Bioresource Technology 128 (2013) 731–737

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

The role of anaerobic sludge recycle in improving anaerobic digester performance

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HIGHLIGHTS

- ▶ A pilot WWTP is operated with and without anaerobic digester sludge recycle.
- ▶ Anaerobic digester sludge recycling in traditional WWTPs increases CH_4 production.
- ▶ Anaerobic digester sludge recycling decreases net solids yield.
- ▶ More consistent *Archaea* concentrations occurred system wide with sludge recycling.

ARTICLE INFO

Article history:
Received 13 September 2012
Received in revised form 14 November 2012
Accepted 19 November 2012
Available online 28 November 2012

Keywords:
Solid retention time (SRT)
Anaerobic digestion
Methanogenesis
Hydrolysis
Sludge yield

ABSTRACT

Solids retention time (SRT) is a critical parameter for the performance of anaerobic digesters (AD) in wastewater treatment plants. AD SRT should increase when active biomass is input to the AD by recycling anaerobic sludge via the wastewater-treatment tanks, creating a hybrid aerobic/anaerobic system. When 85% of the flow through the AD was recycled in pilot-scale hybrid systems, the AD SRT increased by as much as 9-fold, compared to a parallel system without anaerobic-sludge recycle. Longer AD SRTs resulted in increased hydrolysis and methanogenesis in the AD: net solids yield decreased by 39–96% for overall and 23–94% in the AD alone, and AD methane yield increased 1.5- to 5.5-fold. Microbial community assays demonstrated higher, more consistent *Archaea* concentrations in all tanks in the wastewater-treatment system with anaerobic-sludge recycle. Thus, multiple lines of evidence support that AD-sludge recycle increased AD SRT, solids hydrolysis, and methane generation.

1. Introduction

While treatment of municipal and industrial wastewater is essential in the preservation of water environments, conventional wastewater treatment plants (WWTPs) are not necessarily sustainable. It is estimated that up to 1% of the annual United States electricity consumption is applied to wastewater treatment and that energy consumption by WWTPs will increase 20% over the next fifteen years (Carns, 2005). In addition, WWTPs produce 8 million dry tons per year of biosolids (Center for Sustainable Systems, 2009), which must be disposed of, and release 28 million tons of CO_2 equivalents to the atmosphere (U.S. Energy Information Administration, 2010).

Anaerobic digestion is a well-established technology that has potential for helping WWTPs become more sustainable. Anaerobic digestion involves three mechanisms (Lawrence and McCarty, 1969; Parkin and Owen, 1986; Rittmann and McCarty, 2001): hydrolysis of particulate and polymeric organic compounds, fermentation of the solubilized, but complex organic substrates to short chain fatty acids including acetate and hydrogen gas (H_2), and methanogenesis of the acetate and H_2 to methane (CH_4). Major benefits from anaerobic digestion are capturing energy in CH_4 and stabilizing and destroying biosolids.

Hydrolysis of microbial biomass and particulate organic compounds is usually considered the rate-limiting step during anaerobic digestion and is generally modeled with first-order kinetics (Eastman and Ferguson, 1981; Lee et al., 2011; Miron et al., 2000; Rittmann and McCarty, 2001). The extent of hydrolysis increases with increasing solids retention time (SRT) in the anaerobic digester (AD), and this generates additional soluble organic matter for fermentation and methanogenesis. A long-enough SRT also is critical for ensuring that the slow-growing methanogenic microorganisms are stably maintained in the digester (Parkin and Owen, 1986; Rittmann and McCarty, 2001). In a conventional AD, SRT equals the hydraulic retention time (HRT).

SRT, the reciprocal of the net specific growth rate of active biomass in a system, is computed as the ratio of active biomass in the system divided by the production rate of active biomass (Rittmann

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0960-8524/$ - see front matter
http://dx.doi.org/10.1016/j.biortech.2012.11.079

and McCarty, 2001). Because it often is difficult to quantify the amount of active biomass in a system during typical WWTP operations, WWTPs often calculate SRT by substituting VSS concentrations, which comprise active biomass, inert biomass, and particulate COD (PCOD) (Metcalf & Eddy, Inc., 2003). In addition to retaining slow-growing microorganisms, a long SRT should enhance hydrolysis of complex organics, thus increasing biosolids reduction and CH_4 production (e.g., de la Rubia et al., 2002; Miron et al., 2000; Parkin and Owen, 1986). Lee et al. (2011) analyzed the performance and microbial community in bench-scale ADs fed with thickened municipal wastewater mixed sludge and operated over an SRT range of 20–4 days. Although the apparent first-order hydrolysis rate increased when the SRTs declined to 4 days from 20 days, VSS destruction, CH_4 stabilization, and the number of *Archaea* 16S rDNA gene copies were greater with larger SRT.

