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Anaerobic mesophilic co-digestion of ensiled sorghum, cheese whey and liquid cow manure in a two-stage CSTR system : Effect of hydraulic retention time

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Abstract

The aim of this study was to investigate the effect of hydraulic retention time (HRT) on hydrogen and methane production using a two-stage anaerobic process. Two continuously stirred tank reactors (CSTRs) were used under mesophilic conditions (37°C) in order to enhance acidogenesis and methanogenesis. A mixture of pretreated ensiled sorghum, cheese whey and liquid cow manure (55:40:5, v/v/v) was used. The acidogenic reactor was operated at six different HRTs of 5, 3, 2, 1, 0.75 and 0.5d, under controlled pH 5.5, whereas the methanogenic reactor was operated at three HRTs of 24, 16 and 12d. The maximum H_2 productivity (2.14 $L/L_R \cdot d$) and maximum H_2 yield (0.70 mol H_2/mol carbohydrates consumed) were observed at 0.5d HRT. On the other hand,

the maximum CH_4 production rate of 0.90 $L/L_R{\cdot}d$ was achieved at HRT of 16d, whereas at lower HRT the process appeared to be inhibited and/or overloaded.

Keywords : anaerobic digestion, hydraulic retention time, agro-industrial wastewaters, ensiled sorghum, two-stage system

1. INTRODUCTION

Renewable energy sources have received great interest from the international community during the last decades. Biomass is one of the oldest and the most promising energy sources. Organic wastes such as animal wastes, wastewaters with high organic content, energy crops, agricultural and agro-industrial residues can be used for the production of power, heat and biofuels (**Claassen et al., 1999**). Among agro-industrial wastewaters, cheese whey and liquid cow manure can be used for the production of biogas.

Cheese manufacturing industry generates large amounts of high strength wastewater, with associated high biological (BOD_5) and chemical oxygen demand (COD) with the BOD_5/COD ratio being commonly higher than 0.5 (**Prazeres et al., 2012**). Cheese whey (CW) is a by-product of cheese manufacturing which mainly contains a significant amount of carbohydrates (4–5%), mainly lactose (45-50 g/L), proteins (6-8 g/L), lipids (4-5 g/L) and mineral salts (8-10% of dried extract); mineral salts include NaCl and KCl (>50%), calcium salts and others. CW also contains appreciable quantities of lactic (0.5 g/L) and citric acid and B-group vitamins (**Prazeres et al., 2012**). Hence, this substrate is easily amenable to bioconversions (**Prazeres et al., 2012**). However, despite its high carbohydrate content, the anaerobic treatment of raw

CW is quite problematic due to its low bicarbonate alkalinity (50 meq/L), high COD concentration (up to 70 g COD/L) and tendency to rapid acidification (**Prazeres et al., 2012**).

Liquid cow manure (LCM) is one of the most polluting agro-industrial wastes. Intensive dairy farming produces large amount of manure which, when not properly managed, can cause severe environmental problems due to its high organic matter, nitrogen and phosphorous concentrations, such as eutrophication of water receptors (**Carpenter et al., 1998**), air pollution due to volatilization of ammonia and other compounds (**Ryden et al., 1987**) and soil degradation when manure is applied in excess.

Sorghum is a C_4, heat- and drought - tolerant highly productive crop, with a high photosynthetic efficiency. It can be considered as replacement to corn since it requires less water and exhibits better yields than corn in hotter and drier areas. Sorghum is one of several plant species that has been identified as a promising "energy crop" and has thus generated great interest as a feedstock for biogas production (**Sambusiti et al., 2013b**), due to its high yields and biodegradability. Sorghum is a quite diverse species but generally falls into four categories, including grain sorghum, forage sorghum, sudangrass and sudangrass hybrids sorghum. Its biomass, as lignocellulosic grass, is composed mainly of cellulose, hemicelluloses and lignin, while its fraction of soluble sugars is rich in glucose and sucrose. Fermentable soluble sugars are the primary source for bioethanol and biogas production. Normally, cellulose and hemicelluloses are degradable by anaerobic microorganisms; nevertheless, their association with lignin, which acts as a physical barrier, limits their degradation. These limitations can be overcome by pretreatment methods, which break down the linkage between

polysaccharides and lignin thus making cellulose and hemicellulose more accessible to hydrolytic enzymes during anaerobic digestion (**Mosier et al., 2005**). Utilization of sorghum stalks during a year-round operation of an anaerobic digester requires a cost-effective and non-destructive, if possible, method of preservation and storage of harvested sorghum stalks for extended periods of time. To this end, sorghum ensiling is the preferred long-term preservation method.

Multiple streams of organic substrates can be anaerobically co-digested to generate a homogeneous mixture increasing both process and equipment performance. The two-stage anaerobic treatment process has several advantages over the conventional single-stage one, since it permits the selection and enrichment of different bacteria in each digester. It increases thus the stability of the whole process by controlling the acidification phase in the first digester and hence preventing overloading and/ or inhibition of the methanogenic population in the second digester (**Nathao et al., 2013**). At the first stage (acidogenesis) generation of biological hydrogen occurs whereas, at the second stage (methanogenesis), methane evolves. Optimum environmental and operational conditions for each microbial community may be achieved in such a separated two-reactor system resulting in the production of significant amounts of gaseous high-energy end-products (CH_4 and/or H_2). A series of operational parameters including pH (**Dareioti et al., 2014a**), temperature, reactor configuration (**Nasir et al., 2012**), organic loading rate (**Mariakakis et al., 2011**) and hydraulic retention time (**Rincón et al., 2008**) have been investigated in the literature due to their effect on biogas productivity. Among them, hydraulic retention time (HRT) has been reported as one of the most important parameters significantly affecting microbial ecology in CSTR

digesters and must be thus optimized for the particular feedstock fermented in the digester.

There are many reports in the literature on the continuous anaerobic digestion of cheese whey and liquid cow manure either as mono-substrates (**Ghaly, 1996**), or being co-digested (**Kavacik and Topaloglu, 2010**) or, even more, co-digested with other substrates (**Dareioti et al., 2009**). Notwithstanding, continuous anaerobic digestion experiments using sorghum as substrate, are time-consuming and complex, so methane productivity testing in the literature is generally based on the results of batch tests (**Antonopoulou and Lyberatos, 2013**). The commonly used batch tests, although valuable for establishing methane production potentials under specific conditions may fail to truly predict full-scale anaerobic reactors performance, due to their dependency on inoculum type, the substrate to inoculum ratio, and the batch nature of the test itself. Therefore, in order to monitor possible inhibition effects due to addition of chemicals and evaluate the anaerobic digestion performance in terms of biogas production, tests with continuous reactors are needed in order to confirm and quantify the effect on anaerobic digestion of a specific substrate and/or specific operational conditions (**Carrère et al., 2010**). According to our knowledge, the lignocellulosic substrates treatment and especially the co-digestion of them with other substrates have been poorly studied with continuous anaerobic reactors. For instance, co-digestion of manures, energy crops and agro-wastes, was studied by **Giuliano et al. (2013)** using pilot-scale CSTRs, and proved its viability at all operating conditions tested**.** Furthermore, only few studies are yet available treating raw sorghum on anaerobic digestion in continuous anaerobic reactors and especially in a two-stage system for hydrogen and methane production, respectively. **Sambusiti et al. (2013b)**, for example, studied the effect of

pretreated ensiled sorghum forage in single-stage anaerobic digestion and showed an increase of 25% on methane production in comparison with untreated sorghum.

The objective of this investigation was to study the anaerobic co-treatment of a mixture of pretreated ensiled sorghum (ES), cheese whey (CW) and liquid cow manure (LCM) (in a ratio 55:40:5, v/v/v) in a two-stage continuous anaerobic process. More specifically, our aim was to study the effect of HRT, as one of the most critical operating parameters, on hydrogen and methane productivity and also the contribution of ensiled sorghum which replaced olive mill wastewater (OMW) in the same mixture previously studied by **Dareioti et al. (2014b)**. Replacement of OMW by ES simulates the operation of a decentralized AD plant fed with regional agro-wastes which lacks OMW due to seasonal unavailability.

