

# Evaluation of acidogenic sludge from anaerobic reactors running at low solids retention times to reduce sludge generation and enhance biogas production

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## ABSTRACT

Sludges generated in the biological processing of sewage are complex mixtures, the constituents of which pose risks to public health and the environment. Anaerobic digestion is considered the most sustainable option for treating sludge because it offers the possibility of generating biogas. The aim of this study was to compare the quantities, properties, biodegradabilities and biochemical methane potentials (BMP) of primary sludge (PS) generated by a primary decanter with acidogenic sludges produced by upflow anaerobic (UA) reactors operating at solids retention times (SRTs) of 2, 4, 6 and 8 days (Samples S2, S4, S6 and S8, respectively). Sludges from both pre-treatments were submitted to alkaline solubilization in order to determine the efficiency of the process in disrupting extracellular complexes. Based on the levels of total solids (TS) present, the primary decanter was found to generate higher quantities of excess sludge (yield of 3.1 gTS·d<sup>-1</sup>) than UA reactors operating at low SRTs (yields in the range 1.69 to 0.64 gTS·d<sup>-1</sup>). The concentrations of dissolved materials in PS and Samples S2 and S8 were considerably higher after alkaline solubilization, with respective increases of 8, 14 and 28-fold in dissolved organic carbon, 12, 20 and 40-fold in chemical oxygen demand, 25, 31 and 59-fold in proteins, and 17, 21 and 63-fold in carbohydrates. In addition, the BMP value for S8 was some 13% higher than that recorded for PS while the kinetic constant for gas production by S8 was 1.8-fold greater than that of PS. It is concluded that a pre-treatment combining anaerobic digestion at low SRT and alkaline solubilisation would lead to improved performance in subsequent stages of *anaerobic digestion* and, consequently, increased efficiency in biogas production.

**Keywords:** acidogenic sludge, sludge stabilisation, alkaline solubilisation, methanisation

## INTRODUCTION

Sludge generated by the treatment of sewage is generally classified according to the stage of the process from which it originates. Thus, primary sludge arises from the gravitational sedimentation of suspended solids and organics, secondary sludge derives from the conversion of biodegradable material by microorganisms, and tertiary sludge originates from additional biological or chemical treatment.

Anaerobic digestion is considered the most sustainable option for the treatment of sludge because it is not only a relatively economical process but also produces biogas and a residue (biosolid) that can have green applications (Rani et al., 2012). In this context, decomposition of organic matter in the absence of free molecular oxygen affords some specific advantages such as the reduction of sludge volume by withdrawal of water, the transformation of highly biodegradable organic matter into relatively inert substances, the recovery of energy in the form of methane, and the generation of an end-product that can be disposed of in accordance with applicable legislation (Xu et al., 2014; Tchobanoglous et al., 2014).

The main steps involved in the anaerobic digestion of organic matter, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis, require the balanced interaction of several groups of microorganisms in order to ensure rapid and successful degradation. The limiting step is hydrolysis in which insoluble substances, as well as high molecular weight

compounds such as lipids, polysaccharides, proteins and nucleic acids, are broken down to soluble molecules that serve as substrates for the subsequent steps of the process (Gurje and Zehnder, 1983; Tchobanoglous et al., 2014). The hydrolytic step may also represent a pre-treatment method, since prior breakdown and solubilisation of sludge components accelerates anaerobic digestion and increases the efficiency of the process (Rani et al., 2012). A number of pre-treatment methods based on different operating strategies (i.e. biological, chemical, thermal, mechanical and combinations thereof) have been developed with the aim of improving the solubility of sludge solids (Chen et al., 2007; Cho et al., 2013; Bi et al., 2014; Tian et al., 2014; Xu et al., 2014; Sahinkaya, 2015).

Sludge derived from biological wastewater treatment contains two categories of organic complexes, namely soluble microbial products (SMP) and extracellular polymeric substances (EPS). The SMP comprise a pool of organic compounds that are weakly bound to cells or dissolved in solution, and may include humic acids, polysaccharides, proteins, amino acids, nucleic acids, organic acids, antibiotics, steroids, extracellular enzymes, structural components of cells and products of metabolism. The co-occurring EPS are the natural polymers secreted by microorganisms that play important roles in cell aggregation, cell adhesion, biofilm formation and, ultimately, protection against hostile environments (Sheng et al., 2010; Wang et al., 2014). Since EPS matrices are strongly attached to the cells, their disruption requires the application of powerful procedures such as alkaline solubilisation.

