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New criteria to determine the destabilization of the acidogenic anaerobic co-digestion of organic fraction of municipal solid waste (OFMSW) with mixed sludge (MS)

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Abstract

Effect of hydraulic retention time (HRT) on bio-hydrogen production from co-digestion of organic fraction of municipal solid waste (OFMSW) and mixed sludge (MS) in dry thermophilic conditions (55ºC and 20% total solids) was investigated. A decreasing sequence of six HRTs, from 2.9 to 0.8-days, was performed to evaluate the stability of the system and the influence of HRT on the organic matter solubilization, the daily hydrogen production (HP) and the specific hydrogen production (SHP).

Best results were obtained operating at 1.2-days HRT: HP of 3.67 L $H_2/L_{reactor}$/day, SHP of 33.8 mL H_2/gVS_{added} and hydrogen percentage in biogas of 52.4%. However, HRTs lower than 1.2-days induce failure in the system due to an unbalance of the hydrolytic phase. This fact was corroborated through the evaluation of two indirect parameters, "non-solubilized carbon" (NSC) and "acidogenic susbtrate as carbon" (ASC), and the relationships of NSC/TOC and ASC/TOC.

Keywords**:** Co-digestion; Organic fraction of municipal solid waste; Mixed sludge; Bio-hydrogen; Hydrolytic destabilization.

1. Introduction

As a promising alternative to fossil fuels, hydrogen is considered a clean and renewable source of energy with an energy yield of 122 kJ/g, which is about 2.75 times higher than hydrocarbon fuels (Sreela-or et al., 2011). The production of hydrogen-rich biogas from the organic fraction of municipal solid waste (OFMSW) by means of dark fermentation is an attractive option for renewable energy recovery and sustainable management of this problematic waste. The key advantages are low treatment cost, simple operation and high hydrogen production rate (Benemann, 1997; Chen et al., 2008).

Dark fermentation, or acidogenic anaerobic digestion (AAD), includes two steps: hydrolysis followed by acidogenesis. In the first step (hydrolysis), complex organic polymers are hydrolysed into simple soluble organic compounds. In the second step (acidogenesis), the generation of volatile fatty acids (VFAs), H_2, CO_2 and other intermediates takes place. About the operational conditions recommended for acidogenesis of organic wastes, short hydraulic retention times (HRT) and low pH are necessary to avoid the growth of H_2-utilizing methanogens bacteria (Zahedi et al., 2013).

HRT determines the microorganisms/substrate contact time and, therefore, the efficiency of the substrate removal (Zahedi et al., 2013). It has been reported that lower HRTs promote bio-hydrogen production, and it can be used to attain the wash-out of the methanogenic populations from the reactor and to select the hydrolytic and acidogenic microorganisms (Montero et al., 2008; Zahedi et al., 2013). Indeed, several authors have suggested that the optimal pH, for enhancing the hydrolytic and acidogenic activities, must be ranged between 5 and 6 (Guo et al., 2010; Verrier et al., 1987).

On the other hand, OFMSW is substrate with low nitrogen content, which is an essential macro-nutrient for anaerobic microbial populations and specifically for H_2-producers microorganisms (Kim et al., 2004). Therefore, in order to improve the bio-hydrogen production efficiency, to increase the buffering capacity of the system and to balance the nutrient content by means of the co-digestion of the OFMSW with other organic wastes is considered an interesting option. However, as disadvantage operational costs could be increased (Aboudi et al., 2016; Zhu et al., 2008).

In this sense, the use of mixed sewage sludge (MS) as co-substrate, i.e. primary and secondary sludge from wastewater treatment plant (WWTP), could be an ideal source to balance the requirements of alkalinity and nitrogen of the OFMSW in the AAD process. MS has high protein content, which can provide essential nutrients for metabolic activities and the growth of microorganisms. Earlier studies have reported that the co-digestion of OFMSW and MS could enhance the hydrogen production by providing a more balanced C/N ratio and better control on the pH (Tyagi et al., 2014). This fact is linked to the higher buffering capacity of MS at low pHs in comparison to the OFMSW (Zhu et al., 2008). Therefore, the possibility of co-digestion of OFMSW and MS for hydrogen production is fairly extensive as it could integrate the management, reduction and stabilization of the two most abundant and problematic municipal wastes (Kim et al., 2004; Li et al., 2008; Zhu et al., 2008).

Thus, the first main goal of this research was to evaluate the stability of the co-digestion process and to determine the limits in the improvement of bio-hydrogen production when the HRT is decreased. To reach this target, a destabilization episode was induce in the system by decreasing of the HRT from 2.9 to 0.8 in order to analyze the unbalance of the microbial populations involved in the AAD and the effect on the bio-hydrogen production. Moreover, the second main goal was to analyze the utility of the relationships between two indirect parameters to be used as a stability criterion of the process taking into account their variations when the HRT is decreased. This information will be crucial to the plant operators to predict and prevent failures of AAD systems.

