Characterization and biodegradability of sludge from a high rate A-stage contact tank and B-stage membrane bioreactor of pilot-scale AB system treating municipal wastewaters

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ABSTRACT

In light of global warming mitigation efforts, increasing sludge disposal costs, and need for reduction in the carbon footprint of wastewater treatment plants, innovation of treatment technology has been tailored towards energy self-sufficiency. The AB process is a promising technology to achieve maximal energy recovery from wastewaters with minimum energy expenditure and therefore inherently reducing excess sludge production. Characterization of this novel sludge and its comparison with the more conventional B-stage sludge are necessary for a deeper understanding of AB treatment process design. This manuscript presents a case study on a pilot-scale AB system treating municipal wastewaters as well as a bio- (biochemical methane potential and adenosine tri-phosphate analysis) and physico-chemical properties (chemical oxygen demand, sludge volume index, dewaterability, calorific value, zeta potential and particle size distribution) comparison of the organic-rich A-stage against the B-stage activated sludge. Compared to the B-sludge, the A-sludge yielded 1.4 to 4.9 times more methane throughout the 62-weeks operation.

Key words | AB process, anaerobic digestion, biodegradability, contact tank, energy recovery, sewage sludge

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INTRODUCTION

In the recent years, research efforts aiming to improve energy efficiency of wastewater treatment processes at large centralized wastewater treatment plants (WWTPs) have been increasing. Concerns over global warming impacts, energy sustainability, and biosolids generation are among several key drivers towards the establishment of energy-efficient WWTPs. WWTPs have been recognized as major contributors of greenhouse gas emissions as these are significant energy consumers in the industrialized world (Chai *et al.* 2015). In addition, the large quantity of biosolids – a by-product of wastewater treatment processes – would pose solid waste disposal problems as a result of the limited capacity of landfill sites or air pollution problems from incineration sites. Furthermore, the biosolids management system is considered cost-intensive as it typically accounts for 25–60% of the total operational costs of conventional activated sludge (CAS)-based WWTPs (Canales *et al.* 1994; Verstraete & Vlaeminck 2011). Innovative design and treatment strategies, therefore, are required to achieve the cost-effective and energy self-sufficient WWTPs by minimizing energy consumption while increasing recovery.

One of the approaches towards an energy-neutral, if not -positive, wastewater treatment process is to recover the

doi: 10.2166/wst.2016.346

potential energy available in raw municipal wastewaters (Shizas & Bagley 2004). A two-stage process, the so-called AB process, has been suggested for the recovery of caloric energy content from sewage organics (Böhnke 1977; Versprille *et al.* 1984; Meerburg *et al.* 2015). The first stage is primary treatment in an extremely high loaded biosorption stage (A-stage), which is subsequently followed by secondary treatment in low loaded biological stage (B-stage) to ensure the removal of dissolved organics and ammonia. The A-stage treatment allows biological concentration of sewage organics with minimum oxidation to CO_2, and consequently producing a concentrated sludge stream to be channeled to the anaerobic digester. The entrapped organics (chemical energy) could then be recovered through conversion to biogas without significant energy losses (Verstraete *et al.* 2009). A characteristic feature of the A-stage reactor is operation with high Food to Microorganism (F/M) ratio, short hydraulic retention time (HRT), and short solid retention time (SRT), to achieve high removal rate of sewage organics (Boehnke *et al.* 1997). Indeed, treatment with short SRT has been demonstrated to significantly improve the biodegradability of sludge in the downstream anaerobic digester (Ge *et al.* 2013). The separation of excess sludge in the A-stage can be achieved through an intermediate clarifier (henceforth referred to as 'A-stage clarifier') or dynamic membrane filtration unit (Ersahin *et al.* 2012; Roest *et al.* 2012).

Several carbon capture mechanisms during A-stage treatment have been suggested in the literature, namely organic removal by conversion to biomass by fast growing microorganisms, sorption/bio-flocculation, and microbial storage (Boehnke *et al.* 1998; Haider *et al.* 2003; Makinia *et al.* 2006). Among these mechanisms, sorption/bio-flocculation processes have been commonly applied to enhance the A-stage primary treatment (Yu *et al.* 2009; Meerburg *et al.* 2015). Wett *et al.* (2007) presented a successful case of net energy-positive municipal WWTP in Strass, Austria, with a two-stage AB process implementing sorption-based carbon entrapment in the primary step. During the biosorption process, the A-sludge retains particulates and colloidal organic substances within the biomass matrix, and therefore leaving mainly dissolved organics in the effluents. Readily degradable dissolved organics are typically removed very rapidly and depending on the SRT, the A-stage generally leaves behind inert or difficult to degrade dissolved organics (Haider 2003). This would mean a reduced aeration energy requirement and low sludge production in the following B-stage (Versprille *et al.* 1984), and therefore may lead to considerable energy savings and overall reduction in biosolids generation.

