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Short communication

The production of hydrogen by dark fermentation of municipal solid wastes and slaughterhouse waste: A two-phase process

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Abstract

A two-phase fermentation process for the treatment of waste, intended for the recovery of hydrogen for energy use, was investigated in its initial fermentation phase. Hydrogen production was obtained from a mixed culture based on an active mesophilic inoculum without any selective treatment being applied. The liquid stream generated by the hydrogen fermentation process was stabilized in the following, methanogenic, phase for the recovery of methane and further breaking down of the waste stream. The whole process was carried out at a temperature in the mesophilic range (34 °C). The substrate used was an unsterilized mixture of the organic fraction of municipal solid wastes (OFMSW) and slaughterhouse waste from a poultry-processing plant. The hydrogen-producing phase was capable of stable performance under the hydraulic retention times (HRTs) evaluated (3 and 5 days). No methane was detected in the first phase at any point during the whole period of the experiment and the hydrogen yield showed no symptoms of declining as time elapsed. The amount of hydrogen obtained from the fermentation process was in the range of 52.5–71.3 N L kg^{-1} VS_{rem}.

Keywords: Dark fermentation; Hydrogen; Biogas; Mixed culture; Wastes

1. Introduction

The problems associated with the burning of fossil fuels have drawn attention to more sustainable sources for the production of energy. Hydrogen is a clean fuel with a high energy yield (142.35 kJ g^{-1}) [1,2]. Hydrogen has the highest gravimetric energy density than any other known fuel and is compatible with electrochemical and combustion processes for energy conversion without producing the carbon-based emissions that contribute to environmental pollution and climate change [3]. There are several methods of producing this clean fuel. Among them, biological techniques are a promising option. When coupled to the treatment of wastes, they are able to solve two problems: the reduction of pollution from the uncontrolled degradation of waste and the generation of a clean alternative fuel.

The production of hydrogen from wastes by dark fermentation is a relatively new process that has been studied by several authors [4–8]. When dealing with mixed microflora for the treatment of biowaste, the main problem to overcome is that of maintaining an active population during continuous operation, while at the same time avoiding as far as possible a need for costly techniques. The production of hydrogen through continuous fermentation utilizing a non-sterile substrate with readily available mixed microflora would be commercially desirable [9]. Sewage sludge, compost and soil have been used to provide seeds cultures for hydrogen-producing microflora [4–6,10–13]. The use of anaerobic microflora is an attractive option when the aim is to install a hydrogen-recovery unit in existing waste-treatment plants.

The traditional anaerobic digestion process is a well-known technique for the stabilization of wastes and recovery of methane as a fuel gas. Two-phase anaerobic digestion (2PAD) includes a first stage for hydrolysis and acidogenesis of the residue and a second stage for stabilization of the residue through methanogenesis. In two-stage systems, in addition to proper control of acidification and optimization of hydraulic retention time (HRT), biomass concentration can be adjusted independently for

Abbreviations: GPR, gas production rate; HRT, hydraulic retention time; OFMSW, organic fraction of municipal solid wastes; PEMFCs, proton exchange membrane fuel cells; SW, slaughterhouse waste; TS, total solids; VFA, volatile fatty acids; VS, volatile solids

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each stage [14,15]. The pH value and HRT are the parameters most suitable for manipulation in order to obtain the appropriate conditions for hydrogen-producing microflora. The recovery of hydrogen would involve only minor modifications to the process and practical application of this could be carried out in short order. Kraemer and Vagley [16] studied continuous fermentation in a two-phase system for the production of hydrogen with a recycling stream. Although the use of the recycling stream from the methanogenesis phase reduced the amount of alkali needed for pH control, it also caused a reduction in hydrogen-producing micro-organisms. However, it seems that coupling hydrogen and methane production is a sustainable alternative for the treatment of biowastes and so is the application of these systems to the generation of fuels to be used by fuel-cell systems.

