Process Safety and Environmental Protection 131 (2019) 235–245

Contents lists available at ScienceDirect

Process Safety and Environmental Protection

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

Co-digestion of tannery waste activated sludge with slaughterhouse sludge to improve organic biodegradability and biomethane generation

A.B. Mpofu[a,b], O.O. Oyekola[a], P.J. Welz[b,*]

[a] Department of Chemical Engineering, Cape Peninsula University of Technology, Bellville Campus, Symphony Way, Cape Town, 7535, South Africa
[b] Institute of Biomedical and Microbial Biotechnology, Cape Peninsula University of Technology, Bellville Campus, Symphony Way, Cape Town, 7535, South Africa

ARTICLE INFO

Article history:
Received 22 July 2019
Received in revised form 4 September 2019
Accepted 16 September 2019
Available online 18 September 2019

Keywords:
Co-digestion
Tannery waste activated sludge
Slaughterhouse sludge
Inhibition
Optimization
Kinetics

ABSTRACT

A novel approach was used to overcome inhibition of anaerobic digestion of tannery waste activated sludge by co-digesting with slaughterhouse sludge. The seasonal characteristics of the tannery sludge varied significantly. Macronutrient ratios were typically not ideal for anaerobic digestion, mainly due to an over-abundance of nitrogen. In addition, potentially inhibitory concentrations of metals were present. Optimal co-digestion with 50% (vol./vol.) slaughterhouse sludge achieved a CH_4 yield of 215 $mLCH_4$/gVS, and biodegradability of 55.7% VS, 45.5% TS and 48.2% COD. This resulted in a synergistic increase of ≥59.4% in biodegradability and 156% in CH_4 yield compared to mono-digestion of tannery waste activated sludge. Furthermore, the regression of the cumulative CH_4 yield using the Logistic, Cone and modified Gompertz model (adj $R^2 \geq 0.998$) showed an increase of 20.4–21.9%, 58.6–252%, and 164% in the ultimate CH_4 yield, maximum CH_4 production rate and the specific rate constant respectively, and 59.3% reduction of the lag phase. An alternative cleaner production technique that vertically integrates the value chain improves solid waste management and provides an income stream was demonstrated through this co-digestion strategy.

1. Introduction

The industrial processes involved in tanning are waste intensive, and the industry faces escalating costs for wastewater remediation and disposal of hazardous solid waste (Buljan and Král, 2015; Subramani and Haribalaji, 2012). Primary solid waste consists of fleshings, trimmings, and primary wastewater sludge (TPWS). The latter emanate from physical settling of solids or physicochemical treatment processes employed to reduce the concentrations of hazardous components, most notably sulphide (S^{2-}) and chromium (Cr), from effluent streams (Swartz et al., 2017).

The conventional activated sludge (CAS) process is typically employed to reduce the organic load of tannery effluent after primary treatment, but the process is susceptible to operational instability, bulking and foaming (Buljan and Král, 2011). In addition, large quantities of tannery waste activated sludge (TWAS) are generated, which may be laden with toxic metal salts, organics, and inorganic substances, including Cr, sodium (Na), chlorides (Cl), nitrogen (N), sulfates (SO_4^{2-}), S^{2-}, ammonia (NH_3), lipids, dissolved solids, and suspended solids (Buljan and Král, 2015; Subramani and Haribalaji, 2012; Swartz et al., 2017).

Anaerobic digestion (AD) is widely used to reduce the volume and stabilise waste activated sludge (WAS) from domestic wastewater treatment facilities. However, although TWAS contains high concentrations of suitable organic substrates, the AD process is inhibited by high concentrations of metals, SO_4^{2-}, S^{2-}, and long chain fatty acids (LCFA), and accumulation of volatile fatty acids (VFAs) and NH_3 [consequent to the inherent low carbon to nitrogen (C:N) ratios] (Akyol et al., 2014; Sri Bala Kameswari et al., 2014; Thangamani et al., 2009; Zupančič and Jemec, 2010). Although some biogas may be generated via AD, the TS is not significantly reduced (Akyol et al., 2015).

In attempts to overcome process inhibition, studies have been conducted on anaerobic co-digestion (AcoD) with high C:N wastes such as sewage sludge (C:N 25) and manure (C:N 20) (Sri Bala Kameswari et al., 2014; Basak et al., 2014; Shanmugam and Horan, 2009). When choosing a co-substrate, it is important to consider

* Corresponding author.
E-mail addresses: ashtonmpofu@gmail.com (A.B. Mpofu), oyekolas@cput.ac.za (O.O. Oyekola), welzp@cput.ac.za (P.J. Welz).

https://doi.org/10.1016/j.psep.2019.09.018
0957-5820/

that AcoD with protein/nitrogen or lipid rich-waste may lead to ammonia or LCFA inhibition, respectively, and/or ammonia–LCFA synergetic co-inhibition (Girault et al., 2012; Wang et al., 2016). In addition, in order to be cost-effective, the origin of a co-substrate should be in close proximity to the tannery concerned.

Studies focussing on AcoD of different forms of tannery solid waste with tannery wastewater sludge (TWS) have been conducted (Table 1). It is clear that a suitable inoculum is required to maximise process efficiency. For example, in studies focussing on AcoD of sludge with leather shavings, extensive retention times of >169 days were required, and low methane yields were achieved when no inoculum was employed (Agustini et al., 2017, 2018), but when digestate from AD of SO_4^{2-} rich industrial effluent was employed as an inoculum, good biodegradability and specific biogas production rates were achieved with short retention times (20 days) (Di Berardino and Martinho, 2009). Combined with a suitable inoculum, optimisation of the inoculum to substrate ratio (ISR), substrate to substrate ratio (SSR), organic loading rate (OLR) and temperature, and long term acclimation are also key for efficient AD. In a study using domestic WAS as an inoculum, it was found that: (i) increasing the ISR to 1 improved biogas generation rates, but increases thereafter did not warrant the additional reactor footprint required, (ii) biogas production and %VS reduction improved at higher TPWS/TWAS ratios, which was assumed to be because of the presence of higher amounts of recalcitrant exo-polysaccharides in the TWAS, and (iii) biogas production increased with successive runs in a semi-continuous reactor (Sri Bala Kameswari et al., 2014, 2012; Sri Bala Kameswari et al., 2011). In un-replicated, small-scale (70–110 mL) batch experiments using the combined digestate of limed fleshings, TPWS and cow dung as the inocula, greater specific biogas generation [5.9 mL/g total volatile solids (TVS)] was achieved by increasing the loading rate from 1.58 to 5.19 g/100 mL), despite high concentrations of VFAs (>10 000 mg/L), ostensibly due to microbial acclimation to VFAs (Thangamani et al., 2009). The authors later achieved >10 fold higher specific gas generation when the retention time (RT) was increased, but the published results are difficult to interpret because the concentrations of TVS were anomalously higher than the TS concentrations (Thangamani et al., 2010). In another study, it was concluded that thermophilic conditions are critical for AD of fats from tannery fleshings because high specific methane generation (596 mL/g TVS) was achieved after 100 day start-up of a thermophilic anaerobic sequencing batch reactor operated with optimised OLR (Zupančič and Jemec, 2010).

To our knowledge, apart from fleshings and shavings, no other readily available (on-site) substrates have been used for the AcoD of TWS (TPWS/TWAS). Primary sludge from slaughterhouse effluent (SHS) is available at some tanneries that are situated adjacent to slaughterhouses because it vertically integrates the value chain and promotes cleaner production by circumventing the need for preserving skins/hides in NaCl. In this study, it was hypothesised that SHS may be a viable co-substrate for AcoD of TWAS because it: (i) is readily available in large quantities on-site at tanneries with integrated slaughterhouses, (ii) has a good biomethane potential (BMP) due to a high organic content (Pitk et al., 2013) (iii) contains lower concentrations of inhibitory substances compared to TWAS (Mpofu, 2018), and (iv) is currently being disposed to landfill. The practical emphasis of this study is to reduce the amount of TWAS and SHS sent to landfill, with biogas generation being seen as a secondary advantage. The composition of TWAS, and accordingly it's degree of toxicity, depends on the tanning processes at each individual tannery, as well as the effectiveness of primary treatment. Some toxic substances may be degraded during AD, while others may remain in the sludge. Accordingly, although the toxicity of TWAS may be significantly reduced by AD, it will still be categorized as potentially hazardous. However, the volume for disposal to landfill can be reduced considerably.

The primary aims of this study were: (i) to comprehensively characterise SHS and TWAS from a tannery wastewater treatment plant (TWTP), (ii) to determine the seasonal variability of the TWAS, (iii) to determine whether the BMP and/or anaerobic biodegradability (B_o) could be improved by increasing the ISR (gTVS/gTVS) for anaerobic mono digestion (AmoD) and AcoD of TWAS and SHS, (iv) to determine the AmoD and AcoD kinetics through fitting existing kinetic models for predicting cumulative methane yields.

2. Materials and methods

2.1. Sludge sampling

Fresh TWAS and SHS samples were taken from the TWTP of a full-house tannery in South Africa that uses the Cr tanning process. The tannery is equipped with a CAS reactor preceded by pre- and primary treatment units (screens, equalisation basin, primary sedimentation tank).

