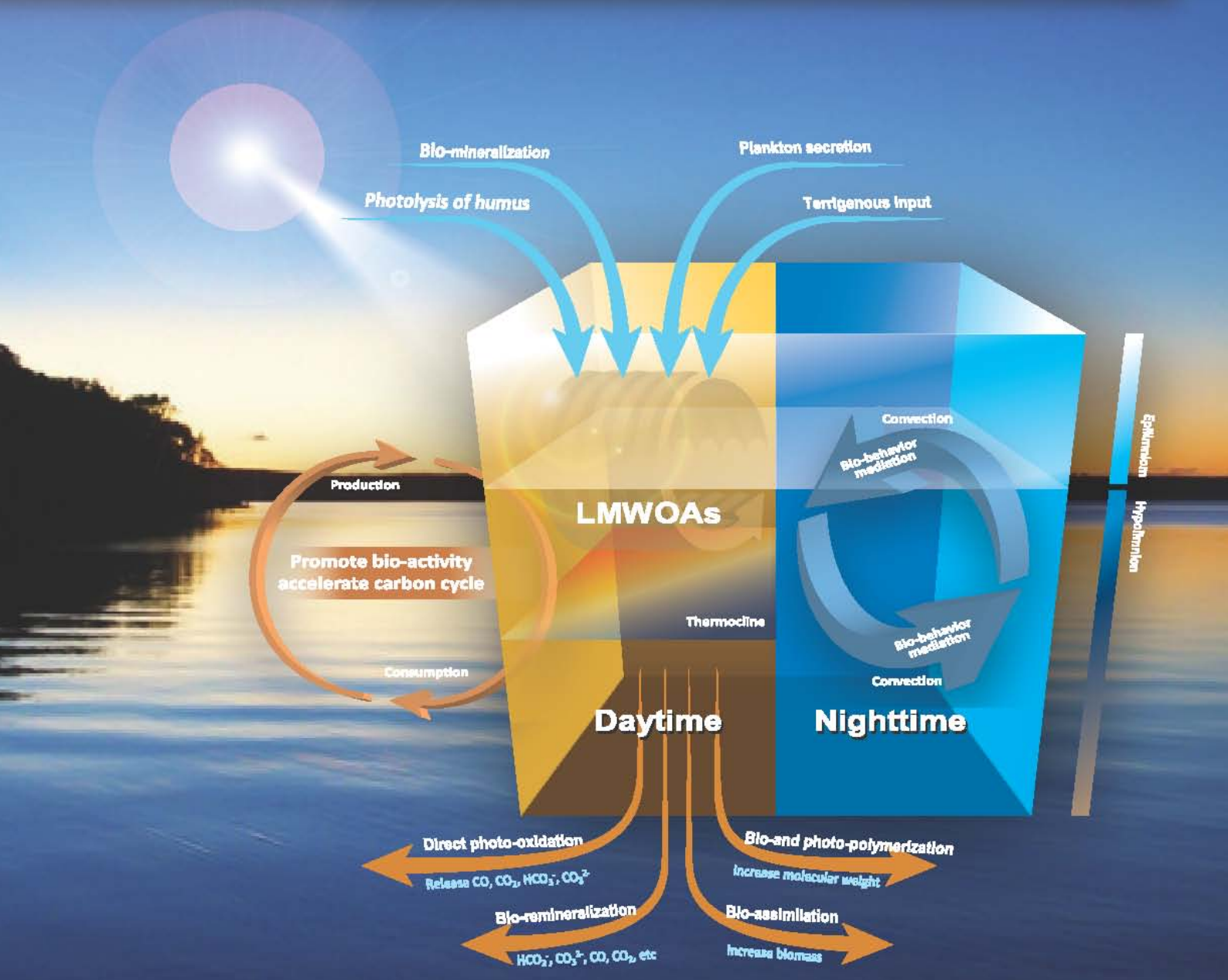

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Settling and dewatering characteristics of granulated methane-oxidizing bacteria

