Inoculum Seed for Simultaneous Biological Removal of Sulfate and Nitrogen Rich Wastewater

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Abstract: High concentrations of sulfate and nitrogen have an impact to anaerobic digestionfor biogas production via hydrogen sulphide and ammonia produced by sulfate reducing bacteria and ammonifying bacteria, respectively, have been found to inhibit the methanogenesis. Furthermore, their removal from digested wastewater is required. Biological treatment by sulfate reducing bacteria (SRB), sulfide oxidizing bacteria (SOB), nitrifier and denitrifieris the most widely used microorganisms for this process. The selection of microbial sources for high activities of SRB, SOB, nitrifier and denitrifier was interested in this study. Four sources of microbial seed sludge from anaerobic wastewater treatment system of concentrated latex (CL) in close UASB and open pond (AOP) while the other two sources from open ponds of swine manure (SM) and cassava starch (CS) were used to evaluate the microbial performances. The activities of SRB, SOB, nitrifier and denitrifier were monitored. Between seed sludge from UASB and AOP of CL, AOP had higher activities of SOB, nitrifier and denitrifier except SRB than UASB. Therefore, seed sludge from AOP of CL, SM and CS were chosen to study. These results were found that seed sludge from SM provided the most high activities of SRB, SOB, nitrifier and denitrifier at 0.159 g-SO_4^{2-}-$S_{consumed}$/g-VSS/d, 0.384 g-S_2O_3-$S_{consumed}$/g-VSS/d, 0.131 g-NH_4^+-$N_{consumed}$/g-VSS/d and 0.182 g-NO_3^--$N_{consumed}$/g-VSS/d, respectively. The denitrifier activity of SM had 1.48 and 1.35 times than CL and CS, respectively. Furthermore, the nitrifier activity of SM was higher 3.28 and 1.15 times than CL and CS, respectively. The SOB activity of SM was higher 1.96 times than CS, while lower 1.27 times than CL. In addition, SM provided higher activity of SRB 2.06 times than CS while lower 1.13 times than CL. This study indicated that seed sludge from SM was suitable for simultaneous sulfate and nitrogen removal in wastewater before entering to anaerobic reactor for biogas production.

Keywords: Inoculum seed, microbial activity, sulfate reducing bacteria, sulfide oxidizing bacteria, nitrifier, denitrifier.

1. Introduction

Contamination of ground and surface water with sulfur and nitrogen compounds is a major environmental concern [1]. The discharge of sulfate rich wastewater into surface water contributes to the increasing of the corrosion potential of receiving water due to the biological reduction of sulfate to sulfide under anaerobic conditions [2]. In addition, nitrogen compounds afford risks associated with toxicity and bad odor in water, leading to eutrophication and serious ecological damage tothe receiving water bodies [1, 3].Furthermore, high concentrations of sulfate and nitrogen such as in concentrated latex wastewater have an effect on methane production in methanogenesis. The production of sulfide and ammonia from the activities of sulfate reducing bacteria and ammonifying bacteria, respectively, could be inhibited methanogens. In order to remove sulfate and nitrogen contaminants, biological treatments are preferred technologies rather than physical-chemical methods, which are expensive and may generate toxic residuals [4].The conventional biological process to treat sulfate consists of two processes, sulfate reducing to sulfide by sulfate reducing bacteria (SRB) under anaerobic condition and followed by sulfide oxidizing to elemental sulfur (S^0) by sulfide oxidation bacteria (SOB) under micro-aeration conditions [3]. Additionally, conventional biological nitrogen removal processes consist of the nitrification process under aerobic conditions and the denitrification process under anaerobic conditions [5]. A common drawback of these processes is the need of separated units for anaerobic and aerobic conditions, which increases capital and operation costs. However, previous literatures have reported that anaerobic and aerobic microorganisms can work together in a single reactor under micro aeration conditions [5-8]. SRB, SOB, nitrifier and denitrifier bacteria are the important bacteria in simultaneous sulfate and nitrogen removal system. The selection sources for these microorganisms with high activities in removal of sulfate and nitrogen from rich wastewater was in concern. Therefore, the batch experiments were carried out to study these specific microbial activities for seed sludge selection from various sources of anaerobic wastewater treatment.

