Resource recovery and nitrogen removal from piggery waste using the combined anaerobic processes

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Abstract The combined ADEPT (Anaerobic Digestion Elutriated Phased Treatment)- SHARON (Single reactor system High Ammonium Removal Over Nitrite) – ANAMMOX (Anaerobic Ammonium Oxidation) processes were operated for the purpose of resource recovery and nitrogen removal from slurry-type piggery waste. The ADEPT operated at acidogenic loading rates of 3.95 gSCOD/L-day, the SCOD elutriation rate and acid production rate were 5.3 gSCOD/L-day and 3.3 gVFAs(as COD)/L-day, respectively. VS reduction and SCOD reduction by hydrolysis were 13% and 0.19 $gSCOD_{prod.}/gVS_{feeding}$, respectively. Also, the acid production rate was 0.80 $gVFAs/gSCOD_{production}$. In the methanogenic reactor, the gas production rate and methane content were 2.8 L/day (0.3 $m^3CH_4/kgCOD_{removal}$@STP) and 77%, respectively. With these operating condition, the removal of nitrogen and phosphorus were 94.1% as NH_4-N (86.5% as TKN) and 87.3% as T-P, respectively.

Keywords Anaerobic digestion elutriated phased treatment; nitrogen removal; piggery waste; resources recovery; SHARON – ANAMMOX process

Introduction

Korea has probably the most stringent effluent nitrogen guidelines in the world for piggery wastewater: less than 60 mg/L. The slurry-type piggery waste in Korea has the characteristics of high strength (up to 140 gCOD/L, 96 g TSS/L and 12 g TKN/L) with poor solid/liquid separation. Because of the strong nitrogenous nature and organic content, piggery waste is often considered a very difficult subject for nitrogen and phosphorus removal.

Most of piggery waste in Korea is generated in rural areas where the small-scale sewage treatment plant is also located. The influent to the small-scale sewage treatment plant often exhibits an unfavourable C/N/P ratio for nutrient removal. The small-scale sewage treatment plants usually use SBR, so that biological nutrient removal (BNR) is more difficult without an external introduction of organic carbon. Because of the circumstantial requirements, the extraction of carbon energy for the use of BNR in sewage treatment followed by autotrophic removal of nitrogen in piggery waste has been studied in this paper.

The Anaerobic Digestion Elutriated Phased Treatment (ADEPT) process, which is a new high rate anaerobic treatment for a particulate-type waste, consists of an acid elutriation slurry reactor for hydrolysis/acidification, followed by a high rate methanogenic reactor such as the upflow anaerobic sludge bed (UASB) reactor (Kim *et al.*, 2001). The added advantage of this process is the production of useful biogas at short HRT. The effluent of this process has very low C/N ratio so that the ANAMMOX process would be

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an alternative method for nitrogen removal of ADEPT effluent containing a large amount of nitrogen.

In the ANAMMOX process, ammonium can be converted to dinitrogen gas using nitrite as electron acceptor in anaerobic conditions (Mulder *et al.*, 1995). Compared with the conventional nitrification/denitrification process, the ANAMMOX process combined with SHARON process has many advantages: no aeration and organic carbon is needed with lower sludge production. The combined SHARON–ANAMMOX process is represented as (Equation 1).

$$2\,NH_4^+ + 2\,HCO_3^- + 1.5\,O_2 \rightarrow N_2 + 2\,CO_2 + 5\,H_2O \qquad (1)$$

During the last several years, we have focused on examining the feasible alternatives rather than conventional nitrification–denitrification processes to remove nitrogen from piggery wastewater (Min *et al.*, 2002; Ahn *et al.*, 2004; Hwang *et al.*, 2004, 2005). Although the development of the ANAMMOX organism was considered as a difficult task, a previous study has demonstrated that an inoculation of anaerobic granules could reduce the acclimation period to develop the ANAMMOX organism.

This paper mainly discusses the operational results of the combined ADEPT–UASB followed by the SHARON–ANAMMOX process configuration aimed at resource recovery (organic carbon for BNR and biogas) and anaerobic autotrophic nitrogen removal for piggery wastewater treatment. The results of microbial community analysis (scanning electron microscopy) on anaerobic granules in the ANAMMOX reactor are also presented.

Material and methods

Laboratory reactors

A lab-scale sequencing ADEPT with an acid elutriation slurry reactor followed by a methanogenic UASB reactor and combined SHARON–ANAMMOX process were operated with mesophilic (35 °C) temperature (Figure 1).