Siemens Water Technologies (SWT) has developed and pilot tested a hybrid process that has goals of increasing CH_4 production and decreasing net sludge production while being easily retrofit into existing WWTPs. The hybrid process links typical activated sludge processing with AD in a novel manner by recycling a minimum of 85% of AD sludge back to the activated sludge system. As we show quantitatively below, recycling a majority of the sludge can significantly increase the AD SRTs. SRT increases ought to cause the hybrid system to have much lower AD net sludge yield and higher CH_4 production.

In this study, we evaluate the ability of AD-sludge recycle to increase biomass and VSS SRTs in the AD and gain the sludge- and CH_4-yield benefits. We perform non-steady-state mass balance analyses of the pilot-plant data for the hybrid process and a conventional process (i.e., without sludge recycle) operated side-by-side. In particular, we focus on quantifying the actual biomass AD SRTs; comparing performance based on total COD removal, CH_4 production, and solids reduction in the AD; and assessing the impacts of recycling on the methanogenic community using quantitative real-time PCR (qPCR).

2. Methods

2.1. Hybrid and conventional processes

SWT installed three pilot-plant trains at Singapore's Public Utilities Board WWTP: two hybrid trains and one conventional train. Each train was supplied with 600 L/day of primary-settled wastewater from the same influent stream. Fig. 1 is a schematic drawing of each train. Each train was comprised of an anoxic tank, aerobic contact tank, clarifier, aerobic stabilization tank, sludge thickener, and AD. Influent entered the system at the anoxic tank. Outputs of the system were the clarified effluent, wasted sludge from the digester, and CH_4 gas.

The hybrid systems differ from the conventional system in the way they exchange biomass between the components treating the wastewater flow and the components handling the biosolids removed from the treatment components. In the conventional approach, WAS is sent to the thickener and AD, and then the entire flow from the digester is wasted from the system. In the hybrid approach, at least 85% of the flow through the digester is routed back to the stabilization tank. This means that the aerobic and anaerobic components of the overall system exchange biomass, thereby creating an overall system that is a hybrid of aerobic and anaerobic processing. As we illustrate below, exchanging anaerobic-digester biomass, instead of wasting all of it, can increase the digester's SRTs significantly. Higher SRTs should increase the degree to which the biomass removed from the aerobic treatment components is hydrolyzed and converted to CH_4.

The target operating parameters are summarized in Tables 1 and 2. According to the definitions of flows used by SWT, waste activated sludge (WAS) is the flow rate from the clarifier underflow to the sludge thickener and is expressed as a percentage of the recycled activated sludge (RAS) flow rate. The pilot plants were operated for 11 operational periods that ranged in duration between 7 days to 4 months. Several of the early periods were start up and shake down phases in which the results were not consistent. However, operation of the three pilots stabilized by the ninth phase, and we present results for three operational periods during which the processes were operated under the constant conditions outlined in Table 2. These phases evaluated the differences in performance between the hybrid and conventional processes, as well as the effects of changes in the nominal AD SRT (ranging from 25 to 30 days) and RAS flow rates (ranging between 100% and 120% of the influent flow rate). Depending upon the phase, the WAS flow rate varied between 8.2% and 8.3% of the RAS flow rate in the hybrid and 3.6 and 4.3% in the conventional configurations. These changes brought about adjustments to the AD influent and wasting sludge flow rates. While the flow rates were held constant, influent conditions varied daily, resulting in non-steady state conditions in terms of concentrations.

2.2. Mass balance analyses

We carried out non-steady-state mass-balance analyses to document the fate of COD and VSS in the systems overall, the wastewater-treatment components, and the AD, as well as to compute AD SRTs. Mass-balances were applied to the overall system and the AD of each train, and Fig. 1 identifies which variables are associated with specific streams for the hybrid and conventional processes. For example, the AD is characterized by an influent volumetric flow rate $Q_{SL\text{-}AD}$ (L^3/t), wasting sludge and sludge recycle volumetric flow rates Q_w and Q_{AD} (L^3/t), influent and effluent concentrations $C_{SL\text{-}AD}$ and C_w, respectively (with units of M/L^3), and the tank volume V_{AD} (L^3). The same variables are defined similarly for all tanks and the overall system. A net reaction rate was calculated between each data point of a phase based on measured concentrations, flow rates, and volumes by subtracting inlet and outlet mass balance terms from the accumulation term for system under evaluation. These individual net reaction rates were averaged over the phase to determine the phase's net reaction rate.

2.3. On-site sample measurements

SWT analyzed the system for TCOD, SCOD, and VSS concentrations in the liquid streams, as well as the biogas content from the AD over the three steady-state operational phases. TCOD and SCOD samples were obtained from each influent and effluent line and all tanks. TCOD and SCOD were measured twice weekly using a HACH 8000 COD kit and COD vials (concentration ranges of 3–1500 mg/L). VSS was measured weekly using *Standard Methods* 2540D and E. Biogas content was analyzed daily for CH_4, H_2, N_2 and CO_2 using a Shimadzu GC-17A with a thermal conductivity detector. The biogas flow rate was measured twice per week using a Sierra mass flow meter. Experimental data for the three phases are presented in the Supplemental Information.