2. MATERIALS AND METHODS

2.1. Substrates

The substrates used in the present study were two typical agro-industrial wastewaters, namely cheese whey (CW) and liquid cow manure (LCM), and an energy crop, i.e. ensiled sorghum (ES). CW was provided from a local cheese factory located in the area of Patras (Western Greece) producing mainly "feta" cheese with daily production of 30 m^3 of wastewaters. LCM was collected from a dairy farm in the same region, breeding 230 cows. Due to their high tendency for fermentation, all wastewater samples were collected fresh and stored immediately in the freezer at −18°C until subsequent use throughout the experimentation period. **Table 1** represents the average values measured during the characterization of each wastewater.

Sorghum (*Sorghum Sudangrass hybrid - HoneyGraze BMR*) was cultivated through biological farming techniques according to European Regulation EC 2092/91. Geo-coordinates of the fields were 38°06΄42.27΄΄N 21°38΄26.37΄΄E. It was harvested in November 2011 from a farm near Patras (Western Greece), chopped into pieces (particle size ranged between 1-3 cm) and finally the fresh chopped sorghum was ensiled by enclosement for 60 days in 30 L plastic bins at ambient temperature. After ensiling, sorghum samples was dried at 55°C, ground into 1 mm particle size with a kitchen blender and sieved to powder of < 315 μm diameter. The effect of mechanical pretreatment on methane production and hydrolysis kinetics has already been investigated by many authors. **Sambusiti et al. (2013a)**, for example, found no significant differences in terms of methane yields and kinetic constants using ensiled sorghum forage milled into 2, 1, 0.5 and 0.25 mm particle sizes. The chemical composition of the ensiled sorghum used in this study, after drying and milling, is given in **Table 2.**

A mixture of pretreated ES, CW and LCM (in a ratio of 55:40:5, v/v/v) was used, based on our previous study (**Dareioti et al., 2014b**). In the present work, the ensiled sorghum was used to replace olive mill wastewater in the mixture because of its seasonal unavailability.

2.2. Alkaline pretreatment

In this study, ES was chemically pretreated using a solution mixture of 1.0% NaOH and 1.0% KOH (w/w dry matter), whereas the solid : liquid ratio was 8% (w/v). The concentration of alkaline solution was chosen so that the concentrations of sodium and potassium in the final mixture remained lower than 5.5 g Na^+ /L and 0.15 M K^+,

respectively, in order to avoid irrecoverable inhibition to methanogens (**Chen et al., 2008**). The alkaline pretreatment was carried out in closed bottles maintained in an orbital shaking water bath (Grant OLS200) continuously agitated at 70 rpm. Sorghum was soaked in the alkaline solution at different temperatures (25, 37, 50 and 80°C) and contact times (0.5, 1, 2 and 3 h) in order to select the optimal conditions for maximization of sugars solubilisation. No further increase in temperature was tested in order to avoid possible inhibition of methanogens from furfural (**Benjamin et al., 1984**). Due to alkaline processing the pretreated ES had a final pH ranging between 12 and 13. It was thus neutralised to pH 7.0 with a concentrated HCl (37%) solution prior to its use in anaerobic digestion.

2.3. Reactors configuration

Experiments were carried out in two CSTR reactors, one used for acidogenesis and the other one for methanogenesis. A schematic description of bioreactors' setup is shown in **Fig. 1**. The two anaerobic reactors were cylindrical in shape, made entirely of stainless steel (INOX 316) with a double wall, having an operating volume of 0.5 L and 4 L, respectively and were operated at constant temperature (37 ± 0.2°C) via a thermocouple controller. Agitation was performed by a geared motor drive unit which was installed on the top of each reactor. The feedstock was stored in a tank inside a refrigerator to maintain constant temperature at 4°C. Both reactors were fed via a double-headed precise peristaltic pump (Watson Marlow Bredel 323). Biogas production was measured separately in each one of the reactors by automatic tailor-made devices comprising of a combination of an engine oil filled U-tube, an electron – valve and a counter. The measurement was based on counting the number of displacements of constant oil volume by the produced biogas in each biogas line. In this study, the acidogenic reactor

was operated under controlled pH throughout the experimentation phase via automatic control (using a Hach PID-controller) by the addition of a solution mixture of NaOH/KOH (1.5 N/1.5 N) via a peristaltic pump, whereas the methanogenic reactor was operated at non-controlled pH conditions.

2.4. Reactors start-up and operation

For the start-up of the system the acidogenic and the methanogenic reactor was filled up with anaerobically digested sludge removed from the acidogenic and methanogenic reactor, respectively, of a two-stage lab-scale system fed with a waste mixture of olive mill wastewater (OMW), CW and LCM in a ratio 55:40:5 (v/v/v). The acidogenic reactor was initially operated batchwise for 48 h and then switched to continuous mode at HRT of 5 d and low organic loading rate (OLR), 17.16 kg COD/m^3·d, which was subsequently increased during the course of the experiment. The continuous operation of the methanogenic reactor started at HRT of 24 d, while the reactor was being fed with acidified effluent from the acidogenic one. Anaerobic conditions in both anaerobic reactors were ensured by sparging with nitrogen gas their liquid content at the beginning of each experiment. Aliquots of mixed liquor were withdrawn periodically from both reactors under continuous stirring conditions and analysed, at least in duplicate, for monitoring each reactor's performance.

Experiments were conducted successively to determine the optimum HRT for maximum hydrogen and methane production. To this end, OLR was increased by decreasing the operating HRT. The acidogenic reactor was thus operated at six different HRTs of 5, 3, 2, 1, 0.75 and 0.5 d with a feeding mixture of pretreated ES, CW and LCM in a ratio 55:40:5 (v/v/v), respectively. The pH in the acidogenic reactor was kept

constant throughout the experimentation phase at pH 5.5, based on previous results. Effluent from the acidogenic reactor was used for feeding the methanogenic one which was operated at three different HRTs (24, 16 and 12 days) equivalent to OLRs of 3.58, 5.36 and 7.15 kg COD/m^3·d, respectively. The tested operating conditions in the two-stage system are summarized in **Table 3**.

2.5. Analytical determinations

The off-line pH measurements were carried out using an electrode (Orion 3-Star), while TS, VS, total and soluble COD, TKN, ammonium nitrogen, total and ortho – phosphates and alkalinity were determined according to *Standard Methods* (**APHA, 1995**). Proteins were determined by multiplying the TKN content by 6.25. Total organic carbon (TOC) was analyzed with a Carbon TOC-V analyzer (Shimadzu). For the measurement of soluble compounds (soluble organic carbon, lactic acid, VFAs etc), the insoluble residue was separated from the supernatant through Whatman® glass microfiber filters, Grade GF/F. For the determination of carbohydrates, a colored sugar derivative was produced through the addition of L-tryptophan, sulfuric and boric acid, which was subsequently measured colorimetrically at 520 nm, as described in detail by **Dareioti et al. (2009)**. The concentrations of VFAs, lactic acid, alcohols and produced biogas composition were measured as reported in our previous study (**Dareioti et al., 2014a**). Cellulose, hemicellulose and lignin contents in the ES were also determined according to **Sloneker (1971)**, **Myhre and Smith (1960)** and **Panagiotopoulos et al. (2010)**, respectively. The gas volume, produced from each reactor, was converted at standard temperature (0 ºC) and pressure (760 mm Hg) conditions.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of raw substrates

The composition analysis of each agro-industrial waste, i.e. CW and LCM, is presented in **Table 1**. Significant differences in the composition of wastewater streams were detected. In particular, CW presented higher organic content (93.21 ± 2.99 g COD/L), compared to LCM (43.14 ± 2.56 g COD/L). CW was characterized by high organic load mainly due to carbohydrates (lactose) and low nitrogen content (0.81 g/L) in contrast with LCM (2.78 g/L). LCM contributes in the buffering capacity of the mixture as a consequence of its neutral pH (7.24 ± 0.18) and alkalinity in high levels (12.38 ± 0.32 g $CaCO_3$/L). It is important to take into consideration the fact that alkalinity should be high enough to avoid destabilization of the system caused by potential accumulation of volatile fatty acids.