The work being reported here was intended to study the fermentation process in a two-phase system, producing hydrogen in an initial phase and methane in the following phase. As substrate, it used the organic fraction of municipal solid wastes (OFMSW) admixed with slaughterhouse waste (SW) from a poultry-processing plant.

2. Materials and methods

2.1. Experimental set-up

Mesophilic anaerobic sludge from a laboratory-scale reactor treating slaughterhouse waste from a poultry-processing plant was used as an inoculum. No previous treatment was applied to the inoculum because this would be impractical if the processes were to be scaled up with a view to applying it to the stabilization of wastes. The total solid and volatile solid concentrations of the inoculum were 19.52 ± 0.47 and $10.92 \pm 0.33\,g\,dm^{-3}$, respectively (from three replicates).

A simulated OFMSW was used as substrate, this being composed of the following components: 10% banana, 10% apple, 10% orange, 35% cabbage, 25% potatoes, 8% bread and 2% paper. This mixture was ground to obtain a particle size of less than 3 mm. The total solid and volatile solid concentrations of the substrate were 70.52 ± 0.65 and $64.87 \pm 0.27\,g\,dm^{-3}$, respectively (from three replicates). The combined mixture with slaughterhouse waste was prepared with a dry weight proportion of 10:1. This mix as prepared had a total solid concentration and a volatile solid concentration of 74.49 ± 0.46 and $68.53 \pm 0.32\,g\,dm^{-3}$, respectively (from three replicates). Once the mixtures used as substrate were ready, they were stored at below 4 °C prior to use.

The reactors used for the hydrogen-production phase were Erlenmeyer flasks with a working volume of 100 cubic centimetres (cm^3). The reactors were kept submerged in a water bath at 34 °C and were provided with magnetic stirrers. Four reactors were employed for the study. Of these, two were for treating OFMSW with HRTs of 3 days and 5 days. These reactors were denoted OF-3 and OF-5. The other two reactors treated the mixture of OFMSW and slaughterhouse waste and were denoted OF:S-3 and OF:S-5, having the same HRTs as the former pair. This stage of the experiment was performed under two conditions for the redox potential. The first stage was set at an average of -350 ± 20 mV and the second at -250 ± 20 mV. These potentials were achieved by allowing the reactors to have contact with the atmosphere while stirring was carried out, until the required potential was attained. The pH level was controlled manually, being kept in the range 5.0–6.0 by the addition of an alkaline solution during the feeding process and by initializing the pH at 6.0. The alkaline solution was prepared by mixing $NaHCO_3$, K_2HPO_4 and Na_2HPO_4. The reactors worked on a semi-continuous basis, being fed daily.

The methanogenic phase was made up of reactors of 600 dm^3 volume. The reactors were kept at 34 °C in a water bath under static conditions. The HRT was set at 15 days, and the systems were run for a period equating to two HRTs. The reactors were fed with the effluent obtained from the first phase.

To check that the hydrogen fermentation process was sustainable on a larger scale, a reactor of 1 L capacity with a 3-day HRT was operational for 20 days. Initialization of this system was performed in the same way as had previously been described for the other reactors. This reactor worked under static conditions and was fed semi-continuously with the mixture of OFMSW as prepared for the previous experiments. Contact with the atmosphere was also allowed after each feeding process.

2.2. Analytic techniques

During the digestion process the following parameters were monitored: pH, alkalinity, total solids (TS), volatile solids (VS), daily gas production, gas composition and the concentration of volatile fatty acids (VFA). The element and immediate analyses for the substrates and the samples obtained from the fermentation process were carried out according to standard ASTM procedures. The values for pH, TS and VS were determined in accordance with standard methods [17]. Daily gas production was measured using a reversible liquid displacement device with a wet-tip counter. Biogas composition was analyzed using a gas chromatograph (Varian CP 3800 GC) equipped with a thermal conductivity detector. A 4-m long column packed with HayeSep Q 80/100 followed by a 1-m long molecular sieve column were used to separate methane (CH_4), carbon dioxide (CO_2), nitrogen (N_2), hydrogen (H_2) and oxygen (O_2). The carrier gas was argon and the columns were operated at 331 kPa and a temperature of 50 °C. Volatile fatty acids (VFA) were analyzed using a gas chromatograph (Varian CP 3800 GC) equipped with a capillary column (from Supelco) and a flame ionization detector. The carrier gas was helium and the temperature of the injector was 250 °C. The temperature of the oven was set at 150 °C for 3 min and thereafter increased to 180 °C.