2. Experimental

2.1 Determination of specific microbial activities

The determination of microbial activity was conducted to select the suitable source of seed sludge for simultaneous sulfate and nitrogen removal. Various sources of seed sludge were from anerobic latex wastewater treatment system (close system of UASB and anaerobic open pond (AOP)), and the other sources from AOP of swine manure (SM) and cassava starch (CS) were chosen to evaluate microbial activities of SRB, SOB, nitrifier and denitrifier. Microbial activity experiments of batch system were conducted in triplicate. The controls were performed by supplement medium without seed sludge. Batch system was done in 120 mL vessel with 100 mL working volume of medium pH 7.0, containing biomass concentration of 2 g-VSS/L and incubated at 30±2°C. Mineral medium for SRB activity contained, in mg/L, 270 KH_2PO_4; 350 K_2HPO_4; 530 NH_4Cl; 100 $MgCl_2\cdot6H_2O$; 75 $CaC1_2\cdot2H_2O$; 1,200 $NaHCO_3$ [9]. The composition of mineral medium for SOB, nitrifier and denitrifier activity was listed by Rattanapan et al. [10].

2.1.1 Sulfate reducing bacteria (SRB) activity test

Determination of sulfate reduction ratefor calculation of SRB activity was performed in serum vial with the mineral medium supplemented 6.0 g-COD/L of lactic acid and 2.0 g-SO_4^{2-}/L of Na_2SO_4. Anaerobic condition was controlled by helium gas flush for 5 minutes to expel oxygen gas and subsequently sealed with butyl rubber stoppers and aluminum seals [3, 9]. 10 mL samples were taken and centrifuged, 5 mL of supernatant was then analyzed for sulfate, sulfide and thaiosulfate. H_2S (gas) production was monitored. SRB activity was calculated according to the following equation;

$$\text{SRB activity} = [\text{Substrate}_{removed}(\text{g-}SO_4^{2-}\text{-}S_{removed}/\text{L/d})] / [\text{Biomass concentration (g-VSS/L)}]$$

2.1.2 Sulfide oxidizing bacteria (SOB) activity test

The SOB activity was tested in Erlenmeyer flask containing mineral medium with 4.13±0.01 g/L of thiosulfate [10]. The liquor samples were collected to determine thiosulfate consumption, and SO_4^{2-}-S and S^0 production during the activity test. SOB activity was calculated according to the following equation;

$$\text{SOB activity} = [\text{Substrate}_{removed}(\text{g-}S_2O_3\text{-}S_{removed}/\text{L/d})] / [\text{Biomass concentration (g-VSS/L)}]$$

2.1.3 Nitrifier activity test

The nitrifier activity test was performed in Erlenmeyer flask. The mineral medium was supplemented with 1.00±0.01 g/L of NH_4^+-N. The liquor samples were analyzed ammonium nitrogen, nitrate and nitrite daily. Nitrifier activity was calculated according to the following equation;

$$\text{Nitrifier activity} = [\text{Substrate}_{removed}(\text{g-}NH_4^+\text{-}N_{removed}/\text{L/d})] / [\text{Biomass concentration (g-VSS/L)}]$$