Table 2 summarizes the chemical composition of dried, milled and sieved ensiled sorghum (ES). The sorghum mainly consisted of polysaccharides (37.60% cellulose, 25.51% hemicellulose), whereas its total lignin content was 17.28%. These results are in accordance with the typical composition of sweet sorghum reported by **Panagiotopoulos et al. (2010)**. The ash content of the total dry matter of ensiled sorghum stalks was rather low (5.93%). During the ensiling procedure soluble carbohydrates are utilized by fermentative bacteria for the production of volatile fatty acids, lactic acid and ethanol, depending on the followed metabolic pathway. Thus, the amount of soluble carbohydrates of ES is low in contrast to the increased content of lactic acid. Prior to its use as feeding material in mixture with CW and LCM, ES was subjected to alkaline pretreatment at 80°C for 2 h with the addition of alkaline solution 1.0% NaOH and 1.0% KOH (w/w dry matter).

3.2. Effect of HRT in the acidogenic reactor

As shown in **Table 3**, the acidogenic reactor was operated at different HRTs, i.e. 5, 3, 2, 1, 0.75 and 0.5 d. After reactor start-up, the initial HRT was set at 5 d for a period of 114 days. HRT was then decreased to 3 d, 2 d, 1 d, 0.75 d and 0.5 d for a period of 54, 55, 65, 36 and 19 days, respectively, reaching steady-state conditions in the reactor. The biogas produced from the acidogenic reactor consisted exclusively of hydrogen and carbon dioxide, whereas no methane was detected indicating the absence or complete inhibition of methanogens under the tested operating conditions. **Fig. 2(a)** illustrates the net biogas and hydrogen production rate as a function of experimental time, at standard temperature and pressure (STP) conditions. The fluctuation of produced biogas may be attributed to microbial population shifts taking place during the extended period of operation, whereas the complexity of the feeding medium and in particular the presence of various organic and inorganic materials may have also caused temporary inhibitory effects.

Initially, the biogas production rate at HRT of 5 d was fluctuating with a mean value of 0.31 $L/L_R \cdot d$ containing 22.16% of hydrogen at the steady state (with the rest being mainly CO_2). The biogas and hydrogen production rate systematically increased when the HRT was decreased from 5 to 0.5 d, where the highest biogas and hydrogen production rates were obtained (5.69 and 2.14 $L/L_R \cdot d$, respectively). A characteristic of the system under consideration is the strong fluctuations of biogas production and hence all relevant parameters not only during the transition from one phase to another, but also from one day to the next even within the same phase. Such fluctuations can be attributed to the instability of such a system operated as CSTR and were also observed in other studies (e.g. **Mariakakis et al., 2011**). It has been widely reported that the

hydrogen productivity increases with decreasing HRT, which is however expected in continuously operating systems without kinetic limitations. For instance, **Scoma et al. (2013)** investigated the anaerobic acidogenic process of dephenolized olive mill waste using Packed Bed Biofilm Reactor and observed a significant increased production of a hydrogen-rich biogas when shorter HRTs (7, 5, 3 and 1 d) were applied. Moreover, **Dareioti et al. (2014b)** conducted experiments in the same system configuration in order to investigate the effect of HRT using the same mixture with olive mill waste instead of pretreated ensiled sorghum. Studying five different HRTs ranging from 5 to 0.75 d, an increase in hydrogen productivity with decreasing HRT was also observed.

Hydrogen yield (calculated as mol H_2/mol carbohydrate consumed) is a good indicator of the microbial populations' effectiveness for hydrogen production and represents the capability of microorganisms to convert carbohydrates into hydrogen gas. The variation of average hydrogen production rate, hydrogen content and hydrogen yield observed at different HRTs is shown in **Fig. 3**. When operating at low HRTs, i.e. 0.75 and 0.5 d, a significant increase was observed in all these parameters. The hydrogen content increased with a decrease in HRT, up to 33.42% at HRT of 0.75 d. The maximum hydrogen yield 0.70 mol H_2/mol carbohydrate consumed was achieved at HRT 0.5 d, whereas the hydrogen yield was very low at higher HRT (0.25 mol H_2/mol carbohydrate consumed).

In summary, shortening the HRT to 0.5 d was sufficient to achieve the highest hydrogen productivity and reduce the diversity of microbial populations associated with elimination of product inhibitors. This low productivity at higher HRTs was likely due to bacteria limitation by the low substrate concentration supplied, which facilitates

microbial population shift and growth of homoacetogens, mainly homoacetogenic clostridia, in the reactor (**Chen et al., 2009**). As regards soluble end-products concentration, significant production of volatile fatty acids (namely acetic, propionic, isobutyric, butyric and caproic acid) and lactic acid were noted as the most prominent ones at all tested HRTs (**Fig. 2(b)**). Valeric acid and ethanol were also measured in concentrations, though, less than 1000 ppm, whereas isovaleric acid was measured in trace concentrations. The complex and fluctuating end-product distribution of this reactor is likely attributed to the complex nature of the wastewater mixture tested. As shown in **Fig. 2(b)** an appreciable amount of propionic acid was produced, especially at HRT of 3 d, which may be one of the reasons of low hydrogen productivity. The production of soluble end-products was a result of carbohydrates degradation. For all HRT studied, carbohydrate utilization efficiency was not affected by HRT changes and was over 34 and 86% for total and soluble, respectively (**Fig. 2(c)**). This is consistent with previous results that carbohydrates degradation of dairy wastewater was not affected by HRT changes (**Fang and Yu, 2000**), suggesting no inhibition or limitation of the acidogenic population to consume carbohydrates even at low retention time of microorganisms. In our study, the low degradation of total carbohydrates was due to the lignocellulosic structure of ensiled sorghum. However, maximization of soluble organic matter and VFAs concentration was crucial for the following methanogenic phase.

Fig. 2(d) presents the evolution of total (TCOD) and soluble COD (SCOD), in the effluent from the acidogenic reactor during the 343 days of its operation. No significant decrease was observed comparing their mean values in the influent and effluent stream. In our previous works operating acidogenic reactors at fixed HRT of 3 d without pH control, no COD removal was observed using a mixture of olive mill waste, cheese

whey and liquid cow manure (**Dareioti et al., 2009**), which is consistent with the low biogas production reported under these conditions. **Fig. 2(e)** depicts the TS and VS concentration in the acidogenic reactor effluent, which remained constant at 77.92 ± 7.59 g TS/L and 46.83 ± 5.97 g VS/L, respectively. VS removal of 18.99% was reached for all values of HRT tested, whereas negligible difference in TS concentration between influent and effluent was observed.

3.3. Effect of HRT in the methanogenic reactor

A methane bioreactor was used for treating the acidified effluent of the first stage (acidogenic reactor) in order to assess the rate and extent of methanogenesis at three different HRTs (24, 16 and 12 d). **Table 4** presents the experimental results, including methane yields and removal efficiencies obtained at steady-state conditions for the different HRTs. Firstly, at HRT of 24 d, biogas and methane production rates increased until the 17th day of operation up to 1.24 and 0.81 $L/L_R \cdot d$ respectively, as shown in **Fig. 4(a)**, whereas after that, the rates fluctuated and stabilized at mean values of 1.02 ± 0.18 and 0.63 ± 0.11 $L/L_R \cdot d$, respectively. Switching to the lower HRT of 16 d (and thus higher OLR of 5.36 kg $COD/m^3 \cdot d$) at the 78th day, an increase in the produced volume of biogas was observed, which then stabilized at 1.52 ± 0.22 L $biogas/L_R \cdot d$ and 0.90 ± 0.12 L $CH_4/L_R \cdot d$. Besides the increase in biogas volume a slight increase in methane content was also noticed, from 58.27 ± 1.03% to 58.58 ± 1.87% at the steady-state conditions of HRT of 24 and 16 d, respectively (**Fig. 4(b)**). **Kavacik and Topaloglu (2010)** obtained the highest biogas (1.51 $L/L_R \cdot d$) at HRT 5 d from the co-digestion of 50% cheese whey with 50% dairy manure (diluted 1:1) with methane content of 60% and suggested that co-digestion of these two wastes is more advantageous than processing each one separately. Finally, lowering the HRT from 16

to 12 d resulted to instability of reactor performance and also decrease of biogas and methane productivity. The reduction of methane production rate took place simultaneously with the increase of volatile fatty acids (VFA) concentration indicating inhibition of the methanogenic biomass by VFA accumulation (**Fig. 4(c)**). The high concentration of total VFA, during the operation at HRT of 12 d, was mainly due to the high acetic acid concentration (up to 10.17 g/L), while the concentration of propionic, butyric and caproic acid were also increased at a lower extent (up to 2.56 g/L, 3.72 g/L and 1.77 g/L, respectively), leading to methanogenesis inhibition with a consequent reduction of methane production. The effect of HRT on anaerobic digestion of poultry slaughterhouse wastes was examined by **Salminen and Rintala (2002)** who observed a gradual accumulation of total VFA with simultaneous decline in the methane yield in the range of HRT from 25 to 13 d. On the other hand, as shown in **Fig. 4(c)**, total VFAs concentration remained lower than 0.5 g/L at HRT values of 24 and 16 d, indicating process stability. Some peaks were however periodically observed, especially during the initial start-up (day 1-48), which was overtaken after that. **Koutrouli et al. (2009)** studied the effect of HRT on two-stage digestion of two-phase olive mill waste (water-diluted 1:4) and observed that methane yield increased with decreasing HRT. However, methane productivity reached the maximum value of 1.13 ± 0.08 $L/L_R{\cdot}d$ at HRT 10 d, while the reactor failed at lower HRT tested (5 d).

