Evaluation of anaerobic digestion processes for short sludge-age waste activated sludge combined with anammox treatment of digestate liquor

Huoqing Ge, Damien Batstone and Jurg Keller

ABSTRACT

The need to reduce energy input and enhance energy recovery from wastewater is driving renewed interest in high-rate activated sludge treatment (i.e. short hydraulic and solids retention times (HRT and SRT, respectively)). This process generates short SRT activated sludge stream, which should be highly degradable. However, the evaluation of anaerobic digestion of short SRT sludge has been limited. This paper assesses anaerobic digestion of short SRT sludge digestion derived from meat processing wastewater under thermophilic and mesophilic conditions. The thermophilic digestion system (55 °C) achieved 60 and 68% volatile solids destruction at 8 day and 10 day HRT, respectively, compared with 50% in the mesophilic digestion system (35 °C, 10 day HRT). The digestion effluents from the thermophilic (8–10 day HRT) and mesophilic systems were stable, as assessed by residual methane potentials. The ammonia rich sludge dewatering liquor was effectively treated by a batch anammox process, which exhibited comparable nitrogen removal rate as the tests using a control synthetic ammonia solution, indicating that the dewatering liquor did not have inhibiting/toxic effects on the anammox activity.

Key words | anaerobic ammonium oxidation (anammox), anaerobic digestion, dewatering liquor, short sludge-age, waste activated sludge

Huoqing Ge
Damien Batstone
Jurg Keller (corresponding author)
Advanced Water Management Centre (AWMC),
The University of Queensland,
St Lucia,
Queensland,
4072,
Australia
E-mail: *j.keller@uq.edu.au*

Jurg Keller
CRC for Water Sensitive Cities,
PO Box 8000,
Clayton,
Victoria,
3800,
Australia

INTRODUCTION

The current focus of wastewater treatment processes is evolving from only achieving organic matter removal towards gaining the additional benefits of energy and nutrient recovery. The main driving force for this change is the increased cost of energy and the fluctuating cost and availability of some nutrients (e.g. phosphorus). Thus there is strong interest in retrofitting existing treatment processes to produce an effluent that complies with water discharge regulations, but with a reduced energy consumption and physical footprint. It is also highly desirable that the new processes can increase opportunities to recover maximal energy and nutrients from wastewater (Batstone & Virdis 2014).

One promising option in this regard is the high-rate activated sludge process (A-stage), which is highly loaded, with short hydraulic and solids retention times (HRT and SRT, respectively) (Chase & Eddy 1944). This process utilizes the maximum biomass growth potential as a means of removing carbon and partial nutrients (i.e. nitrogen, phosphorus) from wastewater, thereby generating a low carbon effluent. More importantly, such a process preserves and captures the removed carbon and nutrients in the biomass (also called activated sludge), rather than being oxidized to carbon dioxide or converted to nitrogen gas and chemical precipitates (e.g. aluminium phosphate) compared with conventional activated sludge processes, which offers a huge potential for energy and nutrient recovery. In particular for phosphorus, high-rate biological phosphorus (Bio-P) removal can allow phosphate to be effectively captured from wastewater (>90%) in the A-stage configuration and be concentrated in a form that is easily amendable for struvite generation, thus maximizing the concurrent carbon and phosphorus recovery (Ge *et al.* 2015). It has been estimated that approximately 66% of the influent phosphorus can be recovered through downstream anaerobic sludge digestion and struvite crystallization (Ge *et al.* 2014). Another advantage of the high-rate process is that

doi: 10.2166/wst.2015.582

the short-contact between wastewater and activated sludge reduces the amount of carbon being oxidized aerobically, and thus requires much less aeration (hence less energy consumption) compared with a conventional activated sludge process with a longer SRT (e.g. 10–15 days) (Ge *et al.* 2013).

Since the activated sludge generated in the A-stage is likely to be relatively degradable, the ideal option for the subsequent sludge stabilization is anaerobic digestion, which can convert the accumulated carbon to methane as a renewable energy source. While the SRT-degradability relationship has been well established some time ago (Gossett & Belser 1982), most previous research focused on the investigation of medium to long SRT (>5 days) activated sludge, or the evaluation of existing full-scale domestic wastewater systems operating under a particular set of conditions, such as the Strass wastewater treatment plant (WWTP) (0.5 day SRT activated sludge) (Wett & Alex 2003). As a result, the expected methane production of an A-stage sludge with different SRTs, as well as the digestibility variations between thermophilic and mesophilic conditions, has not been determined systematically. In response to this, Ge *et al.* (2013) assessed the degradability of short sludge-age (2–4 days) activated sludge using biochemical methane potential (BMP) tests. However, this study was conducted in batch mode, and therefore could not evaluate the volatile solids (VS) destruction nor digestate nitrogen and phosphorous contents, which can only be evaluated in a continuous feeding system. This meant that energy balance could not be estimated. Anaerobic sludge digestion results in nitrogenous and phosphorus organic compounds contained in the sludge to be released as ammonia and orthophosphate, of which particularly the ammonia will flow into the subsequent sludge dewatering liquor stream. Again, the extent of nitrogen and phosphorus release has not been determined. There is also limited information about whether the sludge dewatering liquor can be treated by anaerobic ammonium oxidation (anammox) which is an emerging low-energy process used for nitrogen removal (Strous *et al.* 1997).