Composite grab samples (24 h) were collected over 12 months into polyurethane airtight containers and immediately transported to the laboratory. On arrival, samples were thoroughly homogenised and aliquots were removed for baseline solids and chemical analyses, which were performed within 48 h. The remaining samples were immediately frozen at −18 °C until required.

2.2. Inoculum source and preparation

A robust, acclimated microbial seed consortium was prepared by mixing sludge from mesophilic reactors in a volumetric ratio of 1:2:2:2, that is pre-digested cow manure: granules from an upflow anaerobic sludge blanket reactor (UASB) treating distillery: UASB granules treating brewery wastewater: TWAS from the TWTP. The inoculum was randomly fed with TWAS until the biogas production rate stabilised (Holliger et al., 2016).

2.3. Characterisation of inoculum, secondary tannery sludge and slaughterhouse sludge

Total solids (TS) and TVS concentrations were determined by standard gravimetric methods (loss on ignition at 105 °C and 550 °C, respectively). A Merck (Darmstadt, Germany) Spectroquant Pharo® spectrophotometer together with Merck cell tests or kits were used to determine chemical oxygen demand (COD) (cat no: 14555), total volatile organic acids (VOA_t) as acetic acid equivalents (AAE) (cat no: 01763), total organic carbon (TOC) (cat no: 14879), total nitrogen (TN) (cat no: 14537), total alkalinity as $CaCO_3$ (cat no: 101758), total phosphorous (TP) (cat no: 14729), total ammonia nitrogen as NH_3-N (cat no: 00683), SO_4^{2-} (cat no: 14791), total sulfide as HS- (cat no: 14779), and Cl- (cat no: 14789) concentrations according to manufacturers' instructions. The concentration of free ammonia nitrogen (FAN/NH_3-N) was calculated using Eq. (1) (Kayhanian, 1999).

$$C_{NH_3} = \frac{\boldsymbol{TAN} \times \boldsymbol{pK_a}}{\boldsymbol{C_H} \times \left(\frac{\boldsymbol{pK_a}}{\boldsymbol{pH}} + 1\right)} \tag{1}$$

Where TAN is in mg/L, pKa is the temperature dependent dissociation constant (1.097×10^{-9} at 35 °C), and C_H is the concentration of hydrogen ions.

Metal concentrations [Cr, aluminium (Al), cadmium (Cd), iron (Fe), nickel (Ni), lead (Pb), and zinc (Zn)] and earth elements [Na, calcium (Ca), potassium (K), magnesium (Mg)] were extracted using ethylenediaminetetraacetic acid (EDTA) and ammonium acetate, respectively and analysed at Bemlab (Somerset West, South Africa) using a Varian® MPX (Agilent Technologies, Palo Alto, USA) inductively coupled optical emission spectrophotome-

Table 1
Operational parameters and performance of studies focusing on digestion and co-digestion of tannery wastewater sludges.

	TWAS&TPWS	TWAS&TPWS	TWAS&TPWS	TPWS	TPWS	TWAS&TPWS	TWS	TWS	TWS	TWS
Sludge (TVS %wt.wt)	6.7&60	7.5&67.5	7.5&67.5	50	50	Various	50	-	-	100
Co-substrate (type/s)	TF	TF	TF	TF	TF	GW	TF&LS	LS	LS	nil
Added nutrients	no	no	no	no	no	no	no	yes	yes	no
Operation mode	Batch	Batch	SC	Batch	Batch	CUF	ASBR	Batch	Batch	Batch
Inoculum	AD-DWAS	AD-DWAS	AD-DWAS	PDS+dung	PDS+dung	AD-IWAS	AD-DWAS	No inoc.	No inoc.	AD
ISR (TVS/TVS)	1	1	1	0.67	-	-	-	-	-	0.1
RT (days)	50	50	50	35	56	20 (HRT)	-	200	170	40
Temp (°C)	37±2	37±2	28±5	mesophilic	30±3	35	NG	35	35	37±2
Biogas (mL/gTVS)	383	385	~300-450	27.9	419-635	318-466	-	-	19.6	172
CH_4 yield (mL/gTVS)	268	269	195-329	-	-	-	596	15.0	8.15	86.0
CH_4 (%)	70	70	-	-	71-77	68-77	-	57	-	~40-50
B_o (%TVS)	-	51	-	35	41-52	55 (ave)	71	-	4.8	16
B_o (%TS)	-	-	-	-	-	48 (ave)	-	-	-	20
B_o (%COD)	-	-	-	-	-	55 (ave)	76	85 (TOC)	55 (TOC)	-
References	(Sri Bala Kameswari et al., 2011)	(Sri Bala Kameswari et al., 2012)	(Sri Bala Kameswari et al., 2014)	(Thangamani et al., 2009)	(Thangamani et al., 2010)	(Di Berardino and Martinho, 2009)	(Zupančič and Jemec, 2010)	(Agustini et al., 2017)	(Agustini et al., 2018)	(Akyol et al., 2015)

TWAS = tannery waste activated sludge TPWS = tannery primary wastewater sludge TWS = tannery wastewater sludge (unspecified) TVS = total volatile solids - = not given or unable to calculate from data given TF = tannery fleshings GW = green waste (TF + LS etc.) SC = semi-continuous CUF = continuous upflow ASBR = anaerobic sequencing batch reactor AD = anaerobic digestate (aetiology not specified) AD-DWAS = anaerobic digestate of domestic waste activated sludge AD-IDWAS = anaerobic digestate of industrial waste activated sludge PDS = pre-digested substrate ISR = inoculum to substrate ratio RT = retention time B_o = Biodegradability (%TVS, %TS, %COD reduction) TS = total solids COD = chemical oxygen demand TOC = total organic carbon.

ter. Where applicable, 0.45 μm membrane filters and deionised water were used for filtration and dilution, respectively.

Selected VFAs [acetic acid (HAc), propionic (HPr), and butyric (HBu)] were quantified by high performance liquid chromatography (HPLC) using a Merck Hitachi LaChrom instrument with an L-7400 UV detector and an Agilent (California, USA) refractive index detector, a Phenomenex Rezex RHM-monosaccharide H + column (California, USA) under isocratic conditions with 0.001 mol/L H_2SO_4 as the mobile phase. The sample acquisition time, flow rate and injection volume were set at 30 min, 0.55 mL/min, and 10 μL, respectively.

The pH was measured using a Eutech instrument (Ayer Rajar, Singapore) and cross-checked with pH strips.

2.4. Determination of anaerobic biodegradability and biomethane potential

The BMP tests were conducted using the standard procedure (Holliger et al., 2016). The B_o of sludge was determined by the amount of substrate (% TVS, %TS, %COD) degraded to generate CH_4 (Angelidaki and Sanders, 2004). The substrates consisted of well-blended batches of SHS and TWAS ($TWAS_b$). Experiments (500 mL final volume) were conducted in a water bath (37 ± 2 °C) in gas-tight borosilicate reactors containing 24.5 gVS inoculum.

For the AcoD experiments, optimisation of 2 numeric factors: volumetric composition of TWAS (0–100%) and ISR (1–4) were studied by assessing 2 response variables: maximum methane (CH_4) yield (mLCH_4/gVS) and anaerobic biodegradability (%TS, %VS and %COD reduction), using response surface methodology (RSM). The RSM was based on a full factorial central composite experimental design (CCD) with 13 runs and 5 levels for each factor. The experimental design matrix was generated using Design-Expert® Software Version 10 (Stat-Ease, Inc., Minneapolis, USA).

Biogas volume was measured using the water displacement method. Inverted burettes were filled with saturated NaCl solution to inhibit the dissolution of biogas constituents. The volume of biogas generated was measured daily and thereafter, the contents of the bioreactors were mixed by gently agitating for 1 min. A 100 mL gas tight syringe was used for collecting and transferring biogas from the burettes to separate 1 L Tedlar bags, from which the CH_4, CO_2, H_2S, and O_2 content (%vol) of the biogas was analysed using a Geotech biogas 5000 analyser (Warwickshire, England). The BMP experiments were terminated when <1% of the cumulative biogas volume was produced for 3 consecutive days.

Before and after digestion, the pH in each reactor was measured and the contents sampled for analyses of VS, TS, VOA/VFAs, COD, NH_3-N, and HS^- in order to determine process stability and efficiency. The B_o in terms of %VS, %TS, and %COD were calculated using Eq. (2) (Kafle et al., 2013).

$$\boldsymbol{TS\ or\ VS\,(\%)} = \frac{(\boldsymbol{F}+\boldsymbol{I})\times\boldsymbol{a}-\boldsymbol{I}\times\boldsymbol{b}}{\boldsymbol{F}}\times 100\boldsymbol{\%} \tag{2}$$

where F is total TS or VS of sludge added to reactor (g); I is total TS or VS of inoculum added to reactor (g); a is the calculated TS or VS reduction of sludge plus inoculum based on total initial and final mass of TS or VS after AD (%); b is calculated TS or VS reduction from inoculum in blank controls (%).