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Thus far, the published literature has documented the biodegradability of the enhanced A-stage sludge at several plants treating municipal wastewaters, but there is virtually no report on the sludge characteristics and its comparison with the B-sludge, a more conventional type of sludge. Moreover, as both biological and physical processes play a crucial role in sewage and biosolids treatment, understanding of the change in bio-/physico-chemical properties of the sludge before and after anaerobic digestion (AD) would also be of relevance. In this study, the above-mentioned characteristics of the A- and B-sludge and its changes during the digestion process were examined. This study was a part of Singapore's initiative to advance towards energy-efficient WWTPs through a demonstration of energy recovery efforts at a pilot-scale AB system treating real wastewater. The ultimate aim of the effort was to achieve energy self-sufficiency without compromising effluent quality.

MATERIALS AND METHODS

Process configuration and operating conditions

A pilot plant was operated with an AB process (Singapore) and was run in a continuous flow mode at ambient temperature (28–32 °C) with an average wastewater flow of 1,000 m^3/d. The pilot plant comprised an equalization tank, a coarse (5 mm) screen, a high-rate A-stage, a primary/A-stage clarifier, a fine (2 mm) screen, and an ultrafiltration membrane bioreactor (MBR) system which comprised 5 biological tanks (2 anoxic tanks and 3 aerobic tanks), a membrane tank and a deoxygenation tank. A schematic diagram of the pilot plant is shown in Figure 1. The raw influent was held in the equalization tank. It was drawn through submersible pumps operating in constant flowrate mode. Initial screening was subsequently performed through 5 mm slot-size screen units followed by a screw conveyor type grit removal system. The A-stage was designed with an SRT of 0.5 d (calculated over the entire contact tank and clarifier) and a total HRT of 2 h, consisting of 0.5 h and 1.5 h for the contact tank and clarifier, respectively.

To protect the downstream MBR process, 2 mm fine screens were provided for the removal of smaller solid particles. The B-stage was operated with a 5-h HRT in the Modified Ludzack – Ettinger (MLE) configuration with a step-feed of 50% influent to the first anoxic zone and the other 50% to the second anoxic zone. A target SRT of 5 days was set in order to maintain the slow-growing nitrifying

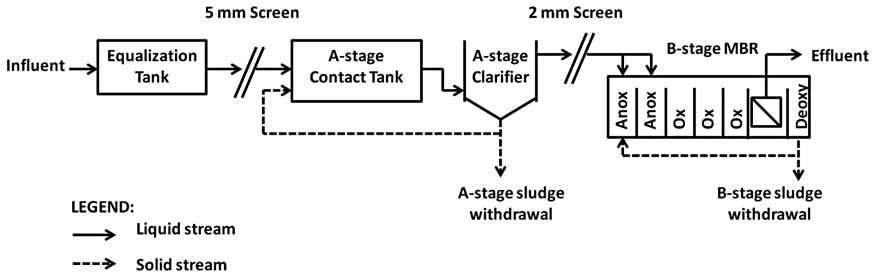

Figure 1 | Schematic diagram of the pilot plant.

organisms for N removal. The mixed liquor suspended solids (MLSS) in B-stage was in the range 0.54–6.1 g/L (Average: 2.2 ± 0.9 g/L). The deoxygenation tank was installed after the membrane tank to deplete the dissolved oxygen (DO) concentration in the mixed liquor prior to recirculation to the first anoxic tank. DO concentrations were maintained at 0.3 and 1 mg O_2/L in the corresponding contact tank and aerobic tanks.

Biochemical methane potential

Biochemical methane potential (BMP) was determined in batch assays using an Automatic Methane Potential Test System (AMPTS II, Bioprocess Control, Sweden). The assay was performed to examine the biodegradability of the A-stage and B-stage sludges through the measurement of its cumulative methane production. The AMPTS reactor was seeded with anaerobic sludge which was collected from a mesophilic digester at Ulu Pandan WRP. The assay was conducted at 35 °C for approximately 28 days. Prior to the assay, the inoculums were degassed at 35 °C for 1 week to eliminate indigenous methane production. Biomedium containing nutrients and vitamin was prepared in accordance with Owen *et al.* (1979). 200 mL of inoculum, 100 mL of substrate, and 50 mL of biomedium were added to each reactor which was subsequently flushed with nitrogen gas at 5 psi for approximately 5 min. For comparison purposes all bottles contained 100 mL of samples. The results were then normalized by dividing by the mass of volatile solids (VS) fed in each bottle and reported as mL CH_4/g VS_{fed}. A batch reactor without substrate addition was used as negative control. Methane produced from that negative control was subtracted from the cumulative methane produced from sludge samples. All assays were performed in duplicate. The biogas composition (CH_4, CO_2 and H_2) was determined using GC (Agilent, USA) with a thermal conductivity detector after the assay was completed as previously reported (Tian *et al.* 2014).