This paper addresses these limitations on an activated sludge stream with 2 day SRT produced from a laboratory-scale A-stage reactor treating meat processing wastewater. Anaerobic digestion effectiveness of the activated sludge was evaluated under continuous thermophilic and mesophilic conditions. The overall chemical oxygen demand (COD) balance as well as the residual nitrogen removal from the sludge dewatering liquor via the anammox process are also assessed to provide a comprehensive analysis for the short SRT activated sludge treatment.

METHODS

Anaerobic sludge digestion

The substrate used in this study was waste activated sludge generated from a high-rate sequencing batch reactor (SBR) based process with 2 day SRT (organic loading rate of 24 gCOD d^{-1}) treating meat processing wastewater. The details of the SBR process were described in Ge *et al.* (2013). The activated sludge collected from the SBR was centrifuged to increase the total solids (TS) concentration prior to feeding. The feed sludge was prepared every second day and stored at approximately 4 °C to minimize degradation and preserve integrity of the material. Regular analysis was performed to determine the characteristics and consistency of the feed materials, and the results are summarized in Table 1.

Two identical anaerobic digesters (0.8 L working volume, Figure 1) were operated in a semi-continuous mode throughout the study to stabilize the waste activated sludge stated above. One digester was operated at 35 °C with a constant HRT of 10 days (referred as the mesophilic digester), while the other was operated at 55 °C with HRT as the experimental variable (referred as the thermophilic digester). The temperature in both digesters was maintained by circulating temperature-controlled water through the water jacket. Each digester was continuously mixed using a magnetic stirring bar. Volumes of biogas production in each digester were measured using tipping bucket gas meters, and online recorded by a process logic control system. The pH in each digester was measured daily with a calibrated glass body probe and recorded online.

At the start-up, the thermophilic and mesophilic digesters were inoculated from a full-scale anaerobic digester (35 ± 1 °C, 20-day HRT) in a municipal WWTP in Brisbane, Australia. Both digesters were fed at intervals of 6 hours

Table 1 | Characteristics of the waste activated sludge used in this study

Parameter	Feed sludge values
TS (g L^{-1})	24.8 ± 0.5[a]
VS (g L^{-1})	21.6 ± 0.3
COD (g L^{-1})	34.7 ± 5.2
VFAs (g L^{-1})	0.09 ± 0.05
NH_4^+-N (g N L^{-1})	0.1 ± 0.04
PO_4^{3-}-P (g P L^{-1})	0.07 ± 0.02

[a]Values are means ± standard deviations for all the feed sludge materials used in the study over 12 months.

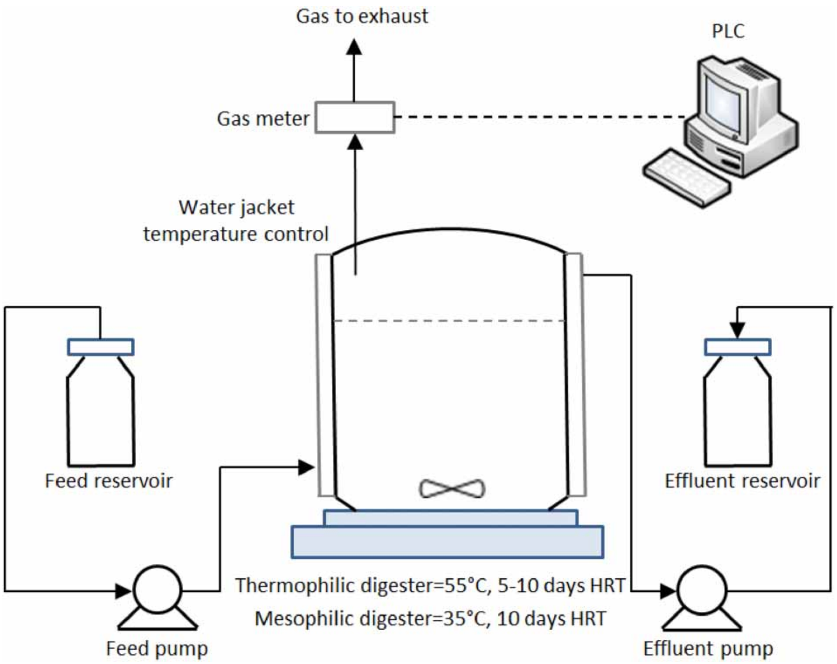

Figure 1 | Set-up of the thermophilic and mesophilic anaerobic digesters for stabilizing the 2 day sludge-age activated sludge.