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Abstract

We evaluated the settling ability and dewaterability of granulated methane-oxidizing bacteria (GMOB) after granulation using a continuous-flow reactor. A comparative analysis on settling and dewatering characteristics due to changes in sludge retention time (SRT, 10, 15 and 20 days) during cultivation of GMOB was conducted. In assessing dewaterability, the specific resistance to filtration (SRF) of activated sludge and GMOB was found to be 8.21×10^{13}–2.38×10^{14} and 4.88×10^{12} –1.98×10^{13} m/kg, respectively. It was confirmed that as SRT decreased, SRF of GMOB increased. In the case of bound extracellular polymeric substance (EPS), activated sludge registered 147.5 mg/g-VSS while GMOB exhibited 171–177.2 mg/g-VSS. In the case of extracellular polymeric substance soluble EPS in effluent, activated sludge measured 62 mg/L and GMOB had 17.4–21.4 mg/L. The particle size analysis showed that mean particle diameters of GMOB were 402, 369, and 350 μm, respectively, at SRTs of 20, 15 and 10 days. In addition, it was found that GMOB had a larger mean particle diameter and exhibited much better settleability and dewaterability than activated sludge did.

Key words: methane-oxidizing bacteria; dewaterability; specific resistance to filtration; extracellular polymeric substances
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Introduction

Methanotrophs are aerobic bacteria that oxidize methane as an energy source and use methane and some 1-carbon compounds as electron donors and carbon sources, which are required to generate energy. They are known to be widely distributed in soil and water in nature (Knowles, 2005; Trotsenko and Khmelenina, 2005).

Recognizing methane oxidizing bacteria's (MOB) excellent ability to decompose non-biodegradable pollutants such as trichloroethylene (TCE), dichloroethylene and vinyl chloride, many researchers carried out experiments to eliminate non-biodegradable pollutants from soil and groundwater, etc. using methane oxidizing bacteria. Chang and Alvarez-Cohen (1997) conducted experiments to remove TCE and cis-1,2-dichloroethylene from water using MOB and obtained more than 99% removal efficiency. Wilson et al. (1985) performed experiments to decompose TCE in soil and groundwater. Based on their results, most of TCE in soil was found to be easily decomposed while TCE of 150 ng/mL was decomposed into carbon dioxide within 2 days. In addition, Nelson et al. (1993) conducted tests to remove vinyl chloride by attaching methanotrophic bacteria in biofilm form.

Microorganisms produce extracellular polymeric substances (EPSs), and the production of EPSs plays an important role in aggregation and settling of microorganisms because they could form a crosslink to connect microbial cells. They can also create granules by binding cells between microorganisms (Ross, 1984; Shen et al., 1993). In general, EPSs can be classified into bound EPS and soluble EPS. The bound EPSs are located close to cells, while the soluble EPSs are weak in coherence between cells or dissolved in a solution. These two kinds of EPSs can be separated via centrifugation (Comte et al., 2006; Sheng et al., 2010). In addition, the bound EPSs can be classified into tightly bound EPSs (TB-EPSs), which maintain a constant shape on cell surface, and loosely bound EPSs (LB-EPSs), which form a slime layer with no clear-cut boundaries in outer layer, and which can affect the dewaterability of microorganisms (Li and Yang, 2007). The study of EPS demonstrated that settleability

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has an effect on dewaterability. In an experiment using activated sludge, TB-EPS was not affected by changes in sludge retention time (SRT). However, LB-EPS decreased with the increase in SRT, which leads to the conclusion that an increase in LB-EPS has a negative effect on bioflocculation, sludge settleability and dewaterability (Li and Yang, 2007; Chen et al., 2010; Xu et al., 2011).

Aerobic granulation was studied to improve the settling and dewaterability of suspended growth-activated sludge. Research results showed that aerobic granulation has the advantages of excellent settling ability, high residence time of microorganisms, resistance to shock loads and simultaneous nitrification-denitrification (Gao et al., 2011). Research to form aerobic granulation in activated sludge has been carried out by means of sequencing batch reactors (SBR) in a variety of ways (Xu et al., 2011; Gao et al., 2011; Chen et al., 2010). Juang et al. (2010) succeeded in the formation of aerobic granulation through the operation of continuous-flow reactor after forming granules using SBR. Chen et al. (2009) observed that granules were maintained steadily when mixing anaerobic and aerobic granules at low dissolved oxygen using continuous-flow reactor.

