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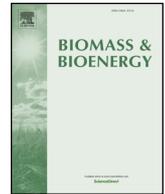
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Research paper

Comparative evaluation of the sludge characteristics along the height of upflow anaerobic sludge blanket coupled ultrafiltration systems

Hale Ozgun^{a,b,*}, Mustafa Evren Ersahin^{a,b}, Zhongbo Zhou^{a,c}, Yu Tao^{a,d}, Henri Spanjers^a, Jules B. van Lier^a

^a Department of Watermanagement, Section Sanitary Engineering, Delft University of Technology, PO Box 5048, 2600, GA, Delft, the Netherlands

^b Istanbul Technical University, Civil Engineering Faculty, Environmental Engineering Department, Ayazaga Campus, Maslak, 34469, Istanbul, Turkey

^c School of Environmental Science and Engineering, Sun Yat-sen University, Guangzhou, 510275, PR China

^d State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin, 150090, China



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ABSTRACT

This study provides a comparison of the sludge characteristics along the height of an upflow anaerobic sludge blanket (UASB) reactor in terms of sludge morphology, activity and stability. The main aim of this study was to identify the best location (i.e. where sludge is of lowest stability and/or highest concentration) in the sludge bed for conveying the sludge from the low temperature UASB reactor to a digester. The sludge profile was investigated by collecting sludge samples along the different heights of the UASB-anaerobic membrane bioreactor treating municipal wastewater. Results showed that total solids and volatile solids concentrations decreased with height, and the highest chemical oxygen demand concentration was observed at the bottom of the reactor. Active biomass remained near inlet of the reactor; whereas, non-active biomass consisted of loose, suspended particles and floculents moved towards the top. This was confirmed by the high specific COD consumption rate near the inlet and poor specific COD biodegradation in the remaining portions of the bioreactor. Apparently, the assumption of a completely mixed sludge bed behavior for the UASB reactor, being part of an AnMBR system, does not hold for this type of reactor systems even at low temperatures, which makes the location in sludge bed from where the sludge is to be conveyed to the digester of operational importance. Considering the observed sludge bed stratification, the sludge to be recirculated from the UASB reactor to the digester is recommended to be taken from 40 to 50% of the sludge bed height.

1. Introduction

Membrane coupled upflow anaerobic sludge blanket (UASB) reactors have been considered as an alternative of interest for the treatment of municipal wastewater in anaerobic membrane bioreactor (AnMBR) systems [1–4]. UASB reactors can be used upfront as biofilters before membrane treatment, which prevents the membrane from excessive exposure to high suspended solids (SS) concentration since the sludge bed would entrap most of the particulate matter by adsorption and biodegradation [5–9]. Enhanced interception of solids in the sludge bed limits the solids and colloidal load to the membrane and thus, membrane flux may become less dependent on the reactor mixed liquor suspended solids (MLSS) concentration, leading to high membrane fluxes [3,10,11].

Results in the study of Ozgun et al. [4] elucidated the potentials of

membrane coupled UASB reactors for the treatment of municipal wastewater under mesophilic conditions. However, under sub-mesophilic conditions, hydrolysis of the retained particulates likely becomes the rate-limiting step and particulate matter accumulation in the sludge bed will occur leading to activity loss [12,13]. Low temperature based limitations in the growth of methanogens will lead to further sludge bed deterioration resulting in a poor soluble chemical oxygen demand (COD) removal, which will lead to more severe pore clogging problems. The latter was confirmed in other studies [13]. Therefore, for municipal wastewater treatment at low and/or fluctuating temperatures, membrane integration to a UASB-Digester system [14] can be an attractive option in order to overcome the hydrolysis limitation induced by low temperature and to reduce non-stabilized particulate matter accumulation in the sludge bed [15,16].

In the UASB-Digester system, the non-degraded particulate COD

* Corresponding author. Istanbul Technical University, Civil Engineering Faculty, Environmental Engineering Department, Ayazaga Campus, Maslak, 34469, Istanbul, Turkey.

E-mail address: ozgunha@itu.edu.tr (H. Ozgun).

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entrapped in the sludge bed of the UASB reactor is further stabilized in a separate completely stirred tank reactor (CSTR) type digester operated under optimal mesophilic conditions (30–35 °C) [14]. By recirculating the digested solids to the UASB reactor, the sludge bed specific methanogenic activity (SMA) is increased resulting in enhanced removal of soluble organics. System performance is affected by several factors related to the sludge recirculation such as sludge characteristics, recirculation rate and sludge volume. Therefore, careful optimization is required in UASB-Digester systems in order to maintain complete conversion of biodegradable dissolved COD.

Sludge characteristics generally change over the height of UASB reactors. Variation of sludge concentration along the UASB reactor depends on several factors such as gas production, COD load per unit area, upflow velocity, hydraulic retention time (HRT) and sludge settling characteristics [17]. As a consequence, a number of studies investigating the effects of applied upflow velocity and/or entrapped or rising gas bubbles on the change in sludge characteristics over the height of UASB reactors have been carried out at bench, pilot and full-scale UASB installations [14,18–23]. Agrawal et al. [18] determined the SS and volatile suspended solids (VSS) concentrations along the reactor height. Maximum SS concentration in the sludge bed was found at 30–70 cm height from the bottom of the reactor, which had a total height of 180 cm. SS concentration was lower at the bottom of the reactor due to the floating mode of sludge related with the occlusion of gas in the bed, especially during the wintertime. Operating a bench-scale UASB reactor treating raw sewage at relatively low temperatures, Mahmoud et al. [14] reported a declining trend in total solids (TS) and volatile solids (VS) values with the increase in sludge bed height and observed a clear solids content stratification around 40% height. Reactor scale effects might have played a role in the obtained results. However, VS/TS ratio was almost constant through the sludge bed height. Uemura and Harada [19], operating a UASB treating sewage, observed an increase in soluble COD at the bottom levels of the sludge bed due to the hydrolysis of entrapped solid organics. However, soluble COD decreased afterwards due to the further progress of methanization.

All these studies about the sludge characteristics were investigated on solely UASB reactors. However, following the results in the study of Ozgun et al. [4], significant changes were observed in the sludge bed when membranes are coupled to UASB reactors due to elimination of hydraulic selection pressure, which avoids the washout of flocculent sludge with poor immobilization characteristics. Membrane incorporation induced an accumulation of fine particles and a decrease in extracellular polymeric substances (EPS) concentration in the sludge. Therefore, a significant change in sludge stratification along the UASB reactor height is expected when membrane units are coupled to the effluent. However, so far, there is no information in the literature available concerning this possible impact.