2.5. Application of kinetic models

Cumulative CH_4 yields were simulated using different model equations (Table 3). Where P (mLCH_4/gVS) is the cumulative CH_4 yield at retention time (RT) t (day); A (mLCH_4/gVS) is the CH_4 potential of a substrate; μ_m (mLCH_4/gVSd) is the maximum CH_4 production rate; K (day^{-1}) is the specific rate constant; λ (day) is the lag phase; HRT (days) is the hydraulic RT; K_{CH} and n (dimen-

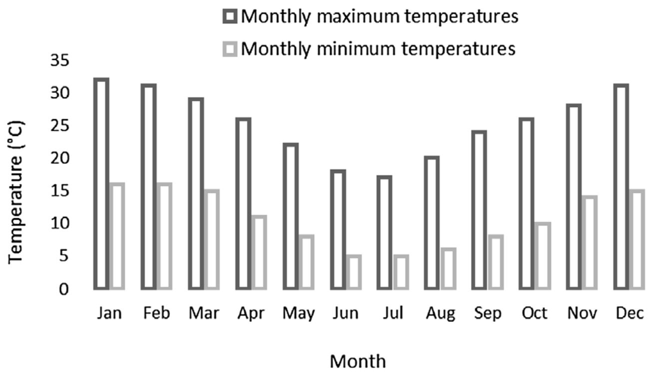

Fig. 1. Temperatures recorded over the period of sampling the tannery waste activated sludge.
Summer = Dec-Feb; Autumn = Mar-May; Winter = Jun-Aug; Spring = Sep-Nov (adapted from www.weather-and-climate.com).

sionless) are the Chen and Hashimoto kinetic constant and shape factor, respectively.

2.6. Statistical analyses

All statistical analyses were performed using Microsoft Excel. The seasonal concentrations of the measured variables in the TWAS samples were summarised using measures of central tendency and dispersion. Analysis of variance (ANOVA) was used to study the significance of the concentration variations and the interactions between process variables and the responses at a confidence interval (CI) of 95%. Nonlinear regression techniques were used to fit known kinetic models onto the observed cumulative CH_4 production data. The adjusted coefficient of determination (adjR^2), root mean square error (RMSE), Akaike's information criterion (AIC) and F Test at 95% CI were used to assess the goodness of fit of the models. Optimisation analysis according to the model of best fit was performed to find combinations of process variables that would maximise CH_4 yield and B_o. Pearson's correlation coefficient (r) was used to test for linear relationships.

2.7. Determination of synergistic effects

Synergistic effects on BMP and B_o were determined using Eq. (3) (Nielfa et al., 2015)

$$\propto = \frac{\boldsymbol{C}}{\boldsymbol{A}+\boldsymbol{B}} \tag{3}$$

where,

A = Experimental CH_4 yield of $TWAS_b$ or B_o x composition (%vol/vol) of TWAS in the mixture

B = Experimental CH_4 yield of $TWAS_b$ or SHS B_o x composition (%vol/vol) of SHS in the mixture

C = Experimental CH_4 yield of $TWAS_b$ or B_0 + SHS co-digestion.

The result of α denotes:

$\alpha > 1$; synergistic effect in the CH_4 yield or B_o.

$\alpha = 1$; the substrates work independently from the mixture.

$\alpha < 1$; antagonistic effect in the CH_4 yield or B_o.

3. Results and discussion

3.1. Characteristics of tannery waste activated sludge, slaughterhouse sludge, and inoculum

The seasonal TWAS samples were obtained during periods of the year with varying average temperatures (Fig. 1). These samples, and the substrates used in the AmoD and AcoD experiments ($TWAS_b$ and SHS) were well-characterised (Table 2). The results were analysed, and only those parameters most likely to exert a significant influence on AD efficiency are discussed in this manuscript.

With the exception of the VOA_t concentration, there was a significant ($p < 0.05$) seasonal variation in the characteristics of TWAS (Table 2). Notably, the TS, VS and COD were highest in winter. It was hypothesised that this may have been due to reduced efficiency of the CAS process because of higher fat content in the influent, and/or inhibition by high metal concentrations or lower ambient temperatures expected in winter. The $TWAS_b$ and autumn TWAS samples were more lipid-like in character (2.45 and 2.34 gCOD:gVS, respectively), while the others were more protein-like (0.495–1.46 gCOD:gVS) (Angelidaki and Sanders, 2004). Lipids have high B_o and CH_4 yields, although for AD, long RTs are required because degradation rates are low. High concentrations of lipids in TWAS could also theoretically result in LCFA inhibition (Appels et al., 2011; Rasit et al., 2015). In the seasonal TWAS samples, the measured short-chain VFAs [acetate (0.15–1.13 g/L), propionate (0.077-0.66 g/L), butyrate (0.035-0.372 g/L) accounted for only ≤13% of the VOA_t and were below the reported inhibitory thresholds (Boe et al., 2010; Wang et al., 2009).

Apart from the TWAS samples taken in winter, where the TOC was significantly higher ($p < 0.05$), the C:N ratios were lower than the optimal range (20–30) for efficient AD (Sri Bala Kameswari et al., 2014; Basak et al., 2014). The SHS, $TWAS_b$ and winter and spring TWAS samples were categorised as dry/high solids sludges (>15% TS) and therefore had better potential for AD (Yan et al., 2015) than the TWAS summer and autumn batches. With the exception of the summer TWAS samples, the TVS:TS ratios (0.55-0.74) were below the recommended ratio of >0.8 for efficient digester performance (Zhang et al., 2007). Apart from the winter TWAS samples and the inoculum, the VFA:Alkalinity ratios were >0.4 (Table 2), indicating the likelihood of reactor instability and failure during AD (Gao et al., 2015). Overall, the seasonal concentrations of TAN and NH_3 were below the AD inhibiting range of 2–14 g/L TAN and 53–1450 mg/L NH_3 (Rajagopal et al., 2013).

As expected, high concentrations of SO_4^{2-}, Na, and Cl were found in the TWAS and $TWAS_b$ samples. Concentrations of toxicants were significantly lower ($p < 0.05$) in the SHS samples, and it was presumed that this would have a positive effect on AcoD. There were significant seasonal differences ($p < 0.05$) in relative SO_4^{2-} and S^{2-} concentrations in the TWAS/$TWAS_b$, which were assumed to emanate mainly from tanning operations rather than differences in the tannery wastewater treatment plant (WWTP) performance. The high COD: SO_4^{2-} ratios (>64) of all the samples were well above the ratio (>1) reported by (Guerrero et al., 2013) for favouring methanogenesis over sulfidogenesis. However, using IC_{50} ranges for H_2S of 43–125 mg/L at pH 7–8, for methanogens, and 57–184 and 14–60 mg/L at pH 7.2–7.4 and 8.0–8.5, respectively for aceticlastic methanogens (AMs) in suspended sludge (Rasit et al., 2015), the H_2S concentrations (9.5–97.6 mg/L) were deemed inhibitory for AD. The macronutrient C:N:P:S ratios did not meet the optimal ratio of 500-600:15:5:1 (Deublein and Steinhauser, 2008), and the Ni concentrations were also below the proposed optimum for AD (11.4 mg/L) (Ortner et al., 2015).

3.2. Experimental determination of anaerobic biodegradability and biomethane potential

3.2.1. Mono-digestion of tannery waste activated sludge and slaughterhouse sludge

The theoretical CH_4 yields for complex wastes such as TWAS are usually overestimated as AD is typically inhibited by NH_3, Cl, SO_4^{2-}, H_2S, metals (particularly Cr, K, Na, Ca, Mg, Fe, Zn, Ni) and metabolites (Appels et al., 2008; Chen et al., 2008). Prior to the AcoD experiments, the performance indicators for AmoD of $TWAS_b$, and

Table 2
Characteristics of inoculum, slaughterhouse and seasonal tannery waste activated sludge.