2.1.4 Denitrifier activity test

Determination of nitrate reduction rate was performed in serum vial. The mineral medium was added with 1.00 g-NO_3^--N/L. Lactic acid was added to give final concentrations of 3.5 g COD/L. Vials were flushed with helium gas for 5 min to remove oxygen from both the aqueous phase and headspace, then sealed with butyl rubber stoppers and aluminum crimps [3]. Gas samples were collected and analyzed for N_2 gas production. 10 mL of samples were taken and centrifuged, and supernatant was then analyzed for daily NO_3^--N consumption and NO_2^--N production. Denitrifier activity was calculated according to the following equation;

$$\text{Denitrifier activity} = [\text{Substrate}_{removed}(\text{g-}NO_3^-\text{-}N_{removed}/\text{L/d})] / [\text{Biomass concentration (g-VSS/L)}]$$

2.2 Analytical procedures

Consumption of sulfate and nitrogen compounds and metabolic products from sulfate and nitrogen removal process were measured to determine the activity of microorganism from various sources. At the end of the experiment, concentrations of SO_4^{2-}, S^{2-}, $S_2O_3^{2-}$, NH_4^+, NO_3^- and NO_2^- in liquor samples were measured after centrifugation at 4500 rpm for 10 minutes. Sulfate was analyzed according to Turbidimetric method [11]. Aqueous sulfide ($H_2S_{(aq)}$+ HS^- + S^{2-}) and thiosulfate were determined by Iodometric method [11]. Macro-Kjeldahl method [11] was used to measure ammonium nitrogen. Nitrate and nitrite were determined by spectrophotometric method. S^0 inmixed liquor was determined by technical development based on the sulfite method [12].Gas samples were collected and analyzed for H_2S and N_2. Concentrations of H_2S and N_2 gas was determined by gaschromatography (GC-2010, Molecular sieve, 50°C, He 25 mL/min).

3. Results and Discussion

3.1 Microbial activity of seed sludge from anaerobic close and open wastewater treatment systems

3.1.1 SRB activity

Seed sludge from close UASB and anaerobic open pond (AOP) of concentrated latex industry (CL) were used to compare their activities. The results showed that sulfate was mostly removed within 4 days by seed sludge from UASB while AOP completely consumed within 7 days as shown in Figure 1a. Seed sludge from UASB can remove sulfate faster than AOP. SRB is anaerobic bacteria [13], so under anaerobic condition in UASB is suitable for SRB more than AOP which not be absolutely anaerobic condition. Furthermore, influent of UASB was found sulfate concentration (substrate for SRB) higher than AOP. Seed sludge from UASB and AOP had activities of SRB at 0.251 and 0.180 g-SO_4^{2-}/g-VSS/d, respectively, as shown in Figure 2. This study also showed that the main metabolic product from sulfate reducing process was sulfide as shown in Table 1.