The main aim of this study is to identify the best location in the sludge bed, i.e. agreeing with sludge having the lowest stability and/or highest concentration, for conveying the sludge from the UASB reactor to the digester. Within this concept, the sludge profile was investigated by collecting sludge samples along the different heights of the UASB reactor in an AnMBR treating municipal wastewater. The sludge characteristics were comparatively evaluated in terms of solids content, PSD, sludge morphology, SMA and stability. Besides, pyrosequencing was carried out for the samples from each location in order to compare the microbial community composition including both archaeal and bacterial communities and the relative abundance of microbial species.

2. Materials and methods

2.1. Wastewater source

Synthetic municipal wastewater was used as feed, having the same composition as the one described in the study of Ozgun et al. [9]. The composition of the concentrated substrate solution and the

Table 1

Composition of the concentrated synthetic municipal wastewater.

Macronutrient Solution			Micronutrient Solution		
Compound	Unit	Value	Compound	Unit	Value
Urea	mg.L ⁻¹	1200	FeCl ₃ .6 H ₂ O	mg.L ⁻¹	1000
NH ₄ Cl	mg.L ⁻¹	2000	CoCl ₂ .6H ₂ O	mg.L ⁻¹	1000
CH ₃ COONa.3H ₂ O	mg.L ⁻¹	7400	MnCl ₂ .4H ₂ O	mg.L ⁻¹	250
Ovalbumin	mg.L ⁻¹	450	CuCl ₂ .2H ₂ O	mg.L ⁻¹	15
MgSO ₄ .7H ₂ O	mg.L ⁻¹	180	ZnCl ₂	mg.L ⁻¹	25
KH ₂ PO ₄ .3H ₂ O	mg.L ⁻¹	1400	H ₃ BO ₃	mg.L ⁻¹	25
CaCl ₂	mg.L ⁻¹	264.9	(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	mg.L ⁻¹	45
Starch	mg.L ⁻¹	6400	Na ₂ SeO ₃ .H ₂ O	mg.L ⁻¹	50
Milk Powder	mg.L ⁻¹	1500	NiCl ₂ .6H ₂ O	mg.L ⁻¹	25
Yeast Extract	mg.L ⁻¹	600	EDTA	mg.L ⁻¹	500
Sunflower Oil	mg.L ⁻¹	1000	HCl 36%	mL.L ⁻¹	0.5
Micronutrients	mL.L ⁻¹	26.6	Resazurin Sodium Salt	mg.L ⁻¹	250
			Yeast Extract	mg.L ⁻¹	1000

Table 2

Characterization of the synthetic municipal wastewater at the inlet of the UASB reactor.

Parameter	Unit	Concentration (Average ± Range)
COD	mg.L ⁻¹	530 ± 30
Soluble COD	mg.L ⁻¹	159 ± 25
Total nitrogen (TN)	mg.L ⁻¹	54 ± 5.2
Ammonium nitrogen	mg.L ⁻¹	36 ± 5.5
Total phosphorus	mg.L ⁻¹	12 ± 0.8
Alkalinity	mg CaCO ₃ .L ⁻¹	300 ± 40
Total Suspended Solids (TSS)	mg.L ⁻¹	230 ± 25
Turbidity	NTU	75 ± 15
pH	–	7.4 ± 0.2

characterization of the synthetic municipal wastewater at the UASB inlet are presented in Table 1 and Table 2, respectively.

2.2. Seed sludge

The reactor was seeded with flocculent anaerobic sludge obtained from a pilot-scale UASB reactor treating black water (Sneek, the Netherlands). The characteristics of the seed sludge are presented in Table 3 [9].

2.3. Experimental setup

The sludge samples were derived from a laboratory-scale AnMBR consisting of a UASB reactor with an effective volume of 7 L coupled to an external membrane module (Fig. 1). Peristaltic pumps (Watson Marlow 120U/DV) were used for circulating the flow between the UASB reactor and the membrane module, and obtaining permeate through the membrane. Furthermore, recirculation over the membrane surface was performed by a peristaltic pump (Watson Marlow 620UN/R) in order to maintain a cross-flow velocity of 1 m s⁻¹ across the

Table 3

Characteristics of the seed sludge.

Parameter	Unit	Value
Total Solids	mg.L ⁻¹	22000 ± 300
Volatile Solids	mg.L ⁻¹	16900 ± 235
Total Suspended Solids	mg.L ⁻¹	20200 ± 75
Volatile Suspended Solids	mg.L ⁻¹	16900 ± 225
COD	mg.L ⁻¹	27100 ± 330
pH	–	7.9
Capillary Suction Time	s	285
Specific Methanogenic Activity	g CH ₄ -COD.g VS ⁻¹ .d ⁻¹	0.3