TWAS (seasonal characteristics)					$TWAS_b$	SHS	INOC
Parameter	Summer	Autumn	Winter	Spring			
TS (g/L)	90.2 ± 4.24	96.5 ± 0.751	373 ± 6.36	153 ± 29.7	178 ± 133	123 ± 5.51	114 ± 1.68
TVS (g/L)	73.2 ± 0.283	63.2 ± 0.212	232 ± 4.67	113 ± 34.8	121 ± 77.4	67.7 ± 2.21	70.0 ± 1.29
TOC (g/L)	11.4 ± 0.40	28.4 ± 3.79	67.2 ± 3.11	5.67 ± 0.659	25 ± 20.7	11.1 ± 1.93	18.9 ± 1.00
COD (g/L)	85.3 ± 2.30	155 ± 17.4	339 ± 23.6	138 ± 23.2	153 ± 75.6	33.5 ± 2.05	150 ± 2.72
TN (g/L)	1.70 ± 0.116	4.83 ± 0.892	2.83 ± 0.793	1.46 ± 0.398	2.88 ± 1.57	1.32 ± 0.166	3.05 ± 0.446
TP (mg/L)	80.1 ± 7.11	130 ± 18.3	53 ± 1.41	19.3 ± 5.68	86.2 ± 49.0	54.0 ± 11.9	74.0 ± 2.04
VOA_t (gAAE/L)	2.01 ± 0.258	3.99 ± 0.809	4.05 ± 0.283	1.56 ± 0.386	3.11 ± 1.23	2.70 ± 0.426	1.91 ± 0.125
$SO_4{}^{2-}$ (g/L)	1.32 ± 0.03	1.31 ± 0.157	2.40 ± 0.113	1.080 ± 0.17	1.53 ± 0.592	417 ± 12.5	671 ± 62
HS^- (mg/L)	43 ± 1.41	97.6 ± 25.6	18.4 ± 5.62	9.50 ± 1.91	42.1 ± 39.6	95.3 ± 36.0	66.6 ± 3.06
Cl (g/L)	7.4 ± 0.819	14.1 ± 1.05	21.8 ± 0.404	21.6 ± 3.85	21.6 ± 6.87	1.13 ± 0.090	5.00 ± 0.346
NH_3-N (mg/L)	175 ± 20	278 ± 33	373 ± 57	278 ± 70	0.2761 ± 0.081	166 ± 12.2	1201 ± 115
$CaCO_3$ (g/L)	3.76 ± 0.967	6.47 ± 0.808	10.8 ± 3.47	2.83 ± 0.557	5.97 ± 3.59	1.50 ± 2.21	6.49 ± 1.17
Na (g/L)	2.83	5.72	11.1	6.36	6.50	0.433	16.4
K (mg/L)	21.3	10	20	0	12.8	208	700
Mg (mg/L)	183	10	70	10	68.3	19.0	600
Zn (mg/L)	<0.03	2.74	31.3	4.34	12.8	0.06	101
Fe (mg/L)	0.6	59.7	2026	241	582	1.00	713
Ni (mg/L)	<3.0E-5	0.41	2.69	0.62	1.24	<3.0E-5	3.69
Cr (mg/L)	0.03	155	519	145	205	<0.027	392
Pb (mg/L)	<0.015	0.4	0.8	0.22	0.47	<0.015	0.894
Density (g/L)	1.04 ± 0.02	1.02 ± 0.02	1.10 ± 0.02	0.98 ± 0.01	1.05 ± 0.020	1.01 ± 0.0136	1.05 ± 0.015
pH	7.17	7.26	5.86	8.24	7.20	7.50	7.68
TVS:TS	0.81	0.65	0.62	0.74	0.68	0.55	0.61
C:N	6.71	5.88	23.75	3.88	8.33	8.41	6.20
VFA:Alk	0.53	0.62	0.38	0.55	0.52	1.8	0.29
$COD:SO_4$	64.6	118	141	128	100	80.3	224
C:N:P:S	24:4:0.2:1	53:9:0.2:1	82:4:0.07:1	13:3:0.04:1	49:6:0.2:1	116:14:0.6:1	85:13:0.3:1
COD:VS	1.17	2.45	1.46	1.22	1.26	0.495	2.14

TWAS/$TWAS_b$ = tannery waste activated sludge/blended; SHS = slaughterhouse sludge; INOC = inoculum; TS = total solids; TVS = total volatile solids.
TOC = total organic carbon; COD = chemical oxygen demand; TN = total nitrogen; TP = total phosphate; VOA_t = total volatile organic acid.

Table 3
Models applied in order to simulate the bio-methane potential and evaluate the kinetic parameters of anaerobic digestion of tannery waste activated sludge.

Equation	Characteristics	Reference
First order $\boldsymbol{P = A\left[1 - e^{(kt)}\right]}$	-Assumes hydrolysis as rate limiting and CH_4 production is simulated using exponential rise to a maximum due to depletion of the biodegradable fraction of the substrate.	(Buljan and Král, 2015; Subramani and Haribalaji, 2012)
Logistic $\boldsymbol{P = \frac{A}{1+e^{\left[\left(\frac{4\mu_m}{A}\right)(\lambda-t)+2\right]}}}$	-Assumes proportionality between CH_4 production rate and the amount of gas already produced, the biodegradable substrate concentration and the specific microbial growth rate.	(Swartz et al., 2017)
Modified Gompertz $\boldsymbol{P = Ae^{\left[-e^{\left(\frac{\mu_m e}{A}(\lambda-t)+1\right)}\right]}}$	-Assumes CH_4 production is proportional to the specific growth rate of microbes and this proportionality reduces with increasing RT.	(Swartz et al., 2017)
Cone $\boldsymbol{P = \frac{A}{1+(kt)^{-n}}}$	-Assumes proportionality between CH_4 production and microbial activity and to the biodegradable substrate concentration.	(Buljan and Král, 2011)
Chen and Hashimoto $\boldsymbol{P = A\left(1 - \frac{K_{CH}}{HRT \times k + K_{CH} - 1}\right)}$	-Predicts CH_4 production based on its proportionality with biodegradable substrate utilisation rate and RT.	(Akyol et al., 2014)

SHS were therefore first determined independently in batch reactor experiments (Table 6).

For $TWAS_b$, reactors with ISRs of 2 (ISR2), 3 (ISR3) and 4 (ISR4) were tested. In ISR2 and ISR3, methane generation was irreversibly inhibited after 17 and 15 days RT, respectively (Fig. 2A). In ISR4, the lag phase lasted for $\tilde{6}0$ days (Fig. 2A). Lag phases are common during AD reactor start-up because an acclimation period is required for the selection of functional microbial consortia in a particular substrate. For example, an acclimation period of >27 days was required to overcome inhibition during AD of TWAS (Mpofu et al., 2019). The protracted lag phases can be attributed in part to the long regeneration times required by slow-growing methanogens (5–16 days) and acetogens (3–4 days) (Deublein and Steinhauser, 2008; Zupančič et al., 2012). In ISR4, ultimate corrected cumulative biogas and CH_4 yields of 150 mL/gVS and 84 mL/gVS (56% CH_4 composition) respectively, were achieved, and approximately 5%, 50% and 80% of the CH_4 yield was obtained after the first 40, 90 and 98 days of AD, respectively. The 56% CH_4 composition was within the 51–70% range for proteinaceous substrates (Angelidaki and Sanders, 2004), while the average biogas H_2S and O_2 composition remained <1 ppm and <1%, respectively (data not shown), indicating a dominance of methanogenesis over sulfidogenesis, as expected from the high feed COD:S O_4^{2-} ratio (100) and NH_3 levels (Guerrero et al., 2013). The metal concentrations as well as COD, $CaCO_3$ (alkalinity), NH_3-N, and COD:N ratios in the inoculum were all notably higher than in the $TWAS_b$ (Table 2), suggesting that the improvement in AD at highest (ISR4) could be attributed to the addition of metabolic co-factors (Fe, Ni, Cr and Zn) from the inoculum. It is also possible that the inoculum contained more AD-efficient functional microbial consortia than the $TWAS_{b.}$ Conversely, the inoculum may also have diluted possible inhibitors such as Cl, VOA, and S O_4^{2-} that were highest in $TWAS_b$.

There was an accumulation of VOAs and NH_3 in the contents of ISR2 (5.96 g/L HPr, 2.36 g/L HBu and 296 mg/L NH_3), ISR3 (2.38 g/L, HPr and 272 mg/L NH_3) and ISR4 (244 mg/L HAc and 62.3 mg/L NH_3). It was postulated that this was due to inhibition of methanogens and/or acetogens (HAc, HPr and HBu degrading) by LCFAs and/or NH_3 (NH_3–LCFA synergetic co-inhibition) and/or H_2S. In ISR4, the accumulation of HAc and change in average $\%CO_2$ in the biogas from 23% to 0.2% suggested that there was a selection

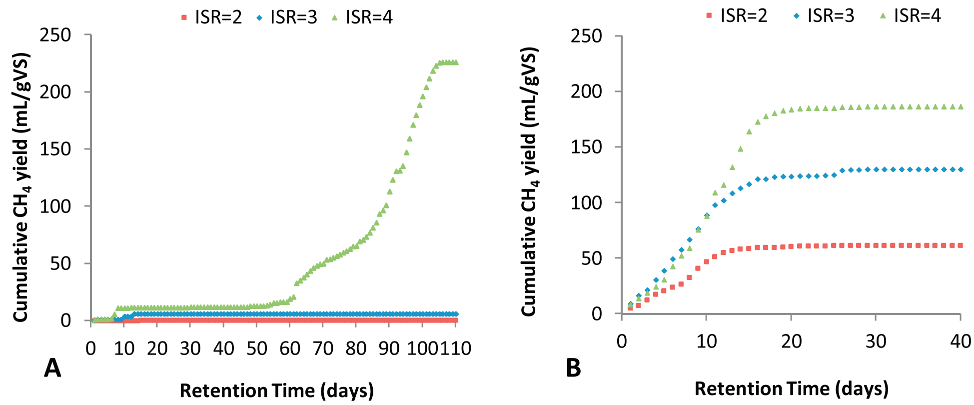

Fig. 2. Uncorrected cumulative methane yields at different inoculum to substrate ratios from: (A) anaerobic mono-digestion of tannery waste activated sludge, (B) anaerobic mono-digestion of slaughterhouse sludge.

of hydrogenotrophic methanogenesis over aceticlastic methanogenesis (Ariunbaatar et al., 2015). Thus, inhibition seem to have impacted CH_4 yields more than the non-ideal nutrient balances, and a higher ISR (= 4) proved successful in alleviating inhibition of AD.