Both acid elutriation and the methanogenic reactor have 2 L of operating volume with a 0.5 L settling tank and retention times of 5 and 2 days, respectively. The substrate (0.4 L/day), which was fed to the top of the sludge blanket in the acidogenic reactor and the refractory slurry which thickened at the bottom of the reactor were wasted daily, thus resulting in a constant sludge blanket level in the acidogenic reactor. The elutriating water (Q_{elu}) to inflow (Q_{in}) ratio in the acid elutriation reactor was 2.5. The effluent of the acid

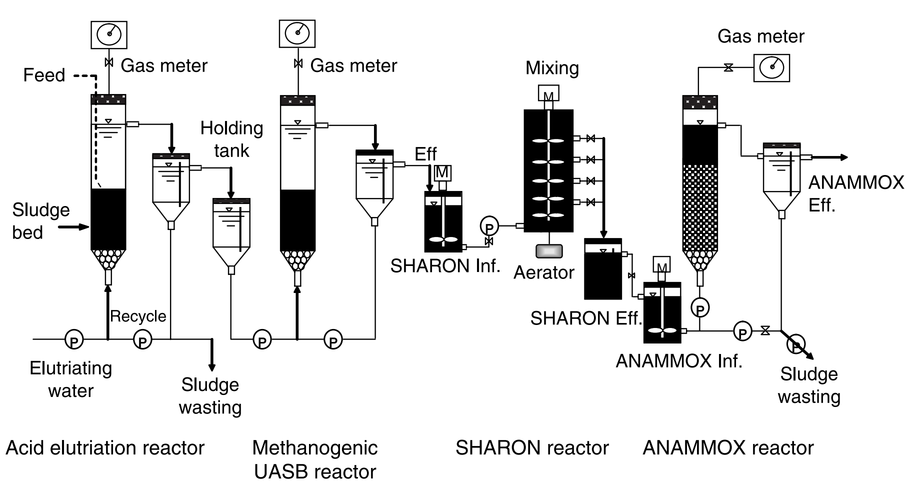

Figure 1 Schematic diagram of sequencing ADEPT and combined SHARON/ANAMMOX processes

elutriation reactor was fed into the UASB reactor, which was inoculated by anaerobic granular sludge from a local brewery with an up-flow sludge blanket (UASB) reactor.

The effective volume of the SHARON reactor was 1 L and was operated with SBR-like, fill-and-draw feeding type at anHRT of 1 day. Nitrifying sludge from a full-scale livestock wastewater treatment plant was inoculated as a seed. The SHARON reactor was operated at a temperature of 35 °C. DO level was maintained within the range of 3–4 mg/L. The ANAMMOX reactor consisted of an upflow-type sludge bed reactor (1 L) and a separate settling tank (0.5 L). The anaerobic granules taken from a local brewery with a UASB reactor were used as seed for the ANAMMOX reactor. The reactor was also operated as a fill-and-draw feeding system like the SBR. The HRT of the ANAMMOX reactor was 2.5 days. The settled sludge from the settling tank was recycled to the ANAMMOX reactor as a ratio of 0.5 Q. In order to prevent adverse effects of DO, the SHARON effluent was stored in an effluent reservoir to remove DO prior to feeding to the ANAMMOX reactor.

Substrate

Slurry type piggery waste screened with 5 mm-opening strainer was used as a substrate. The average pH, COD, TS and VS fraction of the substrate were 8.5, 59 gCOD/L, 66 gTS/L and 71% of TS, respectively. Table 1 shows the characteristics of the piggery waste used in this study. The pH of the system was not artificially manipulated. For nitrification, however, bicarbonate alkalinity ($NaHCO_3$) was added to the SHARON influent, which is the methanogenic effluent.

Water quality analysis

All water quality parameters were measured in accordance with *Standard Methods* (1998). The analysis included pH, COD, TKN, NH_4-N, T-P, PO_4-N, TS, VS, TSS, VSS and alkalinity. The gas production was monitored daily with a wet gas meter (Sinagawa Model W-NK-0.5A). A HPLC (Shimadzu Model LC-10AD, Japan) equipped with a UV detector and an organic acid analysis column (Aminex HPX-87H, Bio-Rad, Inc., USA) was used to monitor volatile fatty acids (VFAs).