Physicochemical analyses

Sludge samples were taken weekly from the pilot plant during 62 consecutive weeks (from 6 March 2014 to 7 May 2015). Total solids (TS), VS, MLSS, mixed liquor volatile suspended solids (MLVSS) and chemical oxygen demand (COD) concentrations were immediately analyzed in accordance with Standard Methods (APHA 2012). The MLSS concentration was also used in sludge volume index (SVI) measurement (APHA 2012) to determine the compactability and settleability of biomass. Calorific value was determined using an oxygen bomb calorimeter (IKA, Malaysia) to measure the energy content in the sludge. The calorimeter unit consisted of a stainless steel bomb, a water jacket, an ignition unit, a thermometer, and a mechanical stirrer. Internal volume of the stainless steel bomb was approximately 350 mL and the volume of water jacket surrounding the bomb was 2 L. The mechanical stirrer was used to keep the water jacket uniformly mixed. After centrifugation, the biomass pellet was frozen at −20 °C and subsequently freeze-dried at 0.01 mbar vacuum and −45 °C overnight. Next, the dried samples were crushed into powder, weighed and combusted using high pressure oxygen (30 bar) in bomb calorimeter. The temperature rise in the water jacket during combustion was used to calculate the energy content of sludge samples. The heat capacity of the bomb was determined using benzoic acid as a standard (Shizas & Bagley 2004).

Sludge dewaterability was determined using the capillary suction time (CST) test. The test was performed using a capillary suction timer (Part No. 294-50, OFI Testing Equipment, USA) as per manufacturer's instruction. CST represents the rate in seconds at which water permeates through the filter paper, which is a measure of filterability of the sludge cake. Sludge dewaterability in seconds was normalized by dividing by the MLSS (g/L) to obtain the specific CST in $s.L.g^{-1}$. The specific CST value was used in order to be able to compare various sludge samples having different solid contents.

Particle size distribution was analyzed by the laser light scattering technique with the Mastersizer 2000 Particle Size Analyzer (Malvern Instruments, UK). The *zeta* potential was determined using the Zetasizer Nano ZS (Malvern Instruments, UK) to measure the surface charge of the biomass. The measurement was based on the electrophoretic mobility of sludge particles in an electric field.

Adenosine tri-phosphate (ATP) concentration was measured on the same day as the sampling using QuenchGone21™ Wastewater Test Kit following the manufacturer's instructions (LuminUltra, USA). The assay was based on the conversion of chemical energy produced from ATP breakdown during luciferase reaction into light energy. The emitted light was quantified using a luminometer in relative light units which were converted to actual ATP concentrations (ng/mL) after calibration with 1 ng/mL ATP standard. Two independent ATP tests were conducted for each sample, i.e. total ATP and dissolved ATP tests. The total ATP includes ATP from living cells (cellular ATP) and extra-cellular ATP from dead biomass. The dissolved ATP represents the extracellular ATP – ATP released from cells as a stress response or dead cells. Hence, cellular ATP was determined by subtracting dissolved ATP from the total ATP. Additionally, the active biomass ratio was also examined by first converting cellular ATP to the active biomass equivalent. The biomass stress index (BSI) was calculated as the ratio of dead-cell ATP to total ATP (Norman & Whalen 2009).

RESULTS AND DISCUSSIONS

Performance of the AB process

Table 1 presents the performance data of the pilot plant. Incoming total raw wastewater COD varied within a wide range (290–1,900 mg/L) and the COD removal by the A-stage varied generally between 20 and 90% (Average 44.6 ± 16%). The MLSS decreased from 510 ± 250 mg/L on average in the influent to 203 ± 106 mg/L due to biosorption/microbial storage and settling in the A-stage. The MLSS removal by the A-stage was 58% ± 16 on average. These COD and MLSS removals rates are similar to those achieved by conventional primary sedimentation tanks (PST). However, up to 90% COD and 83% MLSS removal was observed in this study on certain days which is not achievable by conventional PST. It has been reported that under optimal conditions COD removal in laboratory scale A-stage can be 70–80% (30% of it is SCOD), while MLSS removals can be as high as 80 to 95% (Zhao *et al.* 2000).