2.4. SRT calculations

SRT is the reciprocal of the net specific growth rate of active microorganisms in the system (Rittmann and McCarty, 2001), and it can be quantified as the ratio of the mass of active biomass in a system to the production rate of active biomass. Since most traditional ADs approach completely mixed tanks without biomass

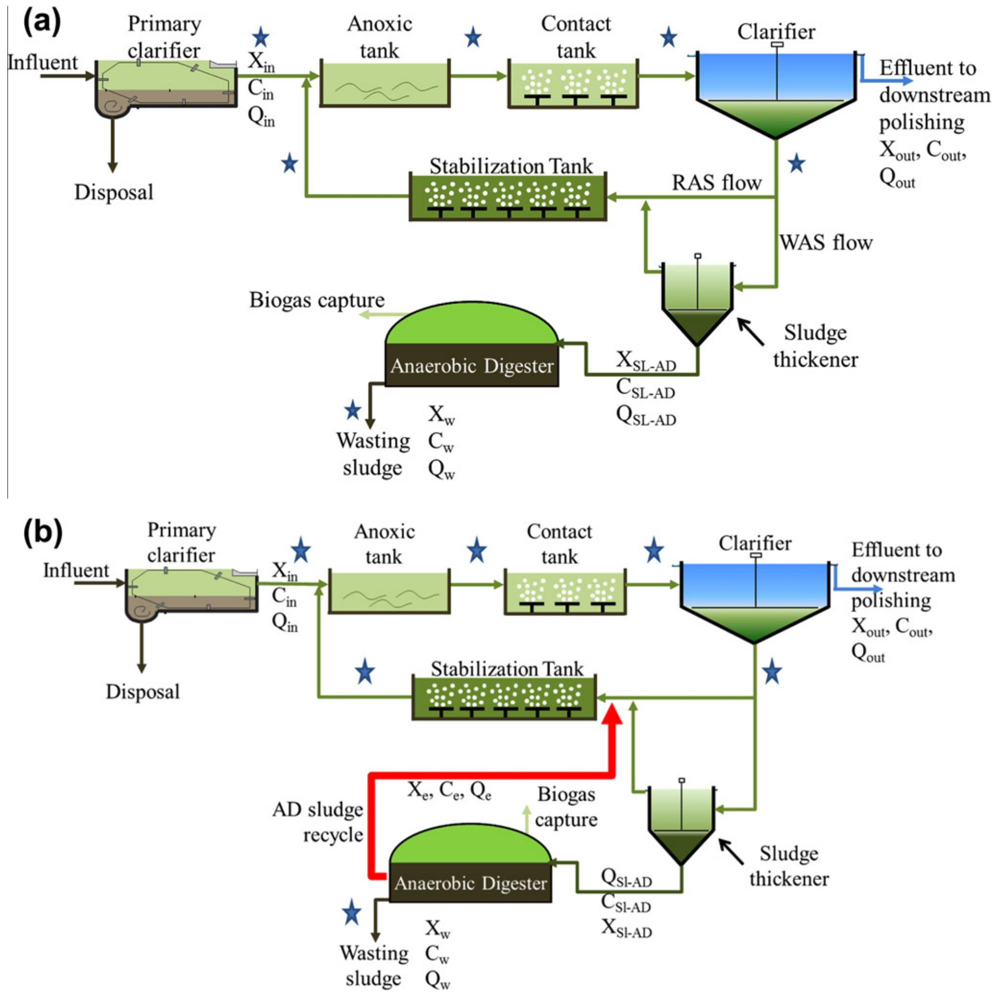

Fig. 1. (a) Conventional process with contact stabilization and anaerobic digestion. (b) Hybrid process with contact stabilization, anaerobic digestion, and digester-sludge recycle (heavy line). Variable labels include *Q* for volumetric flow rate (L^3/t), *C* for concentration (M/L^3), and *X* for biomass concentration (M/L^3). Stars represent sampling points for methanogens assay.

Table 1
Target operating parameters for the hybrid and conventional processes.

	Hybrid	Both systems	Conventional
Influent flow rate (L/day)		600	
RAS rate (% of influent flow rate)		100–120	
WAS rate (% of RAS flow rate)		6–8	
Wasted sludge rate from anaerobic digester (L/day)	2		17
Anaerobic sludge recycle rate to the stabilization tank (L/day)	15		0
Nominal SRTs (days)			
Anoxic/contact/ stabilization tanks	2.5		5
Anaerobic digester		25–30	
Tank volumes (L)			
Anoxic tank		25	
Contact tank		12	
Clarifier		100	
Sludge thickener		100	
AD		650	
Stabilization tank		50	

recycle (Bolzonella et al., 2005; Miron et al., 2000; Parkin and Owen, 1986), the SRT is nominally equal to the HRT, or

$$SRT_{AD} = HRT_{AD} = V_{AD}/Q_{in} \quad (1)$$

where Q_{in} is digester influent flow rate, which is the same as the wasted sludge rate Q_w. SRTs calculated by this method are called the nominal AD SRT regardless of process configuration.