Microbial community analysis

A visual inspection of ANAMMOX granules was conducted using scanning electron microscopy (SEM). In order to observe the microbial diversity in the ANAMMOX reactor, fluorescent *in situ* hybridization (FISH) test was also performed at the beginning and end of operation. The fractions of microorganism were estimated with FISH using

Table 1 Characteristics of slurry-type piggery waste used as a substrate

Items	Range	Average ± S.D.
pH	8.3–8.6	8.5 ± 0.5
TCOD	42,950–81,770	58,600 ± 21,100
SCOD	17,340–38,810	25,490 ± 12,090
TKN	5650–6100	5880 ± 320
NH_4-N	3240–4960	4390 ± 700
T-P	1620–2040	1860 ± 160
PO_4-P	315–772	454 ± 163
TS	55,990–81,840	66,160 ± 8720
VS	40,250–57,210	46,800 ± 6900
Total Alkalinity	12,350–13,830	13,090 ± 1040
Bicarbonate Alkalinity	4250–6250	5250 ± 1410

Unit: mg/L except pH

the 16S rRNA-targeted oligonucleotide probes Pla46, Kst1275, NSO190 and NIT3, which specify *Planctomycetales*, *Candidatus Kuenenia stuttgartiensis*, ammonia-oxidiaing β-subclass proteobacteria and *Nitrobacter* spp., respectively. The mean values for each order, genus and group-specific bacteria and total cell counts were calculated from the counts of 15 randomly chosen fields using an epifluorescence microscope (Zeiss Axioplan, Germany) and the results were expressed in percentile of the number of individual group-specific bacteria to the number of total bacteria.

Results and discussion

ADEPT process

For the acid elutriation slurry reactor, the average organic loading rate (OLR) and HRT were 14.3 gCOD/L-day (8.4 gVS/L-day) and 5 days, resulting in about 4.9 gCOD/L-day (3.1–5.3 gCOD/L-day) and 2 days for the methanogenic UASB reactor.

As shown in Figure 2(a), because of production of organic acids, the pH of the acidogenic effluent decreased to about 7.2–7.5, but was 8.3–8.6 for the influent substrate in the acid elutriation UASB reactor. The total and bicarbonate alkalinity of the effluent were 3800 mg/L (2100–4400 mg/L) and 2700 mg/L (2000–3500 mg/L), respectively, resulting in TA consumption of 27%. Based on the acidogenic effluent in loading rate of 3.95 gSCOD/L-day, the SCOD elutriation rate and acid production rate were 5.3 gSCOD/L-day and 3.3 gVFAs (as COD)/L-day, respectively. This means that the recovered VFA fraction in the effluent SCOD mainly consisted of organic acids such as acetate, propionate and butyrate, and counted as 62% of the produced SCOD as shown Table 2. The TS and VS removal rates based on the effluent were about 37.8% and 46.5%, respectively. VS and VS/TS fraction of the effluent were 7.6 gVS/L and 65.3%, respectively while those in the influent were 42 gVS/L and 74.7%, respectively. VS reduction and SCOD production by the hydrolysis were 13% and 0.19 $gSCOD_{production}/gVS_{feeding}$. Also, acid production rate was 0.80 gVFAs / g $SCOD_{production}$.