Table 1 | Influent and effluent characteristics of A-stage and B-stage

			Concentration		
	Units	N	min	max	Average ± SD
COD parameters					
Influent COD	mg/L	268	290	1,900	775 ± 280
A-stage effluent COD	mg/L	267	180	1,440	440 ± 145
A-stage COD removal	%	240	10	90.2	44.6 ± 16
B-stage permeate COD	mg/L	108	ND	108	20.5 ± 12
B-stage COD removal	%	98	79.2	99.3	95.1 ± 3
AB Process COD removal	%	98	83.5	99.7	97.2 ± 2
BOD parameters					
Influent BOD	mg/L	77	166	1,331	368 ± 180
B-stage permeate BOD	mg/L	63[a]	2	3.8	2.1 ± 0.4
AB Process BOD removal	%	63	98.7	99.9	99.3 ± 0.3
MLSS parameters					
Influent MLSS	mg/L	95	116	1,960	510 ± 250
A-stage effluent MLSS	mg/L	95	88	1,050	203 ± 106
A-stage MLSS removal	%	95	8.3	83	58 ± 16

N = number of samples; ND = non detected. SD = standard deviation.
[a]14 samples had a non-detectable BOD value (<2 mg/L).

Diamantis *et al.* (2013) reported 80% COD removal in a bench scale A-stage treating municipal wastewater with a lower COD content (400–700 mg/L) than this study. However, previous studies in laboratories sometimes report only the best conditions and should therefore be interpreted with caution. Wett *et al.* (2014) reported 40–85% COD removal from a full scale A-stage unit which is similar to the pilot scale data in this study. Despite some occasional high removals, it seems therefore that the A-stage suffers from greater variability at larger scale.

Furthermore, the large variability obtained in this study may be due to the high oil and grease content in the influent which is specific to municipal wastewater in South East Asia, but also from the sludge recycle from the A-stage clarifier to the contact tank. Oil and grease can inhibit the adsorption of SCOD and colloids because it will adsorb onto bioflocs preferentially due to hydrophobicity. This is

consistent with several literature reports suggesting a poor settling performance of primary sludge and hence limiting application of the AB process (Jenkins *et al.* 2003; Frijns & Uijterlinde 2010).

Nevertheless, the A-stage acted as buffer to remove organics and suspended solids and protect the B-stage against the organic shocks from the incoming wastewater.

Because of the membrane in the B-stage, there were no MLSS in the permeate and COD and BOD concentrations were in the non-detectable range up to 108 mg/L (Average: 20.5 ± 12 mg/L) and 2–3.8 mg/L (Average: 2.1 ± 0.4 mg/L), respectively. The total COD and BOD removals were 97.2 ± 2.2% and 99.3 ± 0.3% on average, demonstrating the high quality effluent obtained from the membrane compartment of the B-stage despite the influent variability.

Physico-chemical characteristics of A-stage and B-stage sludges

Figure 2 shows the process parameters monitored over 62 weeks including VS concentration, VS/TS ratio, soluble COD concentration, and calorific energy content (kJ/g TS) in the sludge withdrawn. Higher fluctuation of VS content was observed in the A-sludge ranging from 0.2 to 14.2 g VS/L, while the fluctuation was less pronounced for the B-sludge (1.5–4.8 g VS/L) (Figure 2(a)). Such variation was attributable to suspended solids load in the influent originating from the bottom of equalization tank at low water level which was pumped into the A-stage contact tank. This was due to the diurnal pattern of municipal wastewater flow that contained high amounts of suspended solids every morning.

Two distinct solids spikes were observed in Figure 2(a); the first spike occurred on week 10 and was caused by influent bypassing the contact tank as a result of pump failure, whereas the second spike on week 61 was due to an extreme solids shock load as elaborated above.

A more dynamic VS/TS ratio (range: 36–84%, average: 66% ± 15.6%) was also noted from the A-sludge in comparison with the B-sludge (range: 65–81%, average: 75% ± 3.5%) (Figure 2(b)). The latter ratio is typical for CAS and has often been reported in full-scale WWTPs worldwide (WRC 1984; Cao *et al.* 2013). The low VS/TS ratio in many A-stage sludge samples indicates a high proportion of inorganic material in the influent. These inert particles do not contribute to sorption or microbial storage mechanisms and this may have negatively affected the biosorption process, which would explain the variability in COD and MLSS removals rates by the A-stage.