The summarized average values of methane production rate, methane content and methane yields at steady-state conditions of each HRT tested in this study are presented in **Table 4**. The methane yield shown in **Table 4** was determined from the experimental data at each HRT on the basis of volatile solids added (expressed thus as mL CH_4/g VS $_{added}$) and the COD removed (expressed as mL CH_4/g COD $_{removed}$).

Methane yields of 326.42 and 310.34 mL CH_4/g VS $_{added}$ were thus calculated at HRT of 24 and 16 d respectively, whereas at the lower HRT (12 d) it was almost zero due to the negligible methane productivity. Hence, better performance and higher methane yields were noticed in this study by co-digesting ensiled sorghum with CW and LCM compared to the results obtained in the study of anaerobic digestion of untreated and pretreated ensiled sorghum in two semi-continuous CSTR (**Sambusiti et al., 2013b**). In the latter study, 237 and 297 mL CH_4/g VS $_{added}$ was achieved for untreated and pretreated ensiled sorghum, respectively, suggesting that an alkaline pretreatment step (40°C for 24 h with the addition of 10% NaOH) prior to the anaerobic digestion could have a beneficial effect in enhancing methane production.

Comparing our present results with the ones obtained in a previous study (**Dareioti et al., 2014b**), the presence of pretreated ensiled sorghum in the mixture instead of olive mill waste contributed to higher methane productivity, especially at lower operating HRTs. On the other hand, the methane yield, i.e. the conversion of organic matter to methane, was lower in this study (223.09 and 216.50 mL CH_4/g COD $_{removed}$) compared with the same mixture with olive mill waste (294.37 and 316.08 mL CH_4/g COD $_{removed}$), mainly due to the fact that sorghum is lignocellulosic biomass. Moreover, **Rincón et al. (2008)** found quite similar methane yield (244 mL CH_4/g COD $_{removed}$) obtained in anaerobic digestion of two-phase olive mill solid residue at HRT of 17 d with OLR of 9.2 kg COD/m^3·d.

As shown in **Table 4**, pH remained practically constant at high HRT values (24 and 16 d), with values 8.05 ± 0.06 and 8.00 ± 0.07 respectively, whereas at the lower HRT (12 d) pH decreased at 6.63, as a result of VFA accumulation. The

TVFA/alkalinity ratio can be used as a measure of process stability (**Callaghan et al., 2002**); namely when the ratio is less than 0.3-0.4 (equiv. acetic acid/equiv. $CaCO_3$) the process is considered to be stable without facing any acidification risk. At HRTs of 24 and 16d the TVFA/alkalinity ratio was measured in acceptable values, i.e. ranging from 0.02 to 0.15 which were lower than the suggested failure limit values. However, at HRT 12 d, a considerable increase in the TVFA/alkalinity ratio up to 2.32 was noticed, higher than the safety threshold-value, which was mainly due to VFAs accumulation with a simultaneous decrease in alkalinity to 7.73 g $CaCO_3$/L. Indeed, the process was destabilized and deteriorated due to inhibition of methanogens.

The evolution of total and soluble COD in the methanogenic reactor, as a function of experimental time, is plotted in **Fig. 5(a)**. As can be seen, at the steady-state conditions of both HRTs of 24 and 16 d the COD concentration was almost stable, whereas at the shorter HRT of 12 d the concentration started to rise immediately after the transition, with a concomitant drop in methane production, following the evolution of TVFA. Although at HRT of 12 d most of soluble COD could be accounted for by TVFAs, this was not the case at the higher HRTs indicating the presence of other soluble products which were not detected. The total and soluble COD removal efficiency were both high and similar, namely 84.77% and 84.05% for 24 d HRT and 83.36% and 85.09% for 16 d HRT, respectively. At the lower HRT of 12 d the performance of the reactor deteriorated sharply performing 39.29% removal of total COD and 22.87% of soluble COD. In general, the percentages of organic matter and specifically of COD removal obtained in the present work were higher than those obtained in the anaerobic digestion of the same mixture with olive mill waste instead of ensiled sorghum (**Dareioti et al., 2014b**). This is likely due to the presence of phenolic compounds in olive mill waste,

which are microbiologically toxic and difficult to degrade anaerobically (**Raposo et al., 2003**). **Colussi et al. (2013)** observed a COD removal efficiency of over 80% in a two-stage anaerobic digestion using maize silage as substrate. On the other hand, **Karim et al. (2005)** reported methane production rates of 0.45 L $CH_4/L_R\cdot d$ in the digestion of dairy manure, whereas the removal percentage of TCOD was 50%.

The evolution of solids concentration (TS, VS) during the experimental operation at the three different HRTs is shown in **Fig. 5(b)**. The highest removal efficiencies, in terms of TS and VS, were noticed at HRT of 16 d and were equal to 42.32% and 70.02%, respectively. The degradation of total carbohydrates in glucose equivalents was 90.22% during methanogenesis (data not shown) at HRT of 16 d whereas a slight decrease (86.19%) was observed at HRT of 12 d. The mean value of TKN concentration in the influent for all HRT was 763 mg/L (15% in the form of ammonium nitrogen with the rest being organic N). In the effluent, the mean TKN concentration was 730, 640 and 530 mg/L, whereas the mean ammonium nitrogen concentration was 24, 14 and 31mg/L for the HRTs 24, 16 and 12 d, respectively. This change is due to ammonification process, ammonia nitrogen use in cellular synthesis processes and gaseous emissions of ammonia nitrogen due to increased pH.

4. CONCLUSIONS

Co-digestion of pretreated ES, CW and LCM (55:40:5, v/v/v) was efficiently demonstrated in a two-stage anaerobic system. The highest hydrogen production rate (2.14 $L/L_R\cdot d$) and hydrogen yield (0.70 mol H_2/mol carbohydrates consumed) was achieved when the acidogenic reactor was operated at HRT 0.5d. The highest methane productivity (0.90 L $CH_4/L_R\cdot d$) was achieved at HRT 16d, whereas the methane yield

was 223.09 and 216.50 mL CH_4/g COD consumed at HRTs of 24 and 16d, respectively. Switching to a lower HRT of 12d resulted to reactor instability and inhibition of methane production as a consequence of significant accumulation of VFAs.

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REFERENCES

1. Antonopoulou, G., Lyberatos, G., 2013. Effect of pretreatment of sweet sorghum biomass on methane generation. Waste Biomass Valoriz. 4, 583-591.
2. APHA AWWA WEF, 1995. Standard Methods for the Examination of Water and Wastewater, 19th edition. American Public Health Association, Washington DC, USA.
3. Benjamin, M.M., Woods, S.L., Ferguson, J.F., 1984. Anaerobic toxicity and biodegradability of pulp mill waste constituents. Water Resour. 18, 601-607.
4. Callaghan, F.J., Wase, D.A.J., Thayanithy, K., Forster, C.F., 2002. Continuous co-digestion of cattle slurry with fruit and vegetable wastes and chicken manure. Biomass Bioenerg. 22, 71-77.
5. Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W., Sharpley, A.N., Smith, V.H., 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol. Appl. 8, 559-568.