In this study, we evaluated the settling ability and dewaterability of GMOB after granulation of MOB using a continuous-flow reactor. A comparative analysis on settling and dewatering characteristics due to changes in SRT during cultivation of GMOB was also conducted.

1 Materials and methods

1.1 Cultivation of methane-oxidizing bacteria

The topsoil from landfill, whose adulterants were removed through a series of sieves, was mixed with 200 mL of a modified nitrate-minimal salt (NMS) medium prepared according to Best and Higgins (1981) to cultivate MOB. The opening of the 350 mL Erlenmeyer flask where the mixture was placed was covered with a silicone stopper. Methane gas was injected using a syringe into the flask having 20% head space, which was then agitated in a shaking incubator after sealing. The supernatant (100 mL) was then injected into a new NMS medium after incubation for one day at 30°C and 150 r/min. The process was repeated several times to cultivate MOB. MOBs were transferred to the reactor shown in **Fig. 1**, and the reactor was operated for 30 days to facilitate the formation of granules.

1.2 Activated sludge and GMOB reactor

Activated sludge was collected from an A/O (anaerobic/oxic) process, which consisted of an anaerobic reactor, an aerobic reactor, and a settling tank. The effective volumes of the reactors and tank were 3.5, 6.0, and 2.5 L, respectively. The hydraulic retention time was 8.0 hr, based on the aerobic tank, and SRT was 20 days. The aerobic sludge of a sewage treatment plant was seeded for experiment, and the concentrations of the influent were maintained at COD_{Cr} 200–300 mg/L, NH_4^+-N 50–100 mg/L, and PO_4^{3-}-P 10 mg/L, with alkalinity 300 mg/L as $CaCO_3$.

GMOB system was consisted with a gas dissolution

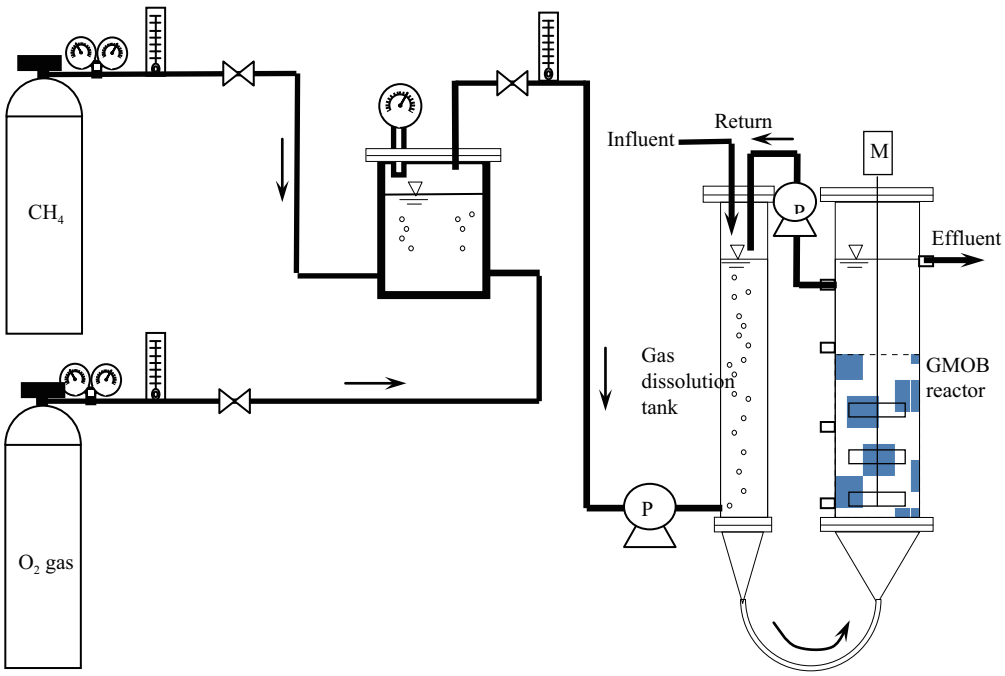

Fig. 1 Schematic diagram of granulated methane-oxidizing bacteria (GMOB) reactor.

tank (1.2 L) and a GMOB reactor (4.5 L), with residence times of 1.6 and 6.0 hr, respectively. Methane and oxygen were injected into the gas dissolution tank at 10 mL/min with influent, and dissolved methane and oxygen were introduced into GMOB reactor through bottom of the reactor, which was then agitated with a stirrer to granulate MOB.