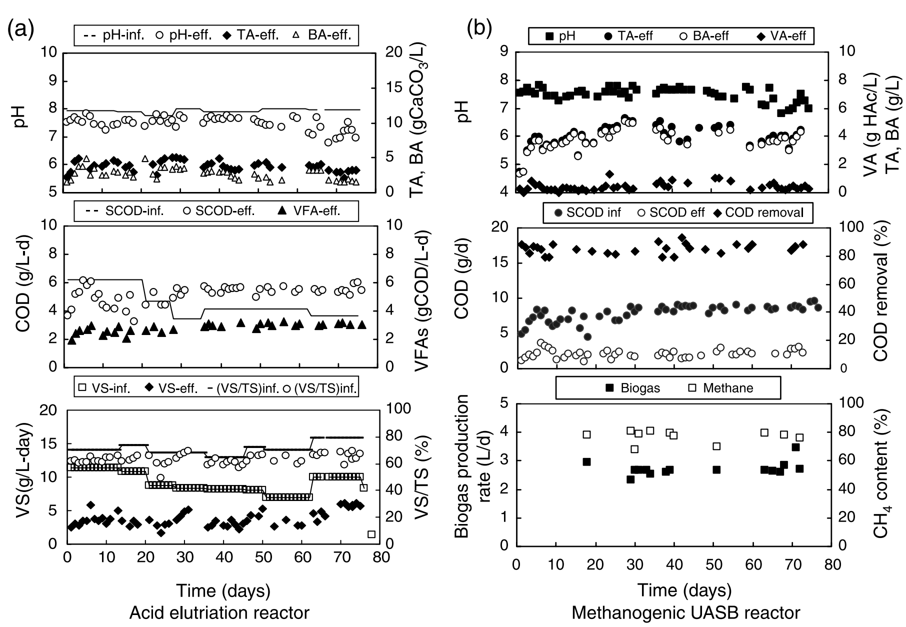

Figure 2 Performance of the ADEPT process

Table 2 VFAs concentration in ADEPT process

Items	Acidogenic effluent		Methanogenic UASB effluent	
	Range	Average ± S.D	Range	Average ± S.D
Formate	N.D	N.D	N.D	N.D
Acetate	2980–3950	3410 ± 300	250–300	290 ± 40
Propionate	790–1090	980 ± 100	96–190	148 ± 38
Butyrate	725–1470	1080 ± 270	72–90	83 ± 7
Valerate	N.D	N.D	0–90	75 ± 21
Lactate	N.D	N.D	N.D	N.D
Tatal VFAs	4860–5900	5470 ± 330	515–564	594 ± 20
Produced SCOD	8350–9570	8880 ± 460	2120–2790	2520 ± 310
VFA recovery	56–66	62 ± 3	20–25	22 ± 2

Unit: mgCOD/L except VFAs recovery (%); N.D, not detected

According to the operational data of the acid elutriation reactor, the influent of the methanogenic UASB reactor was estimated to contain 8600 mg/L (6810–9570 mg/L) of SCOD and 7620 mg/L (3500–10,600 mg/L) of VSS, resulting in about 4.9 gCOD/L-day (3.1–5.3 gCOD/L-day) of OLR (2 days HRT). As shown in Figure 2(b), the methanogenic UASB reactor showed about 88% COD removal and 64% VSS removal rate. The residual VFA in the effluent was about 590 mg/L, which is corresponding to 22% of the effluent SCOD. The main organic acids of the effluent were acetate and propionate but butyrate and valerate were present in a minority. Production capacities and contents of biogas were 2.8 L/day (0.3 m^3 CH_4/$kgCOD_{removal}$ @STP) and 77%, respectively.

SHARON-ANAMMOX reactor

Figure 3 shows the operation results of the SHARON–ANAMMOX reactor. In the SHARON reactor, it appears that a steady state condition to produce a nitrite-accumulated effluent was reached after 65 days from the start-up operation. During the steady

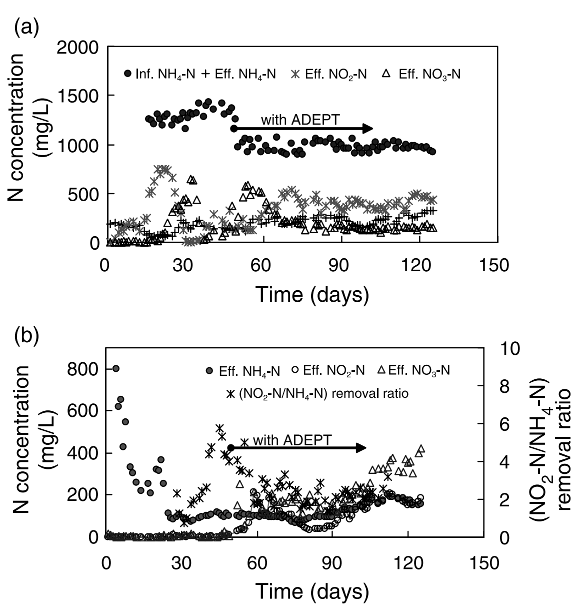

Figure 3 Nitogen conversion behaviour in SHARON and ANAMMOX reactor

state operational period, the SHARON reactor could convert 58.8% of influent ammonium to NO_X-N while 15.8% of influent ammonium was removed by NH_3 stripping and cell synthesis. 25.4% of influent NH_4 was not converted.