6. Carrère, H., Dumas, C., Battimelli, A., Batstone, D.J., Delgenès, J.P., Steyer, J.P., Ferrer, I., 2010. Pretreatment methods to improve sludge anaerobic degradability: A review. J. Hazard. Mater. 183, 1-15.
7. Chen, W.-H., Sung, S., Chen, S.-Y., 2009. Biological hydrogen production in an anaerobic sequencing batch reactor: pH and cyclic duration effects. Int. J. Hydrogen Energy 34, 227-234.
8. Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: A review. Bioresour. Technol. 99, 4044-4064.
9. Claassen, P.A.M., Lopez Contreras, A.M., Sijtsma, L., Weusthuis, R.A., Van Lier, J.B., Van Niel, E.W.J., Stams, A.J.M., De Vries, S.S., 1999. Utilisation of biomass for the supply of energy carriers. Appl. Microbiol. Biot. 52, 741-755.
10. Colussi, I., Cortesi, A., Piccolo, C.D., Gallo, V., Fernandez, A.S.R., Vitanza, R., 2013. Improvement of methane yield from maize silage by a two-stage anaerobic process. Chem. Eng. T. 32, 151-156.
11. Dareioti, M.A., Dokianakis, S.N., Stamatelatou, K., Zafiri, C., Kornaros, M., 2009. Biogas production from anaerobic co-digestion of agroindustrial wastewaters under mesophilic conditions in a two-stage process. Desalination 248, 891–906.
12. Dareioti, M.A., Vavouraki, A.I., Kornaros, M., 2014a. Effect of pH on the anaerobic acidogenesis of agroindustrial wastewaters for maximization of bio-hydrogen production: A lab-scale evaluation using batch tests. Bioresour. Technol. 162, 218–227.
13. Dareioti, M.A., Kornaros, M., 2014b. Effect of hydraulic retention time (HRT) on the anaerobic co-digestion of agro-industrial wastes in a two-stage CSTR system. Bioresour. Technol. 167, 407–415.
14. Fang, H.H.P., Yu, H.Q., 2000. Effect of HRT on mesophilic acidogenesis of dairy

wastewater. J. Environ. Eng. 126, 1145-1148.

15. Ghaly, A.E., 1996. A comparative study of anaerobic digestion of acid cheese whey and dairy manure in a two-stage reactor. Bioresour. Technol. 58, 61-72.
16. Giuliano, A., Bolzonella, D., Pavan, P., Cavinato, C., Cecchi, F., 2013. Co-digestion of livestock effluents, energy crops and agro-waste: Feeding and process optimization in mesophilic and thermophilic conditions. Bioresour. Technol. 128, 612-618.
17. Karim, K., Klasson, K.T., Hoffmann, R., Drescher, S.R., DePaoli, D.W., Al-Dahhan, M.H., 2005. Anaerobic digestion of animal waste: Effect of mixing. Bioresour. Technol. 96, 1607-1612.
18. Kavacik, B., Topaloglu, B., 2010. Biogas production from co-digestion of a mixture of cheese whey and dairy manure. Biomass Bioenerg. 34, 1321-1329.
19. Koutrouli, E.C., Kalfas, H., Gavala, H.N., Skiadas, I.V., Stamatelatou, K., Lyberatos, G., 2009. Hydrogen and methane production through two-stage mesophilic anaerobic digestion of olive pulp. Bioresour. Technol. 100, 3718-3723.
20. Mariakakis, I., Bischoff, P., Krampe, J., Meyer, C., Steinmetz, H., 2011. Effect of organic loading rate and solids retention time on microbial population during bio-hydrogen production by dark fermentation in large lab-scale. Int. J. Hydrogen Energy 36 , 10690-10700.
21. Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M., 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour. Technol. 96, 673-686.
22. Myhre, D.V., Smith, F., 1960. Alfalfa Hemicellulose, constitution of the hemicellulose of alfalfa (Medicago sativa). Hydrolysis of hemicellulose and identification of neutral and acidic components. J. Agr. Food Chem. 8, 359–364.

23. Nasir, I.M., Mohd Ghazi, T.I., Omar, R., 2012. Anaerobic digestion technology in livestock manure treatment for biogas production: A review. Eng. Life Sci. 12, 258-269.

24. Nathao, C., Sirisukpoka, U., Pisutpaisal, N., 2013. Production of hydrogen and methane by one and two stage fermentation of food waste. Int. J. Hydrogen Energy 38, 15764-15769.

25. Panagiotopoulos, I.A., Bakker, R.R., De Vrije, T., Koukios, E.G., Claassen, P.A.M., 2010. Pretreatment of sweet sorghum bagasse for hydrogen production by *Caldicellulosiruptor saccharolyticus*. Int. J. Hydrogen Energy 35, 7738-7747.

26. Prazeres, A.R., Carvalho, F., Rivas, J., 2012. Cheese whey management: A review. J. Environ. Manage. 110, 48-68.

27. Raposo, F., Borja, R., Sánchez, E., Martín, M.A., Martin, A., 2003. Inhibition kinetics of overall substrate and phenolics removals during the anaerobic digestion of two-phase olive mill effluents (TPOME) in suspended and immobilized cell reactors. Process Biochem. 39, 425-435.

28. Rincón, B., Borja, R., González, J.M., Portillo, M.C., Sáiz-Jiménez, C., 2008. Influence of organic loading rate and hydraulic retention time on the performance, stability and microbial communities of one-stage anaerobic digestion of two-phase olive mill solid residue. Biochem. Eng. J. 40, 253-261.

29. Ryden, J.C., Whitehead, D.C., Lockyer, D.R., Thompson, R.B., Skinner, J.H., Garwood, E.A., 1987. Ammonia emission from grassland and livestock production systems in the UK. Environ. Pollution 48, 173-184.

30. Salminen, E.A., Rintala, J.A., 2002. Semi-continuous anaerobic digestion of solid poultry slaughterhouse waste: Effect of hydraulic retention time and loading. Water Res. 36, 3175-3182.

31. Sambusiti, C., Ficara, E., Malpei, F., Steyer, J.P., Carrère, H., 2013a. Effect of Particle Size on Methane Production of Raw and Alkaline Pre-treated Ensiled Sorghum Forage. Waste Biomass Valoriz. 4, 549-556.
32. Sambusiti, C., Ficara, E., Malpei, F., Steyer, J.P., Carrère, H., 2013b. Benefit of sodium hydroxide pretreatment of ensiled sorghum forage on the anaerobic reactor stability and methane production. Bioresour. Technol. 144, 149-155.
33. Scoma, A., Bertin, L., Fava, F., 2013. Effect of hydraulic retention time on biohydrogen and volatile fatty acids production during acidogenic digestion of dephenolized olive mill wastewaters. Biomass Bioenerg. 48, 51-58.
34. Sloneker, J.H., 1971. Determination of cellulose and apparent hemicellulose in plant tissue by gas-liquid chromatography. Anal. Biochem. 43, 539-546.

List of Figures

Fig. 1: Schematic diagram of the two-stage system used in this study for combined hydrogen and methane production.

Fig. 2: Evolution of (a) biogas and hydrogen production rate, (b) main end-products, (c) total and soluble carbohydrates concentration, (d) total and soluble COD concentration and (e) total and volatile solids concentration during acidogenic reactor operation at different HRT.

Fig. 3: Effect of HRT on (a) hydrogen production rate, (b) hydrogen content and (c) hydrogen yield, during acidogenic reactor operation.

Fig. 4: Evolution of (a) biogas and methane production rate, (b) methane content and (c) main volatile fatty acids concentration during methanogenesis operated at different HRT.

Fig. 5: Evolution of (a) total, soluble COD and TVFA (expressed in COD units) concentration and (b) total and volatile solids concentration during methanogenesis operated at different HRT.

List of Tables

Table 1: Chemical composition of CW and LCM. Values correspond to mean ± standard deviation of measurement performed in duplicate.

Table 2: Chemical composition of ES. Values correspond to mean ± standard deviation of measurement performed in duplicate.

Table 3: Operating conditions in acidogenic and methanogenic CSTR.

Table 4: Steady-state characteristics of the methanogenic CSTR at each HRT tested.

