Application of Swim-bed Technology to Enhance Sludge Characteristics of Activated Sludge Process

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Abstract

Biofringe (BF) carrier material creates swimming motion due to velocity of downflow wastewater, a characteristic of swim-bed technology, and provides a high degree of substrate-biomass contact. In this study, BF fiber material was packed in a plug-flow activated sludge reactor, to investigate its feasibility in improving the sludge settleability and enhancing the pollutant degradation performance of the activated sludge process. High SBOD₅, SCOD, and ammonium removal efficiencies of 99.1%, 96.5% and 83.6%, respectively were obtained at volumetric loading rate of 4.5 kg-SCOD/m³/d. SVI values below 50 ml/g and high settling velocities demonstrated satisfactory settling characteristics of the biomass, which were attributed to the increase in the size of the biomass flocs as a result of use of BF carrier material. Relatively low viscosity of mixed liquor also facilitated sludge settling performance as no sludge bulking problems were encountered throughout the experimental period. Existence of a large amount of protozoa and metazoa observed through microscopic examination was considered to contribute to the low sludge yield compared to conventional activated sludge. Microbial analysis demonstrated microbial community shift between the seed sludge and the sludge sample after 378 days of operation with proteobacteria to be predominant. The results demonstrated that use of swim-bed technology enhanced treatment performance and provided process stability to the conventional activated sludge process.

Key Words: plug-flow activated sludge process, Biofringe, granular-like floc, sludge characteristic, swim-bed

INTRODUCTION

There still exist some unsolved problems in conventional activated sludge process, such as difficulties in solid-liquid separation caused by sludge bulking, large space requirement for construction of a settling tank (ST), high excess sludge production and

relatively low pollutant removal capacity¹⁾. Fixed bed and fluidized-bed attached-growth processes have demonstrated high sludge retention capability, reduced sensitivity to toxicity, co-existence of aerobic and anoxic metabolic activities, and reactor compactness²⁾. However, maintenance associated with solids accumulation and possible packing plugging

can affect the performance of fixed bed process. Careful inlet and outlet designs for good flow distribution and higher power to keep fluidized condition are also required in fluidized-bed process^{3, 4)}.

Swim-bed technology involving a novel acryl fiber material, Biofringe (BF) carrier allows for attachment of large amounts of biomass on these carriers in a fixed position. Meanwhile. the high velocity downflow wastewater induces gas-lift in direction, flexing of the BF carriers matrix and creating a "swimming" motion of the flexible carriers with attached biofilm, which provides a high degree of substrate-biomass contact. Using this technology, high reactor mixed liquor suspended solids (MLSS) concentrations from 10 to 15 g/l (excluding the sludge biomass of biofilm), with good sludge settling characteristics, have been achieved without sludge bulking at high volumetric loading rates (VLRs) from 3 to 5 kg-BOD/m³/d with excellent organic pollutant removal performance⁵⁾. Furthermore, long sludge retention time (SRT) can be easily maintained with sludge recycling from settling tank to swim-bed reactor, which reduces sludge production due to long food chain system and predation on bacteria. This biological wastewater treatment method can save operational costs and avoid potential harmful environmental impacts in comparison or chemical physical wastewater treatment methods based on our experimental experience.

In this study swim-bed technology was applied in an activated sludge system. The main purpose of this study was to investigate the possibility of improving sludge characteristic by partially packing BF materials in a plug-flow activated sludge system and to evaluate the treatment capability in terms of organic matter removal under high loading rate conditions.

MATERIALS AND METHODS

Biofringe (BF) biomass carrier The BF carrier material consisted of support filament and fringe yarns (diameter, ca. 3mm), which were made of polyester and hydrophilic acrylic fibers, respectively. The BF had special structure such that the core point had

high density while the outside was loosely knit so that sludge could attach on BF easily and quickly. The fringe yarns waved due to the effect of high velocity water current as shown in Fig. 1. Since the movement of BF imitated swimming motion, we named this treatment process as swim-bed process.

