

Simultaneous removal of organic and strong nitrogen from sewage in a pilot-scale BNR process supplemented with food waste

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Abstract As the sewerage system is incomplete, sewage in Korea lacks easily biodegradable organics for nutrient removal. In this country, about 11,400 tons of food waste of high organic materials is produced daily. Therefore, the potential of food waste as an external carbon source was examined in a pilot-scale BNR (biological nutrient removal) process for a half year. It was found that as the supply of the external carbon increased, the average removal efficiencies of T-N (total nitrogen) and T-P (total phosphorus) increased from 53% and 55% to 97% and 93%, respectively. VFAs (volatile fatty acids) concentration of the external carbon source strongly affected denitrification efficiency and EBPR (enhanced biological phosphorus removal) activity. Biological phosphorus removal was increased to 93% when T-N removal efficiency increased from 78% to 97%. In this study, several kinds of PHAs (poly-hydroxyalkanoates) in cells were observed. The observed PHAs was composed of 37% 3HB (poly-3-hydroxybutyrate), 47% 3HV (poly-3-hydroxyvalerate), 9% 3HH (poly-3-hydroxyhexanoate), 5% 3HO (poly-3-hydroxyoctanoate), and 2% 3HD (poly-3-hydroxydecanoate).

Keywords EBPR; food waste; nutrients; PHAs; sewage; VFAs

Introduction

As an excessive amount of nutrients is discharged into streams and lakes by various routes, eutrophication in water bodies causes a significant water quality problem. There are more than 180 plants treating 19,230,000 tons/day of municipal wastewater in Korea. About 87% of total treatment plants have adopted the biological secondary treatment technology and only 12% uses advanced technology for nutrient removal (MOE, 2002). Untreated or partially treated sewage could be major sources of nutrients.

BNR processes are now widely used in wastewater treatment. For the purpose of simultaneous organic and nutrient removal in the BNR process, the system has been designed to provide different environment of anaerobic/anoxic and aerobic conditions. In BNR processes, nutrient removal efficiency depends highly on the characteristics of biodegradable organics. But the sewage of low C/N ratio lacks carbon source for the biological nutrient removal so that external carbon sources such as acetic acid and methanol are generally added at the advanced wastewater treatment plants. But they are expensive and also could decrease pH of the system (Grabinska-Loniewska, 1991). As a result, other sources including primary sludge (Issacs and Henze, 1995), night soil (Choi *et al.*, 1996), and

biodegradable organic fraction of municipal solid waste (Cecchi *et al.*, 1994) were considered as an alternative carbon source.

Recently, food waste containing high organic content was examined as an alternative (Lim *et al.*, 2000; Lee *et al.*, 2003). Lee *et al.* (2003) reported that as AFLFW (anaerobically fermented leachate of food waste) was supplied, the average removal efficiencies of T-N and T-P increased from 53% and 21% to 74% and 68%, respectively in a lab-scale BNR process treating domestic sewage having low C/N ratio of 4.4. The pronounced improvement in biological nutrient removal, associated with the supplement of AFLFW containing a variety of desirable but undefined biodegradable organic compounds, has left an increased need of investigating the role of specific substances more closely.

In EBPR mechanism, VFAs was known as a precursor of intracellular storage products, PHAs. The reported two representative models such as the "Comeau" model and the "Mino" model (Comeau *et al.*, 1986; Mino *et al.*, 1987; Arun *et al.*, 1998) only describe the biochemical processing of acetic acid to 3HB, one of the PHAs. PHAs are the polyesters of various hydroxyalkanoates that are accumulated as a carbon/energy and/or reducing power storage material in various microorganisms usually under the condition of limiting nutritional elements such as N, P, S, O or Mg in the presence of excess carbon (Anderson and Dawes, 1990; Brandl *et al.*, 1990; Lee, 1996). More than 300 different micro-organisms are known to synthesize and intracellularly accumulate PHAs (Lee, 1996).