2. Material and methods

2.1. Inoculum

The acidogenic inoculum used was the effluent coming from a previous experiments developed by the authors (Angeriz-Campoy et al., 2015). That reactor was operated at dry thermophilic anaerobic co-digestion (55ºC and 20% TS) of organic fraction of municipal solid waste (OFMSW) with food waste (FW) in a mixture ratio of 80:20. The average values of the different monitoring parameters (pH, alkalinity, C/N ratio, total volatile fatty acids (TVFA) and hydrogen yield) were: 5.5, 5.9 g/kg, 15.3, 358 g/kg and 38 mLH_2/g VS_{added}, respectively (Angeriz-Campoy et al., 2015).

2.2. Substrates

OFMSW and MS were used as substrates for the co-digestion experiments. The OFMSW samples were collected from an industrial waste treatment plant. The plant is placed in the south of Andalusia (Angeriz-Campoy et al., 2015). MS was collected from "*Cadiz-San Fernando*" wastewater treatment plant (WWTP) at the same geographical zone. The samples were preserved at -20ºC.

The values of the mixture ratio (OFMSW:MS) and TS concentration of the feed were selected according to the findings reported in the literature, 5:1 and 20%

respectively (Tyagi et al., 2014). The pH of the medium was approximately constant around 5.5 in all the assays without pH regulation. This value is considered favorable for H_2-producers and helps to suppress the growth of methanogenic microorganisms.

2.3. Experimental set-up and monitoring

A 5-liters stirred tank reactor was operated at semi-continuous regime of feeding (SSTR) and 55 ºC (thermophilic range of temperature). The temperature was maintained by recirculation of hot water coming from a thermostatic bath through the jacket of the reactor. A 50-Liters gas bag (manufactured by Tedlar polymer) was used to collect the biogas (Angeriz-Campoy et al., 2015).

Figure 1. Schematic diagram of the semi-continuous stirred tank reactors (SSTR)

To determine the influence of the HRT on the process performance, six values of this variable were selected according to previous papers of the different authors (Angeriz-Campoy et al., 2015; Zahedi et al., 2013): 2.9, 1.9, 1.5, 1.2, 1.0 and 0.8 days. Consequently, the organic loading rates (ORL) were: 17, 26, 30, 37.5, 55 and 75 gVS/L·day.

The assessment of the process was based on the degree of solubilization of the organic matter, daily hydrogen production (HP) and specific hydrogen production (SHP). The showed data are selected from the final stable period of each HRT. The final stable period cover, at least, a duration equivalent to three HRTs. The overall study duration was 170 days.

2.4. Analytical methods

The following parameters were measured according to Standards Methods (APHA, 1999): organic matter as soluble chemical oxygen demand (sCOD), dissolved organic carbon (DOC), solid fractions as total solids (TS) and volatile solids (VS), and other usual variables in anaerobic reactors such as alkalinity, Total Kjeldahl Nitrogen (TKN) and volatile fatty acids (VFAs).

TS, VS and pH were analyzed directly. Moreover, the determinations of VFAs , sCOD and DOC were performed on the leachate obtained by a lixiviation procedure described previously (Álvarez-Gallego, 2005).

DOC was determined in a total carbon analyser (Analytic-Jena multi N/C 3100) and VFAs (acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic, and heptanoic acid) were measured by gas chromatography (Shimadzu GC-2010). The complete details of the applied methods are available in Angeriz-Campoy et al., (2015). Finally, the weighted sum (in molecular weight basis) of the invididual VFAs allows the calculation of the TVFA (Fernández et al., 2008).

All parameters were analyzed by duplicate and three times a week, except for the pH and volume and composition of biogas which were determined daily.

Finally, a gas flow meter (Ritter TG1) and a gas pump (KFN Laboport) were used to quantify the volume of biogas. The biogas composition was measured in a gas chromatograph (Shimadzu GC-2014) with a packed column (Carbosieve SII-SUPELCO) and thermal conductivity detector (TCD) (Angeriz-Campoy et al., 2015).

2.5 Indirect parameters: DAC, NSC and ASC and their ratios

In this study two additional indirect parameters have been used to evaluate the effect of HRT on the balance among the different microbial populations involved in the AAD and, consequently, to analyze its effect on the H_2 production.

The first indirect parameter is called "*acidogenic substrate as carbon (ASC)*". This parameter is the difference between the global dissolved organic matter and the global organic matter in form of VFAs. According to this idea, the parameter is calculated by subtracting the related parameters (Fdez-Güelfo et al., 2012).

The meaning of the ASC could be understood as a measure of the solubilized organic matter that could potentially be metabolized to VFAs and, hence, it may be used to differentiate the extension and degree of coupling of the hydrolytic and acidogenic phases. Furthermore, the evolution of ASC, and its final value, will be different depending on whether the two stages are coupled or decoupled. ASC can be calculated as the difference between two classical parameters: DOC, whose value represents the total concentration of solubilized carbon during the hydrolysis stage and dissolved acid carbon (DAC), whose value represents the concentration of carbon in form of acids, coming from the VFA production in acidogenic phase.