Reactors and operating conditions four-celled reactor made of acryl resin and having a total volume of 34.8 l was used in this study as shown in Fig. 2. The first reactor cell with a working volume of 10.8 l was packed with 0.5m length of BF carrier material. The estimated packing volume of the BF carrier was approximately 5 l resulting in a packing ratio of about 15% of the total reactor volume. The working volumes of the other 3 cells were 8 l, each (named as AS1, AS2, AS3) as shown in Fig. 2. The cells were initially seeded with activated sludge from lab-scale fill-and-draw batch reactor and the initial MLSS concentration was set at about 4,000 mg/l. The air flow rate was fixed at 10 l/min for every reactor and the reactor temperature was maintained at 25°C.

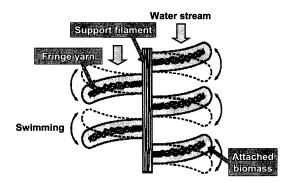


Fig. 1 Schematic diagram of BF and swimming motion.

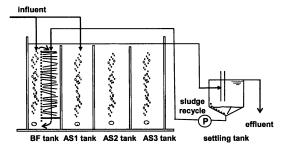


Fig. 2 Schematic diagram of experimental apparatus.

A settling tank, made of acrylic resin, with a working volume of 2 l was also used. Settled sludge in the settling tank was stirred at 4 rpm and returned to the BF reactor at a 100% recycle rate.

The synthetic influent wastewater consisting of meat extract and peptone mixture was prepared as a stock solution and diluted with tap water to obtain the desired concentration. The total nitrogen (TN) was 10% of the soluble chemical oxygen demand (SCOD), while the soluble 5-day biochemical oxygen demand (SBOD₅) was 67 % of the SCOD. NaHCO₃ (1M) was used as the buffer solution. The tap water used for dilution purposes contained 19 mg Na⁺, 6 mg K⁺, 20 mg Ca²⁺, 7 mg Mg²⁺ and 24 mg SO₄²⁻ per liter. VLR was increased by adjusting the influent concentration and the HRT. In order to mitigate the direct influence of VLR on the BF reactor cell, the influent was split and was introduced to AS1 reactor cell on day 23. On day 200, pH controller was installed in the AS2 reactor cell to ensure that the effluent pH was more than 5 by adding 1.25 N NaOH solution.

Analytical techniques SCOD was measured by the closed reflux colorimetric method according to Standard Methods⁶, with prior filtration by a filter paper (No.5B). SBOD₅ was measured by Sewage Analytical Methods (JSWA, 1978) using DO meter (TOX-90i; Toko, Ltd., Tokyo, Japan). Ammonium nitrogen (NH₄-N) was determined by the modified phenol method of Kanda⁷⁾ using ortho-phenyl phenol (OPP), nitrite nitrogen (NO₂-N) was measured by the colorimetric method according to Standard Methods (4500-NO₂ B)6 and nitrate nitrogen (NO₃-N) was measured by brucine method⁸⁾. Total nitrogen (TN) was determined by the persulfate method according to Standard Methods (4500-N_{org} D)⁶⁾. Suspended solids (SS) and MLSS were determined in accordance with Standard Methods⁶⁾. Mixed-liquor volatile suspended solids (MLVSS) and mineral (ash) contents of MLSS were determined following ignition at 600°C for 30 minutes. Viscosity was measured by B-viscosity meter (BL; Toki, Tokyo). Activated sludge flocs in the reactor were examined by light microscopy (ECLIPSE E600; Nikon, Tokyo) and the images were captured by a digital camera (CooLpix 4500; Nikon, Japan). The particle size distribution was analyzed by a laser scattering particle size distribution analyzer (LA-920, Horiba, Kyoto, Japan). pH was measured by pH meter (B-211; Horiba, Japan) and dissolved oxygen (DO) using DO meter (D-55; Horiba, Japan).