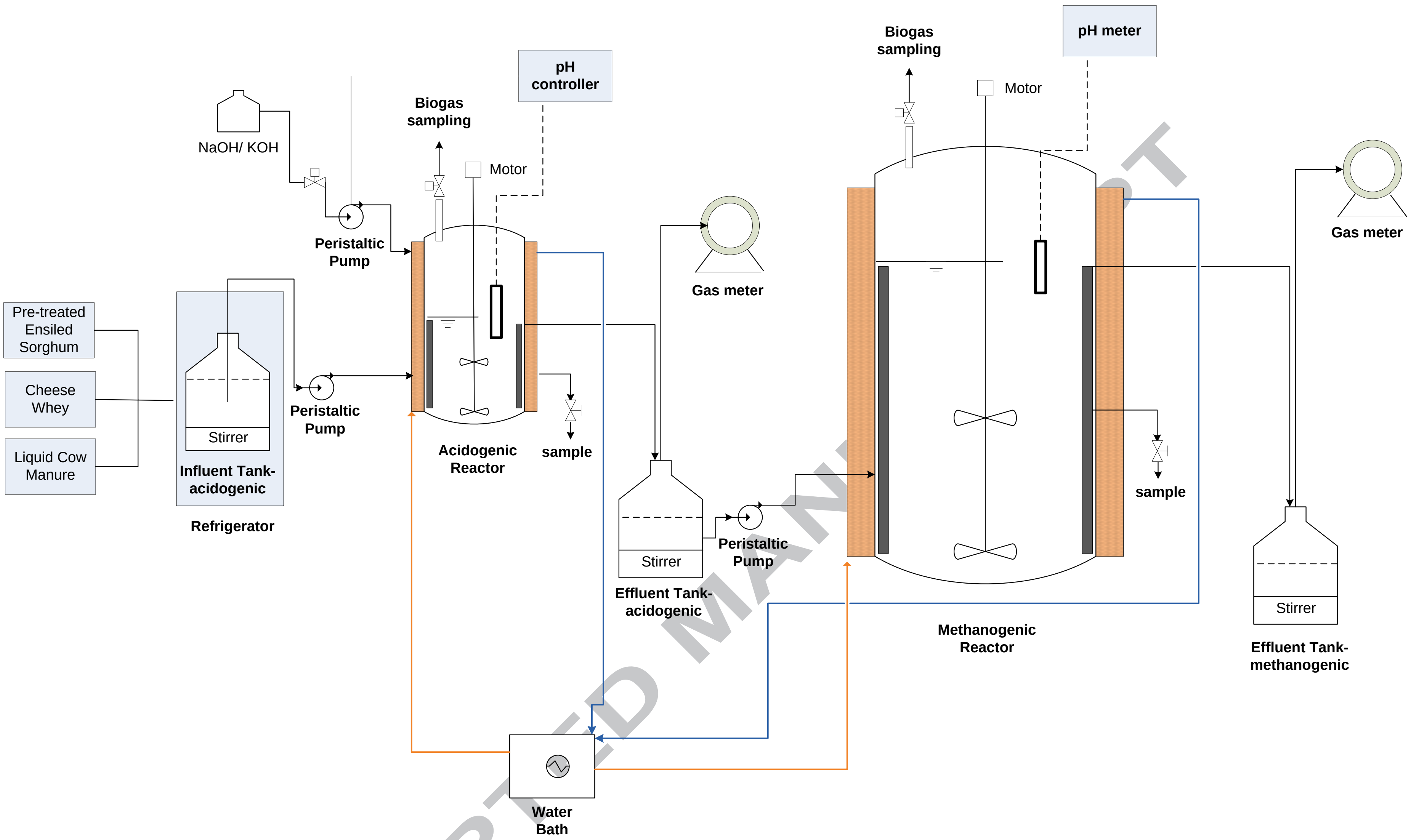

pH controller
pH meter
Biogas sampling
Biogas sampling
Motor
Motor
NaOH/ KOH
Peristaltic Pump
Peristaltic Pump
Peristaltic Pump
Gas meter
Gas meter
Pre-treated Ensiled Sorghum
Cheese Whey
Liquid Cow Manure
Stirrer
Influent Tank-acidogenic
Refrigerator
Acidogenic Reactor
sample
sample
Stirrer
Effluent Tank-acidogenic
Methanogenic Reactor
Stirrer
Effluent Tank-methanogenic
Water Bath

Figure 1.

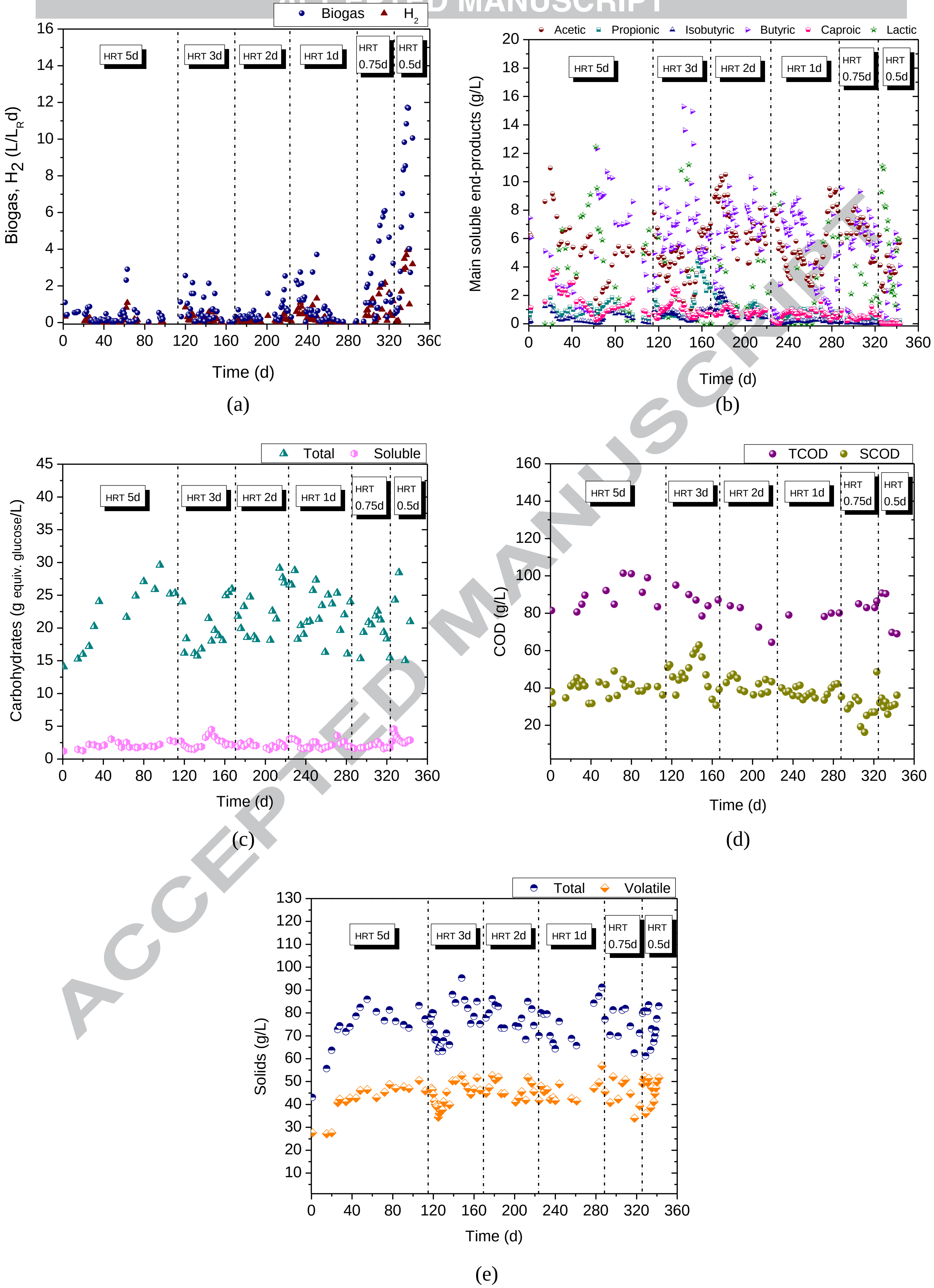

Biogas
H2
Biogas, H2 (L/LR d)
Time (d)
HRT 5d
HRT 3d
HRT 2d
HRT 1d
HRT 0.75d
HRT 0.5d
(a)
Acetic
Propionic
Isobutyric
Butyric
Caproic
Lactic
Main soluble end-products (g/L)
(b)
Total
Soluble
Carbohydrates (g equiv. glucose/L)
(c)
TCOD
SCOD
COD (g/L)
(d)
Total
Volatile
Solids (g/L)
(e)

Figure 2.