(four times daily). During feed events, fresh feed sludge was pumped into the digesters and an equal volume of the effluent was withdrawn simultaneously using a multi-head peristaltic pump. The thermophilic digester was operated at three HRTs: (i) 5-day HRT (240 days); (ii) 8-day HRT (50 days); and (iii) 10-day HRT (53 days). The mesophilic digester had been operated for 90 days before the thermophilic digester commenced operation. All comparisons used in this study were calculated from day 102 forward, where both digesters were stable.

Residual BMP tests

BMP tests were conducted to assess the stability of the digester effluents by determining the residual methane potential from digestion residues. The set-up and operation of the tests are the same as in Jensen *et al.* (2011). Batch tests were performed in 160 mL non-stirred glass serum bottles (100 mL working volume). The inoculum used in the tests was collected from the same full-scale anaerobic digester as stated above. Substrates used in the tests were the digestates collected from the thermophilic digester and the mesophilic digester. The inoculum to substrate ratio used in the tests was 1:1 (VS basis). After loading the substrate and inoculum, the bottles were flushed with high purity nitrogen gas to create anaerobic conditions, sealed and stored in a temperature controlled incubator (35 ± 1 °C). Blanks that contained inoculum and MilliQ water without substrate, were conducted to measure the background methane produced from the inoculum. All tests were carried out in triplicate, and all error bars indicate 95% confidence in the average of the triplicates based on two-tailed *t*-tests. Degradability was estimated by fitting the exponential decay function as described in Jensen *et al.* (2011).

Specific anammox activity tests

Specific anammox activity (SAA) tests were conducted to evaluate the effectiveness of anammox treating the ammonium (NH_4^+) rich sludge dewatering liquor from the thermophilic anaerobic digester. The method is based on the work of Dapena-Mora *et al.* (2007). The anammox biomass used in the tests was collected from a 50 L anammox biofilm reactor (35 °C). This reactor was operated under fed-batch conditions by injecting a concentrated NH_4^+ solution periodically and a nitrite (NO_2^-) solution in small pulses to enrich anammox culture on biofilm carriers (BiofilmChip™ M). After sampling a number of carriers from the parent reactor, the biomass was harvested from the carriers by stirring the carriers in nutrient medium at a low speed and then letting the biomass settle. The medium contains $CaCl_2$ (0.39 g L^{-1}), KH_2PO_4 (0.05 g L^{-1}), $MgSO_4$ (0.2 g L^{-1}), $FeSO_4$ (0.00625 g L^{-1}), EDTA (0.00625 g L^{-1}), trace I acidic solution (1 g L^{-1}) and trace II alkaline solution

($1\ g\ L^{-1}$). The settled biomass was subsequently washed and re-suspended in new nutrient medium. The substrate used in the tests was the dewatering liquor obtained by centrifuging the thermophilic digester effluent.

The SAA tests were performed in 160 mL serum bottles (100 mL working volume). Each bottle contained the pre-determined volumes of the anammox biomass and the sludge dewatering liquor, achieving the initial concentrations of biomass and NH_4^+ at $1\ g\ VSS\ L^{-1}$ and $60\ mg\ N\ L^{-1}$, respectively. The bottles were then flushed with pure nitrogen gas to obtain anoxic conditions, sealed and placed in a thermostatic shaker, at 150 rpm and 30 °C. The initial pH value was fixed at 7.8. NO_2^- was added step-wisely to provide sufficient NO_2^- for anammox but also to maintain the NO_2^- concentration below $30\ mg\ N\ L^{-1}$, which was reported as an inhibition concentration for anammox (Bettazzi *et al.* 2010).

The control SAA tests were also set-up and operated in the same way as described above, with the exception of adding a prepared NH_4^+ stock solution as the substrate. All the tests stated above were conducted in triplicate. Error bars in the figures represent 95% confidence in error based on the average of the triplicate (tests two-tailed *t*-test).

Analysis

Biogas samples were taken daily from the thermophilic and mesophilic anaerobic digesters and the biogas composition (CH_4, CO_2, H_2) was analyzed by a Perkin Elmer gas chromatography (GC) equipped with a thermal conductivity detector. Slurry samples were collected from both digesters periodically and analyzed for total chemical oxygen demand (TCOD), TS and VS. A part of slurry samples were filtered through Millipore filter units (0.45 μm pore size). The filtered samples were then analyzed for soluble COD (SCOD), phosphate (PO_4^{3-}), $NH_4^+$and volatile fatty acids (VFAs). TCOD and SCOD were measured by Merck cell tests (Merck KGaA, Germany). NH_4^+ and PO_4^{3-} were measured using a Lachat Quik-Chem 8000 Flow Injection Analyser (Lachat Instrument, Milwaukee). VFA concentrations were measured by an Agilent Technologies GC with a flame ionization detector.

In the residual BMP tests, biogas production and composition were also monitored throughout each batch test. Accumulated methane production was calculated by the method described in Ge *et al.* (2011) with blank methane production subtracted. For sludge samples collected at the start and end of each batch test, the substrate, inoculum and combined slurry samples were analyzed for TCOD, SCOD, TS, VS, NH_4^+, PO_4^{3-} and VFAs.