There was difference in the SCOD concentration bandwidth for the two sludges. The SCOD were in the range 44–655 mg/L (average: 178 ± 93 mg/L) and non-detectable to 125 mg/L (average: 43.4 ± 27 mg/L) in the A-stage and B-stage sludge, respectively (Figure 2(c)). This highlights again the capacity of the A-stage to absorb organic shocks so that a reduced and relatively more stable load enters the B-stage. The results also show that low SCOD concentrations (<125 mg/L) were detected in the B-stage supernatant. This is relevant since membrane modules (ultrafiltration) are submerged in the B-stage membrane tank and SCOD represent soluble organics that will affect the fouling because they are the same size as the pore diameter (Mei *et al.* 2014). Therefore, a low SCOD concentration is highly desirable in the B-stage to prolong the membrane operation. This information is very relevant for plant operators and further research needs to be carried out to reduce these SCOD levels further in the B-stage, possibly with more baffled compartments and/or plastic biocarriers.

Furthermore, some variation in the calorific content was observed in the A-sludge due the wide variation in VS/TS composition of the sludge (Figure 2(d)). Zanoni and Mueller (1982) reported the average calorific values of 15 and 13.5 kJ/g TS for primary and secondary sludge, respectively, compared with 15.9 and 12.4 kJ/g for Shizas & Bagley (2004), and 18.2 ± 2.3 and 16.8 ± 1.2 kJ/g TS in this study. The difference between this study and the two previous studies could be due to the inherent characteristics of the wastewaters, i.e. high oils content due to Asian cuisine and combination of municipal and a small amount of wastewater from small businesses for this study and completely municipal in nature for the previous studies. It could also be related to the very short HRT applied in this study which preserves the easily biodegradable compounds. As Figure 2(d) demonstrates, the A-stage sludge generally had higher caloric energy content as compared to the B-stage which is consistent with calorific values from conventional primary and secondary sludges reported elsewhere. This indicates that the A-stage sludge contained organic-rich substrates, and, on the other hand, a significant portion of organic content in the B-stage sludge had already been consumed in biological processes.

The SVI is an important parameter used by plant operators to monitor sedimentation tanks in a WWTP. In this study, a relatively narrow SVI range (61–76 mL/g MLSS) was observed for the A-sludge during week 57 to 62, whereas the B-sludge demonstrated a decreasing trend (from 244 to 102 mL/g MLSS) during the test period. This finding, in general, indicated a good settling property of the A-stage sludge, which would be a relevant controlling