A higher value of ASC indicates that higher amounts of solubilized organic matter could be transformed into VFAs and, consequently, a higher H_2 production could be expected (Romero Aguilar et al., 2013). In addition, in a continuous process, high values of ASC are related to a decoupling between the hydrolysis and acidogenesis rates.

The following equations are used to calculate the values of the ASC:

$$\text{ASC}\ (M/L^3) = \text{DOC}\ (M/L^3) - \text{DAC}\ (M/L^3) \qquad (1)$$

$$\mathrm{DAC}\ (M/L^3) = \sum_{i=2}^{i=7} [A_iH(M/L^3) * n_i * 12 / MW_i] \tag{2}$$

About equation (1), DOC is determined by the above described method and DAC is calculated by the equation (2); the others terms of the equations are A_iH (which represents individual VFAs expressed in concentration units) and the factors for the weighted sum: n_i, and MW_i, (the number of carbon atoms and the molecular weight of each individual VFA respectively).

The second indirect parameter is called "*Non-solubilized carbon (NSC)*" and it can be estimated from the total organic carbon (TOC) obtained experimentally from the organic matter expressed in terms of VS. According to Fdez-Güelfo et al., 2012, NSC can be estimated by subtracting the instrumentally determined DOC from the TOC (estimated from the data of VS using the theoretical ratio "Organic matter/organic Carbon"). NSC represents the organic carbon fraction that has not been solubilized in the hydrolysis stage. Hence, if the hydrolytic bacteria are unbalanced in the process as consequence of the variation of some variable, the expected behavior for the NSC/TOC relationship is an increment. NSC was calculated indirectly from classical parameters from Eqs. (3) and (4) according to Fdez-Güelfo et al., 2012.

$$\mathrm{NSC}\ (M/L^3) = \mathrm{TOC}\ (M/L^3) - \mathrm{DOC}\ (M/L^3) \tag{3}$$

$$\mathrm{TOC}\ (M/L^3) = \mathrm{VS}\ (M/L^3) \cdot 0.51 \tag{4}$$

3. Results and discussion

3.1. Characterization of OFMSW, MS and Co-substrate (5:1)

Table 1 shows the physico-chemical characteristics of the OFMSW, MS and co-substrate i.e. OFMSW+MS (5:1).The average TS and VS concentrations of OFMSW indicate that only a fraction of 69% of TS is susceptible to be degraded. However, in the case of MS this value was 82%, which is indicative of a more biodegradable substrate.

(Table 1 must be placed here)

OFMSW generally has higher concentration in soluble chemical oxygen demand (sCOD) and dissolved organic carbon (DOC) than MS and for this reason their co-digestion continues having a high concentration of solubilized organic matter. This indicates that the mixture of them may be considered a promising substrate for bio-hydrogen production.

On the contrary, about the C/N ratio, the value of this parameter for the co-substrate (5:1) was 17.9, which was close to the optimum value of 20 recommended for anaerobic process (Khalid et al., 2011). Therefore, it may be considered that the co-digestion of OFMSW and MS provides sufficient nutrient balance.

Finally, it should be noted that the alkalinity of OFMSW was relatively low (10.14 g/kg), which is indicative of its low buffering capacity (Tyagi et al., 2014). On the contrary, the alkalinity of MS was 21.8 g/kg, indicating the higher buffering capacity of this waste. This fact shows that MS addition as a co-substrate could help to reduce the drop of pH and to maintain stable pH values during the bio-hydrogen production by AAD (Tyagi et al., 2014). In the course of the experiments, the pH in all the reactors was kept around 5.5 without alkali addition.

3.2. Evolutions of the indirect parameters DAC, NSC and ASC and their ratios

In according with the above-mentioned in section 2.5, NSC and ASC are two indirect parameter closely related with the hydrolytic and acidogenic phases and, therefore, with the hydrogen generation. NSC represents the organic carbon fraction that has not been solubilized in the hydrolysis stage and ASC represents the organic matter solubilized which has not been transformed into VFAs by the acidogenic bacteria.

Based on the above explanations and considering a continuous process, for each HRT tested, the expected evolution of these indirect parameters can reveal three possible scenarios from the microbiological point of view:

1. DAC/TOC increases and [(NSC+ASC)/TOC] decreases with a simultaneous increment of the hydrogen production. This behavior would be representative of a stable reactor in which hydrolytic and acidogenic bacteria are balanced and, therefore, all hydrolyzed and solubilized substrate is converted into VFA (except a fraction resistant to acidogenesis) with significant hydrogen generation.
2. NSC/TOC increases and DAC/TOC decreases with a simultaneous decline in hydrogen production. In this case, this behavior would be representative of an unbalance of hydrolytic bacteria in the process and, therefore, the extension of the acidogenesis would be being limited in upstream.
3. ASC/TOC increases and DAC/TOC decreases with a simultaneous decline in hydrogen production. In this last case, this behavior would be representative of an unbalance of acidogenic bacteria in the process.