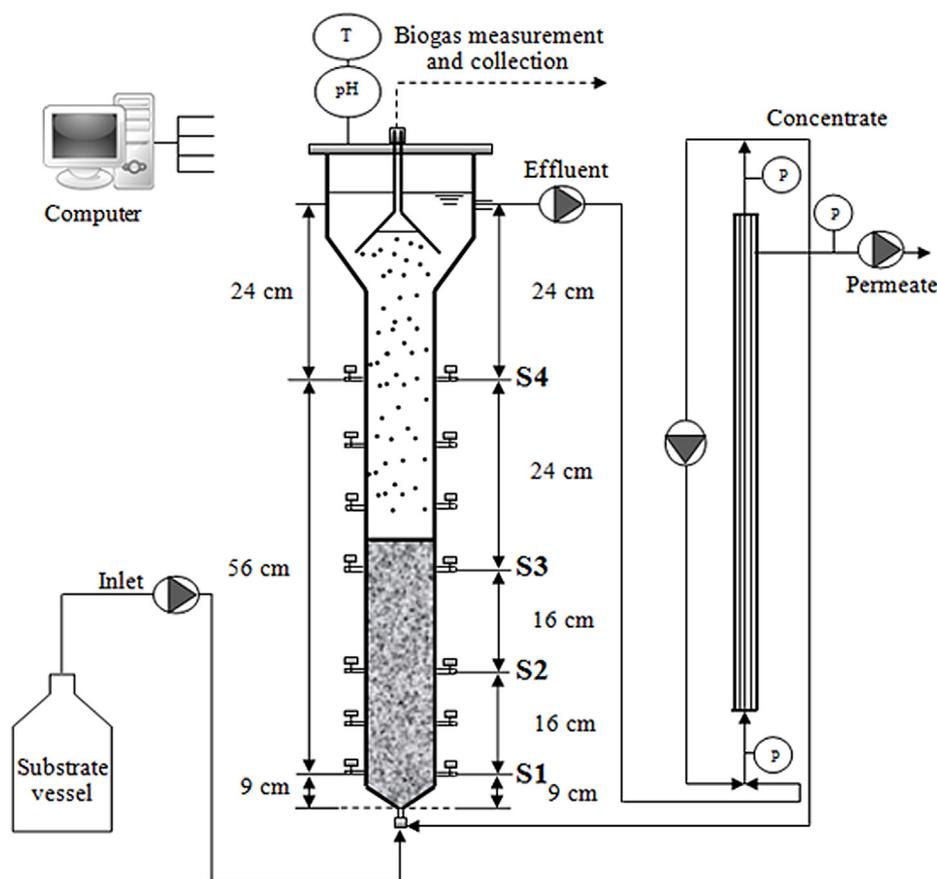


Fig. 1. Schematic overview of the membrane coupled-UASB AnMBR and the sludge sampling ports S1-S4 over the height of the UASB reactor.

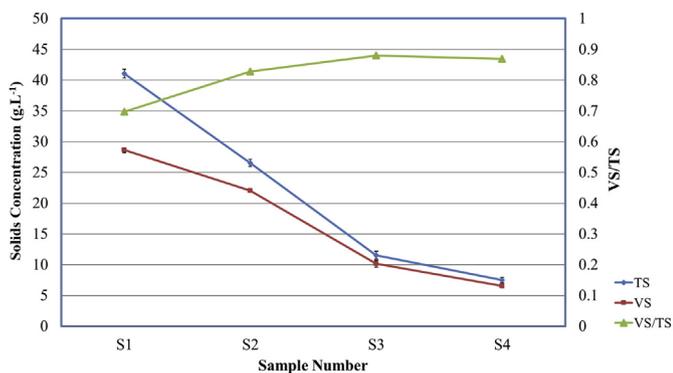


Fig. 2. TS and VS concentrations and VS/TS along the height of the membrane coupled UASB reactor.

membrane surface independently of the circulation flow between the UASB reactor and the membrane module. This concentrate circulation flow-rate was adjusted enabling a stable upflow velocity in the UASB reactor. The biogas was collected by means of a three-phase separator installed at the top part of the UASB reactor. Biogas flow rate was measured with a gas meter (Ritter, Milligas Counter MGC-1 PMMA). Temperature was controlled by means of a water bath (Tamson Instruments, the Netherlands). Temperature and pH inside the bioreactor were monitored on-line by a probe combined with a transmitter (Elscolab, M300 ISM). A computer accessible via LabView software was used to control the pumps and collect the data.

The external cross-flow tubular membrane module contained 28 membrane fibers with an internal diameter of 1.5 mm and a length of 80 cm. Each membrane fiber had 0.0038 m² of total membrane surface area. Pentair X-Flow (Enschede, the Netherlands) PES UF membranes

with a pore size of 30 nm were used in the AnMBR. Pressure sensors (AE Sensors, ATM -800/+600 mbar, the Netherlands) were installed in the membrane feed, concentrate and permeate lines in order to measure the TMP.

2.4. Experimental procedure

Sludge samples were taken from different heights of the UASB reactor on Day 47 and on Day 63 after an operational period of 63 days, representing the middle and end of the experimental period, respectively, in order to study the degree of sludge stratification over the reactor height. Four sampling ports were selected for sludge sampling; the first one at 9 cm above the base of the UASB and the others at 16 cm–24 cm intervals along the height of the reactor (Fig. 1). Sludge samples are denoted as S1, S2, S3 and S4, respectively.

The AnMBR system was operated in a continuous mode at an average HRT of about 6 h and an average organic loading rate (OLR) of 2 kg COD. m⁻³. day⁻¹. The upflow velocity of the UASB reactor was set at 0.6 m h⁻¹ based on results in the study of Ozgun et al. [9] and kept constant during the whole experimental period. pH was in the range of 6.8–7.2. The temperature of the jacketed reactor was controlled at 15 ± 2 °C. Membrane operation consisted of alternating between 3 min filtration and 20 s backwash, at a membrane flux of 12.3 L m⁻² h⁻¹. The cross-flow velocity of the membrane was 1 m s⁻¹.

2.5. Experimental methods

COD, TN, TSS, VSS, TS and VS were measured following Standard Methods [24]. Each analysis was performed in triplicate. The samples for soluble COD measurement were filtered through a 0.45 μm filter.

The PSD of the sludge samples was assessed by using direct image analysis. Image analysis of particles with a size range of 50–5000 μm