In the SAA tests, liquid samples were regularly taken from each bottle throughout the experimental period to measure inorganic nitrogen species (NH_4^+, NO_2^- and NO_3^-). SAA was determined by regression against the linear section of the NH_4^+ degradation curves.

RESULTS

Anaerobic digestion of 2-day sludge-age activated sludge

The sludge digestion performance of the thermophilic and mesophilic anaerobic digesters was evaluated by VS destruction, production of methane, release of NH_4^+ and PO_4^{3-}, and organic acids levels. VS destruction in this study was determined using mass balance and Van Kleeck equations to provide two independently derived measures (Switzenbaum *et al.* 2003), and the results are shown in Figure 2. Consistent results from both VS calculation methods confirm the absence of systematic sampling errors and/or unexpected behaviours. The VS destruction achieved in the thermophilic digester at 5-day HRT was around 50%, and hence similar to that obtained in the mesophilic digester (35 °C) at 10-day HRT. Both processes achieve a digestion performance substantially greater than the legislative level (38%) required by US EPA (EPA 1994), and most Australian legislation. Extending the HRT in the thermophilic digester to 8 days improved VS destruction from 52 to 60%. A further increase of VS destruction to 68% was observed when the HRT in the thermophilic digester was extended to 10 days. Statistical analysis (paired *t*-test, $\alpha = 0.05$, $p = 0.036$) confirmed that VS destruction in the thermophilic digester with longer HRTs (8–10 days) was significantly greater than that achieved in the mesophilic digester at 10-day HRT. Total methane production from both digesters was consistent with the VS destruction results, being higher in the thermophilic digester with 8–10-day HRTs than the 5-day HRT thermophilic digester and the mesophilic digester (Table 2). Methane accounted for approximately 63% of biogas composition in both digesters, with carbon dioxide being the other major component.

The stability of the digester effluents produced at different HRTs was analyzed to further assess the performance of both anaerobic digesters. Figure 3 shows a summary of sludge degradable fractions in the two digesters, which consists of three parts, VS destruction in the digester,

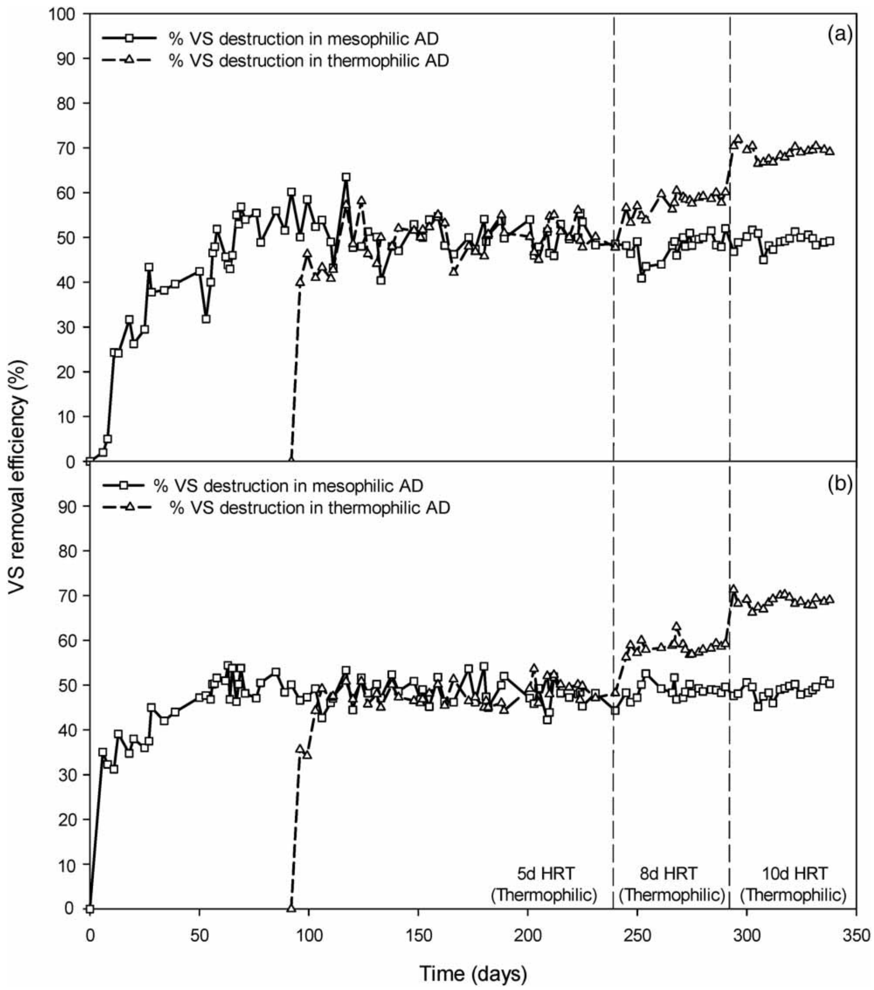

Figure 2 | VS destruction calculated by mass balance equation (a) and Van Kleeck equation (b) in the thermophilic anaerobic digester (55 °C, 5–10 day HRTs) and the mesophilic anaerobic digester (35 °C, 10 day HRT) (% VS destruction is based on the activated sludge feed characteristics).