As it can be seen in Figure 2, when the HRT is decreased from 2.9 to 1.2-days, DAC/TOC increases and the relationship (NSC+ASC)/TOC decreases, which is indicative of a balance between the hydrolytic and acidogenic phases (first scenario). On the contrary, for HRTs lower than 1.2-days, this behavior is the opposite. It can be seen that NSC/TOC ratio clearly increases until its maximum value of 0.7 at 0.8-days HRT and DAC/TOC ratio decreases until its minimum value of 0.28 for the same HRT. Hence, taking into account the three scenarios previously established, the evolution of these ratios is clearly indicative of a failure of the hydrolytic phase (second scenario), which induces a chained failure of the acidogenic step. This chained failure is corroborated thought the simultaneous decreasing of the ASC/TOC ratio and hydrogen production (HP and SHP values) for these HRTs (see Table 3).

Figure 2. Evolution of the ASC/TOC, NSC/TOC, DAC/TOC and (NSC+ASC)/TOC ratios for the different HRTs.

It must be noted that for HRTs lower than 1.2-days, parameters directly related with the organic matter solubilization as DOC and TVFA decrease until their minimum values at 0.8-days HRT, reaching 129 g/kg and 295 gHAc/kg respectively (Table 2).

(Table 2 must be placed here)

3.3. Evolution of the VFA and pH

Table 2 shows the TVFA concentrations for the different HRTs tested. As it can be seen in Figure 3, significant concentrations of acetic and butyric (75-85%) acids were detected in all the assays, which is in concordance with the earlier studies (Hawkes et al., 2002). Acetic and butyric acids are the main co-products in the most efficient pathways for bio-hydrogen production, especially by *Clostridium* sp. (Evvyernie et al., 2001; Singh & Wahid, 2015). Moreover, propionic, valeric and heptanoic acids were detected in very low concentrations for all the HRTs. Intermediate concentrations of caproic acid, between 15 and 30 g/kg, were obtained but no sign of toxicity was observed in contrast with that reported by other authors (Rinzema et al., 1994).

Figure 3. Evolution of VFAs.

An indicator normally used to evaluate the effectiveness of bio-hydrogen production is the butyrate to acetate concentrations ratio (HBu/HAc ratio), which may vary with the increase of the microbial population during fermentation process (Hung et al., 2008; Lin & Hung, 2008). In this study, no significant differences were observed in HBu/HAc ratio during the experiment and the values were maintained between 0.9 and 1.2 for all the different HRTs tested. Moreover, the HBu/HAc values obtained are in agreement with those reported in the literature (0.4-2.1) by other authors (Lee et al., 2004; Ueno et al., 1996).

With regard to pH, this parameter could drop during the AAD process as a consequence of the generation of VFA and, hence, additions of alkaline agents would be required to maintain the pH around the optimum value. However, the enhanced alkalinity in the co-digestion assays kept the pH around 5.5 without requiring external control (Table 2).

3.4. Evolution of the bio-hydrogen production

Data of HP, SHP and biogas composition at the different HRTs tested are shown in Table 3. Presented data correspond to the average values obtained when the system was stabilized for each HRT tested (last HRT of the three tested in each period). As it can be seen, the trends of HP and SHP were completely analogous. From 2.9 to 1.2-days HRT both parameters increase until reach their maximum values of 3.67 LH_2/L_r.d and 33.8 mLH_2/gVS_{added}, respectively. As it was previously mentioned in section 3.2, this behaviour is in agreement with the first scenario raised in which stable operation occurs and the activities of the hydrolytic and acidogenic populations of microorganisms were balanced.

(Table 3 must be placed here)

The inverse relationship between HRT and hydrogen production related-parameters (HP and SHP) in acidogenic anaerobic process has been observed by several researchers. This fact could be related to the selection of H_2-producing bacteria (*Clostridium* sp.) among the different populations of microorganisms involved in the anaerobic digestion, when working at low HRT (Hawkes et al., 2002; Jung et al., 2011; Ueno et al., 2007).

It should be highlighted that the maximum HP obtained in this study (3.67 $LH_2/L_{reactor}$/dayat 1.2-day HRT) was significantly higher than those reported by Kim et al. (2004), 0.79 $LH_2/L_{reactor}$/day, in their studies about acidogenic anaerobic co-digestion of FW and sewage sludge operating at 1-day HRT. The biogas yield can be affected by many factors: type of substrate, microbial composition, reactor design, etc. This improvement in H_2 production may be linked, among other factors, to the supplementation of protein by the addition of MS (type of substrate), which improves the C/N ratio and, therefore, the growth of H_2-producers microorganisms (Zhu et al., 2008).