The SRT is different for the hybrid system, because all or some of the biomass in the sludge recycled to the stabilization tank eventually reenters the ADs. This corresponds to the situation in which input active biomass makes the SRT larger than the nominal SRT from Eq. (1) (Rittmann, 1996; Rittmann and McCarty, 2001). Using the net rate of active-biomass production in the denominator, the SRT with input of active biomass is

Table 2
Average operating conditions by process and phase.

Parameter	Hybrid phase			Conventional phase		
	A	B	C	A	B	C
Phase duration (d)	59	56	119	55	35	140
Mean system influent loading rates						
TCOD (gCOD/d)	380 ± 50	420 ± 90	440 ± 110	380 ± 50	410 ± 100	440 ± 100
SCOD (gCOD/d)	60 ± 20	50 ± 10	50 ± 10	60 ± 20	50 ± 10	50 ± 10
VSS (gVSS/d)	190 ± 30	250 ± 30	250 ± 80	190 ± 40	250 ± 50	260 ± 120
TSS (gTSS/d)	270 ± 70	310 ± 100	300 ± 110	270 ± 70	310 ± 110	300 ± 80
NH_4^+-N (gN/d)	17 ± 3	15 ± 3	17 ± 3	17 ± 3	15 ± 3	17 ± 3
NO_3^--N (gN/d)	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
NO_2^--N(gN/d)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Sludge wasting rate (L/d)	2.9	2.9	5.9	21.6	25.9	21.6
AD sludge recycle rate (L/d)	18.7	23	15.8	0	0	0
RAS ratio (% of influent)	120	120	100	120	120	100
WAS ratio (% of sludge from clarifier)	8.2	8.2	8.3	3.6	3.6	4.3

$$\mathrm{SRT} = \frac{VX}{Q_e X_e + Q_w X_w - Q_{in} X_{in}} \quad (2)$$

where V is the volume of the tank (L^3); Q_e and Q_w are the tank effluent and wasting sludge volumetric flow rates (L^3/t), respectively; Q_{in} is the volumetric flow rate into the tank (L^3/t); and X_{in}, X, X_e, and X_w, and are the influent, mixed-liquor, tank effluent, and wasting sludge biomass concentration (M/L^3), respectively. When Q_e represents recycled sludge, such as the AD sludge being recycled to the AS system, a fraction of that recycled sludge will flow through the AS process and eventually return to the AD as active biomass. If f represents the fraction of active recycled AD biomass that returns to the AD after passing through the AS system of the hybrid process, $Q_{in}X_{in}$ is equivalent to $f Q_e X_e$, and the SRT for a hybrid AD is

$$\mathrm{SRT} = \frac{VX}{Q_e X_e + Q_w X_w - f Q_e X_e} \quad (3)$$

When some or all of the anaerobic biomass retains its activity and is returned to the ADs, then the SRT is larger than that computed by Eq. (1). If all the active biomass from the AD returns to it in active form (i.e., $f = 1$), the AD SRT takes its maximum value:

$$\mathrm{SRT}_{max} = V_{AD}/Q_w \quad (4)$$

where V_{AD} is the volume of the AD (L^3). If only some of the recycled anaerobic biomass re-enters the digester in an active form (i.e., $f < 1$), the hybrid configuration increases the AD SRT to a value between those given in Eqs. (1) and (4).

2.5. Net sludge yield

Net sludge yield is the ratio of net biomass production divided by the amount of electron-donor substrate consumed by the microbial community (Rittmann and McCarty, 2001). Comparing VSS inventory and VSS wasting to changes in COD provides a direct relationship between the amounts of biosolids generated in the system compared to the amount of substrate removed. Since the SW pilots were not always at steady state, we calculated the net sludge yield ($Y_{n,system}$, gVSS/gCOD) by summing the change in the inventory of VSS in the system and the average amount of VSS wasted from the mass balance information, and then we divided the sum by the average COD removal rate:

$$Y_{n,system} = \frac{\frac{\sum \Delta VSS_{inventory\ in\ system}}{\text{Phase duration}} + \frac{\text{Average}\ \Delta VSS_{wasting\ from\ system}}{\Delta t}}{\frac{\text{Average}\ \Delta COD_{system}}{\Delta t}} \quad (5)$$

When performed specifically on contents entering and leaving in the wasting stream from the AD, Eq. (5) becomes

$$Y_{n,low} = \frac{\frac{\sum \Delta VSS_{inventory\ in\ AD}}{\text{Phase duration}} + \frac{\text{Average}\ \Delta VSS_{wasting}}{\Delta t}}{\frac{\text{Average}\ \Delta COD_{AD}}{\Delta t}} \quad (6)$$

While the wasting rate for the conventional process is the same as the influent rate, processes with AD sludge recycle always have a wasting rate smaller than the total flow rate into the process, reducing the value of the numerator. Therefore, Eq. (6) represents the lowest yield ($Y_{n,low}$) obtainable by the hybrid process.