It was found that the SHS was highly biodegradable in comparison to $TWAS_b$ as observed from the exponential rise in the uncorrected cumulative methane production indicating the absence of a lag phase (Fig. 2B). For this substrate, 3 ISRs were applied (2, 3, 4), and optimal performance was found at ISR4, generating ultimate corrected yields of 175 mL biogas/gTVS (40% CH_4) and 70 mLCH_4/gVS, with B_o indices of 70.8%VS, 64.6%TS and 49.6 %COD B_o after 32 days RT.

3.2.2. Co-digestion of tannery waste activated sludge with slaughterhouse sludge

To determine whether AcoD of $TWAS_b$ with SHS could improve AD efficiency, optimisation studies were conducted using 13 reactors (R1-13 with ISRs ranging from 1 to 4, and $TWAS_b$/SHS (%vol) ranging from 0 to 100%. Table 4 shows the experimental design matrix generated using Design Expert® following the central composite design (CCD) and the experimental results obtained.

The CH_4 yields and B_o (% reduction of VS, TS and COD) from the CCD experimental data were modelled using a general quadratic polynomial with up to second degree interaction terms, and linear equations, respectively. The fractional design space (FDS = 0.99) was sufficiently greater than the recommended 0.8 (Stat-Ease, Inc., Minneapolis, USA). Furthermore, the signal:noise ratios (adequate precision) for all the models measured were desirably >4. The models were significant ($p < 0.05$) and there was only 0.28–1.05% chance that this may have occurred due to noise (Table 5). However, the F test suggested that ISR and its interaction (ISR^2) were the only significant ($p < 0.05$) model terms for CH_4 yield, whereas $TWAS_b$ composition (%vol/vol) was the only significant factor ($p < 0.05$) affecting B_o (Fig. 4B–D). Results indicated that 10.8%, 44.7%, 32.8% and 25.9% of the variability in CH_4 yield, %VS, %TS and %COD reduction, respectively, were not explained by the models. The models' adj R^2 values which corrected the R^2 values with respect to the sample size and number of terms in the models were 0.815; 0.443; 0.534 and 0.653, respectively (Table 5), suggesting moderate to good predictability of the actual CH_4 yields and B_o. Thus, the models (Eqs 4–7) in terms of actual factors could be used to navigate the design space and to optimise the cumulative CH_4 yield and B_o as plotted in Fig. 4A–D.

$$\boldsymbol{BMP} = 28(\boldsymbol{ISR})^2 - 0.00425\left(\%\frac{\boldsymbol{vol}}{\boldsymbol{vol}}\ \mathrm{TWAS}\right)^2 + 1.43\left(\%\frac{\boldsymbol{vol}}{\boldsymbol{vol}}\ \mathrm{TWAS} \times \boldsymbol{ISR}\right) - 139(\boldsymbol{ISR}) - 3.52\left(\%\frac{\boldsymbol{vol}}{\boldsymbol{vol}}\ \mathrm{TWAS}\right) + 227 \quad (4)$$

$$\%\boldsymbol{VS_{reduction}} = -1.1(\boldsymbol{ISR}) - 0.589\left(\%\frac{\boldsymbol{vol}}{\boldsymbol{vol}}\boldsymbol{TWAS}\right) + 89.5 \quad (5)$$

$$\%\boldsymbol{TS_{reduction}} = 0.409(\boldsymbol{ISR}) - 0.664\left(\%\frac{\boldsymbol{vol}}{\boldsymbol{vol}}\boldsymbol{TWAS}\right) + 77.0 \quad (6)$$

$$\%\boldsymbol{COD_{reduction}} = 7.11(\boldsymbol{ISR}) - 0.382(\%\frac{\boldsymbol{vol}}{\boldsymbol{vol}}\boldsymbol{TWAS}) + 38.9 \quad (7)$$

Despite a considerable improvement in AD with some combinations of ISR and/or %$TWAS_b$/SHS, severe inhibition occurred in reactors with low ISR and/or high %$TWAS_b$ (Fig. 3). It was hypothesised that in 3 of the 4 reactors where permanent inhibition of methane generation occurred (Fig. 3), the primary cause was high NH_3 concentrations, as the digestates of R6, R7, and R8 accumulated NH_3 to inhibitory levels of 255, 143, and 272 mg/L, respectively. Other factors that may have exacerbated the situation in these reactors were (i) the VFA:Alkalinity ratios of 0.591; 0.473; and 0.448, respectively, which were beyond the optimal <0.3-0.4 and indicated reactor instability (Feng et al., 2009; Martín-González et al., 2013), and, (ii) the high final pH values consequent to NH_3 accumulation (8.66, 8.32, and 8.75, respectively) which were above the optimal pH range (6.5–7.8) for methanogens (Gutierrez et al., 2009; Hulshoff Pol et al., 1998).

The highest specific biogas (431 mL/gVS) and methane generation (215 mL/gVS) yields were obtained in R10 with ISR = 4 and 50% $TWAS_b$/SHS (Tables 1 and 4). This was >2.5 fold higher than during AmoD of $TWAS_b$ at the same ISR. The gas yields were accompanied by increases in B_o indices of 21.7% (TVS), 32.5% (TS) and 20.2% (COD). The highest B_o for reactors that contained ≥50% $TWAS_b$ with ISR < 4, was in R1 at ISR = 2.5 and 50% $TWAS_b$. In this reactor, there were only small increases in specific biogas and methane generation, but notable increases in B_o of TVS, TS and COD of 23.5%, 38.5% and 12.2%, respectively when compared to AmoD of $TWAS_b$. This is not surprising, because the B_o of the SHS was higher than that of $TWAS_b$ during AmoD of each of these substrates at a comparative ISR (Table 6). In addition, it was previously found that the intial pH and variation of temperature within the mesophilic range insignificant ($p > 0.05$) effect on AcoD (Mpofu, 2018; Mpofu et al., 2019).

Possible synergistic effects in terms of substrate composition ($TWAS_b$/SHS) on CH_4 yield and B_o were determined mathematically using α cut-off values (Section 2.7). Results indicated (i) synergistic effects for CH_4 yield in reactors R3 and R10, both operating at ≥50%

Table 4
Performance indicators for anaerobic co-digestion of tannery waste activated sludge and slaughterhouse sludge at different substrate to substrate and inoculum to substrate ratios.

Reactor	$TWAS_b$ (%vol/vol)	ISR	Biogas yield (mL/gTVS)	CH_4 yield ($mLCH_4$/gTVS)	Biogas Composition		Biodegradability indicators (% reduction)		
					CH_4 (%v/v)	CO_2 (%v/v)	(%TVS)	(%TS)	(%COD)
R1	50	2.5	214	77	36.1	0.2	57.5	51.5	40.2
R2	25	2	73.8	24	32.9	0.1	63.9	54.9	47.2
R3	75	3	234	108	46.2	0.1	60.1	48.3	36.3
R4	50	2.5	135	56	41.1	0.2	54.2	47.0	34.5
R5	0	2.5	175	70	39.0	0.3	70.8	64.6	49.6
R6	50	1	0	0	0	0	58.7	46.1	29.7
R7	75	2	0	0	0	0	51.6	14.7	15.1
R8	100	2.5	0	0	0	0	1.90	1.50	15.4
R9	50	2.5	134	48	36.0	0.2	53.0	32.1	45.1
R10	50	4	431	215	49.9	1.1	44.2	27.7	47.6
R11	25	3	160	61	38.1	0.1	86.8	81.0	50.5
R12	50	2.5	112	43	38.1	0.1	63.7	57.7	50.2
R13	50	1	0	0	0	0	78.4	56.2	27.0

$TWAS_b$ = blended tannery waste activated sludge; ISR = inoculum to substrate ratio; R = reactor; TS = total solids; TVS = total volatile solids; COD = chemical oxygen demand.

Table 5
Summary of the statistical results of the fitted models.

Models	Std dev	p-value	F test (LOF) p value	R^2	Adj R^2	Pred R^2	Adeq Prec	AIC
CH_4 Quadratic	25.5	0.0028	0.660	0.892	0.815	0.571	13.2	139
TVS Linear	15.1	0.0221	0.165	0.534	0.443	0.0009	8.12	113
TS Linear	14.5	0.0088	0.184	0.612	0.534	0.225	9.55	112
COD Linear	12.6	0.0017	0.834	0.741	0.653	0.58	10.9	125

Pred = predicted; Std dev = standard deviation; Adj = adjusted; TS = total solid; LOF = lack of fit; AdeqPrec = adequate precision; AIC = Akaike's information criterion; TVS = total volatile solids; COD = chemical oxygen demand.

Table 6
Comparison of operating parameters and performance indicators for anaerobic digestion at optimal inoculum to substrate ratios.