In contrast, for HRTs lower than 1.2 days, the HP decreases from 3.67 to 1.46 $LH_2/L_{reactor}$/day and the SHP from 33.8 to 9 mLH_2/gVS_{added}. However, it should be noted that the theoretical maximum SHP reported for the acidogenesis of glucose (solubilized substrate) was obtained for HRTs between 8h and 12h (Fang & Liu, 2002; Hawkes et al., 2002). Therefore, the data of the present study suggest that the imbalance of the hydrolytic microorganisms occurs, as mentioned in section 3.2 (second scenario). This behavior could be associated to the difficulty of the hydrolytic microorganisms to colonize and hydrolyze the solid particles of the OFMSW.

As it can be seen in Table 3, the biogas composition was exclusively constituted by hydrogen and carbon dioxide. The hydrogen percentages were in the range of 49-52%, which is analogous to the hydrogen percentages reported by other authors in their experiments about bio-hydrogen production from OFMSW, FW or slaughterhouse waste (Gómez et al., 2006; Kim et al., 2004).

4. Conclusions

- Indirect parameters NSC, ASC and their relationships NSC/TOC and ASC/TOC, it can be concluded that for HRTs from 2.9 to 1.2-days the system remains stable, with well-balanced activities between hydrolytic and acidogenic phase. However, for HRTs lower than 1.2-days, a failure occurs in the hydrolytic phase.

- Best results for HP and SHP were obtained at 1.2 days-HRT; 3.67 L $H_2/L_{reactor}$/day and 33.8 mL H_2/gVS_{added} and the hydrogen percentage in biogas was 52.4%. At HRTs lower than 1.2-days, HP and SHP decrease until their minimum values, 1.46 L $H_2/L_{reactor}$/day and 9 mL H_2/gVS_{added} respectively.

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Author Contributions

All the authors designed research, performed research, analyzed data and wrote the paper.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Aboudi, K., Álvarez-Gallego, C.J., Romero-García, L.I. 2016. Evaluation of methane generation and process stability from anaerobic co-digestion of sugar beet by-product and cow manure. *Journal of Bioscience and Bioengineering*, **121**(5), 566-572.

[2] Álvarez-Gallego. 2005. Testing Different Procedures for the Start Up of a Dry Anaerobic Co-Digestion Process of OFMSW and Sewage Sludge at Thermophilic Range (Doctoral thesis). University of Cádiz.

[3] Angeriz-Campoy, R., Álvarez-Gallego, C.J., Romero-García, L.I. 2015. Thermophilic anaerobic co-digestion of organic fraction of municipal solid waste (OFMSW) with food waste (FW): Enhancement of bio-hydrogen production. *Bioresource Technology*, **194**, 291-296.

[4] APHA, A. 1999. WEF, 2005. *Standard methods for the examination of water and wastewater*, **21**, 258-259.

[5] Benemann, J.R. 1997. Feasibility analysis of photobiological hydrogen production. *International Journal of Hydrogen Energy*, **22**(10–11), 979-987.

[6] Chen, Y., Cheng, J.J., Creamer, K.S. 2008. Inhibition of anaerobic digestion process: A review. *Bioresource Technology*, **99**(10), 4044-4064.

[7] Evvyernie, D., Morimoto, K., Karita, S., Kimura, T., Sakka, K., Ohmiya, K. 2001. Conversion of chitinous wastes to hydrogen gas by clostridium paraputrificum M-21. *Journal of Bioscience and Bioengineering*, **91**(4), 339-343.

[8] Fang, H.H.P., Liu, H. 2002. Effect of pH on hydrogen production from glucose by a mixed culture. *Bioresource Technology*, **82**(1), 87-93.

[9] Fdez-Güelfo, L.A., Álvarez-Gallego, C., Sales, D., Romero, L.I. 2012. New indirect parameters for interpreting a destabilization episode in an anaerobic reactor. *Chemical Engineering Journal*, **180**, 32-38.

[10] Fernández, J., Perez, M., Romero, L. 2008. Effect of substrate concentration on dry mesophilic anaerobic digestion of organic fraction of municipal solid waste (OFMSW). *Bioresource technology*, **99**(14), 6075-6080.

[11] Gómez, X., Morán, A., Cuetos, M.J., Sánchez, M.E. 2006. The production of hydrogen by dark fermentation of municipal solid wastes and slaughterhouse waste: A two-phase process. *Journal of Power Sources*, **157**(2), 727-732.

[12] Guo, X.M., Trably, E., Latrille, E., Carrere, H., Steyer, J.-P. 2010. Hydrogen production from agricultural waste by dark fermentation: A review. *International Journal of Hydrogen Energy*, **35**(19), 10660-10673.

[13] Hawkes, F.R., Dinsdale, R., Hawkes, D.L., Hussy, I. 2002. Sustainable fermentative hydrogen production: challenges for process optimisation. *International Journal of Hydrogen Energy*, **27**(11–12), 1339-1347.