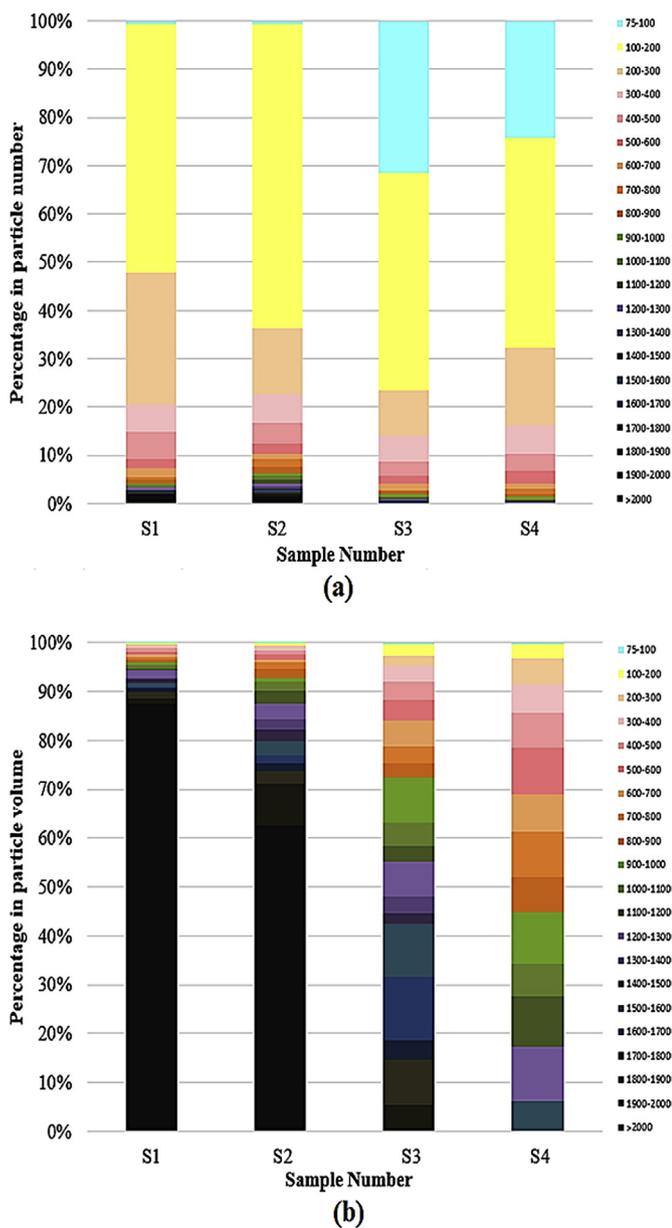


Fig. 3. PSD of the sludge samples in terms of (a) particle number and (b) particle volume along the membrane coupled UASB reactor.

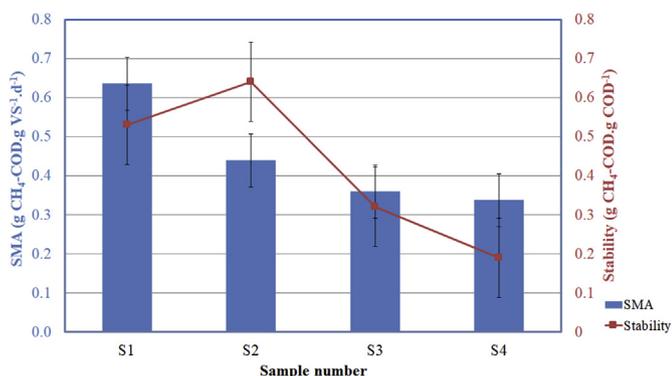


Fig. 4. Morphological changes of sludge samples along the UASB reactor.

was obtained by fluorescence stereo microscope (Leica M205 FA, Germany) with QWin image analyzing software (QG2-32, version V 3.5.1). For assessing the SMP content of sludge samples, a volume of 5 mL sludge was washed by phosphate buffered saline (PBS) (pH = 7.2) and centrifuged at 7000 g for 7 min at 4 °C. The supernatant was filtered using a 0.45 µm filter and the filtrate was collected for soluble microbial products (SMP) analysis. The pellet was re-suspended and vigorously washed with 10 mL PBS and then ultrasonicated at 40 kHz (Cole-Parmer Ultrasonic, the Netherlands) for 3 min. A high speed centrifuge (17000 g) was applied for 20 min under 4 °C and the supernatant obtained was filtered using a 0.45 µm filter for EPS assessment. The phenol-sulfuric acid method was used to quantify polysaccharides [25]. The concentration of protein was determined using the Bradford Method [26].

Automated methane potential test system (AMPTSII) (Bioprocess Control, Sweden) [27] was used to determine SMA of the sludge samples. SMA tests were carried out at 35 °C in 500 mL serum bottles (with a working volume of 400 mL). Sludge and growth medium were included in the serum bottles. Amounts of the sludge and growth medium added into the bottles were determined by the ratio of sludge VS to substrate COD, which was 2:1. The growth medium for the blanks consisted of a mixture of macronutrients, trace elements and phosphate buffer solution [4]. The growth medium for the samples additionally included 0.5 g COD. L⁻¹ as sodium acetate. The nutrient stock solution consisted of (g.L⁻¹): NH₄Cl (170), CaCl₂.2H₂O (8), MgSO₄.7H₂O (9), CoCl₂.6H₂O (2), MnCl₂.4H₂O (0.5), CuCl₂.2H₂O (30), ZnCl₂ (50), H₃BO₃ (50), (NH₄)₆Mo₇.O₂.4H₂O (90), Na₂SeO₃.5H₂O (100), NiCl₂.6H₂O (50), EDTA (1), HCl 36% (1 mL L⁻¹), Resazurine (0.5). pH buffer stock solution was composed of K₂HPO₄.3H₂O (45.65 g L⁻¹) and NaH₂PO₄.2H₂O (31.20 g L⁻¹).

Specific ultimate methane production was measured to determine the stability of sludge samples. Stability tests were carried out in 120 mL serum bottles at 35 °C without substrate addition in order to degrade the accumulated particulate matter. The anaerobic medium was prepared by dissolving 3.5 g L⁻¹ sodium bicarbonate (NaHCO₃) in tap water without the addition of extra nutrients. The head space of the bottles was flushed with mixture of N₂:CO₂ (70:30%). Degradable compounds were converted to biogas during the stability test. A pressure transducer (Centre Point Electronics PSI-30) was used to measure pressure increase in order to monitor the biogas production. The bottles were sealed with butyl rubber stoppers and aluminium caps. Stability tests lasted till the cumulative biogas production reached to a plateau. Stability of the sludge was calculated by dividing the ultimate methane produced to COD of sludge. A high stability value indicates poorly stabilized sludge, which means that high amount of anaerobic biodegradable organic compounds is still present in the sludge.

454-pyrosequencing was used to investigate microbial community structure. UASB sludge samples were taken from different sampling ports (S1-S4) over the height of the UASB reactor. All samples were washed twice with PBS and then centrifuged at 10000 x G for 3 min. To reduce biomass decay during sludge storage, the supernatant was removed. All samples were stored at -25 °C until deoxyribonucleic acid (DNA) extraction [4].