Upflow anaerobic (UA) reactors operating at a low solids retention time (SRT) generate less sludge than conventional

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primary decanters and could replace decanters in pre-treatment processes in order to minimise the volume of sludge to be treated. Hence, the aim of this study was to compare the quantities, properties, biodegradabilities and biochemical methane potentials (BMP) of primary sludge (PS) generated by a primary decanter with acidogenic sludges produced by UA reactors operating at SRTs of 2, 4, 6 and 8 days. Sludges from both pre-treatments were submitted to alkaline solubilization in order to determine the efficiency of the process in disrupting extracellular complexes.

## METHODS

### Pre-treatment of sanitary sewage

Two routes of pre-treatment of sanitary sewage were studied in parallel, as outlined in Figure 1, and the characteristics and energy potential of the sludges so-produced were compared.

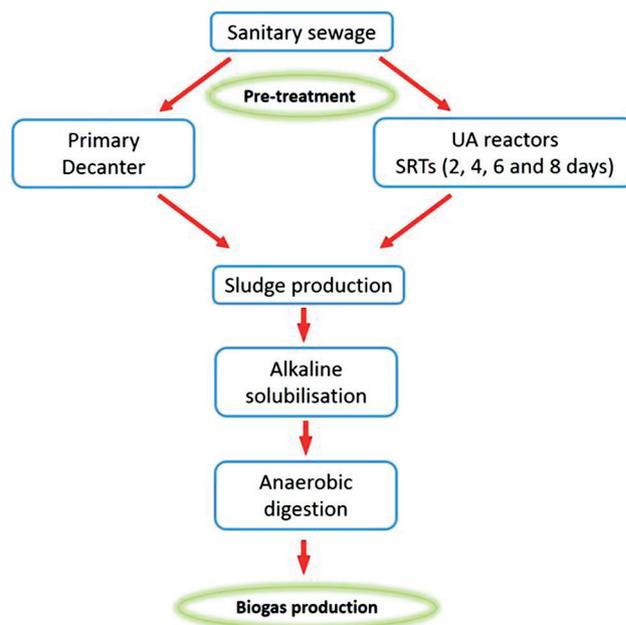
The sanitary sewage used in the experiment originated from the eastern interceptor of the Companhia de Água e Esgoto da Paraíba (CAGEPA; Campina Grande, PB, Brazil) and was characterized prior to treatment as follows: pH 7.62, total alkalinity 454.8 mgCaCO<sub>3</sub>·L<sup>-1</sup>, total solids (TS) 820 mg·L<sup>-1</sup>, volatile suspended solids (VSS) 360 mg·L<sup>-1</sup>, total chemical oxygen demand (COD<sub>T</sub>) 764 mg·L<sup>-1</sup>, soluble COD 272 mg·L<sup>-1</sup>, total phosphorus (TP) 14.5 mg·L<sup>-1</sup>, and total Kjeldahl nitrogen (TKN) 87.8 mg·L<sup>-1</sup>.

Acidogenic sludges were obtained from four UA reactors (each of volume 2 L) that were fed daily with sanitary sewage and operated simultaneously with hydraulic retention times (HRT) of 4 h, flow rates of 12 L·day<sup>-1</sup> and SRTs of 2, 4, 6 or 8 days (S2, S4, S6 and S8 sludges, respectively). For sludge disposal, all of the reactor contents (mixed liquor) were withdrawn and, after homogenisation, quantities equivalent to 1/2, 1/4, 1/6 and 1/8 of the total volume were removed for physicochemical analysis with the remainder being returned to the reactor to maintain SRTs of 2, 4, 6 and 8 days, respectively. Primary sludge was obtained by sedimentation of 12 L of sanitary sewage for 1 h. The supernatant was removed from the 1 L of thickened sewage so-formed, and the sediment that remained was characterized as PS.

Samples of sludges were characterized daily over a 2-month experimental period and excess sludge production of the primary decanter and UA reactors was calculated on the basis of the measured values of TS.

### Physicochemical analyses of sludges

Viscosities of sludge samples were measured using a Q860M26 microprocessor-controlled rotational viscometer (Quimis, Diadema, SP, Brazil), while values of the specific resistance to filtration (SRF) were established according to methodology described by Almeida et al. (1991). Total dissolved solids (TDS), total volatile solids (TVS), total suspended solids (TSS), volatile dissolved solids (VDS), fixed dissolved solids (FDS), dissolved organic carbon (DOC), TS, VSS, COD<sub>T</sub>, TKN and TP were determined following the methodologies recommended by the American Public Health Association (2012). Concentrations of SMP and EPS were assessed in terms of protein and carbohydrate content established according to the methods of Lowry et al. (1951) (as modified by Frølund et al., 1995) and Dubois et al. (1956), respectively. For this purpose, samples of sludge were centrifuged at 4°C for 15 min at 15 455 g and the supernatants were filtered through 0.45 µm glass fibre membranes and employed in the SMP assays, while the sediments were extracted with 0.05% NaCl solution for 30 min at 60°C (Li and Yang, 2007) and submitted to EPS assay.