When the numerator contains all solids entering and leaving the AD in the wasting and recycle streams, Eq. (5) becomes

$$Y_{n,high} = \frac{\frac{\sum \Delta VSS_{inventory\ in\ AD}}{\text{Phase duration}} + \frac{\text{Average}\ \Delta VSS_{wasting}}{\Delta t} + \frac{\text{Average}\ \Delta VSS_{AD\ recycle}}{\Delta t}}{\frac{\text{Average}\ \Delta COD_{AD}}{\Delta t}} \quad (7)$$

Eq. (7) represents the highest yield ($Y_{n,high}$) obtainable in the AD process, and Eqs. (6) and (7) indicate the yield range of the AD.

2.6. Methanogens assay

We assayed for the presence of methanogens throughout each train by targeting *Archaea* 16S rDNA. Two complete sets of samples, referred to as sample sets A and B, were obtained on two different dates at the pilot plant when Phase C had constant operating conditions and stable performance. Each set of DNA samples consisted of one sample from each of six sampling points (illustrated in Fig. 1) for each train. The samples were then shipped to Arizona State University's Swette Center for Environmental Biotechnology for quantitative polymerase chain reaction (qPCR) analysis. The shipping procedure used dry ice to keep samples frozen until they reached the Swette Center. Six points were sampled from each train: the influent stream (common among all pilot plant trains), the line leaving the anoxic tank, the line leaving the contact tank, the line leaving the clarifier, the line leaving the stabilization tank, and the wasting sludge from the AD.

We employed the TaqMan-based qPCR methods established by Yu et al. (2005) to target the 16S rDNA sequence of general *Archaea*. The qPCR reactions were carried out in an Eppendorf Realplex gradient cycler with an initial 3 min denaturation at 94 °C, 45 cycles of denaturation at 94 °C, and a combined annealing and extension for 15 s at 60 °C. qPCR reactions set-up and conditions were as described by Parameswaran et al. (2009). Each sample was analyzed in triplicate.

Archaea concentrations in mg cells/L were obtained using conversion factors based on the number of gene copies per cell and cell volume. Literature-obtained conversion factors were 2 16S rDNA copies per cell (Yu et al., 2005) and average cell volumes of 2.14 μm^3 (Zellner et al., 1998) for *Archaea*.

Table 3
AD SRTs by process and phase.

Calculation method	Phase	Calculated SRT (d)	
		Hybrid	Conventional
Nominal SRT (Eq. (1))	A	30	30
	B	25	25
	C	30	30
Maximum SRT (Eq. (4))	A	220	30
	B	220	25
	C	110	30

3. Results and discussion

3.1. AD SRTs

Table 3 presents AD SRTs computed for all operational phases, including the nominal (Eq. (1)) and maximum (Eq. (4)) SRTs. For the hybrid systems, the maximum SRTs (110–220 days) were significantly larger than the nominal SRTs (25–30 days). Longer maximum SRTs for the hybrid configuration should lead to enhanced retention of methanogens and a greater degree of hydrolysis, which should result in a higher degree of COD conversion to CH_4. These impacts are evaluated in the following sections.

3.2. Methane production

Fig. 2 shows that the pilot hybrid ADs produced 1.5–5.5 times more CH_4 than did the AD of the conventional process. The strong increase in CH_4 production correlated with increasing maximum SRT. As illustrated in Fig. 3a, the hybrid processes converted 12–22% of the system-influent COD to CH_4, while the conventional train converted only 5–12%. Both conversions to CH_4 are low when compared to 30–35% for typical WWTP ADs (Metcalf & Eddy, Inc., 2003). These low conversions to CH_4 may be the result of fermentation and methanogenesis in other portions of the pilot WWTPs, including the clarifiers and sludge thickeners. Fig. 3b also illustrates the hybrid processes converted 12–25% of the COD entering the AD to CH_4, compared with 7–8% in the conventional processes. Even though all conversion ratios are low in absolute terms, they clearly illustrate increased TCOD conversion with increasing maximum AD SRT.

3.3. Net sludge yield

Fig. 4a illustrates the average net sludge yields, based on VSS measurements for the treatment systems overall, as a function of the AD SRTs. The overall system yields (Eq. (5)) for the hybrid-

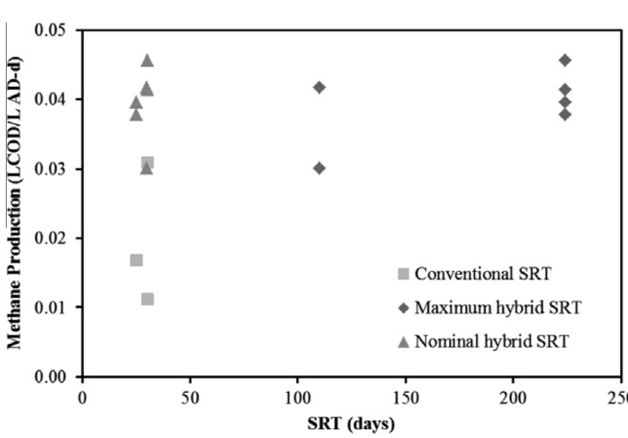

Fig. 2. Average methane production by phase from the hybrid and conventional processes as functions of nominal and maximum SRTs. Nominal SRT is calculated from Eq. (1), and maximum SRT is calculated from Eq. (4).