Table 2 | Summary of the sludge digestion performance in the thermophilic and mesophilic anaerobic digesters

Anaerobic digester	Operating conditions	VS destruction (%)	Methane production (mL gVS_{fed}^{-1})	Total residual methane (mL gVS_{fed}^{-1})
Thermophilic digester (55 °C)	5-day HRT	52 ± 3[a]	231 ± 3	154 ± 18
	8-day HRT	60 ± 2	273 ± 6	95 ± 6
	10-day HRT	68 ± 4	295 ± 5	83 ± 5
Mesophilic digester (35 °C)	10-day HRT	50 ± 2	226 ± 4	97 ± 5

[a]Error bars are 95% confidence across different measurements.

degradable methane from the digester effluent and the non-degradable recalcitrant materials (based on the material not converted to methane). This showed that the mesophilic digester degraded approximately 60% of the available material, while the 5-day HRT thermophilic digester achieved the total conversion of approximately 65%, but leaving a less stabilized effluent that exhibited a higher residual methane potential (Table 2). This was also consistent with the residual organic acids levels in the digesters effluents (Table 3). A more stable effluent was obtained in the thermophilic digester with 8-day and 10-day HRTs (<100 mL residual methane production per $VS_{effluent}$ added), thus enhancing the total conversion to 70–80%. This is again consistent with the significantly higher VS destruction achieved by the thermophilic process compared with the mesophilic operation with similar HRTs.

The extent of NH_4^+ and PO_4^{3-} release during anaerobic digestion was another important performance indicator. In this study, the NH_4^+-N concentrations increased with each HRT step change in the thermophilic digester, which was 8–16% higher than that in the mesophilic digester, except

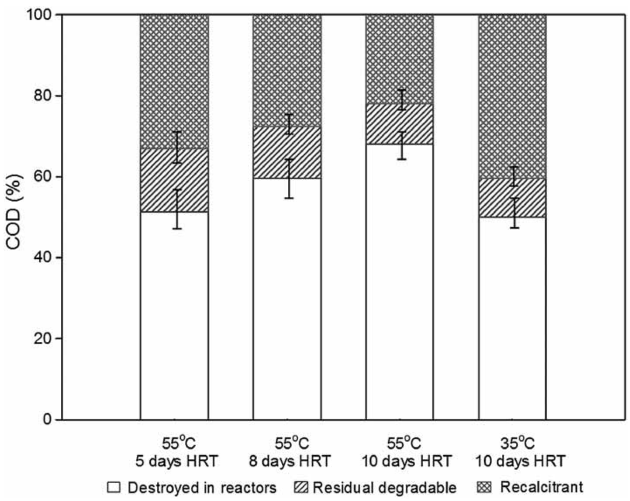

Figure 3 | Summary of degradable fractions of the 2 day sludge-age activated sludge in the thermophilic (55 °C, 5–10 day HRTs) and mesophilic (35 °C, 10 day HRT) digesters Error bars indicate 95% confidence intervals across different measurements.

Table 3 | Summary of the sludge digestion performance in the thermophilic and mesophilic anaerobic digesters according to VFAs, NH_4^+ and PO_4^{3-}

Anaerobic digester	Operating conditions	VFA (mg COD L^{-1})	NH_4^+ (mg N L^{-1})	PO_4^{3-} (mg P L^{-1})
Thermophilic digester (55 °C)	5-day HRT	370 ± 30[a]	$1,240 \pm 40$	210 ± 40
	8-day HRT	50 ± 20	$1,330 \pm 30$	190 ± 20
	10-day HRT	30 ± 10	$1,430 \pm 20$	200 ± 20
Mesophilic digester (35 °C)	10-day HRT	40 ± 10	$1,230 \pm 20$	200 ± 50

[a]Error bars are 95% confidence in mean VFA, NH_4^+ and PO_4^{3-} concentrations.

the thermophilic digester at 5-day HRT (Figure 4(a) and Table 3). However, the PO_4^{3-}-P concentration was similar in the two digesters and was also not affected by increasing the HRT in the thermophilic digester.

Anammox treatment for nitrogen removal from the sludge dewatering liquor

The effectiveness of anammox treating the sludge dewatering liquor from the 10-day HRT thermophilic digester was evaluated based on the SAA test. The external addition of NO_2^- in the SAA tests was performed through successive feedings to prevent excessive NO_2^- accumulation. As shown in Figure 5, after each NO_2^- addition, complete NO_2^- consumption was observed within approximately half a day and concurrently a portion of NH_4^+ was removed. After five successive additions of NO_2^-, NH_4^+ was removed to a low level, resulting in a total nitrogen removal rate of approximately 75 mg N L^{-1} day^{-1}. The average ratio of NH_4^+ and NO_2^- degradations was around 1.3 and is similar to the stoichiometric ratio of 1:1.32 reported previously (Strous *et al.* 1997). The production of NO_3^- in the tests was also monitored (data shown in the Supplementary Material, available with the online version of this paper) and the production rate was close to the theoretical value (1:1.3:0.255 for NH_4^+/ NO_2^-/NO_3^-).