**Figure 1.** Routes of sludge production by pre-treatment of sanitary sewage and subsequent evaluation of the potential for the generation of biogas

### Statistical analysis

Data were submitted to one-way analysis of variance (ANOVA) with post-hoc Tukey multiple comparison tests in order to detect significant differences ( $p < 0.05$ ) between mean values of the parameters determined for the sludge samples. Based on the results of the statistical analysis, sludge samples PS, S2 and S8 were selected for further investigation as described below.

### Alkaline solubilisation of sludges

The selected samples were submitted to alkaline solubilisation in triplicate in order to compare the degree of solubilisation of their constituents. Aliquots (100 mL) of sludges were adjusted to pH 12.0 with 1 M NaOH solution and maintained under constant agitation at 200 r·min<sup>-1</sup> on a gyrotory shaker mixer model G-33 (New Brunswick Scientific, Edison, NJ, United States) for 48 h at approximately 28°C (Monte et al., 2017). Treated samples were subsequently brought to neutral conditions (pH 7.0) by the addition of 1 M HCl and concentrations of carbohydrates, proteins, TDS, VDS, FDS, COD and DOC determined. The masses of NaOH and HCl required to adjust the pH values of sludge samples to those specified in the pre-treatment protocol were determined in a preliminary experiment in which 20 mL aliquots of each sample were titrated sequentially with 1M NaOH and 1M HCl solutions under constant monitoring of pH (Table 1).

### Determination of biochemical methane potential

Assessments of BMP were performed using 250 mL borosilicate flasks containing inoculum (from an upflow anaerobic sludge blanket reactor) and substrate sample (PS or S8 sludge) in the proportion of 2:1. The flasks were placed in an incubator at a constant temperature of 35±2°C (appropriate for mesophilic bacteria) with regular agitation. Assays were continued for 21 days, at which point the cumulative biogas curves had entered the plateau region (Angelidaki et al., 2009).

## RESULTS AND DISCUSSION

### Effect of low SRT on the chemical characteristics of acidogenic sludges

The mean values of solids, COD<sub>T</sub>, TKN and TP, as determined from daily measurements of PS samples and on the accumulated levels in sludges collected during the acidogenic process in UA reactors operating at low SRTs, are shown at Table 2. The concentrations of TS and TSS in the sludges showed a general tendency to rise as SRT increased, even though the levels in S2 and S4 were similar. The overall pattern can be explained by the consumption of organic matter present in the sewage by metabolic processes of the microorganisms, in that the removal of organic matter from the effluent of the reactor and the quantity of sludge biomass produced were maximal at an SRT of 8 days. The volatile fraction accounted for the greater part (on average 75%) of the TS present in all of the sludge samples analysed. Regarding COD<sub>T</sub>, the lowest concentration was observed in S2 while the highest was recorded in S8. TKN also tended to increase with increasing SRT, which may be explained by the assimilation of the nitrogen present in the sewage resulting from anabolism of the sludge biomass growing inside the reactors and by assimilation of nitrogen compounds by the EPS biofilm formed on the sludge flakes. No variations were detected in the concentrations of TP in any of the sludge samples, probably because all four reactors operated at low SRTs and the anaerobic process does not effectuate phosphorus removal.

Based on the TS values shown in Table 2, it is possible to estimate the amount of sludge generated in the primary decanter and the UA reactors. At a sewage flow of 12 L·d<sup>-1</sup>, the primary decanter produced 1L of PS with a TS concentration of 3.1 g·L<sup>-1</sup>, resulting in a yield of 3.1 g TS·d<sup>-1</sup>. Considering that 1/2, 1/4, 1/6 and 1/8 of the contents of the reactors S2, S4, S6 and S8, respectively, were removed for analysis, the corresponding amounts of excess sludge generated by the UA reactors would be 1.69, 0.82, 0.64 and 0.69 g TS·d<sup>-1</sup>.