The start-up operation of the ANAMMOX reactor had begun with a feed consisting of stock nitrite solution and piggery wastewater for about 300 days. It appears that the reactor reached a steady state after the 66th day of operation. With an influent NO_2-N to NH_4-N ratio for ANAMMOX reactor of 1.56, the effluent NO_2-N to NH_4-N removal ratio averaged 2.13. The soluble nitrogen (NH_4-N + NO_2-N + NO_3-N) loading to ANAMMOX reactor was 1.36 kg soluble N/m^3reactor-day during the steady state condition. Soluble nitrogen conversion rate was 0.72 kg soluble N/m^3reactor-day. The ANAMMOX reactor itself could convert 56.8% of influent NH_4 to cell and N_2 gas.

Table 3 shows the nitrogen and phosphorus conversion for each process. The heterotrophic ADEPT process consisted of the acidogenic phase and the UASB removed most of the N and P in the influent. It is noted that T-P removal in the methanogenic UASB phase was due to precipitation to the biosolids and granules.

In the autotrophic SHARON–ANAMMOX phase, the removal mechanism for nitrogen and phosphorus removal appeared to be different. For phosphorus, the crystallization seems the main removal mechanism in ANAMMOX because of high pH and bicarbonate alkalinity. HAP crystallization was detected in the reactor. Nitrogen removal is mainly due to the stripping in the SHARON reactor and to anaerobic autotrophic oxidation in the ANAMMOX reactor. As a result, the ADEPT–SHARON–ANAMMOX process combination removed nitrogen and phosphorus of 94.1% as NH_4-N (86.5% as TKN) and 87.3% as T-P, respectively.

Microbial characteristics

The methanogenic and ANAMMOX reactor were inoculated by anaerobic granular sludge from a local brewery, with a UASB reactor, as a seed. The average diameter of seed granules was about 1–2 mm. Morphology and structure of the granules was

Table 3 Nitrogen and phosphorus conversion at each process based on 400 mL/day of influent

Description	TKN	NH_4-N	T-P	PO_4-P
Influent (400 mL/d)				
(mg/L)	5730 ± 780	4300 ± 60	1770 ± 300	440 ± 50
(g/d)	2.29 ± 0.31	1.70 ± 0.02	0.71 ± 0.12	0.18 ± 0.02
Acidogenic reactor				
Elutriated effluent (1100 mL/d)				
(mg/L)	1480 ± 210	1330 ± 240	514 ± 390	214 ± 142
(g/d)	1.77 ± 0.25	1.60 ± 0.27	0.62 ± 0.47	0.26 ± 0.17
Wasted sludge (150 mL/d)				
(mg/L)	1690 ± 300	1170 ± 260	910 ± 250	166 ± 70
(g/d)	0.15 ± 0.05	0.12 ± 0.03	0.08 ± 0.03	0.008 ± 0.007
Methanogenic UASB reactor				
Effluent (1100 mL/d)				
(mg/L)	1310 ± 160	960 ± 160	204 ± 105	114 ± 140
(g/d)	1.44 ± 0.18	1.06 ± 0.17	0.22 ± 0.12	0.13 ± 0.02
SHARON reactor				
Effluent (1000 mL/d)				
(mg/L)	564 ± 105	225 ± 49	147 ± 36	51 ± 15
(g/d as 1100 mL/d)	0.62 ± 0.12	0.25 ± 0.05	0.16 ± 0.04	0.06 ± 0.02
ANAMMOX reactor				
Effluent (400 mL/d)				
(mg/L)	285 ± 99	92 ± 14	86 ± 28	46 ± 4
(g/d as 1100 mL/d)	0.31 ± 0.11	0.10 ± 0.02	0.09 ± 0.03	0.05 ± 0.004
Removal based on effluent (%)	86.5	94.1	87.3	72.2

observed with scanning electron microscopy according to reactor type and increasing operation time (Figure 4).