A MoBio UltraClean Microbial DNA isolation kit (MoBio Laboratories, Inc., CA, USA) was used to extract 16S recombinant deoxyribonucleic acid (rDNA) of sludge samples following the protocol suggested by the manufacturer. A combination of heat, detergent and mechanical force was applied in order to increase the efficiency in DNA isolation process. To enhance the lysis efficiency of microbial cells, a minor modification including twice bead-beating (5 min) and heating (65 °C, 5 min) was applied to the protocol in sequence. DNA isolation was confirmed by agarose gel electrophoresis. Nanodrop 1000 equipment (Thermo Scientific, Waltham, MA, USA) was used to measure the concentration of DNA.

Roche 454 GS-FLX system (454 Life Science, Branford, CT, USA)

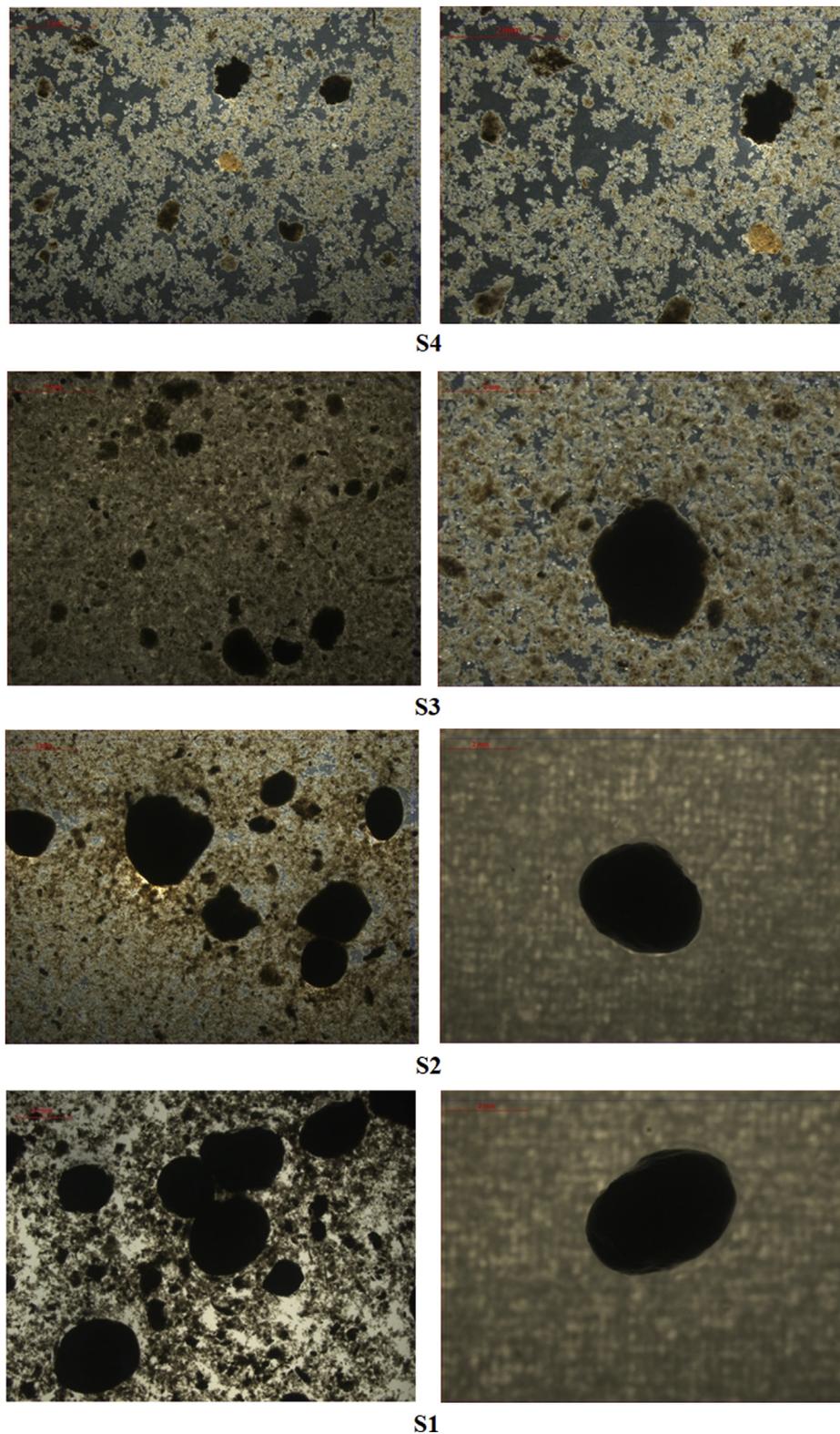


Fig. 5. SMA and stability of sludge samples along the height of the membrane coupled UASB reactor.

with titanium chemistry was used for pyrosequencing the 16 S rDNA gene at Research and Testing Laboratory (Lubbock, TX, USA). Universal primers U515F (GTG CCA GCM GCC GCG GTA A) and U1071R (GAR CTG RCG RCR RCC ATG CA) [28] were used. Forward and reverse primers can cover more than 90% bacterial and archaeal 16 S rDNA by testing on Ribosomal Database Project (RDP) [29].

Different programs from the Quantitative insights into microbial

ecology (QIIME) pipeline, version 1.6.0 [30] were combined in order to analyze the pyrosequencing results, including chimera removal, taxonomic classification and microbial diversity calculation (Chao1 richness, Shannon index and observed species number). The communities' biodiversities within a particular ecosystem were assessed based on α -diversity, that includes four indices: Shannon index, Chao1 richness, phylogenetic diversity and observed species number. The richness was

Table 4
SMP and EPS contents of the sludge samples along the membrane coupled UASB reactor height (Average \pm Standard Deviation).