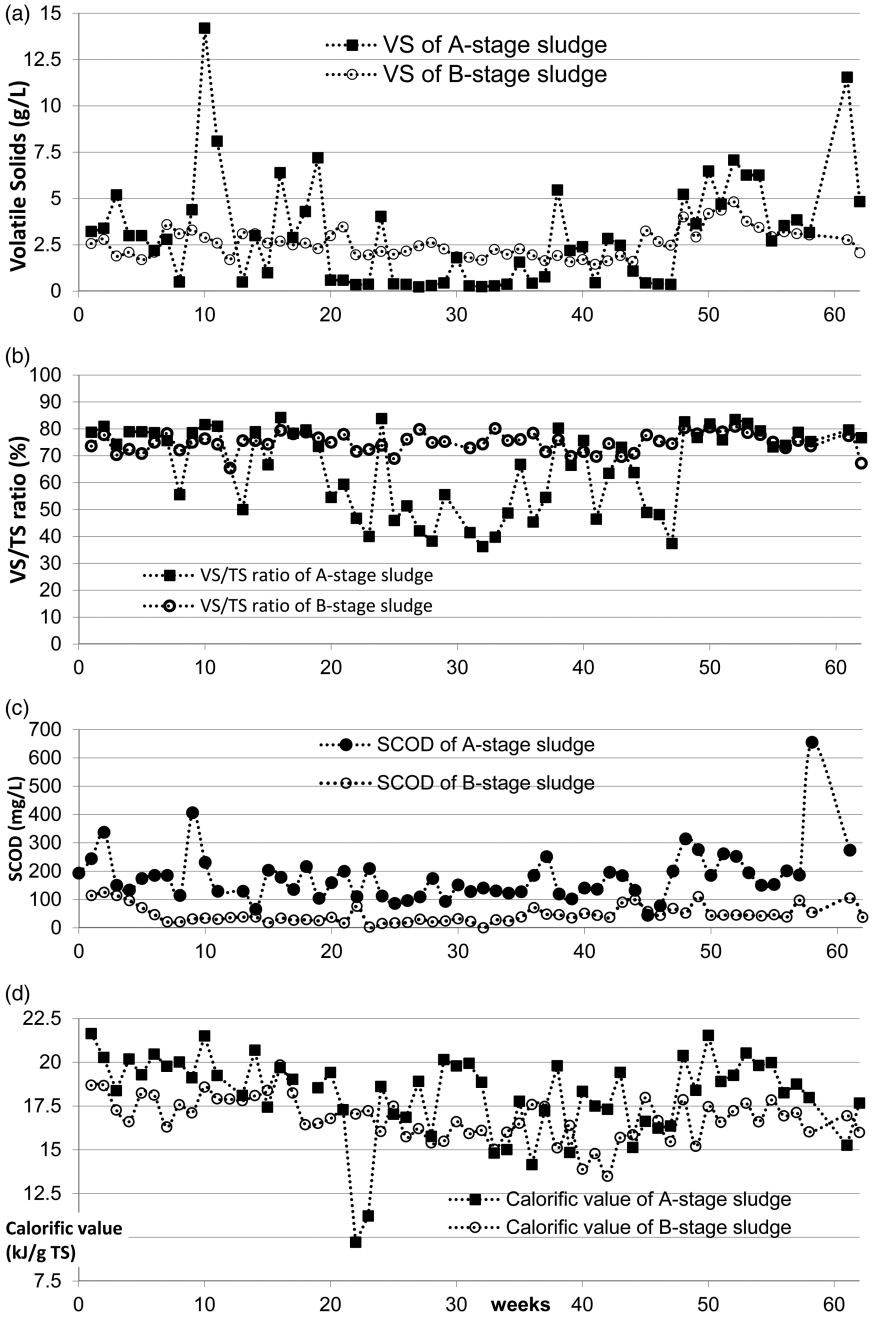

Figure 2 | (a) VS concentrations in A-stage and B-stage sludges. (b) VS to TS ratio in A-stage and B-stage sludges. (c) TCOD and SCOD concentrations in A-stage and B-stage sludges. (d) Calorific values in Joules per gram TS in A-stage and B-stage sludges over the 62 weeks study period.

parameter for the future implementation of the full-scale AB process. The reason for these different SVIs is thought to be due to the presence of some type of extra-polymeric substances (present in raw influent or produced at short SRT) that can increase settleability by enmeshing larger particles together with the bioflocs making them denser. The excretion of microbial products is commonly thought as a stress response of biomass upon environmental changes, high loading or exposure to undesirable toxic/inhibitory substance.

Biochemical methane potential of A-stage and B-stage sludges

The methane yields were in the range 130–775 mL CH_4/g VS_{fed} for the A-stage sludges (Average: 460 ± 152 mL

CH_4/g VS_{fed}) and in the range 120–430 mL CH_4/g VS_{fed} for the B-stage sludges (Average: 256 ± 70 mL CH_4/g VS_{fed}) over the 62 weeks study (Figure 3(a)). The data are plotted in comparison with the corrected calorific value which was defined as the chemical energy content (as measured in kJ) per unit of organic mass (as measured in g VS) calculated using the VS/TS ratio (Figure 2(b)) and the measured calorific value (Figure 2(d)). It is clear from the data in Figure 3 that the methane potential and corrected calorific value followed a similar trend and were consistently higher for the A-stage sludge. From the data obtained in this study, between 14% and 493% more methane (average 97 ± 83%) was obtained from the A-stage sludge in comparison to the B-stage. This was not reported in previous studies because only a single yield taken from a particular day is usually reported. Rapid transfer from aerobic condition in the A-stage to anaerobic conditions preserved the easily degradable organics entrapped in the A-sludge flocs and therefore leading to higher methane yield and corrected calorific values of A-stage sludge. An exception, however, occurred in week 61 where the methane potential of the A-sludge was at its lowest. In week 61, an unusually high solids concentration was observed in the A-stage sludge (Figure 2(a)), which was coupled with a relatively low methane yield – the only measured values which were lower than the B-stage sludge (Figure 3). The BMP curves displayed no lag phase and the hydrolysis constant was generally in the range 0.19–0.23 day^{-1} for both types of sludges indicating no inhibitory compounds. Based on a COD mass balance, the biodegradabilities were found to be 64.2 ± 15.3% and 50.6 ± 14.8% for the A-stage and B-stage sludge, respectively.

In conventional WWTPs, AD is generally applied to mixture of primary and secondary (waste activated) sludge. But waste activated sludge is known to be more difficult to digest than primary sludge (Bougrier *et al.* 2007). For example, Kepp and Solheim (2000) reported a production of methane of 306 mL CH_4/g VS for primary sludge against 146–217 mL CH_4/g VS for secondary sludge. The average methane yield of A-stage sludge in this study was 460 ± 152 mL CH_4/g VS_{fed} which is higher than reported values for conventional primary sludge. A closer look at Figure 3(a) revealed that 16 samples has a BMP close or greater than 600 mL CH_4/g VS_{fed} which is relatively high. This can be due to high oil and grease content in domestic wastewaters especially in Asia. This is linked to the higher calorific