The original granules were irregular and rod-like in shape with smooth granular surfaces (Figure 4(a), (b) and (c)). Also, they mostly consisted of filamentous microbes (Figure 4(b), (c)). Figure 4(d), (e) and (f) shows the shapes of the granules in the methanogenic reactor after 5 months of operation while Figure 4(g), (h) and (i) shows those of the ANAMMOX reactor. Granules in the methanogenic reactor after 5 months of operation showed a rougher surface covered with short-rod organisms than the original granules (Figure 4(d)). The inner structure of the granules showed an increase of fine filamentous microbes (Figure 4(e)). On the other hand, ANAMMOX granules were relatively regular and spherical in shape (Figure 4(g)). The surface of the granules was attached with growing biofilm, and can be seen as having a rough and compact structure. The inner structure revealed less filamentous and complicated microbes (Figure 4(h)). It is interesting to note that both methanogenic and ANAMMOX granules had biogas pores. This observation was similar to that of Wang and Kang's study (2005) with ANAMMOX granules in an expanded granular sludge bed reactor seeded with anaerobic granules.

In FISH analysis of ANAMMOX reactor sludge, the fractions of *Planctomycetales* and *Candidatus Kuenenia stuttgartiensis* were 77% and 59%, respectively (results not shown in here). They were placed in an inner position near the surface. Very small fractions of ammonia-oxidizing β-subclass proteobacteria and *Nitrobacter* spp. were detected by FISH. This means that ANAMMOX bacteria, especially *Candidatus Kuenenia stuttgartiensis*, played a significant role in nitrogen removal in the ANAMMOX reactor.

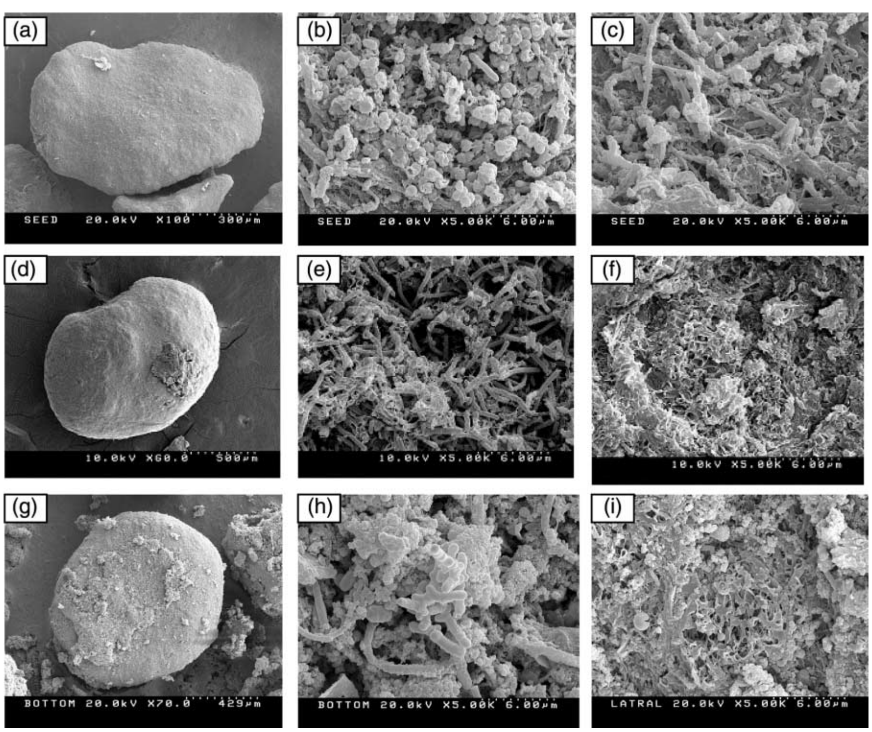

Figure 4 Scanning electron micrographs of granules: (a), (b) & (c), granules at start-up operation; (d), (e) & (f), granules in methanogenic reactor after 5 months; (g), (h) & (i), granules in ANAMMOX reactor after 5 months)

Conclusions

A combined ADEPT–SHARON–ANAMMOX process was applied to resources recovery (organic carbon and biogas) and autotrophic nitrogen removal from slurry-type piggery waste. In the acidogenic reactor, VS reduction and SCOD production by hydrolysis were 13% and 0.19 $gSCOD_{prod.}/gVS_{feed}$. Also, acid production rate was 0.80 $gVFAs/gSCOD_{prod}$. The methanogenic UASB reactor showed about 88% COD removal and 64% VSS removal rate. Production capacities and methane contents of biogas were 0.3 $m^3 CH_4/kgCOD_{removal}$ @STP and 77%, respectively.

In this study, nitrogen and phosphorus were removed 94.1% as NH_4-N (86.5% as TKN) and 87.3% as T-P from slurry-type piggery waste, respectively, in the ADEPT–SHARON–ANAMMOX process.

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