The mixed liquor suspended solids (MLSS) of GMOB reactor was maintained at 5800–7800 mg/L, the dissolved oxygen (DO) concentration in gas dissolution tank at 1.5–1.8 mg/L, and the DO concentration of granulation tank at 0.3–0.8 mg/L. The pH of influent was 6.9–7.6, and the temperature of reactor was maintained at 20°C. The major constituents in the influent added into GMOB reactor are (in mg/L): NH_4^+-N (NH_4Cl) 50–100, NO_3^--N (KNO_3) 10–20, PO_4^{3-}-P (KH_2PO_4, K_2HPO_4) 10, Mg^{2+} ($MgSO_4{\cdot}7H_2O$) 2, Ca^{2+} ($CaCl_2{\cdot}2H_2O$) 5, Fe^{2+} ($FeSO_4{\cdot}7H_2O$) 0.6, Mn^{2+} ($MnSO_4{\cdot}5H_2O$) 1, and Cu^{2+} ($CuSO_4{\cdot}5H_2O$) 1.

1.3 Experimental methods

Aerobic activated sludge was collected and used in this experiment, when A/O process was operating under steady-state conditions and when its MLSS was 4000 mg/L. GMOB were extracted at SRTs of 20, 15, and 10 days. The particle size of MOB increased with the formation of granules, and the biomasses of MOB that were precipitated in the reactor were 9000, 7000, and 5000 mg at SRTs of 20, 15, and 10 days, respectively. Sampling was performed at steady state (15 to 20 days), wherein a constant amount of microorganisms was maintained after changes in SRT. GMOB were evenly collected from top and bottom of the reactor for analysis.

For SRF and dewaterability experiments, activated sludge with MLSS concentration of 4000 mg/L was diluted to concentrations of 1000, 2000, and 4000 mg/L, and GMOB that had been extracted at SRTs of 20, 15, and 10 days were also diluted to concentrations of 1000, 2000, and 4000 mg/L, respectively.

1.3.1 Dewaterability and settleability test

To compare the dewaterability of activated sludge and GMOB, the filterability was determined by measuring the volume of filtrate filtered using Whatman No. 1 paper at 51 kPa as a function of time, using a Buchner funnel and a 250 mL graduated cylinder, which resulted in the specific resistance to filtration (SRF). The correlation between V, the filtered filtrate, and dt/dV, a coefficient, was calculated by obtaining the slope of the plot of dt/dV vs. V (Alam et al., 2003; Fakhru'l-Razi and Molla, 2007; Christensen and Dick, 1985). SRF was calculated as follows. The modulus of volume change in sludge in sludge filtration is

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

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

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

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

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

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

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

In the settleability test, activated sludge and GMOB were injected into a 100 mL graduated cylinder, and sludge blanket that developed over time was measured. To measure sludge volume index (SVI), the amount of sludge precipitated after 30 min was measured.

1.3.2 EPS analysis

For soluble EPS, the mixed liquor containing activated sludge and GMOB were separately centrifuged at 4000×g for 20 min at 4°C for EPS analysis. For bound EPS, EPS of GMOB was extracted according to the procedures for activated sludge at three different SRTs. For extraction, the supernatant was removed via centrifugation at 4000×g for 20 min at 4°C, and the residual microorganisms were extracted by modified method introduced by Comte (2006). The analysis of four EPS components (i.e., protein, polysaccharide, humic substance, and uronic acid) was then carried out. Polysaccharide was analyzed using a glucose standard through the phenol-sulphuric acid method of Dubois et al. (1956), and it was measured as the absorbance at 490 nm (Dubois et al., 1956; Wu et al., 2007). Protein was measured via absorbance at 750 nm by applying the Lowry method and using a bovine serum albumin standard (Raunkjær et al., 1994). The humic substance was measured at 630 nm using the modified Lowry method, in which humic acid was used in standard form (Frølund et al., 1995; Sheng et al., 2007). Uronic acid was measured at 520 nm using the m-hydroxydiphenyl sulphuric-acid method (Blumenkrantz and Asboe-Hanwen, 1973; Mojica et al., 2007).