3. Results

After the systems started up, gas production reached maximum values over the first 3 days and then the production of biogas decreased. This decrease coincided with an increase in the hydrogen content of the biogas. Concentrations of methane as high as 45% were detected in the systems in the early part of the experiments, but once a period of time approximately equal to one HRT had elapsed, no further methane was detected. The

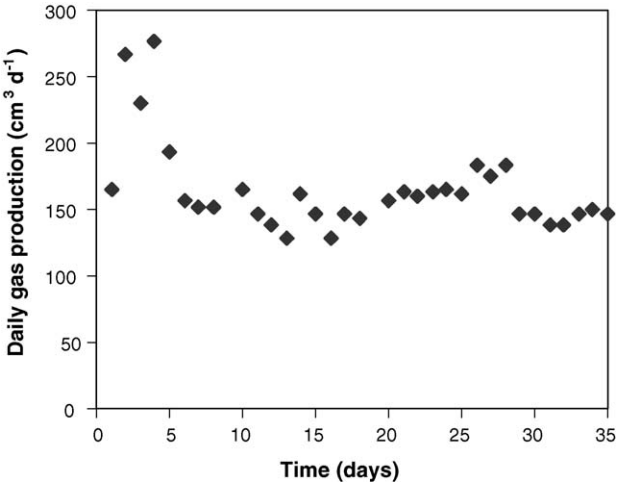

Fig. 1. Daily biogas changes in system OF-5 at a redox potential of −350 mV.

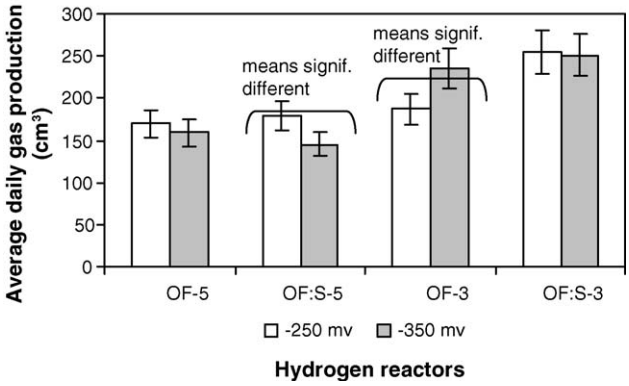

Fig. 2. Average daily gas production for hydrogen reactors.

hydrogen concentration of the biogas stabilized 7 days after the start of the experiment. The hydrogen content of the biogas from all the systems was in the range 25–27%, as an example, Fig. 1 shows the changes in daily biogas production for system OF-5 with its redox potential set at −350 mV. The trend was similar for the other systems studied. During each run, the systems were in operation for 35 days.

The initial pH of the inoculum used for the seed microflora was 7.9. Once the feeding process started, the pH steadily decreased. Alkaline solution was added once the pH reached the value 5.5. From this point on, the pH of the systems was controlled by adding enough alkaline solution to restore the pH value to 6.0 after each feeding process. The pH then fell naturally, reaching values around 5.2, and no further corrections were undertaken until the next feeding procedure. The alkaline solution was sufficiently concentrated to ensure that the volume added was insignificant relative to the amount of substrate fed into the system.

Fig. 2 shows the average daily gas production obtained from all the systems under study. Application of one-way ANOVA to the data obtained from the individual systems, at the two values set for redox potential, revealed that only the means from system OF:S-5 and OF-3 were significantly different one from the other, at a confidence level of 5%.