PCR-DGGE and 16S rRNAcloning techniques were performed for investigating the bacterial community changing. Samples taken from mixed liquor in the BF reactor cell was centrifuged at 9,000×g for 10 minutes and these harvested cells were used for the extraction of DNA. DNA extraction and purification was performed 7 times with Phenol/Chloroform/Isoamyl alcohol. PCR reaction mixture was composed of 30 ng of the extracted DNA, 0.5 unit of KOD-plus-DNA polymerase (Toyobo, Osaka, Japan), and 5 pmol of each forward and reverse primer. The forward primer was 357F with GC-clamp while the reverse primer was $534R^9$. PCR conditions were as follows: 94 °C for 2 min with thermal cycling consisting of 90 cycles each of 15 sec. at 94 °C, 30 sec. at 60°C, and 30 sec. at 68°C. Electrophoresis was performed using Bio-Rad Ccode® system at 100 V and 60 °C for 16 h according to manufacturer's protocol. After electrophoresis, the gel was stained with SYBR Gold Nucleic Acid Gel Stain for 30 min. and DNA bands were verified using EM-20E UV-transilluminator (UVP, Upland, CA, USA). Besides the DGGE analysis, the amplified DNA with 357F and 534R primers were also cloned as follows: the amplified products were inserted into Hinc II site of pBluescript II KS+ (Strantagene) and transformed E.coli.DH10B. Plasmid DNA was extracted and used to sequence the inserted DNA by dideoxy sequencing method with M13 and M4 primers. The DNA sequences obtained were analyzed by homology search with NCBI BLAST program.

RESULTS AND DISCUSSION

General treatment performance The experimental reactor was operated for a period of 382 days. SCOD VLR was increased stepwise by increasing the influent SCOD concentration and the influent flow rate as

shown in Fig. 3 a and b. On day 95, sludge was washed out from the reactor because of clogging of connection tubes, triggering a VLR decrease for about six days (day 95-100). However, after the clogging was cleared, the VLR was increased gradually reaching 4.5 kg-SCOD/m3/day. High SCOD removal rates were consistently achieved throughout the experimental period at VLRs ranging from 0.8 to 4.5 kg-SCOD/m³/day. The average SCOD removal rate was around 96.0%. An average SCOD removal rate of 96.5% was obtained even under the highest VLR of 4.5 kg-SCOD/m³/day. In addition, the average SBOD₅ removal rate was 99.1%, which indicated that most of the influent biodegradable organic matter was removed. Fig. 3c shows the time courses of nitrogen concentrations and nitrification rates during the experimental period. All of the influent nitrogen was composed of organic nitrogen. From the onset of the reactor operation, high effluent NO₃-N concentrations were observed. On day 202 and 232, high effluent NH₄-N concentrations over 100 mg/l were observed due to the high pH levels (7.98-8.28) caused by failure of pH control. Soon after the pH controller was fixed, the system recovered producing high NO₃-N and low NH₄-N concentrations in the effluent. Accumulation of nitrite was not observed with average effluent NO₂-N concentration of about 0.12 mg/l.

$$NE(\%) = \frac{NO_3 - N_{eff} + (TN_{inf} - TN_{eff})}{TN_{inf}} \times 100$$
(1)

Where, NO₃-N means the NO₃-N concentration in effluent and nitrification efficiency (NE) was calculated according to Eq. (1). As shown in Fig. 3c, high NE was achieved during the experimental period with an average value of 83.6 %. This high NE, combined with high effluent NO₃-N concentrations, demonstrated successful accomplishment of nitrification in the reactor. Compared to the results for one-through reactor reported by Rouse et al¹⁰ (effluent SS level about 100 mg/l), the nitrification treatment results of this study are more promising. This could be attributed to the long SRT along with the availability of a

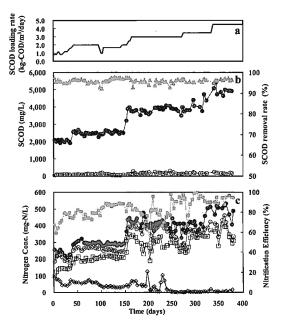


Fig. 3 Treatment performance results during the experimental period.

(a) Time course of SCOD VLR. (b) Time courses of SCOD concentrations and removal rates. Symbols: closed circles, influent SCOD; opened circles, effluent SCOD; closed triangles, SCOD removal rate. (c) Time courses of nitrogen concentrations and nitrification rates. (Symbols: closed circles, influent TN; opened circles, effluent TN; opened squares, effluent

concentrations; opened

effluent NH₄-N concentrations; closed squares.

diamonds

NO₃-N

NE)

settling tank, which was efficient for preventing the washout of nitrifiers. High NEs were also achieved under low effluent pH levels (5.2±0.6) before installing the pH controller. Nevertheless high NO₃−N concentrations were maintained. Simultaneous denitrification was not However it will be possible to get high nitrogen removal by installing denitrification reactor in this treatment system. With the increase in VLR, it was found that the TN removal efficiencies also average increased, e.g., 8.76%, 16.7% and 21.7% at VLR of 3.0, 3.5 and 4.5 kg-SCOD/m³/day, respectively. This could be attributed to the formation of anaerobic zone on the BF media due to the extremely high reactor MLSS concentrations. Based on the results of this

study, simultaneous high SCOD removal and high NE could be obtained in this type of system even at high VLRs.