Parameter	*SHS	*$TWAS_b$	**$TWAS_b$/SHS	***$TWAS_b$/SHS
ISR	3	4	4	2.5
RT (days)	32	108	108	108
Temp (°C)	37 ± 2	37 ± 2	37 ± 2	37 ± 2
Biogas (mL/gTVS)	175	150	431	214
CH_4 yield (mL/gTVS)	70	84	215	77
CH_4 (%)	40	56	50	36
B_O (%TVS)	71	34	56	58
B_O (%TS)	65	13	46	52
B_O (%COD)	50	28	48	40

SHS = slaughterhouse sludge; $TWAS_b$ = blended tannery waste activated sludge; ISR = inoculum to substrate ratio; B_O = biodegradability indicator retention time; *Optimal ISR; **Optimal ISR and $TWAS_b$/SHS ratio for methane generation, and ***optimal ISR and $TWAS_b$/SHS ratio for biodegradability.

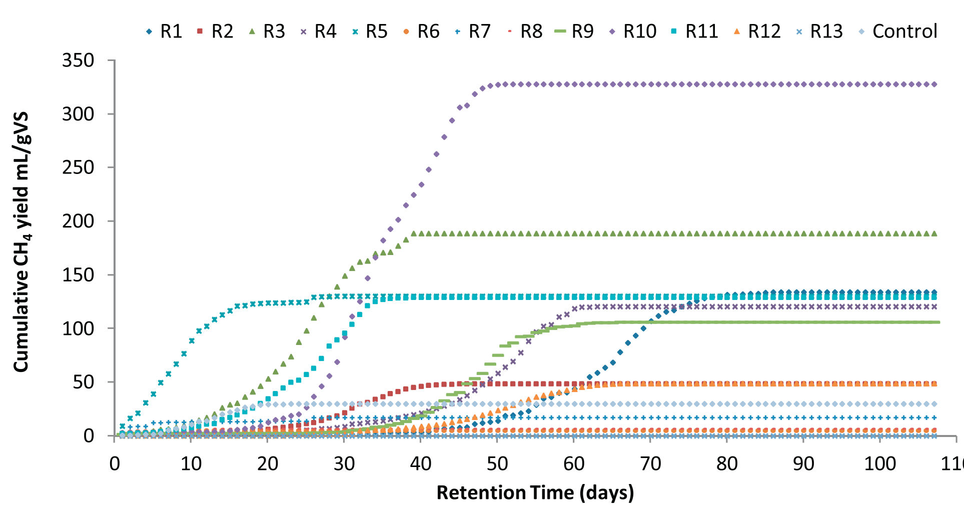

Fig. 3. Uncorrected cumulative methane yields at different inoculum to substrate ratios from the anaerobic co-digestion of different ratios of tannery waste activated sludge and slaughterhouse sludge.
TVS = total volatile solids; ISR = inoculum to substrate ratio; TS = total solids; COD = chemical oxygen demand; BMP = biomethane potential

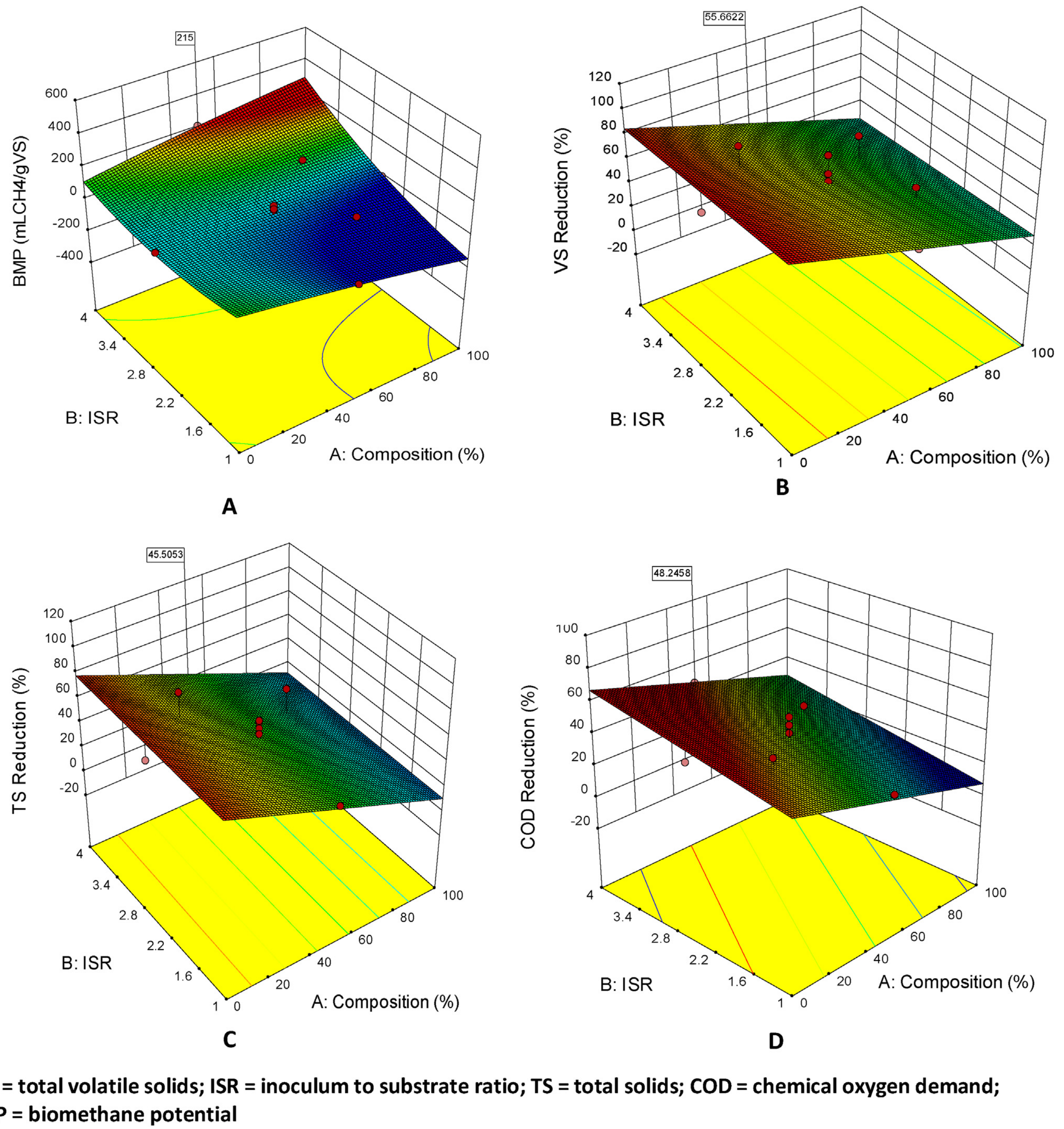

Fig. 4. Effect of inoculum to substrate ratio and %vol/vol of secondary tannery sludge on (A) CH_4 yield, (B) total volatile solids reduction efficiency, (C) total solids reduction efficiency, and (D) chemical oxygen demand reduction efficiency.

$TWAS_b$ (%vol/vol) and ISR ≥ 3, (ii) no effects in R1 (α = 1.01), and (iii) antagonistic effects in the remaining reactors. In terms of B_o, R11 operating at 25% $TWAS_b$ (%vol/vol) and ISR = 3 exhibited relatively higher synergies with α values of 3.0 (VS), 4.6 (TS) and 1.2 (COD). However, the CH_4 yield and %CH_4 in the biogas was low (Table 6). Similarly, the %CH_4 in R10 was 6% lower than that obtained during AmoD of $TWAS_b$. Overall, the results indicated that CH_4 yield could be improved by increasing the ISR and the B_o improved by decreasing the $TWAS_b$ fraction (%vol/vol). It is also possible that besides being more biodegradable, SHS contained more AD-efficient functional microbial consortia than the $TWAS_b$ (Priebe and Gutterres, 2017).

3.2.3. Determination of methane generation kinetics

To evaluate the applicability of predictive models for evaluating AD kinetics, commonly applied models (Cone, modified Gompertz, Logistic, Chen and Hashimoto and First order) were fitted to cumulative CH_4 production from R4 (AmoD of $TWAS_b$), R3 (AmoD of SHS) and R10 (AcoD of $TWAS_b$ with SHS).

For both AmoD and AcoD, the Cone, modified Gompertz, and logistic models fitted the experimental data with high precision and suitability (Table 7, Fig. 5). The sigmoidal shape of these models accurately reflected the lag, exponential, and stationary phases of AD, and were therefore able to predict the uncorrected cumulative CH_4 production. According to the goodness

Table 7
Kinetic parameters and goodness of fit obtained from evaluated models and kinetic parameters from selected experimental anaerobic digesters.