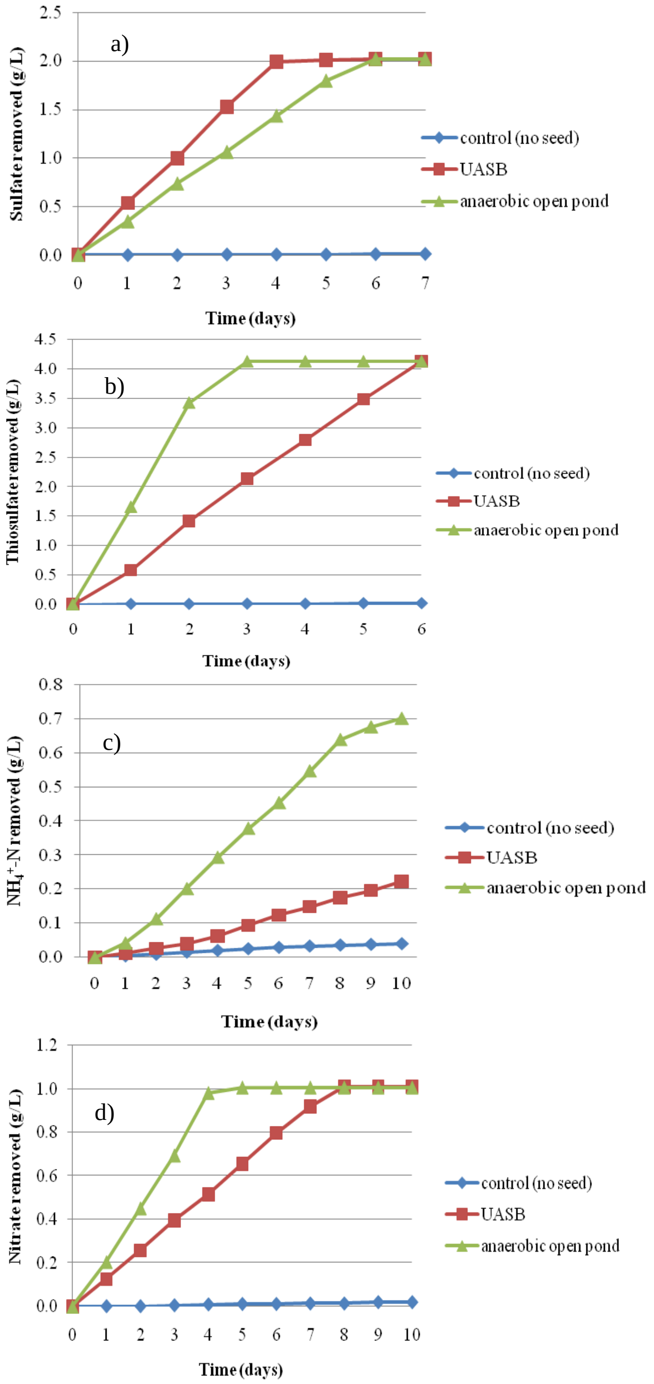

Figure 1. Sulfur and nitrogen compound removal by seed sludge from anaerobic close and open system

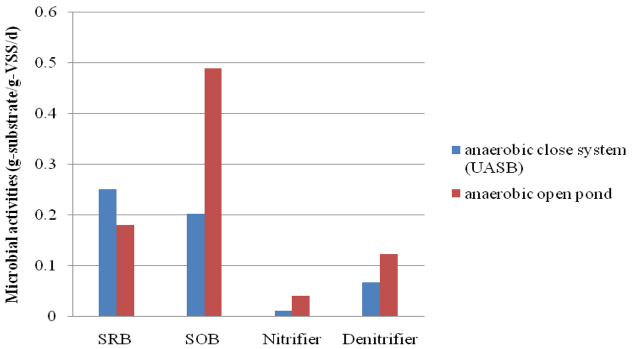

Figure 2. Microbial activity of seed sludge from anaerobic close and open system

3.1.2 SOB activity

The results showed that AOP provided activities of SOB higher than UASB. The SOB activities of sludge from AOP and UASB were 0.489 and 0.202 g S_2O_3-S/g-VSS/d, respectively (Figure 2). SOB works under micro-aerobic condition and use oxygen as electron acceptor [6], so under absolutely anaerobic condition in UASB provided activity of SOB lower than AOP. Thiosulfate was consumed completely in the third day by AOP while it was removed completely within 6 days by UASB as shown in Figure 1b. Table 1 showed that the main metabolic product from sulfide oxidizing process in this study was elemental sulfur.

3.1.3 Nitrifier activity

Seed sludge from UASB and AOP had nitrifier activities at 0.011 and 0.040 g-NH_4^+-N/g-VSS/d, respectively (Figure 2). Nitrifier works under aerobic condition and uses oxygen as energy source [5]. Therefore, AOP which a part of oxygen in air can be dissolved to the system had nitrifier activity higher than UASB. Ammonium was consumed very less by sludge from UASB during in the first three days as shown in Figure 1c. This study also showed that the main metabolic product from ammonium removal process was nitrate nitrogen.