MLSS concentra-Sludge characteristics tions and SVI values in the AS1 reactor cell were targeted and measured to elucidate the function of BF carrier. The results are shown in Fig. 4. MLSS concentrations increased with increase in SCOD VLR soon reaching a concentration of over 10,000 mg/l on around day 70. On day 95, sludge was washed out due to clogging of a connection tube, however, together with increase in VLR, MLSS concentration increased again after the clogging was cleared. The MLSS concentration reached more than 20,000mg/l on day 275 at a VLR of 3.5 kg-SCOD/m³/day and the sludge had an SVI of less than 50ml/g. The MLSS concentration remained at an average value of 20,000 mg/l from day 275 to day 382. Similar characteristics of sludge were also observed in AS2 and AS3 cells indicating that the sludge properties transferred from the BF cell to AS3 cell. Based on these results, it was concluded that BF material was beneficial for the improvement of sludge characteristics. From the measurement of VSS content of about 93%, a large part of sludge was shown to consist of organic matter.

Fig. 5 illustrates the relationship between SCOD VLR, MLSS and SVI in the BF reactor cell. The MLSS concentration increased to more than about 12,000mg/l at a VLR of 4.0 kg-SCOD/m³/day. Furthermore, the MLSS concentration exceeded 20,000mg/l of MLSS at a VLR of 7.0 kg-SCOD/m³/day. On the other hand, the SVI values remained below 50ml/g at a VLR of over 4.0 kg-SCOD/m³/

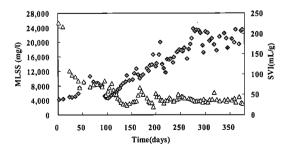


Fig. 4 Daily changes in MLSS and SVI. (Symbols: diamonds, MLSS; triangles, SVI)

day. The trend of high MLSS concentrations and low SVI levels was consistent up to a VLR of more than 4.0 kg-SCOD/m³/day. These results are consistent with the results of Cheng et al⁵. Therefore, it was concluded that application of high VLR to BF reactor was important for getting high reactor MLSS concentrations with low SVI values. It was also suggested that partially packed BF carriers in the treatment process can improve the characteristic of the sludge in conventional activated sludge system.

The SRT and food to microorganisms ratio (F/M) were calculated from equations (2) and (3)⁵, respectively for experimental run with MLSS concentrations of more than 10,000 mg/l:

$$SRT = \frac{V \cdot X_a}{Q_w \cdot X_e} \tag{2}$$

$$F/M = \frac{Q_s \cdot C_s}{X_s \cdot V}$$
 (3)

where V (l) is the reactor volume, X_a (mg/l) is the average MLSS concentration in reactor, Q (l/day) represents the flow rate, X_c (mg/l) is the average effluent SS concentration, and C_a (mg/l) is the average influent SCOD concentration. Table.1 shows the results of these calculations.

Generally, SRTs are controlled between 5 to 10 days in conventional activated sludge process to prevent the problems associated with solid-liquid separation¹¹. Nevertheless, with long SRT ranging from 41 to 66 days in this research, stable operation was still

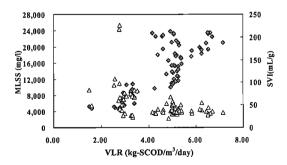


Fig. 5 Relationship between SCOD VLR, MLSS and SVI in the BF reactor cell. (Symbols: diamonds, MLSS; triangles, SVI)