Table 1 gives the performance parameters for the hydrogen-production systems. The yields are expressed in terms of the volume of hydrogen gas produced relative to the amount of VS removed by the system. All the hydrogen yields obtained were in the range 52.5–71.3 N mL g^{-1} VS_{rem}. The highest values attained were for system OF:S-3. The bulk slurry obtained from this stage of the experiment was characterized by its light colour and very repulsive odour. The average VFA content in hydrogen-producing systems is shown in Fig. 3. The high caproic acid content and the low concentration of propionic acid found in all systems are worthy of note. High concentrations of caproic acid were also reported by Chen and Lin [18] using sewage sludge as seed for obtaining the hydrogen-producing microflora, and using as substrate glucose and sucrose.

A reactor 1-L in capacity was used to evaluate the behaviour of hydrogen production when systems are increased in scale. This system was monitored only for gas production, gas composition and the destruction of VS. Under static conditions, the system reached a steady state 6 days after the beginning of the experiment. The hydrogen content of the biogas was once again similar to those of the systems described previously, with a mean value of 27 ± 2.3%, and the average biogas production was 1.71 ± 0.12 N L day^{-1}. The VS content in the reactor was on average 40.3 ± 2.3 g dm^{-3}.

A methanogenic phase was coupled to the hydrogen-production systems working at −250 ± 20 mV. The effluent from the hydrogen phase was used as feed for the methane production phase. Four methanogenic reactors were used for this stage, being given the same label as the systems described above, but with a prefixed letter "M" added to indicate a methane-production phase. Data on the performance of this methanogenic phase are presented in Table 2. The daily biogas production showed higher values in systems coupled to the

Table 1
Performance parameters for biological hydrogen production systems

	System							
	OF-5		OF:S-5		OF-3		OF:S-3	
ORP (mV)	250	350	250	350	250	350	250	350
H_2 yield (N mL g^{-1} VS_{rem})	67.6 ± 14.0	60.8 ± 13.3	68.9 ± 14.1	55.1 ± 9.9	52.5 ± 10.1	62.1 ± 11.4	71.0 ± 14.0	71.3 ± 12.3
VS_{reac} (g dm^{-3})	31.1 ± 0.4	29.5 ± 0.4	36.0 ± 0.3	35.5 ± 0.3	32.2 ± 0.4	30.2 ± 0.3	35.7 ± 0.3	36.3 ± 0.2
TS_{reac} (g dm^{-3})	50.5 ± 0.3	49.0 ± 0.4	60.1 ± 0.3	57.2 ± 0.3	53.1 ± 0.4	51.5 ± 0.2	60.2 ± 0.2	55.5 ± 0.4
% Removal VS	52.2 ± 1.3	54.5 ± 1.3	47.5 ± 1.1	48.2 ± 1.1	50.3 ± 1.2	53.5 ± 1.1	47.9 ± 1.1	47.1 ± 1.0
% H_2 in biogas	27.1 ± 2.1	27.2 ± 2.1	25.0 ± 2.4	25.1 ± 2.2	27.5 ± 1.7	27.5 ± 1.7	27.5 ± 1.5	27.5 ± 1.4

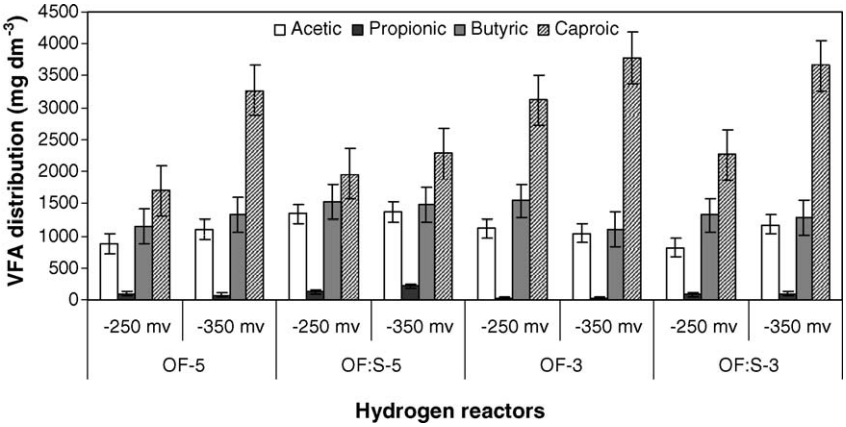

Fig. 3. Volatile fatty acid (VFA) distribution in the hydrogen-production systems.