[14] Hung, C.-H., Cheng, C.-H., Cheng, L.-H., Liang, C.-M., Lin, C.-Y. 2008. Application of Clostridium-specific PCR primers on the analysis of dark fermentation hydrogen-producing bacterial community. *International Journal of Hydrogen Energy*, **33**(5), 1586-1592.

[15] Jung, K.W., Kim, D.H., Kim, S.H., Shin, H.S. 2011. Bioreactor design for continuous dark fermentative hydrogen production. *Bioresource Technology*, **102**(18), 8612-8620.

[16] Khalid, A., Arshad, M., Anjum, M., Mahmood, T., Dawson, L. 2011. The anaerobic digestion of solid organic waste. *Waste Management*, **31**(8), 1737-1744.

[17] Kim, S.H., Han, S.K., Shin, H.S. 2004. Feasibility of biohydrogen production by anaerobic co-digestion of food waste and sewage sludge. *International Journal of Hydrogen Energy*, **29**(15), 1607-1616.

[18] Lee, K.-S., Lo, Y.-S., Lo, Y.-C., Lin, P.-J., Chang, J.-S. 2004. Operation strategies for biohydrogen production with a high-rate anaerobic granular sludge bed bioreactor. *Enzyme and Microbial Technology*, **35**(6–7), 605-612.

[19] Li, M., Zhao, Y., Guo, Q., Qian, X., Niu, D. 2008. Bio-hydrogen production from food waste and sewage sludge in the presence of aged refuse excavated from refuse landfill. *Renewable Energy*, **33**(12), 2573-2579.

[20] Lin, C.-Y., Hung, W.-C. 2008. Enhancement of fermentative hydrogen/ethanol production from cellulose using mixed anaerobic cultures. *International Journal of Hydrogen Energy*, **33**(14), 3660-3667.

[21] Montero, B., Garcia-Morales, J.L., Sales, D., Solera, R. 2008. Evolution of microorganisms in thermophilic-dry anaerobic digestion. *Bioresource Technology*, **99**(8), 3233-3243.

[22] Rinzema, A., Boone, M., Vanknippenberg, K., Lettinga, G. 1994. BACTERICIDAL EFFECT OF LONG-CHAIN FATTY-ACIDS IN ANAEROBIC-DIGESTION. *Water Environment Research*, **66**(1), 40-49.

[23] Romero Aguilar, M.A., Fdez-Güelfo, L.A., Álvarez-Gallego, C.J., Romero García, L.I. 2013. Effect of HRT on hydrogen production and organic matter solubilization in acidogenic anaerobic digestion of OFMSW. *Chemical Engineering Journal*, **219**, 443-449.

[24] Singh, L., Wahid, Z.A. 2015. Methods for enhancing bio-hydrogen production from biological process: A review. *Journal of Industrial and Engineering Chemistry*, **21**, 70-80.

[25] Sreela-or, C., Plangklang, P., Imai, T., Reungsang, A. 2011. Co-digestion of food waste and sludge for hydrogen production by anaerobic mixed cultures: Statistical key factors optimization. *International Journal of Hydrogen Energy*, **36**(21), 14227-14237.

[26] Tyagi, V.K., Angériz Campoy, R., Álvarez-Gallego, C.J., Romero García, L.I. 2014. Enhancement in hydrogen production by thermophilic anaerobic co-digestion of organic fraction of municipal solid waste and sewage sludge – Optimization of treatment conditions. *Bioresource Technology*, **164**, 408-415.

[27] Ueno, Y., Otsuka, S., Morimoto, M. 1996. Hydrogen production from industrial wastewater by anaerobic microflora in chemostat culture. *Journal of Fermentation and Bioengineering*, **82**(2), 194-197.

[28] Ueno, Y., Tatara, M., Fukui, H., Makiuchi, T., Goto, M., Sode, K. 2007. Production of hydrogen and methane from organic solid wastes by phase-separation of anaerobic process. *Bioresource Technology*, **98**(9), 1861-1865.

[29] Verrier, D., Roy, F., Albagnac, G. 1987. Two-phase methanization of solid vegetable wastes. *Biological Wastes*, **22**(3), 163-177.

[30] Zahedi, S., Sales, D., Romero, L.I., Solera, R. 2013. Hydrogen production from the organic fraction of municipal solid waste in anaerobic thermophilic acidogenesis: Influence of organic loading rate and microbial content of the solid waste. *Bioresource Technology*, **129**, 85-91.

[31] Zhu, H., Parker, W., Basnar, R., Proracki, A., Falletta, P., Béland, M., Seto, P. 2008. Biohydrogen production by anaerobic co-digestion of municipal food waste and sewage sludges. *International Journal of Hydrogen Energy*, **33**(14), 3651-3659.