Time	SMP		EPS	
	Protein (mg.g VSS ⁻¹)	Polysaccharide (mg.g VSS ⁻¹)	Protein (mg.g VSS ⁻¹)	Polysaccharide (mg.g VSS ⁻¹)
S1	1.89 \pm 0.05	1.01 \pm 0.09	3.79 \pm 0.10	2.10 \pm 0.14
S2	2.10 \pm 0.03	1.38 \pm 0.04	2.48 \pm 0.03	0.90 \pm 0.05
S3	3.28 \pm 0.19	1.52 \pm 0.25	2.07 \pm 0.07	0.80 \pm 0.11
S4	3.30 \pm 0.08	2.62 \pm 0.11	1.77 \pm 0.08	0.80 \pm 0.13

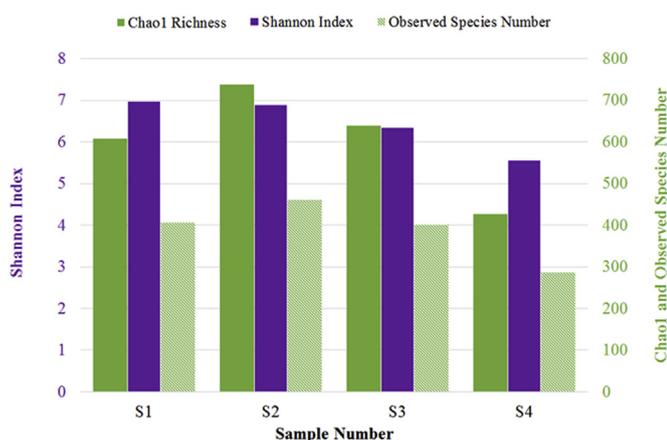


Fig. 6. Alpha-diversity index of each sludge sample along the UASB reactor.

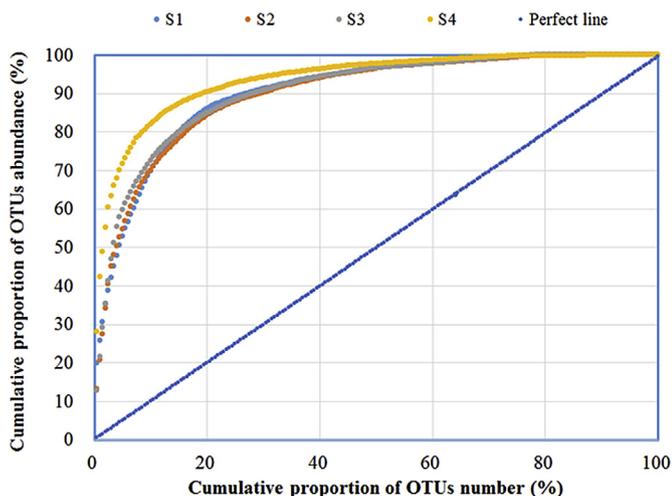


Fig. 7. Lorenz distribution curves of each sludge sample along the UASB reactor.

indicated by Phylogenetic Diversity Index, Chao1 richness and observed species number, while the evenness was shown by Shannon Index [13,31].

3. Results and discussion

Fig. 2 presents the profiles of sludge characteristics along the height of UASB reactor in terms of TS and VS. VS/TS increased from 0.70 ± 0.01 for S1 to 0.88 ± 0.01 for S3, followed by a slight decrease to 0.87 ± 0.02 for S4. The relatively low VS/TS ratio observed at the bottom of the UASB reactor might be attributed to an inorganic sedimentation layer being present there. The maximum concentration of TS in the sludge bed was found to be 41 g L^{-1} at the bottom of the reactor, whereas a decrease was observed at the higher part of the

reactor, which is consistent with the results of Mahmoud et al. [14] treating sewage in a UASB and a UASB-Digester system. However, in contrary to the variations in VS/TS in this study, constant VS/TS ratio was observed along the sludge bed height in the study of Mahmoud et al. [14], which was performed with a sole UASB without membrane. The TS concentration decreased to 8 g L^{-1} in S4. The VS-TS as well as VSS-TSS showed a declining trend in concentration from bottom to top of the UASB with clear stratification at about 46% height of the bottom of the reactor. Highest TSS and VSS concentrations were obtained at the bottom part of UASB reactor followed by a decrease with height, which is in agreement with the results of Singh et al. [32]. Besides, a significant increase in the VSS/TSS ratio from 0.73 ± 0.02 for S1 to 0.97 ± 0.004 for S3 was observed, followed by a slight decrease to 0.93 ± 0.01 for S4. The total COD concentrations decreased from 46 g L^{-1} for S1 to 9 g L^{-1} for S4, in agreement with the TS(S) and VS(S) concentrations.

The number and volume based PSD of each sludge sample are depicted in Fig. 3(a) and Fig. 3(b), respectively. A different size distribution was obtained for each of the sludge samples from different heights. Fig. 3(b) shows that, 85%–90% of the total particle volume for S1 are bigger than 2 mm. The smaller particles with diameters below $100 \mu\text{m}$ represented only 1% of the total volume of particles for S1, but made up a large fraction of the total number in the sludge. An increase in the height led to a shift in the size distribution, with an increase in the volume of smaller sized particles. Fraction of smaller particles with diameters below $100 \mu\text{m}$ increased to 24% of the total volume of particles for S4 and particles bigger than 1.6 mm are not present for S4. Overall, the general trend in size distributions was very similar in both types of PSD graphs with the majority of bigger particles contributing to 85–90% of the total volume at lower levels in comparison to upper levels. The results of PSD analysis along the UASB height are in agreement with the results of Sponza [20], which also confirmed the decrease in particle size in the upper part of the UASB reactor.

Fig. 4 shows the results of stability experiments along different heights of the UASB reactor, which were performed in order to determine whether the sludge was equally stabilized over the bed or not. According to the results, the sludge stability decreased from $0.53 \text{ g CH}_4\text{-COD.g COD}^{-1}$ for S1 to $0.64 \text{ g CH}_4\text{-COD.g COD}^{-1}$ for S2. Following that decrease, an increase in stability was observed for S3 and S4. So according to the sludge stability tests, S2 contains more biodegradable solids accumulated in the sludge bed, which makes it favourable as an extraction point of sludge for the coupled digester.

Morphological changes of the sludge were also visualized along the height of membrane coupled UASB reactor (Fig. 5). More circular flocs with smoother surface and a larger diameter were observed at the lower levels, whereas smaller flocs with rough surface existed at the upper levels, which supported the PSD results.

SMA was also performed for each sludge sample (Fig. 4). According to the results; SMA values of S1 and S2 are higher than those of S3 and S4, which indicates that the biomass in the lower levels is more active than that in the upper level. Highest SMA value of $0.64 \text{ g COD.g VSS}^{-1} \cdot \text{d}^{-1}$ was observed for the sludge collected from the bottom. Following that, SMA decreased gradually to $0.44 \text{ g COD.g VSS}^{-1} \cdot \text{d}^{-1}$ for S2 and then remained constant thereafter for S3 and S4. Large particle sizes may reduce the SMA due to enhanced mass transfer limitations [33]. However, SMA values were found to be higher at the low part of the sludge bed that was characterised by a large particle size. The highest value at 9 cm may be ascribed to the contribution due to the effect of properly controlled wastewater flow, growth of hydrolytic microorganisms capable of decomposing the solid organics accumulated in the reactor bottom part and population shift.