Table 2
Performance parameters of methanogenic systems coupled to a hydrogen-production phase

System	M-OF-5	M-OF:S-5	M-OF-3	M-OF:S-3
pH	7.92 ± 0.2	8.1 ± 0.3	7.97 ± 0.2	8.2 ± 0.2
Biogas production (N mL day^{-1})	449 ± 28	761 ± 41	652 ± 52	989 ± 48
GPR (N mL Lr^{-1} day^{-1})	1.5 ± 0.1	2.54 ± 0.1	1.5 ± 0.2	2.2 ± 0.1
Alkalinity (mg $CaCO_3$ dm^{-3})	11300 ± 1240	11200 ± 1200	11300 ± 1350	11400 ± 1100
VS_{reac} (g dm^{-3})	17.1 ± 0.3	20.4 ± 0.2	20.6 ± 0.2	20.7 ± 0.2
ST_{reac} (g dm^{-3})	31 ± 0.2	36.4 ± 0.3	37.5 ± 0.3	36.4 ± 0.3
% Global removal SV	73.7 ± 1.2	70.3 ± 1.0	68.2 ± 1.1	69.7 ± 1.1
% CH_4 in biogas	60.1 ± 2.4	65.0 ± 1.5	61.2 ± 1.8	64.2 ± 2.2

hydrogen-production phases with shorter HRTs. Those systems fed with the mixture containing slaughterhouse waste delivered a higher gas production rate (GPR) and the quality of biogas was also slightly better for these systems. No great difference was noted in the behaviour of solids in the reactors, all systems having approximately the same percentage of VS removal when this was calculated for the process as a whole. The colour of the digestate obtained from the methanogenic phases was dark brown and its odour no longer repulsive. The VFA content in the supernatant was considerably reduced for all systems. The only acids detected in the supernatant were acetic and propionic, with a mean concentration calculated from all systems of 33.8 ± 25.6 and 9.4 ± 10.7 mg dm^{-3}, respectively.

Representative samples taken from the substrate, together with solids obtained from the hydrogen-producing phase and the methanogenic phase were subjected to immediate and element analysis. The results are shown in Table 3. The feed made up of OFMSW is indicated as Feed-OF and feed composed of a mixture of OFMSW with slaughterhouse waste as Feed-OF:S. The results show a decrease in the volatile content together with an increase in the ash content of the organic matter as the waste is transferred from one stage to the other. The nitrogen content of the digestate increases in parallel with a decrease in the hydrogen content. On the basis of the data obtained from the elemental analysis, the hydrogen utilization efficiency was calculated from the hydrogen present in the feed and the amount present in the biosolids remaining after the fermentation phase. The results are shown in Fig. 4. The greatest level of efficiency was achieved by system OF:S-3.

4. Discussion

The mixed culture obtained from an active anaerobic inoculum was able to maintain stable production of hydrogen in a semi-continuous process with no deterioration in the microflora. Once the process was stable, the average hydrogen concentration in the gas was in the range of 25–27.5%, balanced to CO_2. During the experiment, no methane was produced (in the first phase) by any of the systems. The only systems affected by modifications in the redox potential were OF:S-5 and OF-3. The

Table 3
Element and immediate analyses for samples of feed and digestates obtained from the hydrogen- and methane-producing stages