The control SAA tests were also conducted with externally adding NO_2^- and NH_4^+, and achieved a total nitrogen removal rate of approximately 77 mg N L^{-1} day^{-1}, which is similar to the removal rate obtained in the SAA tests with the real sludge dewatering liquor. It should also be noted that the actual amount of anammox biomass used in the tests was less than 1 g VSS L^{-1}, as the biomass collected from the parent anammox reactor contained some ammonium oxidizing bacteria (converting a part of NH_4^+ to NO_2^- for anammox). It therefore can be expected that the total nitrogen removal rate can be substantially enhanced when the anammox biomass is increased to the level normally seen in full-scale plants.

DISCUSSION

Current changes in the wastewater treatment focus is evolving to energy and nutrients recovery and will result in the production of a (very) short SRT activated sludge stream, the anaerobic degradability of which was determined in continuous operation under mesophilic and thermophilic conditions as part of this study. As measured by VS destruction, the digestion temperature had a very clear effect on the sludge degradability with an increase from $50 \pm 2\%$ degradability at 35 °C (10-day HRT) to $68 \pm 4\%$ degradability at 55 °C (10-day HRT). This is probably due to the thermophilic temperature enabling increased destruction of specific components or dissolution of complex organics (e.g. waxes and fats), and also improving the lysis and hydrolysis rates of microbial cells in the activated sludge to release more cellular organics into the bulk for subsequent degradation, thereby enhancing the overall degradability. Moreover, the determined sludge degradability is consistent with the reverse correlation between SRT and sludge degradability as reported by Gossett & Belser (1982), and also fills the blank where the degradability of the short SRT (<4 days) sludge has been poorly assessed. The higher degradability of 60–70% measured at 8–10 day HRTs (55 °C) is also close to the maximal degradability (nearly 80%) obtained in the BMP tests for the similar sludge-age (2 days) activated sludge (Ge *et al.* 2013).

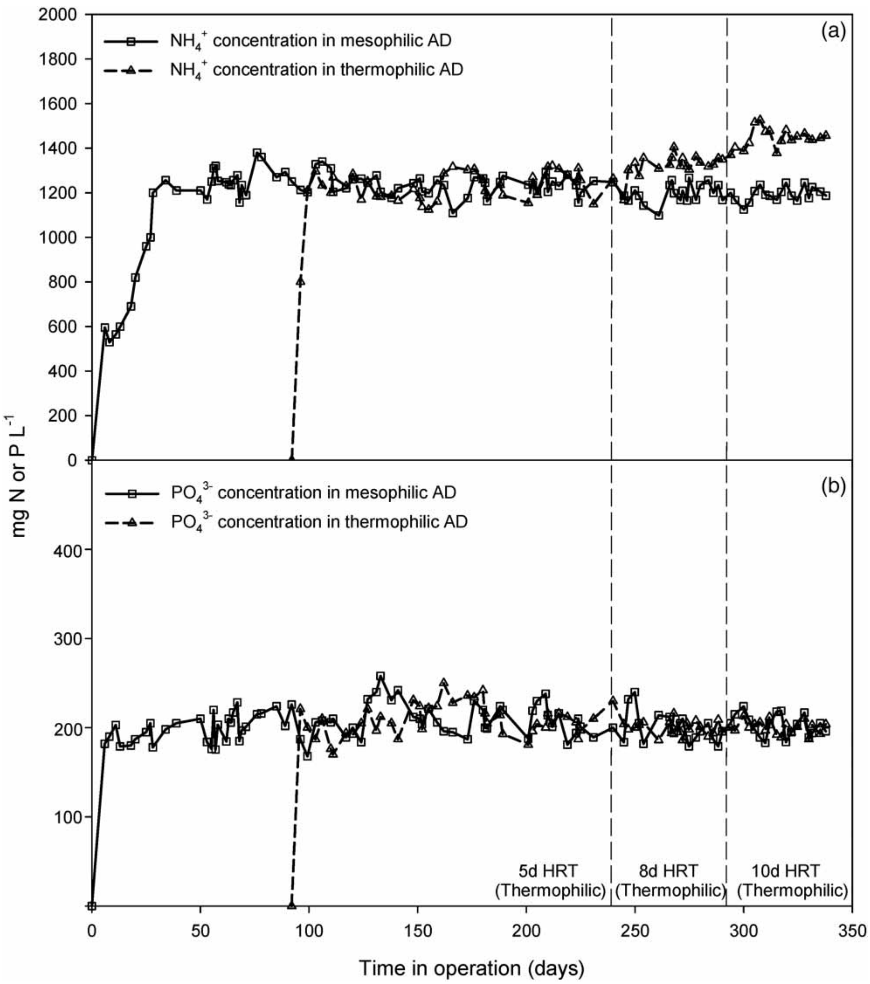

Figure 4 | Concentrations of NH_4^+-N and PO_4^{3-}-P in the thermophilic (55 °C, 5–10 day HRTs) and mesophilic (35 °C, 10 day HRT) digesters.