The structure of PHAs in cells was formed depending on the characteristics of substrates and microorganisms in the system. In fact, PHAs were mainly composed of 3HV instead of 3HB when glucose or propionic acid was used as a sole carbon source (Jeon and Park, 2000; Wang *et al.*, 2002). Recently, Hood and Randall (2001) reported that acetic acid and isovaleric acid formed 3HB while valeric and propionic acids resulted in 3HV and/or 3H2MV (3-hydroxy-2-methyl valeric acid). Lee *et al.* (2003) observed that other PHAs were also accumulated inside the biomass when using AFLFW as an external carbon source in a lab-scale BNR process. The average compositions of 3HB, 3HV, 3HH, 3HO, 3HD, 3HDD (poly-3-hydroxydodecanoate) were 24%, 41%, 18%, 10%, 5%, and 2% respectively.

In this study, the potential of AFLFW as an external carbon source was examined in a pilot-scale BNR process treating sewage containing high nitrogen content. Also the behaviours of intracellular materials affecting EBPR mechanism in this system were studied.

Materials and methods

Characteristics of influent and external carbon source

As shown in Table 1, the influent sewage contained a low organic strength with relatively higher nutrients content indicating a shortage of carbon for nutrients removal. About

Table 1 Characteristics of sewage and external carbon source

Constituents		AFLFW		Sewage
		*5g VS/l/day	*10g VS/l/day	
COD _{cr} (mg/l)	Total	12,574	20,174	85 (±28)
	Soluble	11,440	18,919	55 (±15)
SS (mg/l)	Total	374	710	192 (±22)
	Volatile	232	440	87 (±10)
Nitrogen (mg/l)	TKN	396	753	204 (±27)
	NH ₄ -N	282	520	153 (±22)
Phosphorus (mg/l)	Total	174	330	2.7 (±0.8)
	Soluble	32	57	1.1 (±0.3)
TVFAs as COD (mg/l)		10,021	16,149	-

* These values indicate organic loading rates of food waste to the anaerobic acidification reactor

11,000 tons of food waste has been produced daily in Korea (MOE, 2002), containing vegetable of 53%, fish and meat of 29%, cereals and grains of 13%, and fruits of 5%. Sources are households (53%), restaurants (30%), markets (12%) and fast food eateries (5%). Average total solid, volatile solid, and total COD (chemical oxygen demand) concentration of food waste were 17.4%, 15.7% and 157,000 mg/l, respectively (Lee *et al.*, 2003). Using this food waste, the external carbon source was produced from the anaerobic acidification reactor having effective volume of 63L. The produced VFAs contained 28% acetate, 11% propionate, 43% butyrate, 15% valerate, 2% caporate and 1% lactate. The VFA/TCOD ratios at organic loading rates of 5 and 10g VS/l/day were 79% and 80%, respectively.

BNR process and experimental conditions

For this study, a pilot-scale BNR process that containing pre-anoxic (pre-AX), anaerobic (AN), post-anoxic (post-AX), and aerobic (OX) stage with 668 l of effective working volume was used (Figure 1). As shown in Table 2, HRTs (hydraulic retention times) of each stage were 1, 1.5, 1.5 and 4 hrs, respectively. Table 3 showed operating conditions of a pilot-scale BNR process. Sewage was supplied to the pre-AX stage at the flow rate of 1.4 l/min (2 m³/day). AFLFW was supplemented to the post-AX stage and settled sludge