SMP and EPS concentrations of the sludge samples along the UASB reactor height are presented in Table 4. These samples contained cells, cell flocs bound by EPS, SMP, and un-degraded or partially degraded particulate matter. The protein contents of SMP and EPS were found higher than their polysaccharide contents for all of the sludge samples.

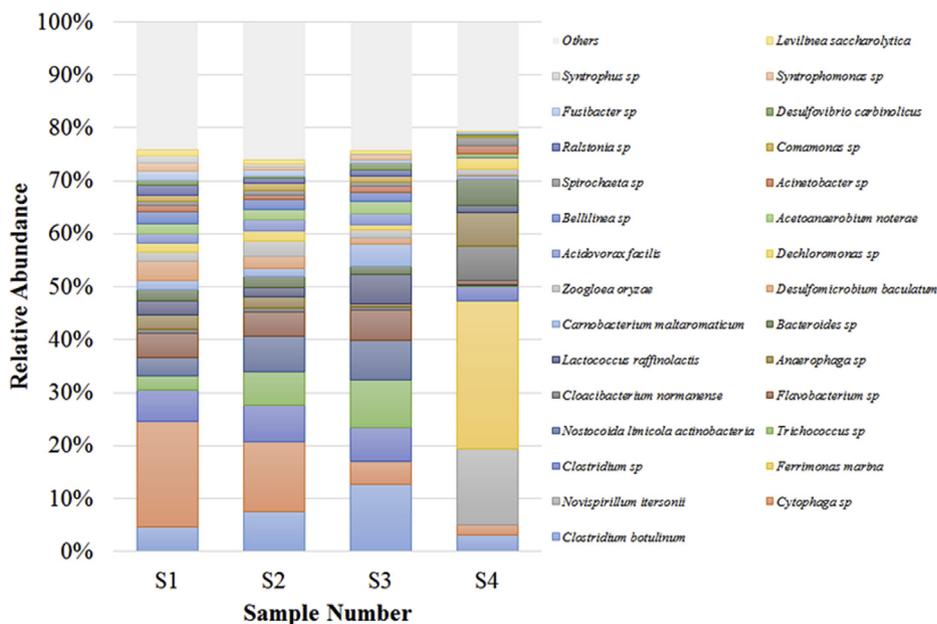


Fig. 8. Relative abundance of major bacterial genera.

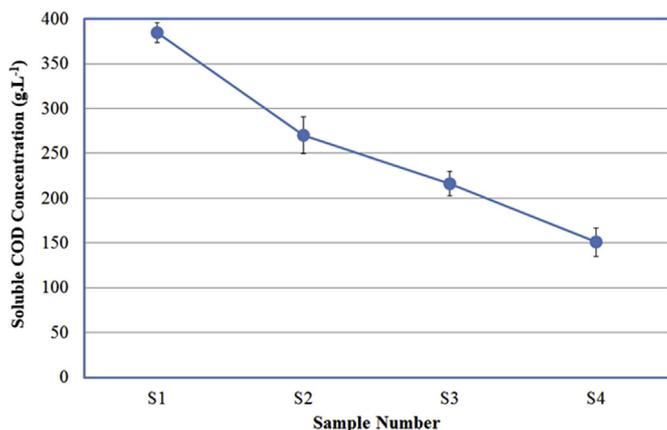


Fig. 9. Soluble COD concentration along the height of the membrane coupled UASB reactor.

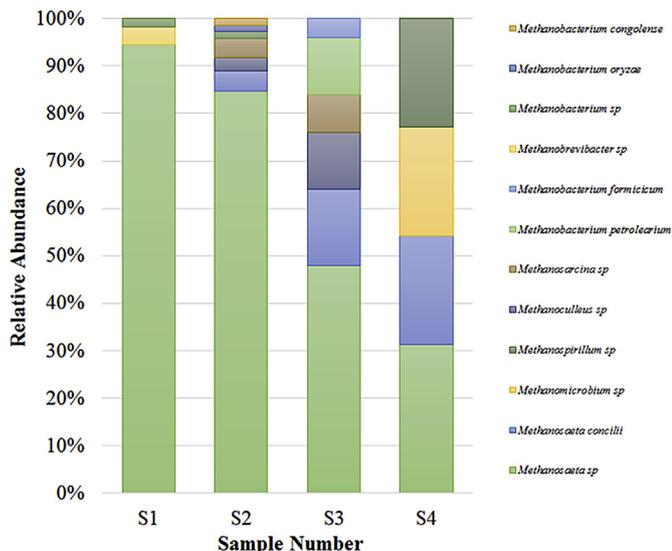


Fig. 10. Relative abundance of all archaeal species.

Higher protein content may be attributed to the presence of exo-enzymes in the sludge flocs and cell lysis compounds [34]. An increasing trend was observed for SMP concentration with the increase in reactor height. Drews et al. [35] indicated that a drop in SMP content can occur due to biodegradation in a MBR. Therefore, SMP increase at higher levels might result from the lower degradation rates observed with the increase in height. Protein and polysaccharide content in the SMP were $1.89 \text{ mg g}^{-1} \text{ VSS}$ and $1.01 \text{ mg g}^{-1} \text{ VSS}$ for S1, which increased to $3.30 \text{ mg g}^{-1} \text{ VSS}$ and $2.62 \text{ mg g}^{-1} \text{ VSS}$ for S4, respectively. However, a decreasing trend was observed for EPS compositions, with the highest concentrations at the down part of the sludge bed, coinciding with the dominance of large particles (Fig. 3).

16S rDNA was extracted from sludge taken from the four sampling points. A total of 10078 sequences were retrieved by 454-tag Pyrosequencing. The clone libraries showed marked differences in microbial community composition at different reactor heights. Remarkable differences in microbial community structure were found between S1-S3 and S4, while such differences between S1, S2 and S3 were small. Details of bacterial and archaeal abundance can be seen in Table S1 and S2, respectively.