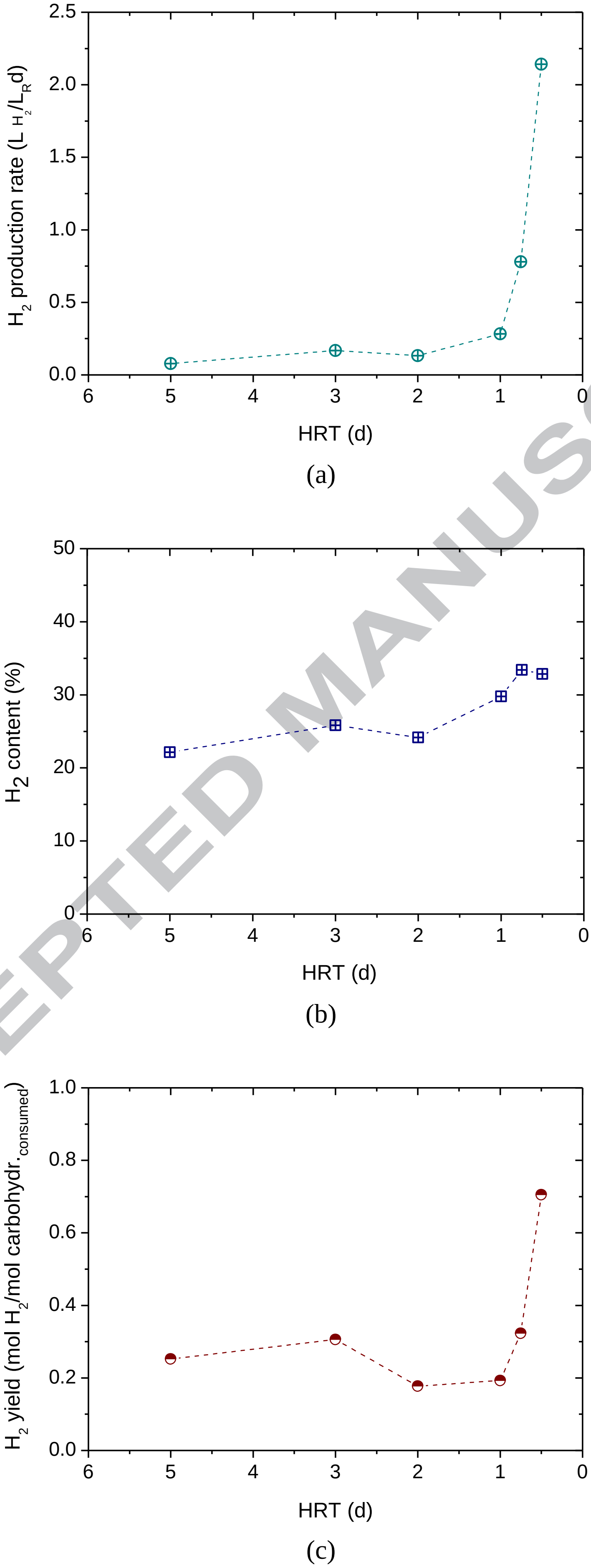
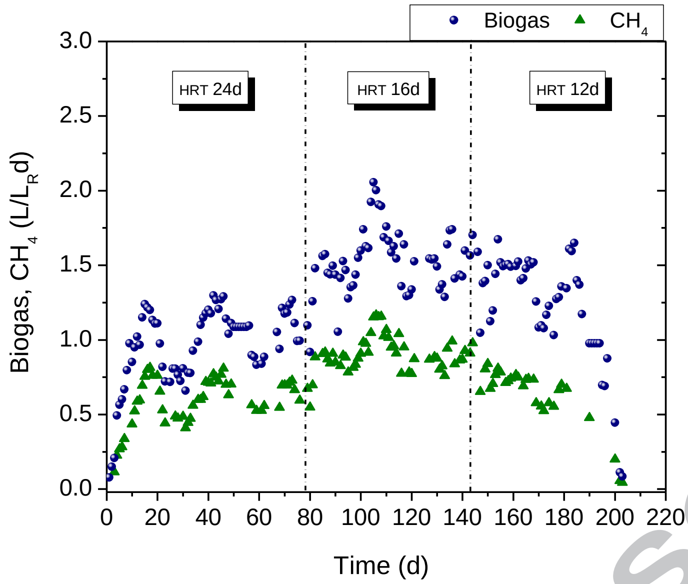
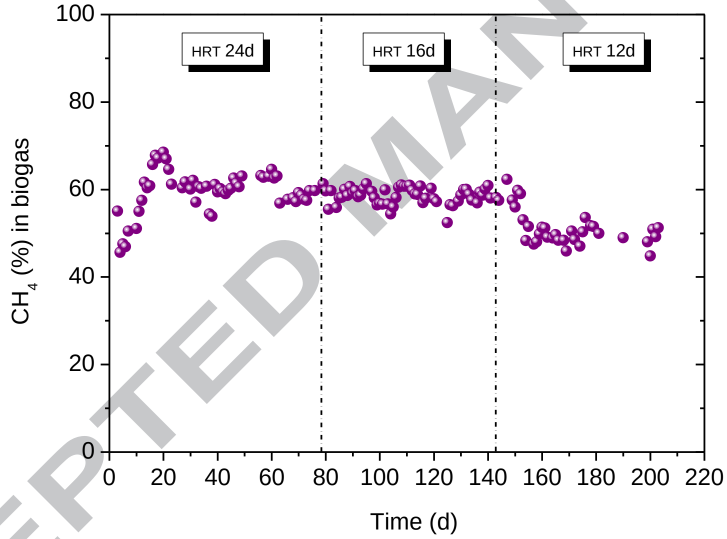

H2 production rate (L H2/LR d)
2.5
2.0
1.5
1.0
0.5
0.0
6
5
4
3
2
1
0
HRT (d)
(a)
H2 content (%)
50
40
30
20
10
0
6
5
4
3
2
1
0
HRT (d)
(b)
H2 yield (mol H2/mol carbohydr.consumed)
1.0
0.8
0.6
0.4
0.2
0.0
6
5
4
3
2
1
0
HRT (d)
(c)

Figure 3.



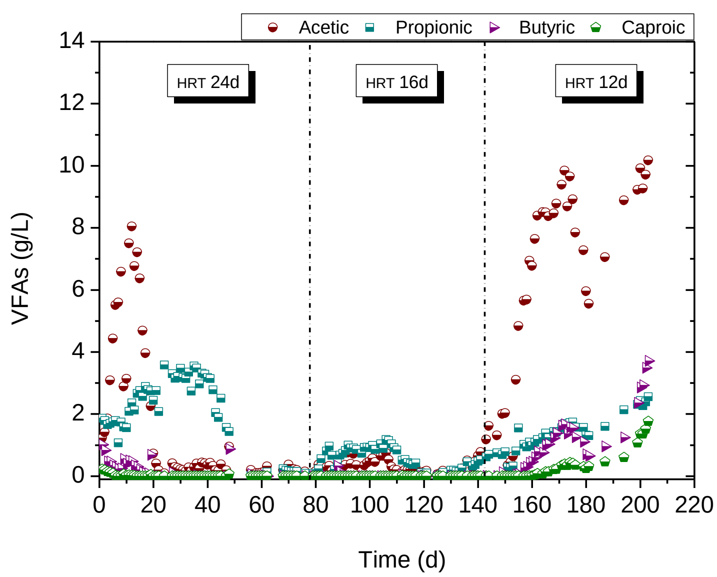
Biogas
CH4
HRT 24d
HRT 16d
HRT 12d
Biogas, CH4 (L/LRd)
Time (d)

(a)



HRT 24d
HRT 16d
HRT 12d
CH4 (%) in biogas
Time (d)