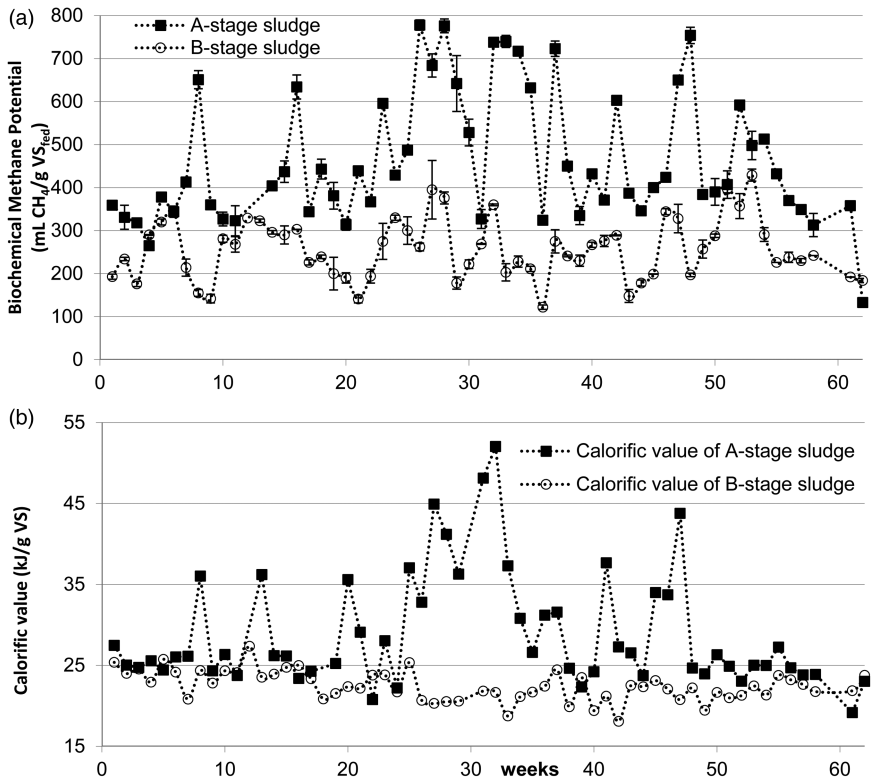

Figure 3 | (a) Comparison between the BMP of A-stage and B-stage sludges. (b) Comparison between corrected calorific value (the biomass-specific calorific value) of A-stage and B-stage sludges over the 62 weeks study.

values of the sludge and can explain the variability in COD and MLSS removals by the A-stage in this study. The occasional presence of oil and grease in the influent may have inhibited the adsorption of SCOD and entrapment of colloids.

Biomass activity

Table 2 presents the comparison of biomass activity between the A- and B-sludge collected in week 62 and their changes following AD. The cellular ATP level reflects the quantity of living biomass and the value was used in the conversion to active VSS and subsequently in the calculation of active biomass ratio. The results showed that the cellular ATP content of A-sludge was significantly lower than the B-sludge prior to AD: 7 ng/mL versus 255 ng/mL. This finding, coupled with higher BSI of the A-sludge (95%), suggested that the biomass experienced a high level of stress in the A-stage. It could be hypothesized that due to the massive solids load, the supposed low-aerobic condition of the A-stage contact tank had turned to a localized anaerobic condition, and leading to a decrease in enzyme activities of the biomass. It can also be related to the very short SRT (0.5 days) and HRT (2 hrs) applied in the A-stage.

On the other hand, the A-stage sludge demonstrated a marginally higher cellular ATP level than the B-stage during post-AD measurement. This observation was also consistent with results of the active biomass ratio wherein the A-sludge contained a significantly lower ratio before AD and a slightly higher post-AD ratio as compared to the B-stage sludge. This could be expected as the A-sludge was rich in carbonaceous materials which were highly biodegradable and hence resulting in a more active biomass in the digester and, as a consequence, higher methane production. This finding indicated that the above ATP-based parameters could be useful in facilitating bioreactor operation, particularly in controlling and maintaining a stable living biomass population as well as in the identification of reactor failure.

Physical properties of sludge before and after AD

Table 3 presents the physical properties of both sludges, namely dewaterability, *zeta* potential, and particle size distribution, and summarizes their changes after AD process. The sludge dewaterability was measured using CST which revealed a better dewatering capacity of B-sludge as compared to the A-sludge before digestion. The inferiority of the A-sludge dewatering property appeared due to the abundance of extracellular organic materials in the liquor which were mostly present in colloidal forms. This finding is in contrast to the typical case of conventional wastewater treatment where primary sludge has been reported to have better dewaterability than secondary sludge. The post-digestion mixed liquor, nonetheless, showed a rather similar CST value which was within 4–5 s.L/g MLSS for both sludges. In general, post-AD sludge dewaterability (CST) was marginally deteriorated and this could be expected as extracellular polymeric substances or EPS were produced during the AD. EPS are highly hydrated and able to bind a large volume of water, therefore contributing to the decrease in dewatering characteristic of the sludge.