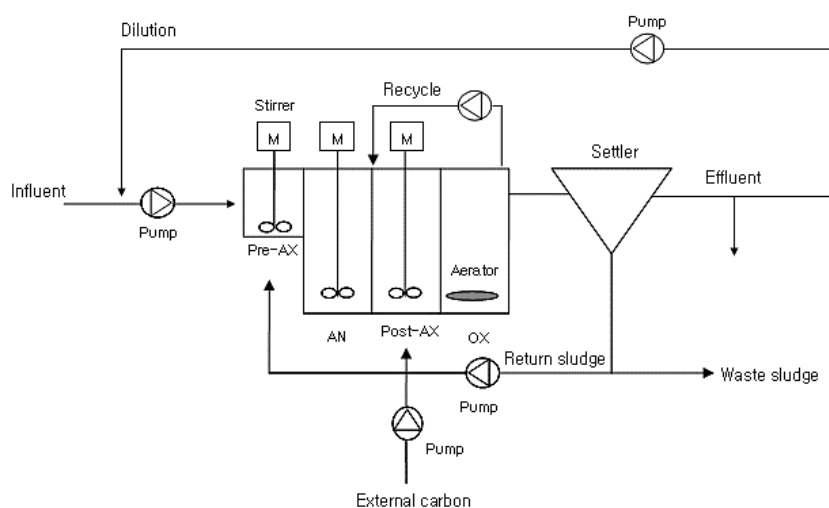


Figure 1 Schematic diagram of a pilot-scale BNR process

Table 2 Dimension of a pilot-scale BNR process

	Pre-AX	AN	Post-AX	OX	Total	Settler
HRT (hr)	1	1.5	1.5	4	8	3
Working volume (l)	84	125	125	334	668	251

Table 3 Operation conditions of a pilot-scale BNR process

Operation conditions	
SRT	30 days
Flow rate of influent	1.4 l/min (Q)
Internal recycle rate	5.6 l/min (4Q)
Return sludge rate	0.7 l/min (0.5Q)
MLSS	3.850 mg/l (± 220)
VSS/SS	0.78–0.81

Table 4 Experimental conditions

Phase	Dilution	Influent: effluent	Operation period	VFAs concentration in AFLFW	AFLFW supply
1	0%	1 : 0	2 months	–	–
2	0%	1 : 0	0–22 days	10,021 mg/L as COD _{cr}	20 L/day
3	200%	0.5 : 0.5	23–50 days		
4	400%	0.25 : 0.75	51–77 days	16,149 mg/L as COD _{cr}	
5	400%	0.25 : 0.75	78–100 days		
6	200%	0.5 : 0.5	101–125 days		

was recycled from the settler to the pre-Ax stage at a flow rate of 0.7 l/min. A flow rate of internal recycle from the OX stage to the post-AX state was 5.6 l/min. Average MLSS concentration was 3,850 mg/l at SRT (sludge retention time) of 30 days.

Table 4 depicted the designed experimental schedule for 185 days. After phase 1 of the acclimation period, the BNR process was operated without dilution at phase 2. In this case, total nitrogen concentration in the influent was about 225 mg/l and nitrogen loading rate was 0.67 kg/m³/day so nitrification and denitrification efficiencies were low. To reduce the nitrogen loading, influent was diluted with effluent after phase 2. Dilution rate was increased from 0% to 400%. From phase 2 to phase 4, VFAs concentration of AFLFW was 10,021 mg/l as COD. It increased to 16,149 mg/l as COD after phase 4. A flow rate of AFLFW was 20 l/day that was 1% of the influent flow rate.

Analytical methods

Suspended solids (SS), COD_{cr}, TKN, NH₄-N, and T-P concentrations were measured according to *Standard Methods* (1992). Concentrations of various ions in solution were analyzed using ion chromatography (DX-120, Dionex, USA). VFAs were analyzed by HPLC (Spectra Physics 2000, USA) equipped with Aminex HPX-87H column (300 mm × 7.8 mm, Bio-Rad, USA). The used solvent was 0.01N H₂SO₄ solution. PHAs concentrations were determined by gas chromatography with FID detector (HP 5890, Hewlett-Packard, USA) equipped with a SUPELCO SPB-5 column (30 M × 0.32 mm, Bio-Rad, USA) using benzoic acid as an internal standard (Barunegg *et al.*, 1978). For glycogen determination, 6M HCl was directly added to the pre-treated sample to a final concentration of 0.6M (Smolders *et al.*, 1994). After digestion in 100 °C oven and centrifugation, the supernatant was analyzed by a BioLC (Dionex, USA) including ED 50 electrochemical detector with a CarboPac PA10 column (4mm × 250mm), 200mM and 18mM NaOH solution as the gradient eluents. Detection of poly-P concentration in biomass followed the acid extraction method suggested by De Hass (1991). For the estimation of molecular size distribution of dissolved organic matter in effluent, an array of ultra filtration membranes (YM1, YM10, YM30, Millipore Co., USA) in stirred cells (Amicon, Co., USA) were used as described by Logan and Jiang (1990).