Table 1. Characterization of the OFMSW, MS and co-substrate OFMSW+ MS (5:1)

Parameter/unit	OFMSW	MS	Co-substrate (5:1)
pH [a]	5.6 (0.35)	5.3 (0.01)	5.6 (0.08)
Density (kg/l) [ab]	1.18 (0.03)	0.98 (0.04)	1.10 (0.04)
Alkalinity (g/kg) [ac]	10.3 (1.8)	21.8 (4.1)	13.8 (1.2)
TS (%) [ab]	52.7 (0.4)	4.5 (0.2)	19.6 (1.5)
VS (%) [ab]	36.1 (0.6)	3.7 (0.2)	13 (1.5)
VS/TS	0.69	0.82	0.66
C/N ratio	18.7 (2.9)	11 (0.6)	17.9 (0.6)
sCOD (g/kg) [ac]	287 (18.4)	43 (15.2)	242 (20)
DOC (g/kg) [ac]	19.3 (1.2)	14.4 (3.5)	18.3 (0.9)
TVFA (g/kg) [ac]	18.6 (0.8)	5.4 (1.3)	15.6 (0.4)

[a] Data are the average value from different replicates. The standard deviation is included into the brackets.

[b] Data calculated on wet basis.

[c] Data calculated on dry basis.

Table 2. Characterization of the effluents for the different HRTs tested.

HRT (day)	**Duration (day)**	**Influent volume [a] (L/day)**	**pH [b]**	**TS [b,c] (g/kg)**	**VS [b,c] (g/kg)**	**DOC [b,c] (g/kg)**	**sCOD [b,c] (g/kg)**	**TVFA [b,c] (gHAc/kg)**	**DAC [d,e] (g/kg)**	**ASC [d.f] (g/kg)**
2.9	65	1.72	5.63 (0.1)	77.6 (14)	49.1 (11)	169 (24)	592 (24.8)	372 (28)	149	20
1.9	62	2.63	5.53 (0.9)	74.9 (18)	44.7 (8)	227 (32)	795 (27.7)	473 (22)	184	38
1.5	21	3.33	5.55 (0.2)	70.3 (17)	44.8 (11)	271 (34)	829 (28.5)	527 (31)	211	61
1.2	8	4.16	5.52 (0.1)	70.3 (16)	44.2 (8)	310 (29)	908 (31.5)	598 (27)	239	71
1	7	5.00	5.43 (0.2)	77.6 (18)	55.4 (14)	173 (18)	461 (22.3)	363 (33)	145	28
0.8	7	6.25	5.43 (0.2)	81.7 (19)	60.4 (12)	129 (22)	380 (21.5)	295 (29)	118	11

[a] The SSTR was fed once daily for all HRTs except for 1 and 0.8-days HRT where it was fed twice daily

[b] Data are the average value from different replicates. The standard deviation is included into the brackets.

[c] Data calculated on dry basis.

[d] Data calculated on wet basis.

[e] DAC has been calculated from VFA through Eq. (2)

[f] ASC has been calculated through Eq. (1)

Table 3. Biogas composition, HP and SHP for the different HRTs tested.

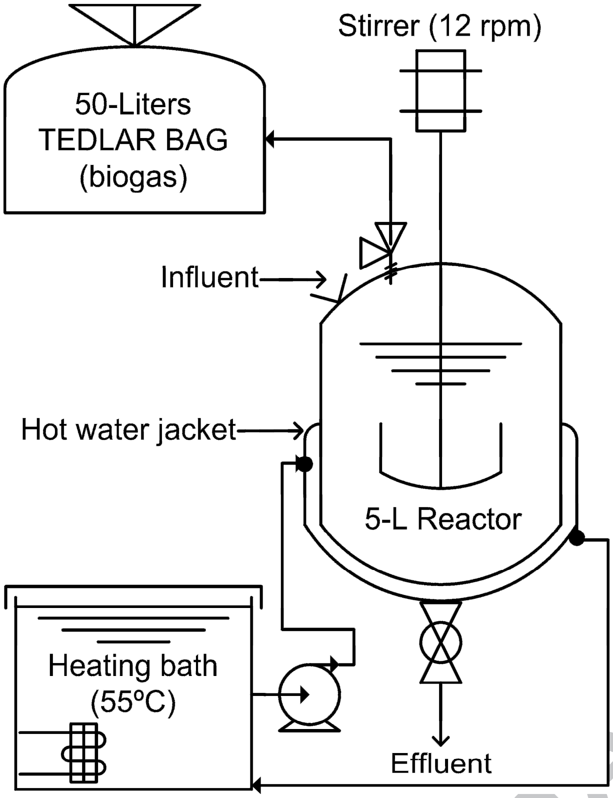
HRT (day)	**H_2 (%)**	**CO2 (%)**	**HP ($LH_2/L_r\cdot day$)**	**SHP (mLH_2/gVS_{added})**
2.9	49.1 (2.4)	50.9 (3.6)	1.19 (0.23)	26.5 (0.1)
1.9	50.2 (2.5)	49.8 (2.8)	1.90 (0.34)	27.7 (0.3)
1.5	50.7 (1.8)	49.3 (1.1)	2.88 (0.22)	33.2 (0.2)
1.2	52.4 (1.3)	47.6 (2.8)	3.67 (0.21)	33.8 (0.1)
1	50.2 (1.6)	49.8 (1.5)	1.49 (0.26)	11.4 (0.3)
0.8	50.3 (1.3)	49.7 (1.3)	1.46 (0.33)	9.0 (0.2)