1.3.3 Particle size analysis

The activated sludge in aerobic reactor was taken at its MLSS concentration of 4000 mg/L. For GMOB, the methane-granulated microbial material at SRTs of 20, 15, and 10 days was taken for particle size analysis, which was

conducted using the Malvern Mastersizer 2000E (Malvern Instruments Ltd., UK). In particle size analysis, all analyses were conducted 10 times, and mean values were presented.

2 Results and discussion

2.1 Filterability and settleability

The volumes of filtrates for activated sludge and GMOB with respect to time elapsed at MLSS concentrations of 1000, 2000 and 4000 mg/L are shown in **Fig. 2**. It was observed that the filtration rates of GMOB were generally much higher than those of activated sludge. For GMOB, the largest amount of filtrate was observed at SRT of 20 days.

The filtrate volume for activated sludge of 1000 mg MLSS/L was 9 mL at 1 min after the initiation of filtration. The corresponding filtrates for GMOB of 1000 mg MLSS/L were 166, 94 and 54 mL, at SRTs of 20, 15 and 10 days, respectively. The filtrate volume for activated sludge of 2000 mg MLSS/L was 8.5 mL at 1 min after filtration was initiated. The filtrates for GMOB of 2000 mg MLSS/L were 100, 68 and 42 mL, respectively, at SRTs of 20, 15 and 10 days. The filtrate volume for activated sludge of 4000 mg MLSS/L was 5.7 mL at 1 min after initiation of filtration. The filtrates for GMOB of 4000 mg MLSS/L were 40, 28 and 22 mL, respectively, at SRTs of 20, 15 and 10 days. The volume of filtrate increased as SRTs increased, but it decreased as MLSS concentrations of reactor increased. With GMOB, the volume of filtrate eventually reached an asymptote of 250 mL, which was the initial volume in 250 mL flask at SRTs of 20 and 15 days. However, for activated sludge, only a small amount of filtrate was obtained at 10 min after filtration was initiated.

The variation in sludge blanket depths for activated sludge and GMOB with time is shown in **Fig. 3**. In activated sludge, the sludge blanket depth became gradually lower, but it rapidly decreased for GMOB due to the latter's superior settleability. In addition, the SVI of activated sludge was 155 mL/g, and those of GMOB were 76, 85, and 72 mL/g at SRTs of 20, 15, and 10 days, respectively, indicating that SVIs of GMOB were much lower than that of activated sludge.

2.2 Specific resistance to filtration

The SRF for evaluating the dewaterability of sludge is closely related to the flocculation and settling characteristics of activated sludge. Granting that the SRF value varies depending on the analytical methods and operating conditions of activated sludge process, the SRFs of activated sludge appear to range from 2.5×10^{13} to 210×10^{13} m/kg (Yu et al., 2010) at domestic wastewater treatment plants, which is similar to that of activated sludge in this study.

The SRFs of activated sludge ranged from 8.21×10^{13} to 2.38×10^{14} m/kg, and those of GMOB ranged from 1.79×10^{12} to 3.58×10^{13} m/kg (**Fig. 4**), indicating that SRFs of GMOB were much lower than those of activated sludge. In the case of GMOB, as SRT decreased from 20 to 10 days, SRFs increased. The dissolution of the granules due to the reduction in SRT for methane-oxidizing bacteria might lead to an increase in SRF. This phenomenon also occurred for activated sludge. When SRT of activated sludge further decreased to five days, filtration became difficult due to increased viscosity. When SRT of activated sludge increased, the concentration of organic materials in effluent decreased and, subsequently, the flocculation and sedimentation of activated sludge improved (Li and Yang, 2007). This study also confirmed in that SRT and