Model	Kinetic parameters					Adj R^2	rMSPE	AIC	p value *
	$A (mLCH_4/gTVS)$	μ_m ($mLCH_4/gTVSd$)	λ(d)	K (d^{-1})	n(K_{CH})				
Anaerobic mono-digestion of TWAS$_b$ in ISR4									
Cone	270	ND	ND	0.011	7.40	0.956	13.2	864	0.389
Logistic	272	4.37	60.0	ND	ND	0.951	13.9	875	0.350
Mod. Gompertz	275	5.12	64.2	ND	ND	0.944	14.8	889	0.416
Chen and Hash	270	ND	ND	0.0571	(7.51)	0.559	41.9	1110	1.43E-08
First order	260	ND	ND	0.00585	ND	0.529	43.7	1115	1.73E-12
Anaerobic mono-digestion of SHS ISR3									
Logistic	128	10.4	1.49	ND	ND	0.997	1.99	134	0.483
Mod. Gompertz	48.0	3.91	5.76	ND	ND	0.997	2.17	140	0.486
Cone	137	ND	ND	0.133	2.22	0.990	3.88	175	0.426
Chen and Hash	176	0.797	ND	7.19	ND	0.958	7.61	215	0.407
First order	141	0.094	ND	ND	ND	0.958	7.69	216	0.289
Anaerobic co-digestion R10									
Logistic	329	18.1	25.5	ND	ND	0.999	5.02	657	0.497
Cone	331	ND	ND	0.0291	7.64	0.998	5.94	693	0.458
Mod. Gompertz	122	6.93	31.7	ND	ND	0.998	6.62	716	0.440

* probability < F; A = ultimate CH_4 yield; μ_m = maximum CH_4 production rate; λ = lag phase; n = shape factor constant; K = specific rate constant; Adj = adjusted; RMSE = root mean square error; AIC = Akaike's information criterion; ND = no data; NA = not applicable; Hash = Hashimoto.

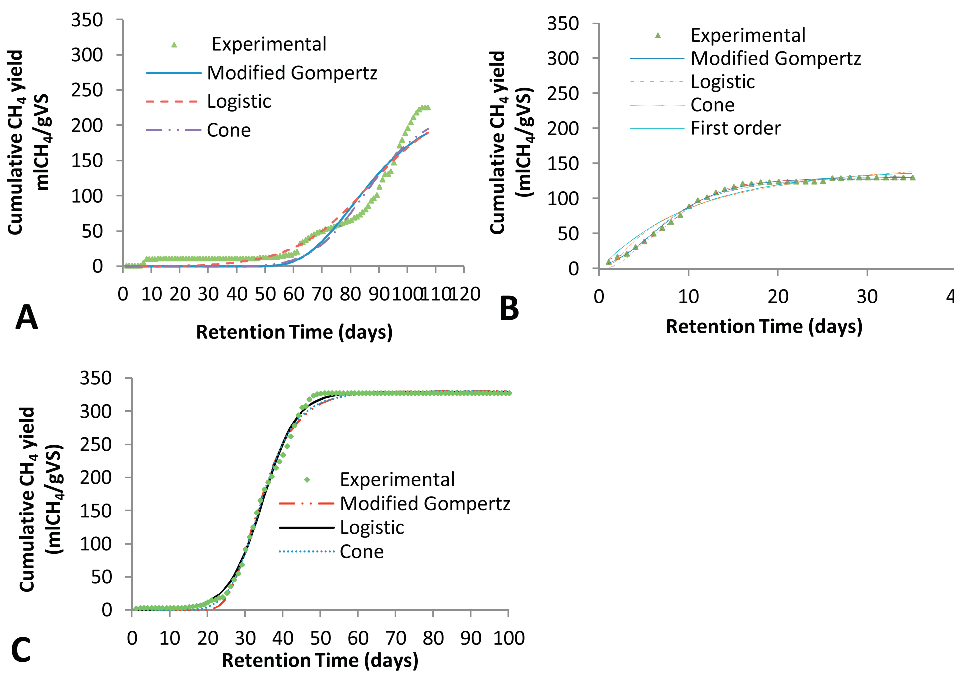

Fig. 5. Uncorrected cumulative methane yield from (A) mono anaerobic digestion of tannery waste activated sludge and slaughterhouse sludge (B) with inoculum to substrate ratios of 4 and 3, respectively, and (C) co-digestion of tannery waste activated sludge and slaughterhouse sludge with inoculum to substrate ratio of 4 and substrate to substrate ratio of 50/50% (v/v).

of fit, the models predicted the experimental data in the order Cone > Logistic > modified Gompertz for AmoD of TWAS$_b$, for SHS Logistic > modified Gompertz > Cone > Chen and Hashimoto > First order and Logistic > Cone > modified Gompertz for AcoD, with insignificant ($p > 0.05$) variation. During the digestion of TWAS$_b$, the lag phase in R4 lasted for $\tilde{6}0$ days, after which it was assumed that functional microbial consortia were selected (i.e. acclimated). This did not occur in R2 and R3, where the consortia were unable to overcome the inhibitory nature of the physicochemical milieu. In contrast, the lag phase in the AD of SHS was estimated as 1.5–5.8 days, demonstrating that the SHS was readily biodegradable and supported microbial activity: the maximum CH_4 production rates (μ_m) were estimated as 3.9–10.4 $mLCH_4/gVSd^{-1}$ and the rate constant (K) as 0.13–7.2 d^{-1}. However, the SHS had a lower ultimate yield (A) compared to TWAS$_b$, which was almost double.

For AcoD, the models predicted maximum CH_4 production rate (μ_m) of 18.1; 6.93 and 6.00 $mLCH_4/gVSd^{-1}$, respectively, and an uncorrected ultimate cumulative CH_4 yield (A) of 331; 329 and 122 $mLCH_4/gVS$. The Logistic and Cone models therefore over-estimated, while the modified Gompertz model under-estimated the experimental value (215 $mLCH_4/gVS$), as found with AmoD (Table 7). Lag phases (λ) of 26 and 32 days were deduced from the logistic and modified Gompertz models respectively, and were comparable to 24 days obtained experimentally ($p > 0.05$). The Cone model estimated the rate constant (K) as 0.0291 $days^{-1}$ and this was verified using the reciprocal relationship between K and RT of 0.0200 day^{-1} (Rittmann and McCarty, 2001).

In contrast to the other models, for both AmoD and AcoD, there was a significant lack of fit for the first order kinetic and the Chen and Hashimoto models (Table 7) because they only consider the exponential stage in CH_4 production. This was not unexpected, because cumulative CH_4 production curves do not follow first order kinetic models when AD is inhibited. Lower K values (range: 0.0185-0.0239 day^{-1}) with shorter λ (range: 3–16 days) (Thangamani et al., 2009, 2010), and lower μ_m (range: 2.04–5.48 $mLCH_4$/gVSd) (Sri Bala Kameswari et al., 2012) have been found when TWS was co-digested with fleshings as co-substrates. In this study, SHS replaced fleshings as a co-substrate for the AcoD of TWAS, and the AcoD of the three substrates combined may be worthy of investigation. Nonetheless, in comparison to the methane generation kinetics obtained during the AmoD of $TWAS_b$ in ISR4 (Table 7), the ultimate CH_4 yield (A), maximum CH_4 production rate (μ_m) and specific rate constant (K) in R10 was 20.4–21.9%, 58.6–252%, and 164% higher, respectively, whilst the lag phase (λ) was reduced by 59.3%.

4. Conclusion

The study demonstrated the feasibility of integrating tanneries and slaughterhouses and the AcoD of TWAS and SHS for cleaner production, biogas generation and solid waste reduction. It was demonstrated that ISR had a significant effect on CH_4 yield whereas TWAS composition (%vol/vol) on B_o. The process was optimal at 50% TWAS and SHS composition (%vol/vol) and at ISR = 4 for maximal CH_4 yield and B_o. The Logistic, Cone and modified Gompertz models accurately fitted (adj$R^2 \geq$99.8%) the experimental data, respectively. AcoD improved the process kinetics and performance, particularly μ_m (252%), K (164%), CH_4 yields (156%) and B_o (59.4–96.7%). The lag phase was reduced by 59.3% and the retention time by 53.7%. This countered the disadvantage of using a high ISR which requires a high reactor capacity. It is possible that with long-term operation, or by changing the mode of operation from batch to pulse-fed, that the process could improve to the extent that a lower ISR could be applied. These results could serve as the basis for further improvements in sludge management for tanneries integrated with slaughterhouses.

Disclaimer

Declaration of Competing Interest

None.

Acknowledgements

The authors would like to thank the Water Research Commission for funding this project and the NATSURV K5/2490 project team.

References

Agustini, C.B., Spier, F., Gutterres, M., 2017. Biogas production for anaerobic co-digestion of tannery solid wastes under presence and absence of the tanning agent. Resour. Conserv. Recycl. 130 (July), 51–59, 2018.

Agustini, C.B., Meyer, M., Da, M., Gutterres, M., 2018. Biogas from anaerobic co-digestion of chrome and vegetable tannery solid waste mixture : influence of the tanning agent and thermal pretreatment. Process Saf. Environ. Prot. 118, 24–31.

Akyol, Ç., Demirel, B., Onay, T.T., 2014. Recovery of methane from tannery sludge: the effect of inoculum to substrate ratio and solids content. J. Mater. Cycles Waste Manage. 17 (4), 1–8.