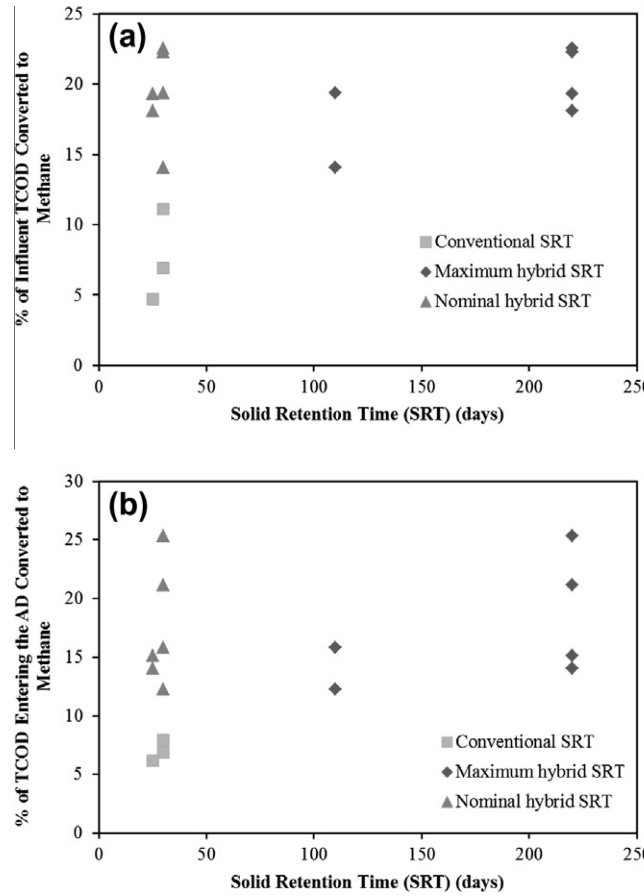

Fig. 3. Average percentages of (a) system-influent TCOD and (b) TCOD entering the AD converted to methane by phase in the hybrid and conventional processes as functions of nominal SRT (Eq. (1)) and maximum SRT (Eq. (4)).

processes ranged from 0.03 to 0.22 g VSS/g COD removed, which were much lower than 0.36–0.69 g VSS/g COD removed in the conventional system. As illustrated in Fig. 4b for the AD alone, the highest amount of sludge reduction (Eq. (6)) that could have occurred in the hybrid AD ranged from 0.00 to 0.20 g VSS/g COD and from 0.53 to 0.96 g VSS/g COD for the conventional AD. The minimum amount of sludge reduction, from Eq. (7), was 0.41–0.73 g VSS/g COD for the hybrid AD alone and 0.61–0.96 g VSS/g COD for the conventional AD alone. Fig. 4b and c demonstrate that, regardless of calculation basis, the yields in the hybrid process ranged from 11% to 96% lower, and this difference correlated with the much high maximum AD SRT of the hybrid systems, along with more COD being converted to methane (Fig. 3). This supports the hypothesis that longer maximum SRTs in the hybrid process provided time to hydrolyze more influent and biomass PCOD, resulting in overall increased CH_4 production and influent COD conversion to CH_4 in the AD.

3.4. Methanogen qPCR analysis

Fig. 5 presents the average concentration of *Archaea* cells in mg cells per liter in the influent, anoxic tank, contact tank, stabilization tank, and AD based on the two sampling sets. For the times at which samples were taken, the nominal SRTs in all processes were 30 days, while the maximum SRT was 110 days in the hybrid processes. Recycling of AD sludge to the aerobic sections of the process should lead to higher and more consistent concentrations of *Archaea* throughout the wastewater-treatment parts of hybrid system. However, the *Archaea* concentrations may not necessarily be