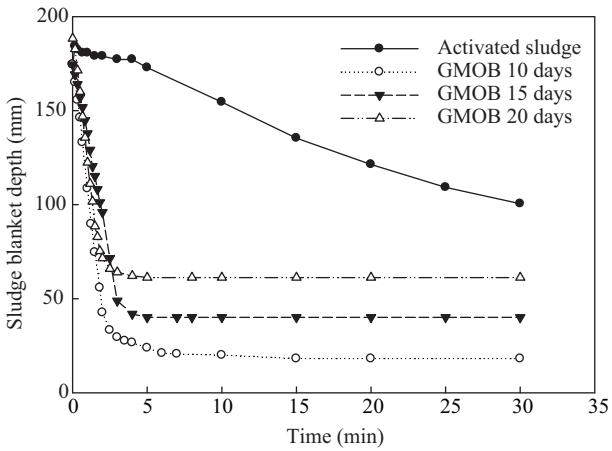

Fig. 3 Sludge blanket depths with respect to time elapsed.

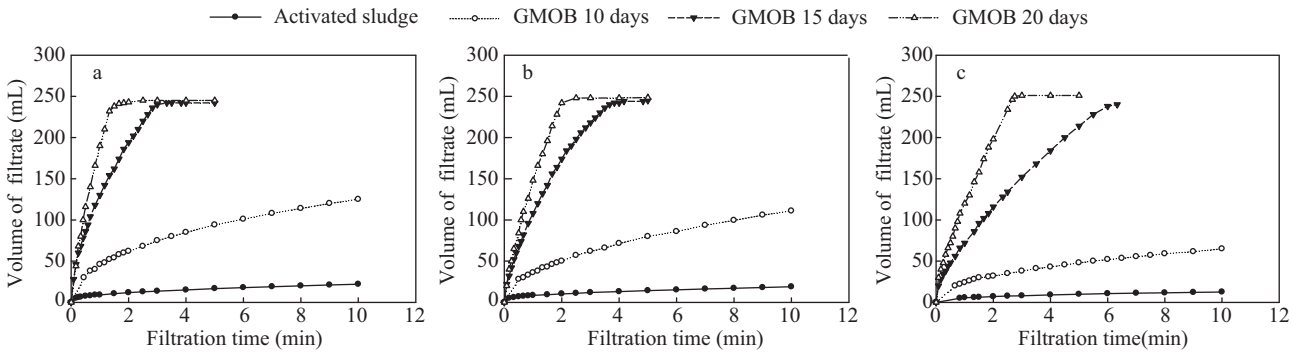

Fig. 2 Filtrate volume of activated sludge and GMOB with respect to time elapsed at MLSS concentrations of 1000 (a), 2000 (b) and 4000 (c) mg/L.

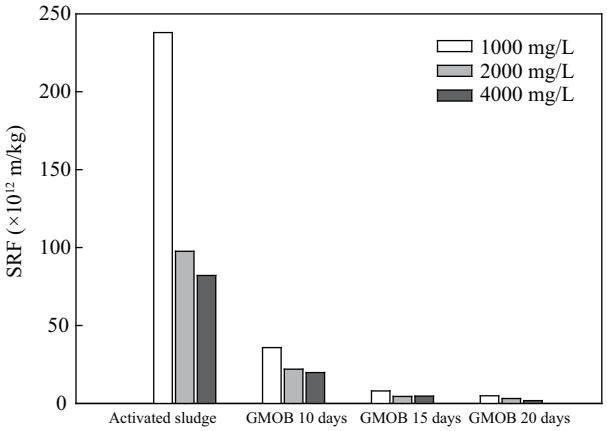

Fig. 4 Specific resistance to filtration of activated sludge and GMOB.

dewaterability were correlated for GMOB.

As concentrations of activated sludge increased from 1000 to 4000 mg MLSS/L, SRFs tended to decrease as shown in **Fig. 4**. C_c is the remaining amount of activated sludge cake after filtration. It is noted that C_c for activated sludge in this study was similar to that of GMOB. When C_s increased from 1000 to 4000 mg MLSS/L, C increased. However, SRF decreased as shown in Eq. (3).