The *zeta* potential was measured in weeks 57, 58, 61, and 62. As shown in Table 3, no significant difference in

Table 2 | Comparison and changes in biomass activity of the A-stage and B-stage sludge before and after AD

	A-stage sludge		B-stage sludge	
Parameter	**Before AD**	**After AD**	**Before AD**	**After AD**
Total ATP[a]	138 ng ATP/mL	62 ng ATP/mL	375 ng ATP/mL	53 ng ATP/mL
dATP[b]	131 ng ATP/mL	13 ng ATP/mL	120 ng ATP/mL	11 ng ATP/mL
cATP[c]	7 ng ATP/mL	49 ng ATP/mL	255 ng ATP/mL	42 ng ATP/mL
Active MLVSS[d]	3.4 mg biomass/L	24.5 mg biomass/L	127.3 mg biomass/L	20.8 mg biomass/L
Active biomass ratio[e]	0.025%	0.912%	4.271%	0.755%
BSI[f]	95%	20%	32%	21%

[a]Includes ATP from living cells and ATP released from dead cells.
[b]ATP that was released from the dead cells (extra-cellular ATP).
[c]ATP content of the living cells (cellular ATP)-direct indication of total living biomass. Calculated as total ATP–dATP.
[d]Total mass of living microorganisms. Calculated as cATP*0.5 where 0.5 is an estimated factor to convert from ng ATP/mL to mg solids/L.
[e]% of total MLSS that are living microorganisms. Calculated as Active MLVSS/MLTSS * 100%.
[f]A measure of stress level of the microbial community. Calculated as dATP/total ATP * 100%.

Table 3 | Comparison and changes in physicochemical properties of the A-stage and B-stage sludge before and after AD

	A-stage		B-stage	
Parameter	**Before AD**	**After AD**	**Before AD**	**After AD**
CST (s.L/g)	4.14 ± 0.27	4.57 ± 0.19	2.78 ± 0.24	4.11 ± 0.19
Zeta potential (mV)	−17.9 ± 1.4	−16.8 ± 0.5	−17.5 ± 1.4	−16.4 ± 0.3
Particle size distribution				
Modal value (μm)	33	79	99	79
D10 (μm)	14	26	37	28
D50 (μm)	58	75	96	79
D90 (μm)	359	247	279	211

zeta potential was observed from the A- and B-sludge of both pre- and post-AD. This occurred as (1) both sludge were in their original state due to the absence of any chemical treatment or surface charge manipulation; and (2) the surface active component was not degraded during AD.

Prior to AD, the A-sludge demonstrated a wider range of particle size than the B-sludge (2–1,000 μm and 10–700 μm for A- and B-sludge, respectively). While the post-AD mixed liquor for both sludges demonstrated a more uniform distribution in a similar range (10–500 μm) (data not shown). It was also observed that the modal value of the A-sludge was 33 μm which was much smaller than that of the B-sludge (99 μm) (Table 3). This suggested fine colloidal particles dominating the A-sludge and contributing to its lower extent of dewaterability. Particles represent a dominant component (≤ 85%) of the total COD in sewage and the size is known to affect both biological and physical processes of sewage treatment (Levine *et al.* 1985; Zeeman *et al.* 1997). Particle size can be converted via hydrolysis and this activity contributing to the changes in size distribution after AD.

CONCLUSIONS

The AB process is a two-sludge system designed to capture energy in the first step, the A-stage, such that minimum energy is required in the second step, the B-stage. Due to the membrane in the B-stage, the AB process achieved 97.2% COD removal, 99.3% BOD removal with permeate COD and BOD values lower than 108 mg/L and 3.8 mg/L, respectively. Throughout the 62 weeks study, results showed that the A-sludge was more biodegradable and delivered higher recovery of chemical energy from sewage organics as compared to the more conventional B-sludge. The ATP analysis revealed that cellular ATP content of A-sludge was two orders of magnitude lower than the B-sludge. This translated to a BSI of 95% suggesting that A-stage biomass experienced a high level of stress due to the massive organic load, low HRT and low-aerobic condition.

ACKNOWLEDGEMENTS

The authors would like to thank the Public Utilities Board (PUB) of Singapore for financial support of this research.

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First received 31 May 2016; accepted in revised form 6 July 2016. Available online 23 July 2016

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Manuscript: WST-EM16757

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