3.1.4 Denitrifier activity

Nitrate reduction rate by seed sludge from UASB and AOP were shown in Figure 1d. The results indicated that AOP provided higher nitrate reduction rate than seed sludge from UASB. Nitrate was removed completely in the fifth day of inoculation for seed sludge from AOP while seed sludge from UASB completely removed nitrate within 8 days. The denitrifier activity of sludge from AOP and UASB were 0.123 and 0.066 g-NO_3^--N/g-VSS/d, respectively (Figure 2). The main metabolic product was nitrogen gas as shown in Table 1. Denitrifier is facultative anaerobic bacteria which very active under anaerobic condition. However, in this study denitrifier activity of seed sludge from UASB was lower than AOP. This may be because denitrifier was inhibited by produced sulfide under anaerobic condition in UASB (about 150 mg S^{2-}/L was found in UASB system). While, produced sulfide can be removed by SOB in AOP (only about 75 mg S^{2-}/L was found in AOP system).

This study showed that seed sludge from AOP of concentrated latex wastewater treatment system (indigenous source) had higher activity of SOB, nitrifier and denitrifier than seed sludge from UASB. AOP had only SRB activity lower than UASB as shown in Figure 2. Therefore, seed sludge from AOP of another sources (swine manure and cassava starch wastewater) were chosen to compare with seed sludge from concentrated latex industry.

3.2 Microbial activity of seed sludge from various source of anaerobic open pond

Seed sludge from anaerobic open pond (AOP) of concentrated latex wastewater (CL), swine manure (SM) and cassava starch wastewater (CS) were used to investigate the activities of SRB, SOB, nitrifier and denitrifier.

3.2.1 SRB activity

Seed sludge from CL, SM and CS completely sulphate removed within 6, 8 and 14 days, respectively, as shown in Figure 3a.

Table 1. Metabolic products of SRB, SOB, nitrifier and denitrifier by seed sludge from concentrated latex wastewater treatment plant

Sources	SRB (g/L)			SOB (g/L)		*Nitrifier (g/L)*		Denitrifier (g/L)	
	S^{2-}-S	S_2O_3-S	H_2S-S(gas)	SO_4^{2-}-S	S^0	NO_3^--N	*NO_2^--N*	NO_2^--N	N_2 (gas)
UASB	0.6412	0.0015	0.0242	0.6773	1.647	0.148	0.073	0.115	0.872
AOP	0.6358	0.0017	0.0285	0.7073	1.617	0.542	0.152	0.075	0.909