2.3 Extracellular polymeric substance analyses

The major constituents of EPS for activated sludge and GMOB are shown in **Fig. 5**. The total bound EPS concentration of activated sludge was 147.5 mg/g-VSS, and total bound EPS concentrations of GMOB ranged from 171 to 177.2 mg/g-VSS, which indicates that the bound-EPS concentration of GMOB was higher than that of activated sludge. The bound-EPS concentrations of GMOB did not noticeably vary with changes in SRT.

The total soluble-EPS concentration obtained from the effluent analysis of A/O process was 62 mg/L, and that of effluent of GMOB reactor ranged from 17.4 to 21.4 mg/L. This indicates that total soluble EPS of activated sludge effluent was about three times higher than that of GMOB reactor effluent, unlike the results for bound EPS that were obtained in the analysis of the microorganisms. It was noted that in a biological wastewater treatment process, dewaterability was affected by soluble EPS, which degraded the filtration performance in a membrane bioreactor (Rojas et al., 2005). The results of current experiment showed that the concentration of soluble EPS in activated sludge effluent was higher than that of GMOB effluent, and that the dewaterability of activated sludge appeared to be lower than that of GMOB. It was further observed that both soluble EPS and viscosity played relatively negative roles in sludge dewatering, whereas no correlation was established between sludge dewaterability and bound EPS (Zhen et al., 2012; Cai et al., 2012).

The concentration ratios of EPSs in activated sludge vary depending on the extraction methods used and the operating conditions of the biological process. The concentration ratio of protein or polysaccharide to total bound EPS was found to be higher than those of other components to total bound EPS in activated sludge (Comte et al., 2006). In this study, the concentration ratio of protein or polysaccharide to total bound EPS was higher than those of humic substance or uronic acid to the total bound EPS in both activated sludge and GMOB. The concentration ratios of protein or polysaccharide to total soluble EPS were also high in activated sludge. However, in the case of the GMOB, the concentration ratios of polysaccharide or humic substance to total soluble EPS were higher than those of other components to total soluble EPS, which might be attributed to the different carbon sources and metabolism of activated sludge and GMOB (Guibaud et al., 2005; Ye et al., 2011). The soluble EPS of GMOB was lowest for SRT of 20 days, while the concentrations of protein increased as SRT decreased from 20 days to 10 days (Liming et al., 2009).

The concentration ratios of protein to polysaccharide were 2.4 and 0.7 for bound and soluble EPSs of activated sludge, respectively. For GMOB, the ratio were 2.1–2.7 and 0.1–0.4 for bound and soluble EPSs, respectively. This indicates that the concentration ratios of protein to polysac-