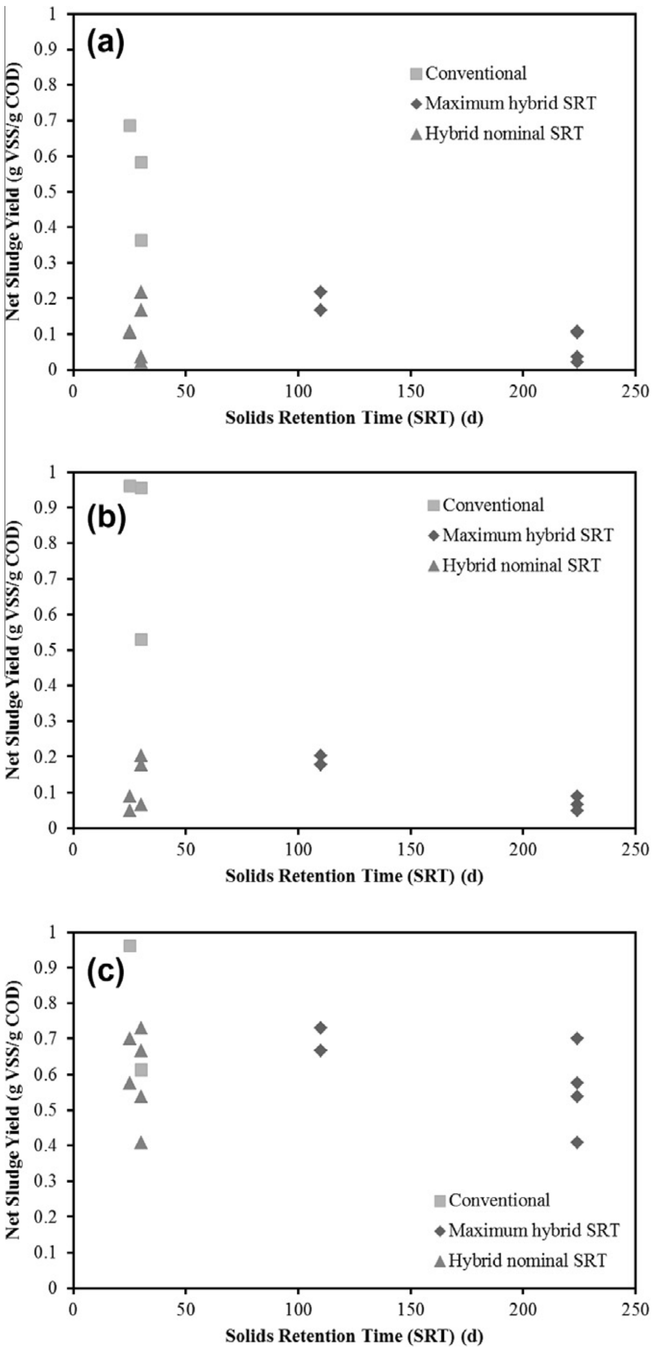

Fig. 4. Net average net sludge yields by phase in the hybrid and conventional processes (a) for the total system (Eq. (5)), (b) from the AD using the wasting sludge only, and (c) from the AD taking into account the total flow leaving the AD. SRTs are calculated based on Eq. (1) for nominal SRT and Eq. (4) for maximum SRT.

higher in the hybrid AD, since its longer AD SRT may lead to more overall decay of methanogenic biomass.

The first and most important finding revealed by Fig. 5 is that *Archaea* were present throughout each system. This was true for conventional and hybrid processes because *Archaea* were present in the influent at a significant concentration, ~46 mg cells/L. The presence of *Archaea* in the influent may have been accentuated by the consistently warm wastewater temperature in Singapore (~33 °C). Finding *Archaea* everywhere supports that methanogens in AD sludge recycle were returned to the AD of the hybrid process. Second, the concentrations of *Archaea* in the wastewater-treatment tanks of the hybrid process were generally higher than for the

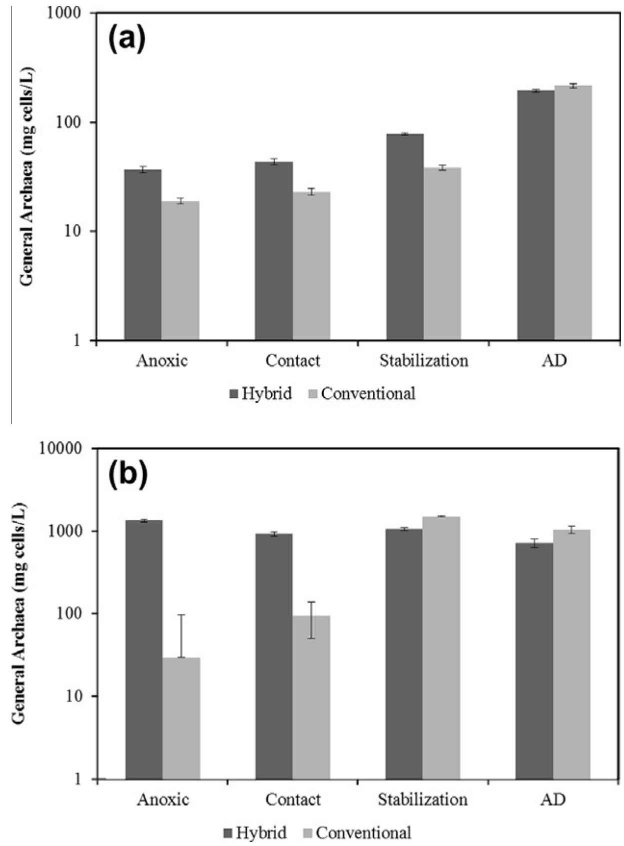

Fig. 5. General *Archaea* concentration (in mg cells/L) by process configuration and tank for sample sets (a) A and (b) B. The bars show the average concentrations, and the error bars indicate the standard deviation in the triplicates performed.

conventional processes, since *Archaea* we input due to AD sludge recycle. Finally, *Archaea* concentrations in the hybrid AD were slightly lower than concentrations in the conventional AD. With significantly longer operating SRTs, the hybrid ADs had greater endogenous decay, resulting in lower methanogen concentrations.