**Table 1.** Concentrations of total solids in primary sludge and acidogenic sludges collected from upflow anaerobic reactors at different solids retention times, and the amounts of NaOH and HCl employed in the alkaline pre-treatment of 100 mL samples of sludges

Sludge sample	TS <sup>a</sup> (g·L <sup>-1</sup> )	NaOH <sup>a</sup> (g)	HCl <sup>a</sup> (mg)
Primary sludge	15 ± 0.1	0.24	0.82
S2 (SRT 2 days)	14 ± 1.4	0.28	0.82
S8 (SRT 8 days)	19 ± 2.8	0.32	0.98

TS = total solids, SRT = solids retention time

<sup>a</sup>Mean values (n = 3) ± standard deviation where shown

### Effect of low SRT on the biochemical composition and physical properties of acidogenic sludges

The concentration of proteins predominated over that of carbohydrates in the SMP and EPS fractions of all sludge samples analysed (Table 3), thereby corroborating the findings of Li and Yang (2007). With regard to SMP, the highest mean concentrations of proteins and carbohydrates were observed in PS and the lowest in S8 signifying that, for SRT > 2 days, the levels of SMP in the sludge decreased substantially with increasing SRT. Li and Yang (2007) also recorded higher SMP concentrations at lower SRTs, a finding that may be ascribed to the assimilation of soluble material by microorganisms present in the liquid fraction resulting in the development of cell aggregates that form part of the structure of the sludge. Since high concentrations of SMP can adversely affect the sedimentation and dewatering characteristics of sludge, an understanding of how factors such as SRT, HRT and organic load influence the production of SMP is important for improving the design and operation of wastewater treatment plants (Aquino et al., 2009; Kunacheva et al., 2017).

In the case of EPS, the highest mean concentration of proteins was observed in S8 and the lowest in PS (Table 3). On this basis, the levels of EPS in sludge would appear to show a tendency to rise with increasing SRT. On the other hand, the levels of carbohydrates in EPS showed relatively small fluctuations between sludge samples, a finding in accordance with the report of Li and Yang (2007) that the amount of EPS does not vary significantly with increasing SRT. According to Ye et al. (2011), the concentration of EPS in sludge depends on a number of variables, including the amount of residual water, level of nutrients, reactor configuration and SRT.

The viscosities of sludges S2, S6 and S8 were similar but showed slight rises with increasing SRT (Table 3). On the other hand, the viscosity of S4 was somewhat lower than that of the other sludges, reflecting the decreased concentration of EPS as indicated by the reduced protein and carbohydrate levels exhibited by this sample. Thus, our results suggest a direct relationship between viscosity and EPS concentration. A similar association can be observed between SRF and EPS, since the lowest resistance value was recorded with S2 and the highest with S8.

Aggregation of dispersed microorganisms leads to increased particle size, and this gives rise to denser flocs and promotes the incrementation of EPS. Moreover, dehydration of the sludge becomes more difficult with higher SRTs by virtue of the increases in SRF, EPS and viscosity (Feng et al., 2016). In the present study, the SRTs adopted were relatively low (maximum 8 days) and the elevated levels of EPS and viscosity observed did not appear to hinder sludge dewatering. Indeed, higher levels of EPS may be advantageous since the larger amount of organic matter present would allow higher biogas generation.

**Table 2.** Chemical characteristics of primary sludge and acidogenic sludges collected from upflow anaerobic reactors at different solids retention times

Sludge sample	TS <sup>a</sup> (g·L <sup>-1</sup> )	TVS <sup>a</sup> (g·L <sup>-1</sup> )	TSS <sup>a</sup> (g·L <sup>-1</sup> )	VSS <sup>a</sup> (g·L <sup>-1</sup> )	COD <sub>T</sub> <sup>a</sup> (gO <sub>2</sub> ·L <sup>-1</sup> )	TKN <sup>a</sup> (g·kg <sup>-1</sup> TS)	TP <sup>a</sup> (g·kg <sup>-1</sup> TS)
Primary sludge	3.1±0.1 <sup>A</sup>	2.1±0.1 <sup>A</sup>	1.9±0.1 <sup>A</sup>	1.5±0.1 <sup>A</sup>	3.3±0.3 <sup>A</sup>	29.3±2.9	9.8±0.5
S2 (SRT 2 days)	13.5±2.5 <sup>B</sup>	9.7±2.0 <sup>B</sup>	12.4±2.6 <sup>B</sup>	9.2±2.0 <sup>B</sup>	20.5±4.4 <sup>B</sup>	41.1±1.8	12.6 ±2.2
S4 (SRT 4 days)	13.1±3.7 <sup>B</sup>	9.2±2.6 <sup>B</sup>	12.4±3.3 <sup>B</sup>	9.0±2.3 <sup>B</sup>	21.1±1.1 <sup>B</sup>	38.4±3.8	11.2 ±1.2
S6 (SRT 6 days)	15.4±5.4 <sup>B</sup>	10.7±3.9 <sup>B</sup>	14.0±5.8 <sup>B</sup>	9.9±4.1 <sup>B</sup>	17.9±7.5 <sup>B</sup>	42.4±2.4	12.2 ±0.7
S8 (SRT 8 days)	22.7±1.6 <sup>C</sup>	16.0±1.2 <sup>C</sup>	21.8±1.3 <sup>C</sup>	15.6±1.2 <sup>C</sup>	28.9±3.1 <sup>C</sup>	46.2±2.9	11.9±1.7

TS = total solids, TVS = total volatile solids, TSS = total suspended solids, VSS = volatile suspended solids, COD<sub>T</sub> = total chemical oxygen demand, TKN = total Kjeldahl nitrogen, TP = total phosphorus, SRT = solids retention time

<sup>a</sup>Mean values (n = 6) ± standard deviation. In each column, values bearing dissimilar uppercase letters are significantly different (p < 0.05) according to the Tukey test.