Table 1	Summarization of the SRT values and the F/M
	ratios at each VI R

COD loading rate (kg-COD/m³/day)	3**	3.5	4.5
(days)	63	63	65
SRT (days)	66	59	41
F/M (kg-BOD/kg-MLSS/day)	0.14	0.11	0.14

possible without problems in the solid-liquid F/M ratios are separation. normally maintained between 0.2 to 0.4 kg-SBOD/kg-MLSS/day form agglomeration providing good settleability of activated sludge floc and to maintain good SBOD removal¹²⁾. In our experiments, good treatment performance was observed at relatively lower F/M ratios of 0.11 to 0.14 kg-SBOD/kg-MLSS/day. The actual F/M ratios were considered to be lower than these calculated values if the amount of attached sludge on BF carrier is taken into consideration. The sludge yield observed (Yob) was calculated according to Eq. (4) and (5)5) for the operational period when the MLSS concentration was more than 10,000mg/l.

$$Y_{ob} = \frac{g\text{-}SS_{end} - g\text{-}SS_{start} + \sum Q \cdot SS_{eff} + \sum Q' \cdot SS_{excess}}{g\text{-}SCOD_{removed}} \tag{4}$$

$$g-SS_{end} = SS_R + SS_{ST}$$
 (5)

where the terms g-SS_{end} and g-SS_{start} are total amount of MLSS in all of reactor cells at the end and beginning of each VLR, respectively including the SS in the reactor (SSR) and in the settling tank (SS_{ST}). Q and Q' represent the influent flow rate and the excess sludge withdrawal rate, respectively. g-SCOD_{removed} is the total amount of SCOD removed at each VLR. Fig. 6 shows observed sludge yields (Yob) for each VLR. The calculated Yob values ranged from 0.116 to 0.190 kg-MLSS/ kg-SBOD_{removed}. These values were compared Y_{ob} values reported for to activated sludge^{13, 14)}. conventional reasons of these low Yob values are attributed to long SRTs.

For sludge settling ability test, $1\ l$ mixed liquor were taken from reactors and mixed

totally. After 30 mins settling, the SV values was observed for performance comparison. Sludge settling capabilities were examined through settling tests using diluted activated sludge taken from the AS1 reactor cell and the seed sludge tank in our laboratory. Sludge A was taken on day 305 from the AS1 reactor cell and diluted to 6,700 mg/l. Sludges B and C were taken from seed sludge tank and diluted to 3,040 mg/l and 6,170 mg/l, respectively to imitate MLSS concentration in common activated sludges (sludge B) and activated sludge havin same MLSS concentration as that of sludge A (sludge C). The results of the sludge settling tests are shown in Fig. 7. It is evident that BF materials improved the sludge settling characteristics due to the improved compressibility and settle ability.

Fig. 8 shows the particle size distribution of the seed sludge and the sludge samples taken from the AS1 reactor cell on days 217,

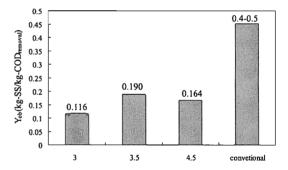


Fig. 6 Sludge yield of each VLR. (Number of 3, 3.5 and 4.5 represents the OLR with unit of kg-SCOD/m³/d)

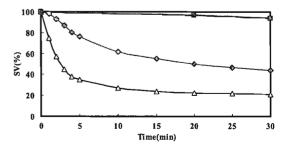


Fig. 7 Comparison of sludge settling curves for sludge in this study with activated sludges.
(Symbols: triangles, sludge A; diamonds, sludge B; squares, sludge C)

309, and 369 at VLRs of 3.0, 3.5, and 4.5 kg-SCOD/m³/day, respectively. The particle size distribution varied with an increase in VLR. Although the average floc diameter decreased with the increase in VLR, the frequency increased significantly. It was interesting to note that the test sludge samples taken from the AS1 reactor cell had same SVI values, but had the tendency to form smaller size floc with the increase in VLR.

Sludge viscosities were also measured using viscosity meter and defined distilled water as 1 Cp (MLSS = 0 mg/l). To compare with the sludge obtained in this study, activated sludge samples were taken from treatment several waste water (WWTP) and their viscosities were measured. Further, Fig. 9 illustrates the relationship between MLSS concentration and viscosity for activated sludge taken from systems BF. operating with and without exponential relationship observed between MLSS concentration and viscosity of sludges is consistent with the results reported by other researchers^{15,16)}. Significantly lower viscosities at high MLSS concentrations were observed in this study. For example, the viscosity was 5 Cp at MLSS concentration of 5,000 mg/l in conventional activated sludge, while 5 Cp at MLSS concentration of 15,000 mg/l for activated sludge with BF carrier (this study). This result shows that high MLSS concentration sludge with low viscosity was only realized using swim-bed technology. Generally, excessively high MLSS concentration, accumulation of untreated organic compounds or presence of excess polymeric flocculant molecules contribute to increase in