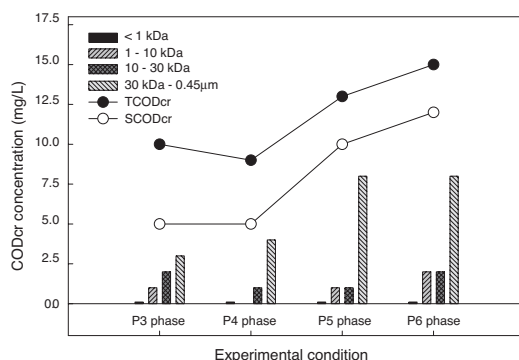
Results and discussion

Performance of a pilot-scale BNR process

To reach the steady-state, the reactor was operated for 60 days until stable operation was achieved. After this acclimation period (phase 1), five different phases (phases 2–6) were studied to estimate the effect of AFLFW on nutrient removal. Table 5 shows the performance of a pilot-scale BNR process during the experimental period. In this table, average total COD removal efficiency was 88% in phase 4 but it decreased to 83% in phase 6. Figure 2 showed that the reason why organic removal efficiency decreased when organic concentration in AFLFW increased from 12,574 to 20,174 mg/l in

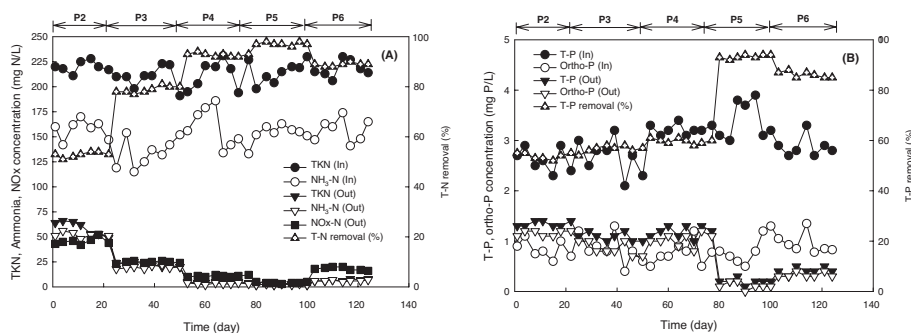
Table 5 Nutrient removal efficiencies under various operation conditions

Phase	TCOD			T-N			T-P		
	In	Out	Removal	In	Out	Removal	In	Out	Removal
	(mg/l)	(%)		(mg/l)	(%)		(mg/l)	(%)	
2	83	9	89	225	105	53	2.9	1.3	55
3	79	10	87	214	47	78	2.5	1.1	56
4	77	9	89	220	14	94	3.2	1.3	59
5	91	13	86	215	6	97	2.7	0.2	93
6	88	15	83	223	23	90	3.1	0.4	87

**Figure 2** COD concentration and molecular size distribution of organics in the effluent

phases 5 and 6. The molecular size distribution of organics in the effluent under various conditions, almost small molecular weight organics were used in the system but relatively large size organics (30 kDa – 0.45 μ m) were not degraded and increased from 5.1 to 10.3 mg/l as COD resulting in the increase of total COD concentration in the effluent.

Figure 3(A) indicated that total nitrogen concentration in the influent was so high that T-N removal efficiency was low in phase 2. The T-N removal efficiency increased to 94% in phase 4 as the influent was diluted with the effluent. After phase 4, organic loading rate of the anaerobic acidification reactor was increased from 5 to 10g VS//day resulting in the T-N removal efficiency over 95% in phase 5. On the other hand, T-P removal efficiency was below 60% from phase 2 to phase 4, however, it increased to 93% in phase 5 as T-N removal efficiency increased to 94% as shown in Figure 3(B). This result was well matched with other previous studies indicating that the nitrate nitrogen was related with biological phosphorus removal. Average MLSS concentration in the system was 3,850 mg/l during the experimental period. VSS/SS ratio was 0.78–0.81 and