Another concern of short SRT sludge stabilization is the degree of residual methane potential from the digestion residues, due to the relatively high degradability of the input material. In this study, except for the 5-day HRT thermophilic digestion process, the residues from all tested digestion processes showed relatively low residual methane potentials, which are all within the commonly applied residue disposal standard of 17% (equivalent to 110 mL methane production per $VS_{effluent}$ added in this case) (EPA 1994). This indicates the digestion residues are suitable for disposal in terms of nutrients regulations. The thermophilic treatment (55 °C), on the other hand, enables the residues to meet the standards for pathogen destruction if semi-batch operation is applied in practise (Paul *et al.* 2012). It should also be noted that some nutrients (e.g. NH_4^+, PO_4^{3-}) are highly concentrated in the digestion residues. For example, the level of PO_4^{3-} reached 200 mg PO_4^{3-}-P L^{-1} in this case, suggesting a strong potential to achieve efficient recovery in a dedicated struvite crystallizer (Yuan *et al.* 2012). While struvite precipitation would also remove some limited amount (approximately 30%) of NH_4^+, the remainder would ideally be eliminated from the sludge dewatering liquor through a dedicated process such as the anammox process tested in this study. The observed total nitrogen removal rate in the SAA tests confirmed that the anammox process operated efficiently with the dewatering liquor, as similar removal rates were measured with real sludge dewatering liquor and pure NH_4^+ solution. It indicates that the SCOD present in the dewatering liquor was not further consumed for denitrification, and the dewatering liquor also did not have inhibiting/toxic effects on the anammox activity. This is very important for the emerging energy self-sufficient high-rate wastewater treatment processes as the low COD:N ratio effluent streams from the A-stage and the digester could be combined and treated by anammox. This also can optimally utilize the infrastructure investments for anammox reactors and reduce the nitrogen

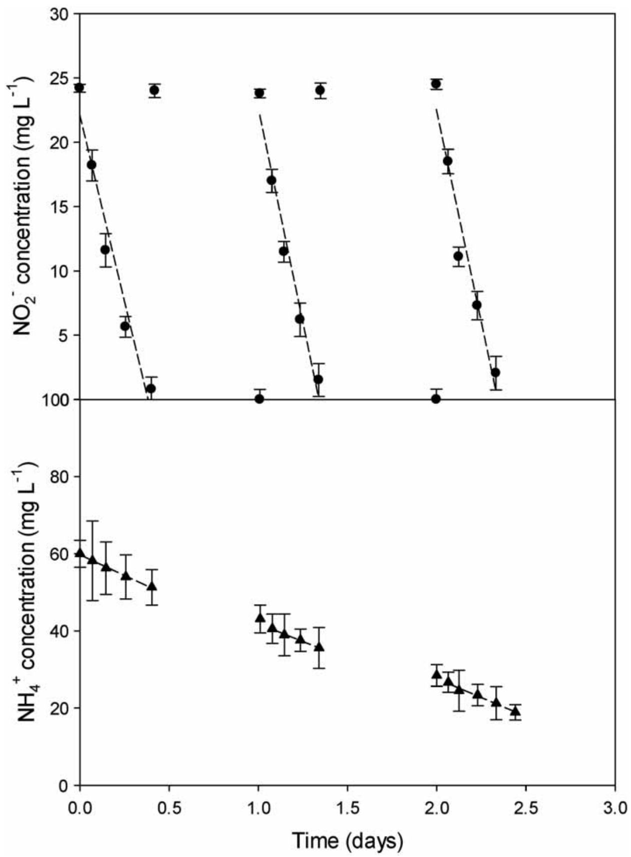

Figure 5 | Nitrite (NO_2^-) and ammonium (NH_4^+) removal profiles after each NO_2^- addition of approximately 25 mg N L^{-1} in the SAA tests on the dewatered anaerobic effluent from the 10-day HRT thermophilic anaerobic digester. Error bars are 95% confidence intervals based on triplicate analyses.

load in the subsequent process following A-stage (by avoiding the nitrogen loading from the dewatering liquor).

The large amount of methane is another valuable product from anaerobic sludge digestion, particularly for the highly degradable short SRT sludge tested in this study. The generated methane can be used in a co-generation process for production of energy (electricity) and heat, which can offset the power and heating demand for thermophilic digestion, as well as other upstream and downstream processes (i.e. high-rate wastewater treatment, secondary thickening, sludge dewatering, anammox). The energy balance of such an integrated high-rate treatment system has been analyzed (details in the Supplementary Material, available with the online version of this paper) based on the method described in Ge *et al.* (2013). Generally, the energy recovered from the methane production exceeded the energy requirements of the integrated high-rate system, suggesting that a net electric power output can be gained. In particular, approximately 3.5 times more net energy gain can be achieved from the integrated high-rate system with thermophilic digestion compared with mesophilic digestion, resulting in the system energy self-sufficiency reaching to approximately 170%. In addition, the usage of renewable energy (methane) can generate renewable energy credits (e.g. $0.034 per kWh in Australia), which is expected to substantially reduce the operating expenses for such an integrated system.