## Solubilisation of acidogenic and primary sludges by alkaline pre-treatment

Since the mean values of most of the parameters determined for Sludges S2, S4 and S6 were not significantly different (Table 2), the SRT that was most applicable from the point of view of cost (namely, 2 days) was selected as representative of these samples for the alkaline solubilization test. After 48 h of alkaline pre-treatment, PS and sludge samples S2 and S8 presented elevated concentrations of dissolved materials with respective increases of 4.7, 3.9 and 5.7-fold in TDS, 5.7, 5.7 and 10-fold in VDS, and 4.3, 3.4 and 4.4-fold in FDS (Table 4).

The amounts of DOC in PS, S2 and S8 after alkaline solubilisation were, respectively, 8, 14 and 28-fold higher than those detected in the samples prior to the procedure (Table 4). It is important to note that, even though S2 had been generated with a very short SRT, the amount of solubilised material present in the sample after alkaline treatment was greater than that detected in solubilised PS. The levels of COD also rose appreciably after alkaline treatment with 12, 20 and 40-fold increases recorded in PS, S2 and S8, respectively. A number of researchers have reported significant enhancements in COD following alkaline solubilisation with recorded increases of 2-times (Cho et al., 2013), 17-times (Chen et al., 2007) and 100-times (Xu et al., 2014) the pre-treatment levels. Thus, alkaline solubilisation is an effective method of destroying cell matrices and increasing the concentration of soluble materials. Moreover, since the values of TDS, VDS, FDS, DOC and COD increased gradually from PS to S8, it would appear that the amount of soluble material produced by alkaline solubilisation is related directly to SRT.

The concentrations of dissolved proteins and carbohydrates increased considerably in PS, S2 and S8 following alkaline solubilisation, with respective gains of 25, 31 and 59-fold for proteins and 17, 21 and 63-fold for carbohydrates (Table 4). Previous studies of the effects of alkaline pre-treatment (pH 12) of sludges have revealed increases in the concentrations of proteins and carbohydrates, respectively, in the order of 9.4 and 7.8-fold (Chen et al., 2007), 2.8 and 1.4-fold (Cho et al., 2013), 3.0 and 2.8-fold (Xu et al., 2014) and 26.3 and 36.9-fold (Monte et al., 2017). Our results are in accord with earlier reports and demonstrate that alkaline solubilisation of acidogenic sludge is efficient in the disruption of EPS, thereby overcoming the limitations of the hydrolytic step of degradation and making available larger amounts of substrate for methanogenesis.

Samples of PS, S2 and S8 showed increased concentrations of dissolved TP and TKN following alkaline solubilisation (Table 4), although the respective gains (i.e. 1.5, 1.7 and 4.0-fold for TP and 2.9, 2.3 and 5.5-fold for TKN) were not as pronounced as those recorded for the other variables studied. Kim et al. (2003) also obtained low values for nitrogen and phosphorus using the alkaline solubilisation process, while Chen et al. (2007) reported that solubilisation at low pH values (4.0 to 5.0) was more effective in increasing the concentrations of these constituents. One explanation for this finding is that the activities of hydrolytic enzymes may be reduced at higher pH values.

Pre-treatment of sanitary sewage using a combination of UA digestion at low SRT and alkaline solubilisation affords a number of advantages considering that the process involves proven technologies that are simple to apply and control, and that the pH of the sludge can be raised using a wide range of

**Table 3.** Biochemical compositions and physical properties of primary sludge and acidogenic sludges collected from upflow anaerobic reactors at different solids retention times

Sludge sample	SMP <sup>a</sup> (g·kg <sup>-1</sup> TS) (n = 6)		EPS <sup>a</sup> (g·kg <sup>-1</sup> TS) (n = 3)		Viscosity (mPa·s) (n = 1)	SRF (cm·g <sup>-1</sup> ) (n = 1)
	Proteins	Carbohydrates	Proteins	Carbohydrates		
Primary sludge	16.6±1.3	2.8±0.7	10.9±6.6	4.2±2.1	1.38	2.00 × 10 <sup>11</sup>
S2 (SRT 2 days)	5.88±2.16	1.09±0.25	17.16±0.6	3.50±0.6	1.45	9.64 × 10 <sup>10</sup>
S4 (SRT 4 days)	6.75±4.27	1.07±0.57	14.22±3.1	3.33±1.1	1.14	1.70 × 10 <sup>11</sup>
S6 (SRT 6 days)	4.54±1.98	0.82±0.34	17.23±1.4	4.41±1.1	1.46	1.04 × 10 <sup>11</sup>
S8 (SRT 8 days)	3.03±1.02	0.50±0.11	23.34±1.5	4.10±0.2	1.47	3.94 × 10 <sup>11</sup>