**Figure 3** Nitrogen (A) and phosphorus (B) removal in a pilot-scale BNR process

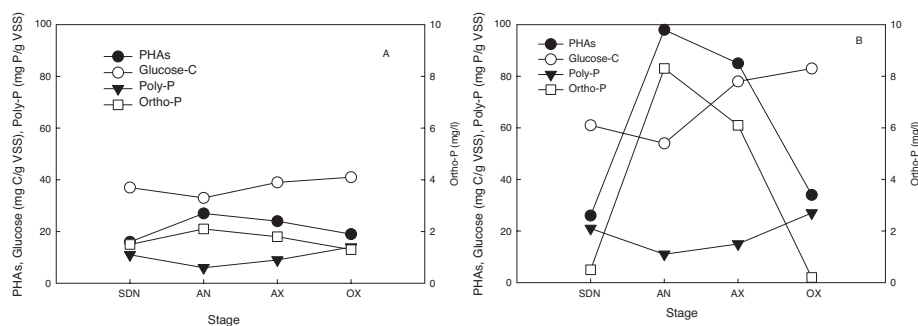


Figure 4 Behaviour of intracellular materials in a pilot-scale BNR process (A = in phase 4; B = in phase 5)

daily sludge production was 12.9 g/day. Biomass yield was 0.031 g/g TCOD that was 5–10% of the biomass yield in the conventional activated sludge process (Mayhew and Stephenson, 1997). This low value indicated that most organics were used for denitrification and phosphorus removal.

EBPR activity at different conditions

As shown in Figure 4(A), EBPR activity (i.e. anaerobic P-release and aerobic P-uptake) of microorganism was low in phase 4 because most organic substance was used for denitrification. But, the active EBPR performance was observed in phase 5 when VFAs concentration of AFLFW increased from 10,021 to 16,149 mg/l (Figure 4(B)). Ortho-P uptake/release ratios in phases 4 and 5 were 0.8 and 1.1, respectively. The anaerobic P-release, anoxic/aerobic P-uptake and net P-removal in phase 5 were 7.5, 8.3 and 0.8 mg/l on average, respectively. Hood and Randall (2001) reported that ortho-P uptake/release ratio was 3.2 when acetate was used as a carbon source. In this case, acetate concentration was 46.8 mg/l in the batch reactor. P uptake/release ratio was strongly linked to the characteristics of the carbon source.

Several kinds of PHAs were observed inside the activated sludge. When acetate was used as a substrate, 3HB was the major compound, but other PHAs were also detected in this study. The average composition of 3HB, 3HV, 3HH, 3HO, and 3HD in cells were 37%, 47%, 9%, 5%, and 2% in phase 5, respectively. It means that microorganisms could store various PHAs through different metabolic pathways when various organic substrates such as sewage and AFLFW were supplemented. Actually, almost *pseudomonads* could synthesize copolymers consisting of MCL (medium chain length)-PHAs from various substrates (Timm and Steinbuechel, 1990; Anderson and Dawes, 1990). The precursors for PHA biosynthesis are derived from de novo fatty acid synthetic pathways. However, the dominant microorganism that played an important role in EBPR mechanism was not identified. Therefore, further study is needed to account for the accumulation of various PHAs in the cells by using molecular methodologies such as PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis) and FISH (fluorescent *in situ* hybridization).

Conclusions

A pilot-scale BNR process that was supplemented with AFLFW as an external carbon source showed good performance for 6 months. Maximum removal efficiencies of T-N and T-P were 97% and 93%, respectively. But there was a side effect from using the new carbon source. When the organic concentration of AFLFW increased, COD concentration in the effluent increased because large molecular organics increased. Denitrification efficiency and EBPR activity were strongly related with VFAs concentration in AFLFW. EBPR

activity increased when T-N removal efficiency increased from 78% to 97%, implying that AFLFW were first effectively used to reduce nitrate concentration. Several kinds PHAs such as 3HB (37%), 3HV (47%), 3HH (9%), 3HO (5%), and 3HD (2%) were detected. From this study, it was concluded that the low concentration of nitrate indicating the enhancement of denitrification efficiency was essential for active EBPR mechanism. Additionally, the desirable organics such as VFAs for the EBPR mechanism should be supplied sufficiently.

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