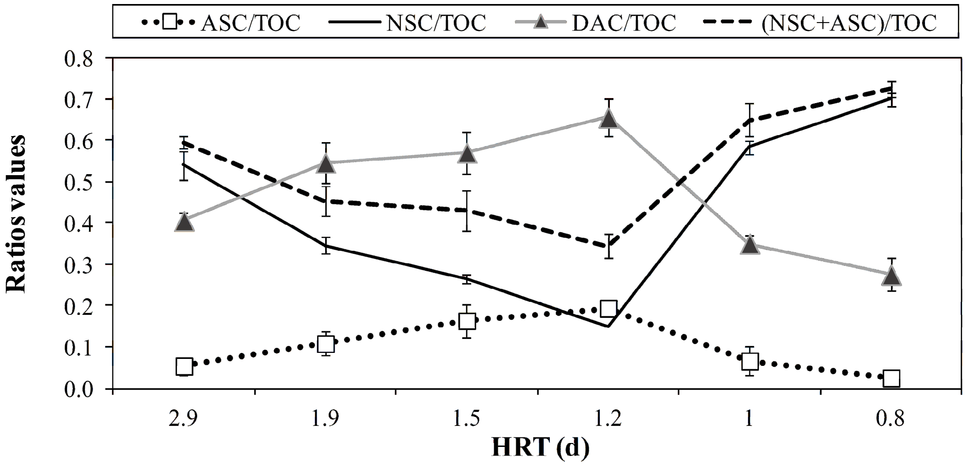
[*] Data are the average value from different replicates. The standard deviation is included into the brackets.

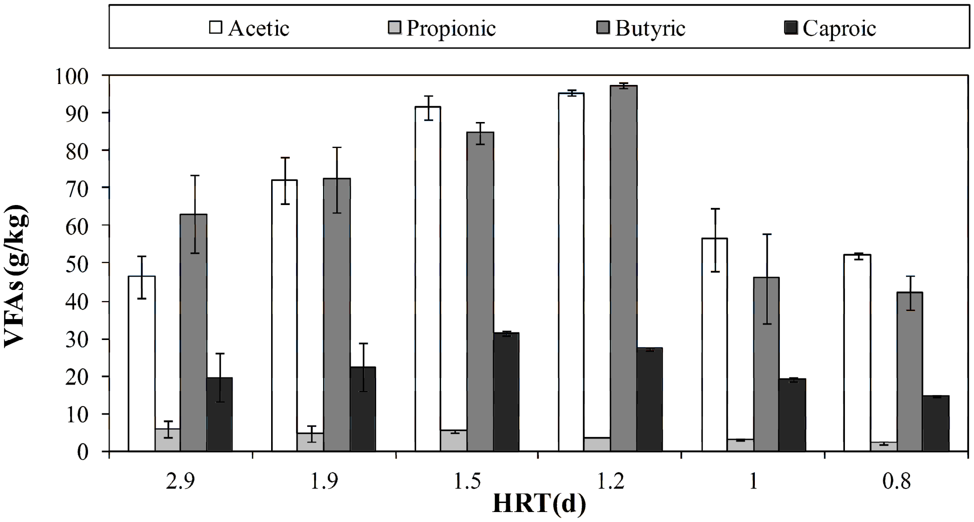
[*] No methane production was detected in the different samples.

Highlights

- HRT of 1.2 days led to the maximum hydrogen production of 3.67 L H_2/L reactor/day.
- Maximum specific hydrogen production was 33.8 mL H_2/gVS$_{added}$ (1.2-days HRT).
- Two indirect parameters NSC and ASC determine the balance in hydrolytic phase.
- At HRTs between 2.9 and 1.2-days the system operates stable.
- Failure of the hydrolytic phase occurs at HRTs lower than 1.2 days.


Stirrer (12 rpm)
50-Liters
TEDLAR BAG
(biogas)
Influent
Hot water jacket
5-L Reactor
Heating bath
(55ºC)
Effluent


ASC/TOC
NSC/TOC
DAC/TOC
(NSC+ASC)/TOC
Ratios values
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
2.9
1.9
1.5
1.2
1
0.8
HRT (d)


Acetic
Propionic
Butyric
Caproic
VFAs (g/kg)
100
90
80
70
60
50
40
30
20
10
0
2.9
1.9
1.5
1.2
1
0.8
HRT(d)