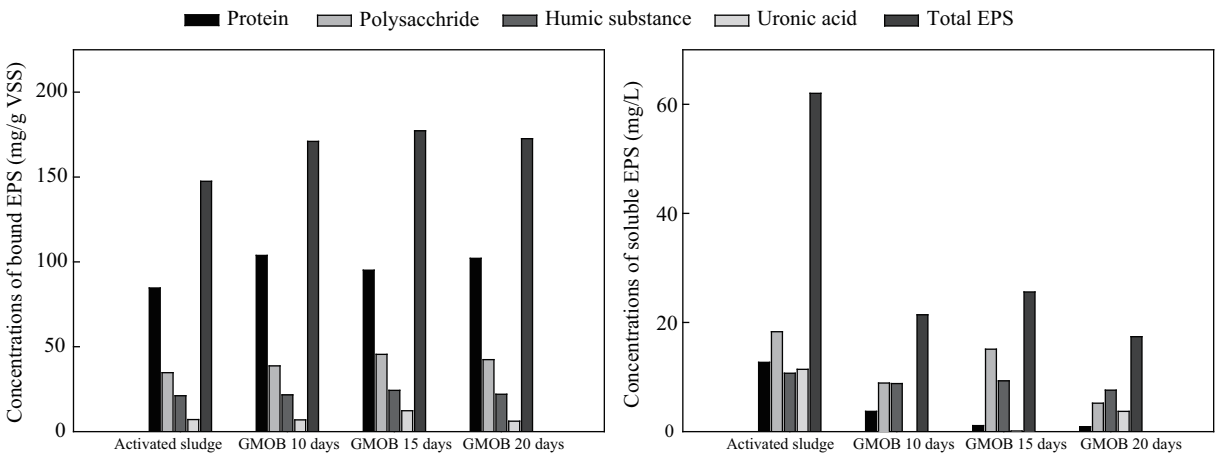

Fig. 5 Major constituents of extracellular polymeric substance (EPS) for activated sludge and GMOB.

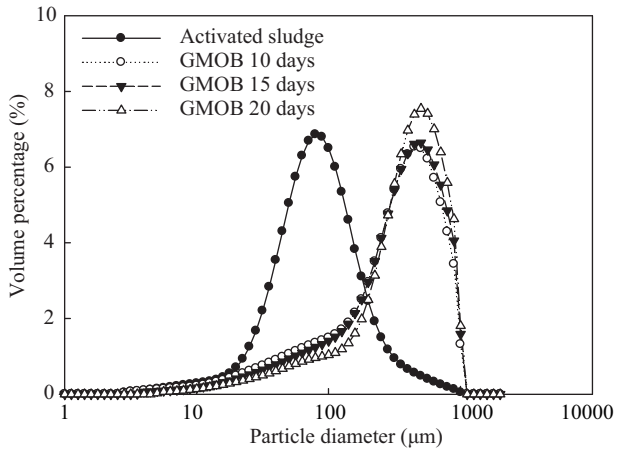

Fig. 6 Particle size distributions of the activated sludge and GMOB.

charide for bound EPS of activated sludge were similar to those for bound EPS of GMOB. The concentration ratios for soluble EPSs of activated sludge were higher than those for soluble EPSs of GMOB throughout this study. It is known that the typical concentration ratio of protein to polysaccharide of activated sludge varies from 0.5 to 21.2 depending on the operating conditions of biological treatment process (Comte et al., 2006; Guibaud et al., 2005).

2.4 Particle size distribution

The particle size distributions of activated sludge and GMOB are shown in **Fig. 6** and 10, 50 and 90 percentiles of distributions are summarized in **Table 1**. The average particle diameter of activated sludge was 104 μm. For MOB it was 402, 369, and 350 μm at SRTs of 20, 15, and 10 days, respectively. These values indicate that the particle diameters of GMOB were generally larger than those of activated sludge.

The 50 percentile of particle size distribution of activated sludge was 77 μm, while for GMOB it was 385, 344 and 326 μm at SRTs of 20, 15 and 10 days, respectively. This indicates a difference between the average particle diameter of activated sludge and 50 percentile of particle size distribution. However, the average particle diameter of GMOB was similar to 50 percentile of particle size distribution of GMOB at three different SRTs. This analytical result also showed that the average particle diameter of GMOB was three times larger than that of activated sludge. In the case of GMOB, as SRT decreased, the particle diameters decreased, which indicated that the longer SRT is, the firmer the status of the microbial granules and the larger the particle diameter becomes, consequently representing higher settleability and dewaterability.

3 Conclusions

As a result of comparative analysis of GMOB and activated sludge, the settleability and dewaterability of GMOB were found to be superior compared to those of activated sludge. This was attributed to the following facts: (1) The bound EPS contents of GMOB were higher than those of activated sludge, while the soluble EPS of GMOB was lower than that of activated sludge. (2) Based on the results of particle size analysis on GMOB and activated sludge, the average particle size of GMOB was found to be three times larger than that of activated sludge.

Table 1 Particle size distribution of activated sludge and GMOB of 10, 50 and 90 percentiles

Particle diameter	Activated sludge	GMOB 20 days	GMOB 15 days	GMOB 10 days
Mean particle size (μm)	104	402	369	350
D10 (μm)	31	81	61	50
D50 (μm)	77	385	344	326
D90 (μm)	194	738	717	694

Acknowledgments

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