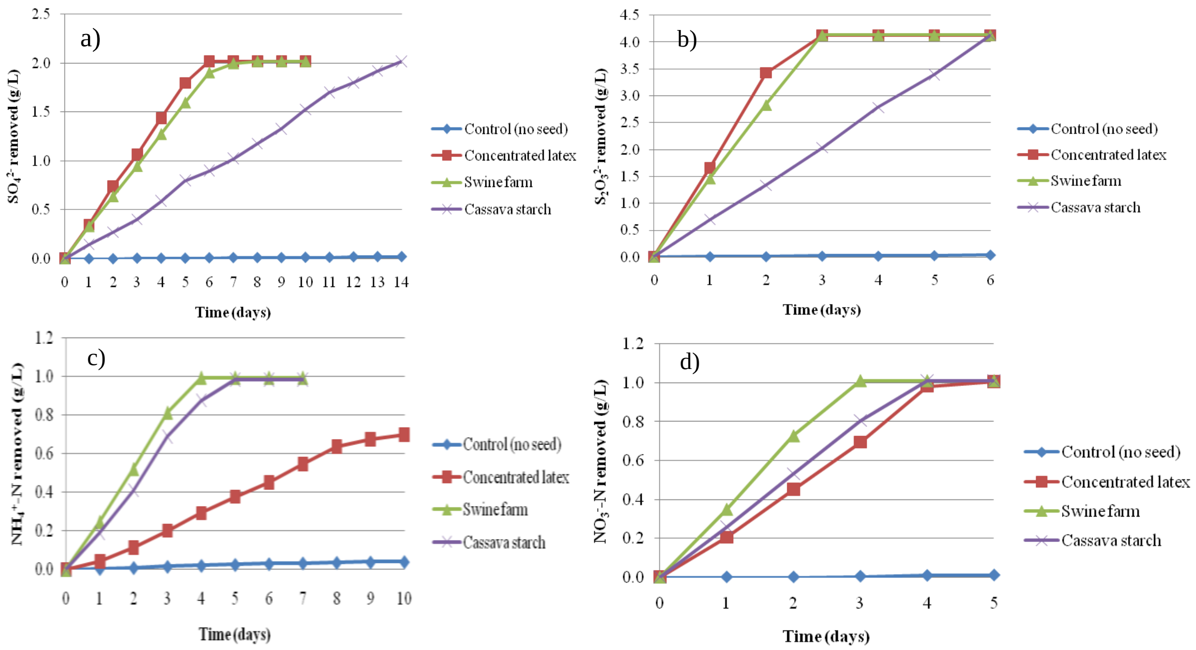

Figure 3. Sulfur and nitrogen compounds removal by seed sludge from AOP of CL, SM and CS.

Table 2. Metabolic products of SRB, SOB, nitrifier and denitrifier by seed sludge from anaerobic open pond of concentrated latex (CL), swine manure (SM) and cassava starch wastewater (CS).

Sources	SRB (g/L)			SOB (g/L)		Nitrifier (g/L)		Denitrifier (g/L)	
	S^{2-}-S	S_2O_3-S	H_2S-S(gas)	SO_4^{2-}-S	S^0	NO_3^--N	NO_2^--N	NO_2^--N	N_2 (gas)
CL	0.6358	0.0017	0.0285	0.7073	1.617	0.542	0.152	0.075	0.909
SM	0.6336	0.0021	0.0323	0.7173	1.615	0.990	Nd	Nd	0.982
CS	0.6162	0.0023	0.0484	0.7207	1.613	0.979	Nd	Nd	0.989

Nd = Not detectable

Figure 4 showed that seed sludge from CL had the highest activities of SRB.It might be due to concentrated latex wastewater contains high concentration of sulfate which as substrate for SRB. The wastewater from concentrated latex industry contains 1,075-4,210 mg-SO_4^{2-}/L [15-16]. The SRB activity of SM was slightly lower than CL. The activity of sludge from CL, SM and CS were 0.180, 0.159 and 0.077 g-SO_4^{2-}/g-VSS/d, respectively (Figure 4). This study also showed that the main metabolic product of SRB was sulfide as shown in Table 2.

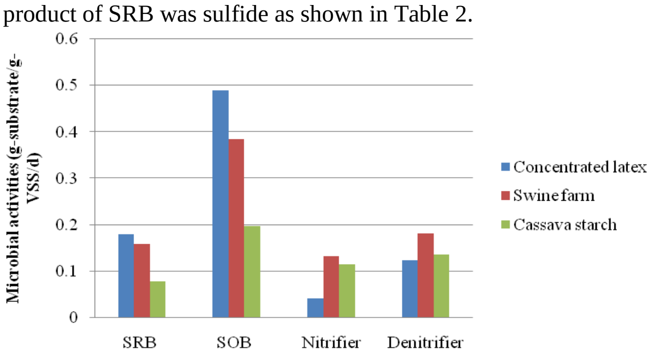

Figure 4. Microbial activity of seed sludge from AOP of CL, SM and CS

3.2.2 SOB activity

The SOB activity of seed sludge from CL, SM and CS were 0.489, 0.384 and 0.196 g S_2O_3-S/g VSS/d, respectively (Figure 4). It indicated that seed sludge from CL provided the highest activities of SOB while SOB activity of SM was slightly lower than CL. Thiosulfate was removed completely in the third day for CL and SM while seed sludge from CS completely consumed thiosulfate within 6 days as shown in Figure 3b. Table 2 showed that the main metabolic product of SOB was S^0.