CONCLUSIONS

The thermophilic (55 °C) digestion process with 8–10 day HRT achieved 60–68% VS destruction of the 2 day SRT activated sludge, which is significantly higher than a comparable 10 day HRT mesophilic process. Even for this highly degradable material, the results indicated an advantage for thermophilic digestion. The NH_4^+-rich dewatering liquor from the sludge digestion was treated by an anammox process, with the comparable nitrogen removal rate as the tests using a control synthetic ammonia solution, indicating that the dewatering liquor did not have inhibiting/toxic effects on the anammox activity.

ACKNOWLEDGEMENTS

This work was funded by the Australian Meat Processor Corporation (AMPC), with matching funds provided from the Australian Government, via Meat and Livestock Australia (MLA). We thank Barbara Wronski for the assistance in digester operation, and the analytical laboratory in the Advanced Water Management Centre for chemical analysis.

REFERENCES

Batstone, D. J. & Virdis, B. 2014 The role of anaerobic digestion in the emerging energy economy. *Current Opinion in Biotechnology* **27**, 142–149.

Bettazzi, E., Caffaz, S., Vannini, C. & Lubello, C. 2010 Nitrite inhibition and intermediates effects on anammox bacteria: a batch-scale experimental study. *Process Biochemistry* **45**, 573–580.

Chase, E. S. & Eddy, H. P. 1944 High rate activated sludge treatment of sewage. *Sewage Works Journal* **16**, 878–885.

Dapena-Mora, A., Fernandez, I., Campos, J. L., Mosquera-Corral, A., Mendez, R. & Jetten, M. S. M. 2007 Evaluation of activity and inhibition effects on anammox process by batch tests based on the nitrogen gas production. *Enzyme and Microbial Technology* **40**, 859–865.

EPA 1994 Pathogen and Vector Attraction Reduction Requirements. In: *A Plain English Guide to the EPA Part 503 Biosolids Rule*.

United States Environmental Protection Agency, Office of Wastewater Management (Walker, J., Knight, L., & Stein, L. eds), Washington, DC, USA, pp. 107–127.

Ge, H. Q., Jensen, P. D. & Batstone, D. J. 2011 Increased temperature in the thermophilic stage in temperature phased anaerobic digestion improves degradability of waste activated sludge. *Journal of Hazardous Materials* **187**, 355–361.

Ge, H. Q., Batstone, D. J. & Keller, J. 2013 Operating aerobic wastewater treatment at very short sludge ages enables treatment and energy recovery through anaerobic sludge digestion. *Water Research* **47**, 173–182.

Ge, H. Q., Batstone, D. J. & Keller, J. 2014 High-rate activated sludge process with Bio-P removal for sustainable wastewater treatment. In: *Conference Proceeding. Global Challenges: Sustainable Wastewater Treatment and Resource Recovery*, Nepal.

Ge, H. Q., Batstone, D. J. & Keller, J. 2015 Biological phosphorus removal from abattoir wastewater at very short sludge ages mediated by novel PAO clade *Comamonadaceae*. *Water Research* **69**, 6546–6557.

Gossett, J. M. & Belser, R. L. 1982 Anaerobic digestion of waste activated sludge. *Journal of the Environmental Engineering Division-ASCE* **108**, 1101–1120.

Jensen, P. D., Ge, H. Q. & Batstone, D. J. 2011 Assessing the role of biochemical methane potential tests in determining anaerobic degradability rate and extent. *Water Science & Technology* **64**, 880–886.

Paul, E., Carrere, H. & Batstone, , D. J. 2012 Thermal methods to enhance biological treatment processes. In: *Biological Sludge Minimisation and Biomaterials/Bioenergy Recovery Technologies* (E. Paul & Y. Liu, eds). John Wiley & Sons, Hoboken, NJ, USA, pp. 373–404.

Strous, M., Gerven, E. V., Zheng, P., Kuenen, J. G. & Jetten, M. S. M. 1997 Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (Anammox) process in different reactor configurations. *Water Research* **31**, 1955–1962.

Switzenbaum, M. S., Farrell, J. B. & Pincince, A. B. 2003 Relationship between the Van Keleeck and mass-balance calculation of volatile solids loss. *Water Environment Research* **75**, 377–380.

Wett, B. & Alex, J. 2003 Impacts of separate rejection water treatment on the overall plant performance. *Water Science & Technology* **48**, 139–146.

Yuan, Z., Pratt, S. & Batstone, D. J. 2012 Phosphorus recovery from wastewater through microbial processes. *Current Opinion in Biotechnology* **23**, 878–883.

First received 21 April 2015; accepted in revised form 30 October 2015. Available online 13 November 2015