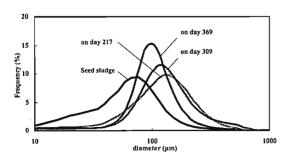


Fig. 8 Changes in sludge particle size distribution.

sludge viscosity¹⁷⁾. Extremely high viscosity of mixed liquor will cause deterioration of sludge settleability and oxygen transfer efficiency. In this research, low sludge viscosities were observed even at high MLSS concentrations demonstrating potential advantage of swim-bed technology.

Fig. 10 illustrates the results of microscopic observation of the reactor sludge on days 18, 189 and 364. At the beginning of the reactor operation, the sludge was observed to have low density and composed of filamentous microorganisms (Fig. 10a). With the increase in VLR, granular-like flocs were confirmed (Fig. 10b). This granular-like sludge exhibited good settleability even at high MLSS concentration and contributed to the stable treatment performance of the reactor. Fig. 10c shows a photograph of the sludge sample taken on day 364 when The concentration was 23,510 mg/l. photograph indicates the dense sludge. Moreover, high population of protozoa and metozoa was generally observed in the sludge sample. The presence of protozoa, e.g., Opercularia.sp, Aspidisca.sp is well known as indicator microorganism observed under high efficiency and high VLR conditions. The presence of large number of mentozoa, e.g., Rotaria.sp and Philodina.sp, Nais.sp.however, is generally observed at low VLRs or occurrence of high nitrification 18). These microorganisms formed a long food chain, maintained stable ecosystem and led to low Y_{ob} and high organic removal efficiency¹⁸⁻²⁰⁾. Especially, massive growth of *Philodina.sp* contributed to agglomeration of activated

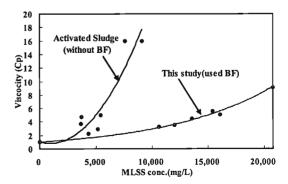
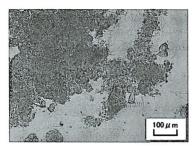
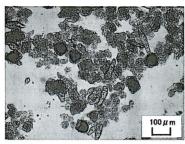
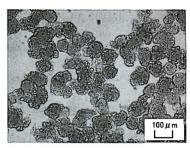


Fig. 9 Relationship of MLSS and viscosity.







a) on day 18

b) on day 189

c) on day 364

Fig. 10 Microscopic photographs of activated sludge in AS1 reactor cell: a) on day 18, MLSS, 4,386 mg/l; SVI, 217m/l/g; b) on day 189, MLSS,13,560 mg/l; SVI, 30m/l/g; c) on day 364, MLSS, 23,510 mg/l; SVI, 31m/l/g.

sludge and, subsequently clear effluent¹⁸). Agglomeration of sludge, however, may be impacted by shear stress and turbulence in the reactor due to aeration or reactor geometry. Liu *et al.*, for example, reported that shear stress generated by mixing water influenced sludge particle diameter and SVI²¹). In our research, it was suggested that the turbulent flow caused by aeration at the rate of 10 *l*/min and the BF carrier material in the four-celled reactor led to the glanular-like floc formation.

Microbial analysis Two sludge samples, one from the seed sludge tank (the seed sludge) and one from the BF reactor on day 378, were taken and subjected to 16S rDNA gene analysis by PCR-DGGE method. The DGGE results are showed in Fig. 11. Since bands in the two lanes gave different patterns, bacterial population seemed to have changed during the long term operation. The cloned DNAs were used to infer the bacterial members in the consortia, as shown in Table 2. A large number of bacteria belonging to phylum Proteobacteriawere commonly observed in both samples. Changes in the day 378 sludge sample were observed as a wide variety of classes in phylum Proteobacteria were detected along with a variety of other phyla.

In both samples, Thermomonas.sp and Xanthomonas.sp belonged to Xanthomonadaceae (family), and Comamonas.sp and Acidovorax.sp belonged to Comamonadaceae (family). Xanthomonadaceae and Comamonadaceae can consume DO and decompose organic substances such as peptone²²⁻²⁴).