3.2.3 Nitrifier activity

Results of nitrifier activity test indicated that seed sludge from SM had higher ammonium oxidation rate than CS and CL. Because wastewater from swine manure contains high concentration of nitrogen at about 1,640-3,200 mg-N/L [17], it could enhance the high activity of nitrifier. The nitrifier activity of CL, SM and CS were 0.040, 0.131 and 0.114 g NH_4^+-N/g VSS/d, respectively (Figure 4). The sludge from SM and CS completely removed ammonium nitrogen (1 g NH_4^+-N/L) within 4 and 5 days, respectively while CL removed only 0.702 g NH_4^+-N/L within 10 days as shown in Figure 3c. CL had very low nitrifier activity due to wastewater from concentrated latex industry contains high sulfate concentration which both sulfate and produced sulfide by SRB might be toxic to nitrifier and denitrifier. Similar result to the study of Beristain et al. (2010), they presented that increasing of sulfide concentration can reduce ammonium oxidation rate and nitrate reduction rate of nitrifier and denitrifier. The main metabolic product of nitifier was nitrate nitrogen (Table 2). Nitrite nitrogen accumulation was found for CL. It indicated that seed sludge from CL could not completely convert ammonium to be nitrate within the time of experiment.

3.2.4 Denitrifier activity

Nitrate nitrogen reducing rate of seed sludge from CL, SM and CS were shown in Figure 3d. It indicated that SM provided the highest nitrate reduction rate. Nitrate was removed completely in the third day of inoculation by SM while the sludge from CS and CL completely removed nitrate within 4 and 5 days, respectively. The denitrifier activity of sludge from CL, SM and CS were 0.123, 0.182 and 0.135 g- NO_3^--N/g-VSS/d, respectively (Figure 4). Table 2 showed that nitrate nitrogen was converted completely to be N_2 by seed sludge from SM and CS. Nitrite nitrogen accumulation was found only denitrifier activity test of seed sludge from CL. Denitrifier from CL might be inhibited by high concentration of sulfide in the system.

This study showed that seed sludge from AOP of CL had the highest activity of SRB and SOB. However, SRB and SOB activity of CL were slightly higher than SM. In addition, seed sludge from SM provided the highest activity of nitrifierand denitrifier as shown in Figure 4. Therefore, seed sludge from anaerobic open pond of SM which had high activity of SRB, SOB, nitrifier and nitrifier at 0.159 g-$SO_4^{2-}{}_{consumed}$/g-VSS/d, 0.384 g-S_2O_3-$S_{consumed}$/g-VSS/d, 0.131 g-NH_4^+-$N_{consumed}$/g-VSS/d and 0.182 g-NO_3^--$N_{consumed}$/g-VSS/d, respectively, was suitable for simultaneous sulfate and nitrogen removal.

This work also showed that seed sludge from AOP of SM had methanogens activity at 0.020 g-$CH_{4(gas)}$/g-VSS/d. The sludge from SM provided higher activity of methanogens than UASB, CL and CS 1.20, 1.67 and 1.43 times, respectively. This indicated that the sludge from SM also suitable to produce biogas from treated sulfate and nitrogen wastewater.

4. Conclusions

Seed sludge from anaerobic open pond (AOP) had higher activities of SOB, nitrifier and denitrifier than seed sludge from anaerobic close system (UASB). Only SRB activity of AOP was found slightly lower than UASB. Seed sludge from open pond of concentrated latex wastewater (CL), swine manure (SM) and cassava starch wastewater (CS) were used to study for their efficiency in sulfate and nitrogen removal. Among these results, it found that SM mostly showed high activities of SRB, SOB, nitrifier and denitrifier compared to CL and CS. Therefore, sludge from AOP of SM was suitable for the simultaneous sulfate, sulfide, ammonium and nitrate removal in rich sulfate and nitrogen wastewater.

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