SMP = soluble microbial product, EPS = extracellular polymeric substance, SRF = specific resistance to filtration,

TS = total solids, SRT = solids retention time

<sup>a</sup>Mean values ± standard deviation.

**Table 4.** Comparison of the chemical and biochemical characteristics of primary sludge and acidogenic sludges collected from upflow anaerobic reactors at different solids retention times as determined before and after alkaline pre-treatment

Variable	Sludge sample					
	Primary sludge <sup>a</sup>		S2 <sup>a</sup> (SRT 2 days)		S8 <sup>a</sup> (SRT 8 days)	
	Before	After	Before	After	Before	After
TDS (g·L <sup>-1</sup> )	1.51±0.02	7.10±0.27	1.99±0.01	7.83±0.16	1.62±0.07	9.29±0.39
VDS (g·L <sup>-1</sup> )	0.42±0.001	2.40±0.31	0.44±0.09	2.53±0.26	0.38±0.10	3.83±0.23
FDS (g·L <sup>-1</sup> )	1.09±0.02	4.70±0.14	1.55±0.10	5.30±0.10	1.23±0.03	5.46±0.25
DOC (g·kg <sup>-1</sup> TS)	2.04	16.12±0.36	1.39	19.80±1.13	0.74	20.90±1.53
COD (mgO <sub>2</sub> ·kg <sup>-1</sup> TS)	17.49±0.36	217.95±21.91	11.93±0.34	242.82±18.25	6.75±0.26	271.18±24.27
Proteins (g·kg <sup>-1</sup> TS)	4.55±0.09	112.59±7.43	4.11±0.02	128.16±9.60	2.27±0.07	134.32±6.71
Carbohydrates (g·kg <sup>-1</sup> TS)	0.97±0.01	17.00±2.63	0.90±0.00	18.98±3.24	0.39±0.01	24.63±0.58
TP (g·kg <sup>-1</sup> TS)	1.47±0.05	2.21±0.13	1.32±0.04	2.30±0.11	0.76±0.12	2.55±0.22
TKN (g·kg <sup>-1</sup> TS)	6.07±0.16	18.39±0.92	10.65±0.70	25.63±1.34	4.22±0.30	24.20±1.70

SRT = solids retention time, TDS = total dissolved solids, VDS = volatile dissolved solids, FDS = fixed dissolved solids, DOC = dissolved organic carbon, COD = chemical oxygen demand, TP = total phosphorus, TKN = total Kjeldahl nitrogen, TS = total solids. <sup>a</sup>Mean values (n = 3) ± standard deviation (except for DOC determined before alkaline treatment).

low-cost materials such as limestone ( $\text{CaCO}_3$ ),  $\text{CaO}$ ,  $\text{Ca(OH)}_2$ ,  $\text{NaOH}$ ,  $\text{Na}_2\text{CO}_3$  and ammonia. The disadvantages include the inclusion of a separate stage in the digestion process and the non-selectivity of the solubilisation stage.

## Biogas production

The generation of biogas was evaluated with PS and with Sample S8 because sludge produced in a UA with an SRT of 8 days contained the highest concentrations of organic matter as indicated by the values of COD and EPS (Tables 2 and 3). As shown in Fig. 2, PS generated more biogas than S8 in the first 8 days, likely because the initial digestion process in S8 increased the lead-time for methanation since soluble materials present in the liquid fraction are first degraded and sludge flakes are formed. The volumes of biogas produced by PS and S8 were similar at Day 9, after which the production of biogas by S8 surpassed that of PS with both sludges reaching a constant rate at Day 16. According to the BMP values for PS and S8 (i.e. 56.8 and  $64.1 \text{ NmL}\cdot\text{g}^{-1} \text{ VSS}$ , respectively), the acidogenic sludge showed a 13% increase in biogas potential that could be explained by the incorporation of soluble material into the biomass, as demonstrated by the increase in EPS values. Kooijman et al. (2017) reported that the addition of flocculants during primary settling enhanced the removal of more readily degradable solids and increased the BMP of PS.