Fig 11 DGGE results of different days from BF reactor.

These organisms might have contributed to the high SCOD removal rate achieved in this study. Nitrosomonas oligotropha, known as ammonia-oxidizing bacteria (AOB), was identified as a member of β-proteobacteria in the day 378 sample. This species is reported to be detected under low NH₄-N concentration conditions²⁶⁾. Although high nitrification efficiencies were obtained in the reactor, none of the nitrite-oxidizing bacteria (NOB) such as Nitrospira or Nitrobacter were identified. It is reported that the AOB

	Seed Sludge		378th Sludge	
Taxon(Phylum-class)	No. of clone	percentage of total clones(%)	No. of clone	percentage of total clones(%)
Proteobacteria	13	65.0	22	57.9
a– $proteobacteria$	_	_	(5)	(22.7)
β-proteobacteria	(6)	(46.2)	(7)	(31.8)
γ–proteobacteria	(7)	(53.8)	(10)	(45.5)
Actinobacteria	_	_	2	5.3
Bacteroidetes	3	15.0	7	18.4
Gemmatimonadetes	_	_	2	5.3
Unclassified	4	20.0	5	13.2
Total	20	100	38	100

Table 2 Summary of analysis of the cloned bacterial 16S rDNA genes

numbers are about ten times higher than that of NOB even that both of them share the same reaction rate^{26,27)}. Therefore, non detection of NOB might be due to low population numbers as Ha et al. reported in their sample²⁸⁾.

CONCLUSION

This study investigated the potential of treatment performance enhancing conventional activated sludge process by installing BF carrier material, known to create characteristics of swim-bed technology. Stable and high organic removal efficiencies were observed at VLRs of up to 4.5 kg-SCOD/m³/day as the average SCOD and SBOD₅ removal efficiencies were 96.5% and 99.1%, respectively. The reactor also exhibited high ammonium removal efficiency as an average removal efficiency of 83.6% was obtained. Packing of BF carriers in the reactor enhanced biomass retention and increased the particle size of flocs as compared to the seed sludge. The low SVI values below 50 ml/g and high settling velocities demonstrated satisfactory settling characteristics of the biomass, which could be attributed to the increase in the size of the biomass flocs. The relatively low viscosity of mixed liquor also facilitated the sludge settling performance as no sludge bulking problems occurred throughout the entire experimental period. Microscopic observation revealed abundance of protozoa and metazoa, which contributed to low sludge yields as compared with the conventional activated

sludge. DNA analysis verified that proteobacteria was predominant in the sludge samples taken on day 378. Although high nitrification efficiencies were obtained, no NOB were detected in the sludge sample due to low population of NOB. The results of this study demonstrate that the use of swim-bed technology has the potential to enhance treatment performance and provide process stability to the conventional activated sludge process.

REFERENCE

- 1) Cornelissen, E. R., Janse, W., and Koning, J.: Wastewater treatment with the internal MEMBIOR, Desalination 146, 463-466 (2002)
- Pastorelli, G., Andreottola, G., Canziani, R., Frangipane, D. F., Pascqalis, F. D., Gurrieri, G., and Rozzi, A.: Pilot-plant experiments with moving-bed biofilm reactors, Water Sci. Technol. 36(1), 43-50 (1997)
- Wang, Y. T., Suidan, M. T., and Rittman, B.
 E.: Anaerobic treatment of phonol by an expanded bed reactor. Journal Water Pollution Control Federation, 58, 227-233 (1986)
- Fox, P., Suidan, M. T., and Pfeffer, J. T.: Anaerobic treatment of biologically inhibitory wastewater. Journal Water Pollution Control Federation, 60, 86-92 (1988)
- 5) Cheng, Y. J., Yazaki, D., Koyama, T., and Furukawa K.: Swim-bed technology as an innovative attached-growth process in the