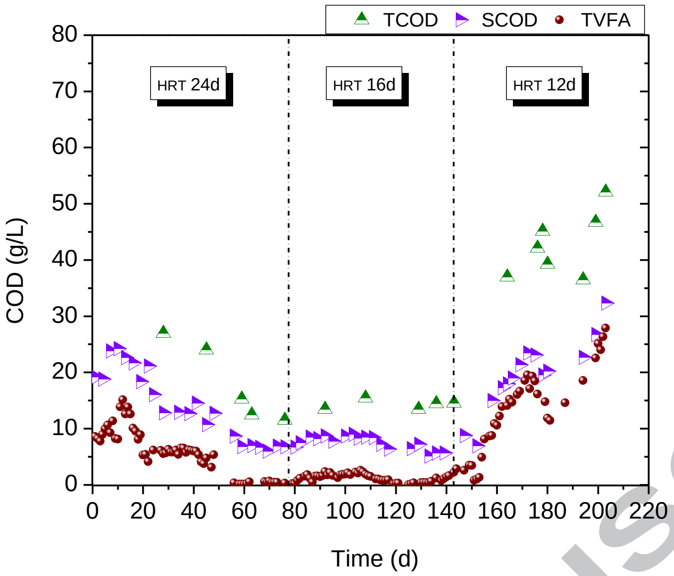
(b)



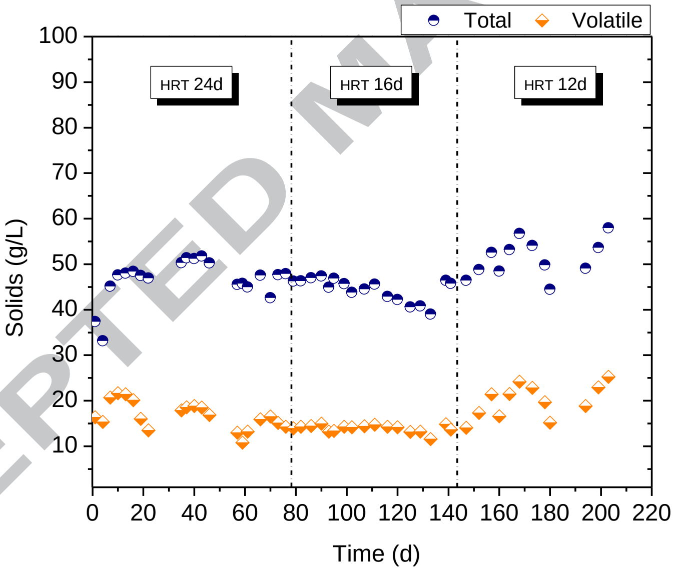
Acetic
Propionic
Butyric
Caproic
HRT 24d
HRT 16d
HRT 12d
VFAs (g/L)
Time (d)

(c)

Figure 4.


TCOD
SCOD
TVFA
HRT 24d
HRT 16d
HRT 12d
COD (g/L)
Time (d)
0
10
20
30
40
50
60
70
80
0
20
40
60
80
100
120
140
160
180
200
220

(a)


Total
Volatile
HRT 24d
HRT 16d
HRT 12d
Solids (g/L)
Time (d)
10
20
30
40
50
60
70
80
90
100
0
20
40
60
80
100
120
140
160
180
200
220

(b)

Figure 5.

Table 1.

Parameters	*Units*	*CW*	*LCM*
pH	-	6.12 ± 0.04	7.24 ± 0.18
TS	g/L	72.33 ± 2.45	33.15 ± 1.98
VS	g/L	62.40 ± 1.62	22.50 ± 0.98
TCOD	g O_2/L	93.21 ± 2.99	43.14 ± 2.56
SCOD	g O_2/L	38.96 ± 3.05	15.05 ± 0.32
TOC	g/L	39.49 ± 0.33	16.72 ± 0.24
Total carbohydrates	$g_{equiv.glucose}$/L	51.37 ± 1.65	6.99 ± 0.45
Soluble carbohydrates	$g_{equiv.glucose}$/L	40.63 ± 1.99	0.45 ± 0.05
Total Nitrogen, TKN	g/L	0.81 ± 0.03	2.78 ± 0.00
Ammonium Nitrogen	g/L	0.11 ± 0.00	1.57 ± 0.02
Proteins	g/L	5.06 ± 0.19	17.38 ± 0.00
Total phosphorus	mg/L	228.03 ± 3.00	463.50 ± 9.49
Soluble phosphorus	mg/L	57.50 ± 0.91	21.68 ± 0.04
Alkalinity	g $CaCO_3$/L	0.50 ± 0.00	12.38 ± 0.32
TVFA	mg/L	0.00 ± 0.00	4986.80 ± 19.61

Table 2.

Parameters	*Value*
pH	4.10 ± 0.00
Moisture (% wet weight)	76.32 ± 0.10
TS (% wet weight)	23.73 ± 0.17
VS (%TS)	94.08 ± 3.15
Ash (%TS)	5.93 ± 3.15
TOC (%TS)	46.18 ± 0.00
Total carbohydrates[a] (%TS)	38.82 ± 1.29
Soluble carbohydrates[a] (%TS)	3.50 ± 0.85
Cellulose (%TS)	37.60 ± 5.37
Hemicellulose (%TS)	25.51 ± 3.66
Lignin (%TS)	17.28 ± 4.93
Total Nitrogen, TKN (%TS)	0.96 ± 0.45
Proteins (%TS)	6.00 ± 0.00
Lactic Acid (%TS)	4.28 ± 0.00

[a]in equivalent glucose

Table 3.

Operating conditions	Acidogenic Reactor					
HRT (d)	*5*	*3*	*2*	*1*	*0.75*	*0.5*
Flow rate (mL/d)	100	167	250	500	667	1000
OLR (kg VS/m^3·d)	11.56	19.27	28.91	57.81	77.08	115.62
OLR (kg COD/m^3·d)	17.16	28.60	42.90	85.80	114.40	171.60

	Methanogenic Reactor		
HRT (d)	*24*	*16*	*12*
Flow rate (mL/ d)	167	250	333
OLR (kg VS/m^3·d)	1.93	2.90	3.87
OLR (kg COD/m^3·d)	3.58	5.36	7.15

Table 4.

Parameter	HRT (d)		
	24	16	12*
pH	8.05 ± 0.06	8.00 ± 0.07	6.63
Biogas ($L/L_R \cdot d$)	1.02 ± 0.18	1.52 ± 0.22	0.08
CH_4 ($L/L_R \cdot d$)	0.63 ± 0.11	0.90 ± 0.12	0.04
CH_4 (%)	58.27 ± 1.03	58.58 ± 1.87	48.88
TVFAs (g/L)	0.30 ± 0.14	0.41 ± 0.28	19.51
Yield CH_4 (mL CH_4/g VS_{added})	326.42 ± 56.9	310.34 ± 41.3	-
Yield CH_4 (mL CH_4/g $COD_{consumed}$)	223.09 ± 38.9	216.50 ± 28.8	-
TCOD removed (%)	84.77 ± 8.71	83.36 ± 4.90	39.29
SCOD removed (%)	84.05 ± 4.64	85.09 ± 9.89	22.87
TS removed (%)	37.48 ± 2.29	42.32 ± 2.44	25.62
VS removed (%)	65.97 ± 6.52	70.02 ± 4.27	45.58

* The parameter values recorded in this operating condition do not correspond to steady-state operation, since the bioreactor was in transition towards washout.

Nomenclature

ES	Ensiled Sorghum	SCOD	Soluble Chemical Oxygen Demand, g/L
CW	Cheese Whey	VFA	Volatile Fatty Acids, mg/L
LCM	Liquid Cow Manure	TVFA	Total Volatile Fatty Acids, mg/L
CSTR	Continuous Stirred Tank Reactor	TS	Total Solids, g/L
HRT	Hydraulic Retention Time, days	VS	Volatile Solids, g/L
OLR	Organic Loading Rate, kg COD/m^3 or kg VS/m^3 d	TOC	Total Organic Carbon, g/L
COD	Chemical Oxygen Demand, g/L	BOD_5	Biochemical Oxygen Demand, g/L
TCOD	Total Chemical Oxygen Demand, g/L	TKN	Total Kjeldahl Nitrogen, g/L

Highlights

- Co-digestion using a mixture of agro-wastes was tested in a two-stage system
- The effect of HRT on biohydrogen and methane production was investigated
- In acidogenesis, the highest H_2 production and yield were observed at HRT 0.5d
- In methanogenesis, the highest CH_4 productivity was observed at HRT 16d
- Methanogenic reactor instability occurred during operation at HRT 12d