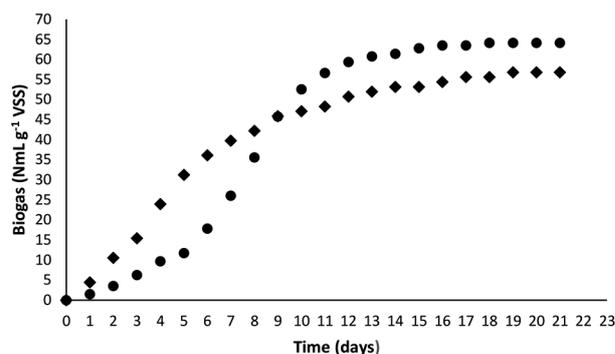
Although the difference in biogas production between PS and S8 was not remarkable, the increase observed with S8 would become more expressive during large-scale processing. More importantly, the values of the kinetic constants, derived from sludge biogas curves on the basis that biogas production obeys first-order kinetics, were established to be  $0.09 \text{ d}^{-1}$  for PS and  $0.165 \text{ d}^{-1}$  for S8. In this sense, the higher rate of conversion of organic matter obtained with S8 is significant because it implies that a shorter time would be required to stabilize the sludge in the reactor. From an engineering viewpoint, this finding is very relevant since it signifies that smaller digesters can be used in the treatment of such sludges with attendant reductions in processing costs.

## CONCLUSIONS

The amount of solid material in sludges produced by four UA reactors running at a fixed HRT (4 h) with a  $12 \text{ L}\cdot\text{day}^{-1}$  flow rate increased with SRTs within the range of 2 to 8 days. The level of EPS also increased with increasing SRT owing to the formation of cell aggregates with concomitant utilisation of soluble proteins and carbohydrates. The concentrations of TDS, VDS, FDS, DOC, COD, proteins and carbohydrates in the sludges increased after alkaline solubilisation according to the order  $\text{S8} > \text{S2} > \text{PS}$ . The results indicate that, in comparison with the primary decanter, UA reactors running at low SRTs produced less excess sludge, sludges with higher amounts of soluble materials and, consequently, higher biogas potential. In addition, alkaline solubilization of the sludge should lead to better performance in the last stages of anaerobic digestion with greater efficiency in biogas generation.

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**Figure 2.** Biochemical methane potential (BMP) per gram of volatile suspended solids (VSS) as determined for primary (◆) and S8 (●) sludges over 21-day period. S8 was generated using an upflow anaerobic reactor operating at a solids retention time of 8 days

## REFERENCES

- ALMEIDA GN, GONÇALVES MC and GUIMARÃES SAZ (1991) Utilização do teste de resistência específica na operação de filtros prensa. *Revista DAE* **160** 20–26. [http://revistadae.com.br/artigos/artigo\\_edicao\\_160\\_n\\_115.pdf](http://revistadae.com.br/artigos/artigo_edicao_160_n_115.pdf)
- AMERICAN PUBLIC HEALTH ASSOCIATION (2012) *Standard Methods for the Examination of Water and Wastewater* (22<sup>nd</sup> edn). APHA, Washington.
- ANGELIDAKI I, ALVES M, BOLZONELLA D, BORZACCONI L, CAMPOS JL, GUWY AJ, KALYUZHNYI S, JENICEK P and VAN LIER JB (2009) Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Sci. Technol.* **59** 927–934. <https://doi.org/10.2166/wst.2009.040>
- AQUINO SF, GLORIA RM, SILVA SQ and CHERNICHARO CA (2009) Quantification of the inert chemical oxygen demand of raw wastewater and evaluation of soluble microbial product production in demo-scale upflow anaerobic sludge blanket reactors under different operational conditions. *Water Environ.* **81** 608–616. <https://doi.org/10.2175/106143008X370386>
- BI W, LI Y and HU Y (2014) Recovery of phosphorus and nitrogen from alkaline hydrolysis supernatant of excess sludge by magnesium ammonium phosphate. *Bioresour. Technol.* **166** 1–8. <https://doi.org/10.1016/j.biortech.2014.04.092>
- CHEN Y, JIANG S, YUAN H, ZHOU Q and GU G (2007) Hydrolysis and acidification of waste activated sludge at different pHs. *Water Res.* **41** 683–689. <https://doi.org/10.1016/j.watres.2006.07.030>
- CHO HU, PARK SK, HA JH and PARK JM (2013) An innovative sewage sludge reduction by using a combined mesophilic anaerobic and thermophilic aerobic process with thermal-alkaline treatment and sludge recirculation. *J. Environ. Manage.* **129** 274–282. <https://doi.org/10.1016/j.jenvman.2013.07.009>
- DUBOIS M, GILLES KA, HAMILTON JK, REBERS PA and SMITH F (1956) Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28** 350–356. <https://doi.org/10.1021/ac60111a017>
- FENG X, TANG B, BIN L, SONG H, HUANG S, FU F, DING J, CHEN C and YU C (2016). Rheological behavior of the sludge in a long-running anaerobic digester: Essential factors to optimize the operation. *Biochem. Eng. J.* **114** 147–154. <https://doi.org/10.1016/j.bej.2016.06.022>
- FRØLUND B, GRIEBE T and NIELSEN PH (1995) Enzymatic activity in the activated-sludge floc matrix. *Appl. Microbiol. Biotechnol.* **43** 755–761. <https://doi.org/10.1007/BF00164784>
- GURJE W and ZEHNDER AJB (1983) Conversion processes in anaerobic digestion. *Water Sci. Technol.* **15** 127–167. <https://doi.org/10.2166/wst.1983.0164>
- KIM J, PARK C, KIM T-H, LEE M, KIM S, KIM S-W and LEE J (2003) Effects of various pretreatments for enhanced anaerobic digestion with waste activated sludge. *J. Biosci. Bioeng.* **95** 271–275. [https://doi.org/10.1016/S1389-1723\(03\)80028-2](https://doi.org/10.1016/S1389-1723(03)80028-2)