Akyol, Ç., Demirel, B., Onay, T.T., 2015. Recovery of methane from tannery sludge: the effect of inoculum to substrate ratio and solids content. J. Mater. Cycles Waste Manage. 17 (4), 808–815.

Angelidaki, I., Sanders, W., 2004. Assessment of the anaerobic biodegradability of macropollutants. Rev. Environ. Sci. Biotechnol. 3 (2), 117–129.

Appels, L., Van Assche, A., Willems, K., Degrève, J., Van Impe, J., Dewil, R., 2011. Peracetic acid oxidation as an alternative pre-treatment for the anaerobic digestion of waste activated sludge. Bioresour. Technol. 102 (5), 4124–4130.

Appels, L., Baeyens, J., Degrève, J., Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energy Combust. Sci. 34 (6), 755–781.

Ariunbaatar, J., et al., 2015. Effect of ammoniacal nitrogen on one-stage and two-stage anaerobic digestion of food waste. Waste Manage. 38 (1), 388–398.

Basak, S.R., Rouf, M.A., Hossain, M.D., Islam, M.S., Rabeya, T., 2014. Anaerobic digestion of tannery solid waste by mixing with different substrates. Bangladesh J. Sci. Ind. Res. 49 (2), 119–124.

Boe, K., Batstone, D.J., Steyer, J.-P., Angelidaki, I., 2010. State indicators for monitoring the anaerobic digestion process. Water Res. 44 (20), 5973–5980.

Buljan, J., Král, I., Vienna 2015. The Framework for Sustainable Leather Manufacture.

Buljan, J., Král, I., Vienna 2011. Introduction to Treatment of Tannery Effluents.

Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: a review. Bioresour. Technol. 99 (10), 4044–4064.

Deublein, D., Steinhauser, A., 2008. Biogas From Waste and Renewable Resources, 1st ed. John Wiley & Sons, Weinhem.

Di Berardino, S., Martinho, A., 2009. Co-digestion of tanning residues and sludge. In: 12th IWA Sludge Conference—Sustainable Management of Water and Wastewater Sludge.

Feng, H., Hu, L., Mahmood, Q., Fang, C., Qiu, C., Shen, D., 2009. Effects of temperature and feed strength on a carrier anaerobic baffled reactor treating dilute wastewater. Desalination 238 (1–3), 111–121.

Gao, S., Zhao, M., Chen, Y., Yu, M., Ruan, W., 2015. Tolerance response to in situ ammonia stress in a pilot-scale anaerobic digestion reactor for alleviating ammonia inhibition. Bioresour. Technol. 198, 372–379.

Girault, R., et al., 2012. Anaerobic co-digestion of waste activated sludge and greasy sludge from flotation process: Batch versus CSTR experiments to investigate optimal design. Bioresour. Technol. 105, 1–8.

Guerrero, L., et al., 2013. Behavior of the anaerobic treatment of tannery wastewater at different initial pH values and sulfate concentrations. Environ. Sci. Heal. 48, 1073–1078.

Gutierrez, O., Park, D., Sharma, K.R., Yuan, Z., 2009. Effects of long-term pH elevation on the sulfate-reducing and methanogenic activities of anaerobic sewer biofilms. Water Res. 43 (9), 2549–2557.

Holliger, C., et al., 2016. Towards a standardization of biomethane potential tests. Water Sci. Technol. 74 (11), 2515–2522.

Hulshoff Pol, L.W., Lens, P.N., Stams, A.J., Lettinga, G., 1998. Anaerobic treatment of sulphate-rich wastewaters. Biodegradation 9 (3–4), 213–224.

Kafle, G.K., Kim, S.H., Sung, K.I., 2013. Ensiling of fish industry waste for biogas production: a lab scale evaluation of biochemical methane potential (BMP) and kinetics. Bioresour. Technol. 127, 326–336.

Kayhanian, M., 1999. Ammonia inhibition in high-solids biogasification: an overview and practical solutions. Environ. Technol. 20 (4), 355–365.

Martín-González, L., Font, X., Vicent, T., 2013. Alkalinity ratios to identify process imbalances in anaerobic digesters treating source-sorted organic fraction of municipal wastes. Biochem. Eng. J. 76, 1–5.

Mpofu, A.B., 2018. Optimisation of the Anaerobic Treatment of Secondary Tannery Sludge for Biogas Production and Solids Reduction. Cape Peninsula University of Technology.

Mpofu, A.B., Welz, P.J., Oyekola, O.O., 2019. Anaerobic digestion of secondary tannery sludge: optimisation of initial pH and temperature and evaluation of kinetics. Waste Biomass Valor.

Nielfa, A., Cano, R., Pérez, A., Fdez-Polanco, M., 2015. Co-digestion of municipal sewage sludge and solid waste: modelling of carbohydrate, lipid and protein content influence. Waste Manage. Res. 33 (3), 241–249.

Ortner, M., Rameder, M., Rachbauer, L., Bochmann, G., Fuchs, W., 2015. Bioavailability of essential trace elements and their impact on anaerobic digestion of slaughterhouse waste. Biochem. Eng. J. 99, 107–113.

Pitk, P., Kaparaju, P., Palatsi, J., Affes, R., Vilu, R., 2013. Co-digestion of sewage sludge and sterilized solid slaughterhouse waste: methane production efficiency and process limitations. Bioresour. Technol. 134, 227–232.

Priebe, G.P.S., Gutterres, M., 2017. Special review: anaerobic digestion of leather industry wastes - an alternate source of energy. J. Am. Leather Chem. Assoc. 112 (2), 59–71.

Rajagopal, R., Massé, D.I., Singh, G., 2013. A critical review on inhibition of anaerobic digestion process by excess ammonia. Bioresour. Technol. 143, 632–641.

Rasit, N., Idris, A., Harun, R., Wan Ab Karim Ghani, W.A., 2015. Effects of lipid inhibition on biogas production of anaerobic digestion from oily effluents and sludges: an overview. Renew. Sustain. Energy Rev. 45, 351–358.

Rittmann, B., McCarty, P., 2001. Environmental Biotechnology: Principles and Applications. McGraw-Hill Companies Inc, New York.

Shanmugam, P., Horan, N.J., 2009. Optimising the biogas production from leather fleshing waste by co-digestion with MSW. Bioresour. Technol. 100 (18), 4117–4120.

Sri Bala Kameswari, K., Kalyanaraman, C., Umamaheswari, B., Thanasekaran, K., 2014. Enhancement of biogas generation during co-digestion of tannery solid wastes through optimization of mix proportions of substrates. Clean Technol. Environ. Policy 16 (6), 1067–1080.

Sri Bala Kameswari, K., Kalyanaraman, C., Porselvam, S., Thanasekaran, K., 2012. Optimization of inoculum to substrate ratio for bio-energy generation in co-digestion of tannery solid wastes. Clean Technol. Environ. Policy 14 (2), 241–250.

Sri Bala Kameswari, K., Kalyanaraman, Chitra, Thanasekaran, K., 2011. Effect of ozonation and ultrasonication pretreatment processes on co-digestion of tannery solid wastes. Clean Technol. Environ. Policy 13 (3), 517–525.
Subramani, T., Haribalaji, D., 2012. Biodegradation of tannery effluent and designing the reactor for clarifier and activated sludge process. Int. J. Mod. Eng. Res. 2 (3), 774–781.
Swartz, C.D., Jackson-Moss, C., Rowswell, R., Mpofu, A.B., Welz, P.J., 2017. Water and Wastewater Management in the Tanning and Leather Finishing Industry. Water Research Commission, Pretoria.
Thangamani, A., Rajakumar, S., Ramanujam, R.A., 2009. Biomethanation potential of animal fleshing and primary sludge and effect of refractory fraction of volatile solids. J. Environ. Sci. Sustain. Soc. 3 (March), 29–34.
Thangamani, A., Rajakumar, S., Ramanujam, R.A., 2010. Anaerobic co-digestion of hazardous tannery solid waste and primary sludge: biodegradation kinetics and metabolite analysis. Clean Technol. Environ. Policy 12 (5), 517–524.
Wang, H., Fotidis, I.A., Angelidaki, I., 2016. Ammonia – LCFA synergetic co-inhibition effect in manure-based continuous biomethanation process. Bioresour. Technol. 209, 282–289.
Wang, Y., Zhang, Y., Wang, J., Meng, L., 2009. Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria. Biomass Bioenergy 33 (5), 848–853.
Yan, Z., Song, Z., Li, D., Yuan, Y., Liu, X., Zheng, T., 2015. The effects of initial substrate concentration, C/N ratio, and temperature on solid-state anaerobic digestion from composting rice straw. Bioresour. Technol. 177, 266–273.
Zhang, R., et al., 2007. Characterization of food waste as feedstock for anaerobic digestion. Bioresour. Technol. 98 (4), 929–935.
Zupančič, G.D., Jemec, A., 2010. Anaerobic digestion of tannery waste: semi-continuous and anaerobic sequencing batch reactor processes. Bioresour. Technol. 101 (1), 26–33.
Zupančič, G.D., Viktor, G., Grilc, V., Slovenia 2012. Anaerobic Treatment and Biogas Production from Organic Waste.