The peaks of alpha-diversity index (Shannon, Chao1 and OSN) appeared for S2 (Fig. 6), indicating the most diverse microbial community at the second bottom sampling port of the UASB. The index of S1 and S3 were slightly lower than S2. The alpha-diversity index of S4 was lowest compared to the other three sludge samples, and decreased by 42% (Chao1), 38% (OSN) and 20% (Shannon) compared to the corresponding maximum. This means that the UASB biomass from the top part of the sludge bed had a much less diversity than the middle and bottom biomass. Fig. 7 presents the Lorenz distribution curves based on the abundance of each operational taxonomic unit (OTU) that originated from pyrosequencing of 16S rDNA of the communities of different heights of UASB. The 45° diagonal is the theoretical perfect evenness line representing an absolutely even microbial community. A curve further away from the diagonal has a lower evenness or higher dominance since evenness quantifies how relative abundance is distributed among species [36]. The Lorenz distribution curves showed that the biomass community of S4 was less even than the other three positions, meaning that several species greatly dominated the upper part of UASB microbial community. The biomass community of S4, dominated by less species, can be considered less diverse in comparison to the ones in which several different species have a similar abundance.

The abundances of Bacteria and Archaea were quantified along the height of the reactor. Remarkable increasing/decreasing trends of several bacterial and archaeal species were discovered along the height of UASB from bottom to top. However, the compositions of the microbial community for S1, S2 and S3 were markedly different from that of S4. For example, the number of *Cytophaga* clones decreased dramatically from 19.9% for S1 to 2% for S4. The most abundant *Clostridium*-related OTU obtained for S1, S2 and S3 was also observed for S4, but the number of clones was significantly less. Instead, OTUs similar to *Ferrimonas marina* (28%), as well as *Novispirillum itersonii* (14.3%) and *Cloacibacterium normanense* (6.4%) appeared to be dominant for S4 (Fig. 8, Table S1).

Likely, availability of specific substrates and/or physical selection had contributed to the varied microbial communities along the UASB height. For instance, as typical primary fermenting bacteria, *Cytophaga* sp. can grow competitively under nutrient rich environments [37,38]. The relative abundance of *Cytophaga* sp. decreased by 90% from bottom to top. This can be related to the gradient decrease of substrate bio-availability (i.e. COD, Fig. 9). The drop in *Cytophaga* sp. abundance in the upper part of the UASB might be related to the lack of granules there. PSD results (Fig. 3) and microscopic pictures (Fig. 4) showed much more granular sludge in the bottom part of the UASB and nearly no granules were found in the middle part. Abundant growth of acetogenic bacteria such as *Cytophaga* sp. is often related to granular biofilms and its abundance decreased when the granules are deteriorated [39].

Water quality profile for soluble COD is shown in Fig. 9. Influent has an average soluble COD concentration of 159 mg L⁻¹. This increased to 385 mg L⁻¹ at a height of 10 cm from the bottom, likely due to hydrolysis of entrapped solid organics that accumulated in the lower portion of the reactor and then decreased along the reactor height due to further progress of methanization. As suggested by the water quality profiles, most of the organic matter in the wastewater was degraded in the bottom part of the reactor, implying that the dominant phylotypes in the bottom part of the UASB could be responsible for removing organic matter. Higher soluble COD removal in the lower granular sludge bed zone was also observed in the study of Ahn et al. [40]. The same authors also measured higher specific uptake rates near the inlet compared to other volume fractions, indicating accumulation of active biomass near the reactor inlet [40]. The most abundant OTU in the S1 belonged to the genus *Cytophaga* (Fig. 8). This OTU, that represented approximately 20% of all of the clones from S1 (344/1721 clones), decreased with reactor height, i.e. 236/1798, 70/1662 and 43/2034 clones in S2, S3, S4, respectively. This drop coincided with the observed soluble COD decrease along the height of the reactor (Fig. 9).

The variation in sludge composition is also illustrated by the variation in archaeal communities over the height of the sludge bed (Fig. 10, Table 2). Species belonging to genus *Methanosaeta* were the pre-dominant archaeal species in the lower parts (S1 and S2) of the UASB, but their abundance decreased to 1/2 for S3 and even down to 1/3 for S4. As a typical acetoclastic methanogen, the growth of *Methanosaeta* sp. is highly related to the presence of acetate and its availability to biomass [41,42]. High abundance appeared in the bottom part, coinciding with the likely presence of the methanogenic precursor acetate that is generated by acidogens and acetogens converting influent organic matter. In addition, *Methanosaeta* sp. are commonly identified as the predominant acetate consuming methanogen in granular sludge, which was particularly present at the bottom of the UASB reactor (Figs. 3 and 4) [39,43–46].

4. Conclusions

Sludge bed stratification manifested in a UASB reactor coupled to a UF membrane filtration unit. Results showed striking differences in sludge characteristics at different reactor heights along the UASB reactor. Even though membrane incorporation led to a deterioration in

sludge bed settleability, the reactor biomass became segregated into several zones. The lower zone had dense granulated biomass, whereas the upper zone had loose active and nonactive biomass. Analysis over the height of the reactor showed that the TS, VS, TSS and VSS concentrations in the reactor decreased with increased height, and highest COD concentration of 46 g L⁻¹ was found at the bottom of the reactor. Image analysis showed significant differences in particle size between each sampling port. Results clearly showed that the highest SMA was obtained with the sludge taken from the bottom parts. The most active biomass remained near the inlet of the reactor; whereas, non-active biomass consisting of loose, suspended particles and flocs were present at the top. Comparative sequence analysis at the phylum level revealed changes in microbial community composition with reactor height. A high diversity of microbial species were present in the lower part of the reactor, whereas less diversity was observed in the middle and upper parts. The observed sludge bed stratification contradicts the pre-assumption that the UASB sludge bed is fully mixed when coupled to a membrane unit in an AnMBR setup. The findings indicate that sludge conveyance from the low temperature UASB reactor to the parallel operating digester for sludge stabilisation is location specific. Considering the low sludge stability and solids content stratification, the sludge to be recirculated from UASB reactor to digester is recommended to be taken from 40 to 50% of the sludge bed height. Overall, this study provided a better understanding about the variations of sludge characteristics in UASB reactors in AnMBRs, which might lead to an increased applicability of this promising technology for the treatment of municipal wastewater.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biombioe.2019.04.001>.

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