Sample	Volatiles (%)	Ash (%)	C (%)	H (%)	N (%)	C/N
Feed-OF	80.2	2.5	45.1	6.4	1.4	32.2
OF-5	66.5	29.0	36.1	4.9	2.6	13.9
M-OF-5	50.4	44.2	29.7	3.8	4.4	6.8
OF-3	69.3	26.6	37.9	5.1	2.6	14.6
M-OF-3	49.6	47.8	26.1	3.5	3.8	6.9
Feed-OF:S	81.3	2.7	46.6	6.7	1.9	24.5
OF:S-5	61.0	32.9	35.4	4.8	3.5	10.1
M-OF:S-5	49.3	46.1	29.1	3.6	4.0	7.3
OF:S-3	72.5	21.3	43.2	5.9	3.0	14.4
M-OF:S-3	51.1	44.0	29.5	3.7	4.2	7.0

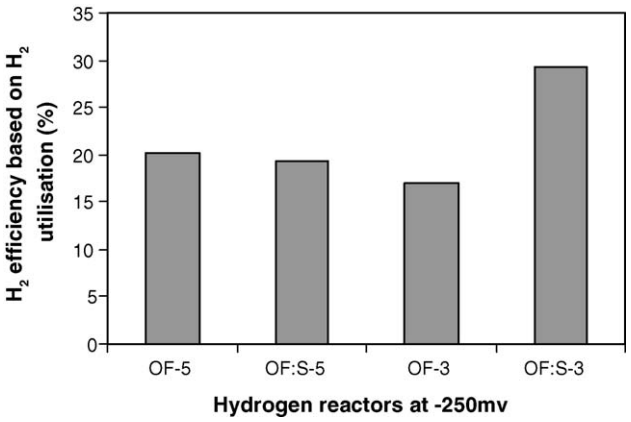

Fig. 4. Efficiency of hydrogen production at −250 mV on the basis of the utilization of hydrogen calculated from element analysis data.

increase in the redox potential, caused by allowing contact with the atmosphere, brought about an increase in biogas production for system OF:S-5. However, this was not the case for system OF-3.

The Tuckey test was applied to data on biogas production collected from the hydrogen-fermentation systems. The data were analyzed in two groups, each containing four fermentation systems, one group with a 5-day HRT and the other with a 3-day HRT. The outcome was that system OF:S-5 at −350 mV. was the only one differing with a probability greater than 95% ($p < 0.05$) within the group of systems with 5-day HRTs, the same applying to system OF-3 at −250 mV, when the systems with HRTs of 3 days were considered. This implies that no effect on the generation of hydrogen was detected when slaughterhouse waste was added to the feed.

A decrease in the HRT of the systems caused an increase in the average daily gas production. No relevant changes were observed in the hydrogen content of the biogas and the hydrogen yield was not affected to any great extent, this indicating that a sufficient population of micro-organisms remained in the system and was able to handle this new organic load. A decrease in the concentration of caproic acid was observed when the redox potential was increased. However, no clear tendency could be seen in the behaviour of the concentrations of acetic and butyric acid, other than the fact that the concentration of these acids was slightly lower in systems with shorter HRTs.

Theoretically, sucrose can be converted directly to hydrogen by anaerobic bacteria through a fermentative butyrate/acetate metabolism (Eqs. (1) and (2)), giving yields of 2–4 mole of hydrogen per mole of hexose converted [6–9,19]:

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (1)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (2)$$

The efficiency of the process can be calculated using the assumption proposed by Valdez-Vazquez et al. [6]. They assumed that 1 g of VS equated to approximately 0.9 g of hexoses. Thus, the calculated hydrogen yield in terms of moles of hexose converted is in the range 0.47 ± 0.09 to 0.64 ± 0.11 mole of H_2 per mole of hexose. That implies an efficiency in the range 11.7 ± 2.2 to $16.1 \pm 2.7\%$, when compared to the maximum yield of 4 mole of hydrogen per mole of glucose. Although this efficiency is rather low, since Valdez-Vazquez et al. [6] reported an efficiency level of $37 \pm 3.3\%$ for a mesophilic system working with mixed cultures, it should also be kept in mind that their system worked with HRTs of 21 days, much longer than those used in the experiment being reported here. Hydrogen yields of 1.25 mole per mole of hexose have been reported from continuous operation with the facultative anaerobe *Enterobacter aerogenes* [20]. However, higher yields are expected from obligate anaerobes such as *Clostridia* [9]. Since the systems in the experiments being reported here were allowed contact with the atmosphere, it would appear likely that the main hydrogen-producing population was of facultative micro-organisms, since *Clostridia* are obligate anaerobic. It should also be kept in mind that under static conditions the larger-scale reactor continued producing hydrogen for the whole of the experimental period, with a hydrogen yield of 62.7 ± 14.1 N mL g^{-1} VS_{rem}. This value falls within the range obtained from the small-scale systems.