- KOOIJMAN G, DE KREUK MK and VAN LIER JB (2017) Influence of chemically enhanced primary treatment on anaerobic digestion and dewaterability of waste sludge. *Water Sci. Technol.* **76** 1629–1639. <https://doi.org/10.2166/wst.2017.314>
- KUNACHEVA C, SOH YNA and STUCKEY DC (2017) Effect of feed pH on reactor performance and production of soluble microbial products (SMPs) in a submerged anaerobic membrane bioreactor. *Chem. Eng. J.* **320** 135–143. <https://doi.org/10.1016/j.cej.2017.03.018>
- LI XY and YANG SF (2007) Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. *Water Res.* **41** 1022–1030. <https://doi.org/10.1016/j.watres.2006.06.037>
- LOWRY OH, ROSEBROUGH NJ, FARR AL and RANDALL RJ (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193** 265–275. <http://www.jbc.org/content/193/1/265.short>
- MONTE FP, BRITO ALM, LOPES WS and CEBALLOS BSO (2017) Aumento da produção de biogás (metano) a partir da solubilização de lodos de esgotos. In: Costa One GM and Albuquerque HN (eds). *Saúde e Meio Ambiente: Os Desafios da Interdisciplinaridade nos Ciclos da Vida Humana*. Instituto Bioeducação, Campina Grande. 276–294. <http://cinasama.com.br/upload/160217040814257614.pdf>
- RANI R U, KALIAPPAN S, KUMAR S A and BANU JR (2012) Combined treatment of alkaline and disperser for improving solubilization and anaerobic biodegradability of dairy waste activated sludge. *Bioresour. Technol.* **126** 107–116. <https://doi.org/10.1016/j.biortech.2012.09.027>
- SAHINKAYA S (2015) Disintegration of municipal waste activated sludge by simultaneous combination of acid and ultrasonic pretreatment. *Process Saf. Environ.* **93** 201–205. <https://doi.org/10.1016/j.psep.2014.04.002>
- SHENG G-P, YU H-Q and LIX-Y (2010) Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review. *Biotechnol. Adv.* **28** 882–894. <https://doi.org/10.1016/j.biotechadv.2010.08.001>
- TCHOBANOGLOUS G, BURTON FL and STENSEL HD (2014) *Wastewater Engineering: Treatment and Resource Recovery* (5<sup>th</sup> edn). McGraw Hill, New York.
- TIAN X, WANG C, TRZCINSKI A P, LIN L and NG WJ (2014) Insights on the solubilization products after combined alkaline and ultrasonic pre-treatment of sewage sludge. *J. Environ. Sci.* **29** 97–105. <https://doi.org/10.1016/j.jes.2014.07.024>
- WANG Z, MA J, TANG CY, KIMURA K, WANG Q and HAN X (2014) Membrane cleaning in membrane bioreactors: A review. *J. Membrane Sci.* **468** 276–307. <https://doi.org/10.1016/j.memsci.2014.05.060>
- XU J, YUAN H, LIN J and YUAN W (2014) Evaluation of thermal, thermal-alkaline, alkaline and electrochemical pretreatments on sludge to enhance anaerobic biogas production. *J. Taiwan Inst. Chem. Eng.* **45** 2531–2536. <https://doi.org/10.1016/j.jtice.2014.05.029>
- YE F, PENG G and LI Y (2011) Influences of influent carbon source on extracellular polymeric substances (EPS) and physicochemical properties of activated sludge. *Chemosphere* **84** 1250–1255. <https://doi.org/10.1016/j.chemosphere.2011.05.004>