To summarize, the consistent concentration of *Archaea* around the treatment tanks of the hybrid system supports that recycling biomass between the aerobic and anaerobic sections of the hybrid process resulted in significant input of active methanogens to the hybrid AD, thereby increasing the AD SRT, which led to greater COD conversion to CH_4 and lower net sludge yield for the entire system.

4. Conclusions

Mass-balance analyses demonstrated that, by recycling ~85% of the AD sludge back to the AS system, the hybrid system achieved maximum AD SRTs up to 9 times greater than for a conventional system. Longer maximum AD SRTs increased input-PCOD hydrolysis and methane production: Hybrid-system net solids yield decreased by 39–96%, and the CH_4 yield increased by 1.5- to 5.5-fold. Recycling AD sludge led to more consistent concentrations of *Archaea* throughout the hybrid systems, but *Archaea* concentrations in the hybrid AD were slightly lower due to greater endogenous decay with the long AD SRT.

Acknowledgements

We thank Siemens Water Technologies for their financial support and for providing pilot-plant operating results and samples for *Archaea* analysis. In particular, we thank Dr. Hongbo Bobby

Ding for his cooperation in obtaining data and samples from the pilot plant. We also thank Giorgio Scarpellini for his work in performing the qPCR assays.

References

Bolzonella, D., Pavan, P., Battistoni, P., Cecchi, F., 2005. Mesophilic anaerobic digestion of waste activated sludge: influence of the solid retention time in the wastewater treatment process. Process Biochem. 40, 1453–1460.

Carns, K., 2005. Bringing Energy Efficiency to the Water and Wastewater Industry: How Do We Get There? EPRI Solutions, Inc.

Center for Sustainable Systems, 2009. U.S. Wastewater Treatment Fact Sheet. University of Michigan.

de la Rubia, M., Perez, M., Romero, L., Sales, D., 2002. Anaerobic mesophilic and thermophilic municipal sludge digestion. Chem. Biochem. Eng. Q. 16, 119–124.

Eastman, J., Ferguson, J., 1981. Solubilization of particulate organic-carbon during the acid phase of anaerobic-digestion. J. Water Pollut. Control Fed. 53, 352–366.

Lawrence, A., McCarty, P., 1969. Kinetics of methane fermentation in anaerobic treatment. J. Water Pollut. Control Fed. 41, R1–R17.

Lee, I., Parameswaran, P., Rittmann, B.E., 2011. Effects of solids retention time on methanogenesis in anaerobic digestion of thickened mixed sludge. Bioresour. Technol. 102, 10266–10272.

Metcalf & Eddy, Inc., 2003. Wastewater Engineering: Treatment and Reuse. McGraw-Hill, New York.

Miron, Y., Zeeman, G., van Lier, J.B., Lettinga, G., 2000. The role of sludge retention time in the hydrolysis and acidification of lipids, carbohydrates and proteins during digestion of primary sludge in CSTR systems. Water Res. 34, 1705–1713.

Parameswaran, P., Torres, C.I., Lee, H., Krajmalnik-Brown, R., Rittmann, B.E., 2009. Syntrophic interactions among anode respiring bacteria (ARB) and non-ARB in a biofilm anode: electron balances. Biotechnol. Bioeng. 103, 513–523.

Parkin, G.F., Owen, W.F., 1986. Fundamentals of anaerobic-digestion of waste-water sludges. J. Environ. Eng.-ASCE 112, 867–920.

Rittmann, B., 1996. How input active biomass affects sludge age and process stability. J. Environ. Eng.-ASCE 122, 4–8.

Rittmann, B.E., McCarty, P.L., 2001. Environmental Biotechnology: Principles and Applications. McGraw-Hill, Boston.

U.S. Energy Information Administration, 2010. Electric Power Annual 2009, 2010.

Yu, Y., Lee, C., Kim, J., Hwang, S., 2005. Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. Biotechnol. Bioeng. 89, 670–679.

Zellner, G., Messner, P., Winter, J., Stackebrandt, E., 1998. *Methanoculleus palmolei* sp. nov., an irregularly coccoid methanogen from an anaerobic digester treating wastewater of a palm oil plant in North-Sumatra, Indonesia. Int. J. Syst. Bacteriol. 48, 1111–1117.