The hydrogen rate in terms of the reactor volume was in average 0.7 L Lr^{-1} day^{-1} for the most favourable scenario, that is, data collected from system OF:S-3. Since the hydrogen obtained in dark fermentation processes should be suitable to fuel a fuel cell, it is used the hydrogen consumption by proton exchange membrane fuel cells (PEMFCs) described by Levina et al. [3] to calculate the volume of the reactor needed to fuel such a cell. It would be needed a working volume of 20 m^3 kW^{-1} to power a cell with an efficiency of 50%, a 95% of H_2 utilization rate and an average cell voltage of 0.779 V.

The results obtained from the methanogenic phase coupled to the hydrogen-fermentation systems showed that the addition of slaughterhouse waste to the systems considerably increased the amount of biogas produced in this second phase, although no effect was noted during the first stage. The GPR increased from 1.5 to 2.2–2.54 N mL Lr^{-1} day^{-1} when slaughterhouse waste was added to the mixture used as substrate. The acid effluent coming from the hydrogen phase was mineralized in the methanogenic phase and the final pH of the digestate reached a value of around 8.0. The large amount of VFAs present in the acid effluent was reduced to a low level in the second stage. Caproic and butyric acids were no longer detected in the supernatant from the methanogenic phase.

From the results of immediate and element analysis, it may be observed that in each stage of treatment the ash content of the samples increases, with a parallel decrease in the content of volatiles, indicating that a biological mineralization process is taking place [21,22]. A further parameter indicating the state of this biological mineralization process is the C/N ratio, which would be expected to decrease as the organic matter in the wastes turns into more stable compounds [23,24]. It is important to bear in mind that waste treatment is carried out in order to avoid pollution arising from the uncontrolled degradation of organic matter. A hydrogen-fermentation unit, when intended for waste treatment, should thus be accompanied by some kind of stabilization step. The anaerobic stage allows the recovery of useful fuel as well as bringing about the mineralization of organic matter.

The overall reduction in VS attained by the two-stage process is between 68.2 ± 1.1 and $73.7 \pm 1.2\%$ under the conditions studied.

Use of the data obtained from the element analysis allows the efficiency of the fermentation process in producing hydrogen to be evaluated in terms of the hydrogen produced and the atomic hydrogen used by the process. This latter value was calculated as the difference between the atomic hydrogen present in the original substrate and what was left in the remaining biosolids. From this, it became clear that the addition of slaughterhouse waste to the feed improved the utilization of hydrogen intended for the production of hydrogen gas when the HRT was reduced to 3 days. However, no effect was seen when the HRT is 5 days. Further, a decrease in the HRT caused a slight drop in efficiency at −250 mV (see system OF-3, Fig. 4).

5. Conclusions

The incorporation of a hydrogen-recovery unit into the traditional anaerobic process for the treatment of waste seems appropriate, since stable hydrogen production was attained by using active mesophilic anaerobic microflora as inoculum, without applying any type of pre-treatment. No prior sterilization of the wastes used as feed was necessary. These two features make the process attractive for large-scale application, thanks to the existence of two-phase systems which could produce hydrogen to be used in fuel-cell systems.

The methanogenic phase coupled to the hydrogen-producing systems allows stabilization of the wastes in a reasonable time.

The hydrogen yield from the fermentation process was in the range 52.5–71.3 $\mathrm{N\,L\,kg^{-1}\,VS_{rem}}$, which translates to a capacity for hydrogen recovery of 26.5–34 $\mathrm{N\,L\,kg^{-1}}$ VS fed into the system.

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