- wastewater treatment, Proceeding of IWA 2005, 295-305, Xi'an, China (2005)
- 6) APHA, AWWA and WEF: Standard Methods for the Examination of Water and Wastewater, 19th Ed. American Public Health Association, Washington D.C. (1995)
- 7) Kanda, J.: Determination of ammonium in seawater based on the indophenol reaction with o-phenylphenol (opp), Water Res. 29, 2746-2750 (1995)
- 8) JSWA (Japan Sewage Works Association): Standard method on wastewater. 92-100, in Japanese (1978)
- Muyzer, G., Dewaal, E. C., and Uitierlinden,
 A. G.: Profiling of complex microbial populations by denatureing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol., 59(3), 695-700 (1993)
- 10) Rouse, J. D., Yazaki, D., Cheng, Y. J., Koyama, T., and Furukawa, K.: Swim-bed technology as an innovative attached-growth Process for high-rate wastewater treatment. Japanese Journal of Water Treatment Biology 40, 115-124 (2004)
- 11) Hashimoto, M., and Furukawa, K.: Study on biological nitrogen removal from wastewater. Water Treatment Technology, 22, 193-199, in Japanese (1981)
- 12) Shimizu, T., Fujita, M., Furukawa, K., and Horiuchi, J.: Microbe and Environmental Protection, Sankyo publishing, 2, 46.
- Metcalf, Eddy, Inc.: Wastewater engineering: treatment, disposal and reuse, 3rd ed., McGraw-Hill Book Company, 529-591 (1991)
- 14) Ghyoot, W., and Verstraete, W.: Reduced sludge production in a two-stage membrane-assisted bioreactor. Water Res. 34, 205-215 (1999)
- 15) Khongnakorn, W., Wisniewski, C., Pottier, L., and Vachoud, L.: Physical properties of activated sludge in a submerged membrane bioreactor and relation with membrane fouling. Separation Purification Technology 55, 125-131 (2007)
- 16) Reid, E., Xingrong, Liu, and Judd, S.J.: Sludge characteristics and membrane fouling in full-scale submerged membrane bioreactors. Desalination 219, 240-249 (2008)

- 17) Nakagaki, M., Shimizu, H., and Arai, K.: Membrane treatment technology, Fujitec Corporation (1991)
- 18) Kojima, S., Sudo, R., and Chihara, M.: Environmental microorganism pictorial book, Kodansha, 63-70, 662-664, 673-674 (1996)
- Lapinski, J., and Tunnacliffe, A.: Reduction of suspended biomass in municipal wastewater using bdelloid rotifers. Water Res. 37, 2027–2034 (2003)
- 20) Wei, Y., Houten, R. T. V., Borger, A.R., Eikelboom, D. H., and Fan, Y.: Minimization of excess sludge production for biological wastewater treatment. Water Res. 37, 4453-4467 (2003)
- 21) Liu, Q., Liu, Y., Tay, J., and Show, K.: Response of sludge flocs to shear strength. Process Biochem. 40, 3213-3217 (2005)
- Vauterin, L., Hoste, B., Kersters, K., Swings,
 J.: Reclassification of Xanthomonas. Int.
 J. Syst. Bacteriol. 45, 472-489 (1995)
- 23) Bergey, D. H., Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., Williams, S. T.: Bergey's manual of systematic bacteriology Vol.2, 230-869 (2005)
- 24) Bergey, D. H., Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., Williams, S. T.: Bergey's manual of systematic bacteriology Vol.3, 62-119 (2005)
- 25) Bollmann, A., Bar-Gilissen, M. J., and Laanbroek, T. A.: Growth at low ammonium concentrations and starvation response as potential factors involved in niche differentiation among ammonia-oxidizing bacteria. Appl. Environ. Microbiol. 68, 4751-4757 (2002)
- 26) Fujita, M., Tsuji, K., Takeda, N., Minakawa, M., Nakamura, M., Mino, T., Akashi, A.: Temporal variation in maximum cell-specific nitrification rates in municipal wastewater treatement plant and controlling factor. Journal of Japan Society on Water Environment 30(12), 723-729, in Japanese (2007)
- 27) Fujita, M.: Development of nitrification model using molecular techniques.

 Journal of Japan Society on Water Environment 31(1), 17-20, in Japanese (2008)
- 28) Ha, D. T., Kusumoto, R., Koyama, T., Fujii, T., and Furukawa, K.: Evaluation of the swim-bed attached-growth process for

nitrification of Hanoi groundwater containing high levels of iron. Japanese Journal of Water Treatment Biology 41(4), 181-192 (2005) (Submitted 2009. 5. 27) (Accepted 2009. 9. 24)