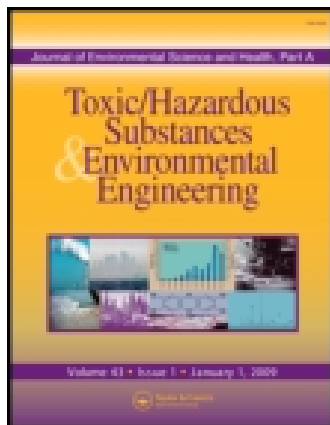


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Application of a packed bed reactor for the production of hydrogen from cheese whey permeate: Effect of organic loading rate

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Application of a packed bed reactor for the production of hydrogen from cheese whey permeate: Effect of organic loading rate

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The production of H₂ was studied using a packed bed reactor with polyurethane foam acting as support material. Experiments were performed using mixed microflora under non sterile conditions. The system was initially operated with synthetic wastewater as the sole substrate. Subsequently, cheese whey permeate was added to the system at varying organic loading rates (OLR). The performance of the reactor was evaluated by applying a continuous decrease in OLR. As a result, a significant decrease in H₂ yields (HY) was observed with the decrease in OLR from 18.8 to 6.3 g chemical oxygen demand (COD)/L d. Microbial analysis demonstrated that the prevalence of non-hydrogen producers, *Sporolactobacillus* sp. and *Prevotella*, was the main reason for low HYs obtained. This behavior indicates that the fermentation under non-sterile conditions was favored by high concentrations of substrate by creating an adverse environment for nonhydrogen producer organisms.

Keywords: Fermentative hydrogen production, sucrose, cheese whey, packed bed reactor.

Introduction

Nowadays, the limited availability of fossil fuels and pollution associated to their use is raising environmental concern in society. According to this, research dedicated to finding alternative energy sources has received growing attention.^[1] H₂ production is attractive in light of zero CO₂ emissions attained upon burning. Moreover, it has a high energy yield (141.8 kJ/g) which is almost three times that of gasoline,^[2] therefore, making it an ideal alternative to fossil fuels. H₂ can be considered as a useful energy vector if it is regarded as a form of stored energy. In this sense, the production of H₂ appears as an essential support for other renewable energy sources, especially for those with an intermittent supply.^[3]

Biological processes dedicated to the production of H₂ are gaining importance as a source of renewable fuels. These processes are considered to be more environmentally friendly and less energy intensive as compared to thermochemical and electrochemical processes.^[4] Amongst them, dark fermentative H₂ production (FHP) is a promis-

ing method for producing biohydrogen due to its high rate of H₂ evolution without the need of a light source,^[5] which greatly facilitates reactor design. Nevertheless, the process is characterized by presenting an effluent stream with high organic content, which should be further treated in a complementary stage.^[6] In this sense, to consider this process as an alternative choice for waste treatment, the complete degradation and stabilization of organic matter should be attained.

Cheese whey is the liquid remaining after the precipitation and removal of milk casein during cheese-making. Due to its high content in biochemical and chemical oxygen demand (BOD and COD), this stream requires treatment and further disposal. FHP may be an appropriate procedure for the valorization of cheese whey. Several studies have been previously conducted to evaluate FHP performance when using this waste stream as substrate.^[7–10] However, limitations of the process should be carefully studied in order to attain economic feasibility. The configuration of the reactor and operating conditions play an important role in the final productivity obtained. Most studies on FHP have been conducted in conventional continuously stirred tank reactor (CSTR). However, immobilized-cell reactors seem to be more advantageous than CSTR in terms of high H₂ productivities.^[11] The feasibility of anaerobic batch reactor containing immobilized biomass on polyurethane foam (PUF) has been demonstrated when treating low-strength

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synthetic wastewater by Ratusznei et al.^[12] Yang et al.^[13] studied different bed materials. Amongst them, PUF as support material presented better performance. Similar results were obtained by Jo et al.,^[14] who reported an improvement in the efficiency of substrate utilization and H₂ productivity when using immobilized *Clostridium tyrobutyricum* JMI in a packed bed reactor with PUF.

In the present research, FHP was studied in a packed bed anaerobic reactor with PUF as support material. Synthetic wastewater (SW) and cheese whey permeate (CWP) were used as substrate and mixed microflora was used as inoculum. Changes in organic loading rate (OLR) were performed with the aim of evaluating the effect over H₂ producing activity.

Materials and methods

Substrates and inoculum

SW was prepared using sucrose (20 g COD/L) supplemented with the following nutrients (adapted from ^[15]): 21 mg/L K₂HPO₄, 60 mg/L NaCl, 7 mg/L MgCl₂·6H₂O, 4.8 mg/L CaCl₂·2H₂O, 50 mg/L FeSO₄·7H₂O and 30 mg/L of yeast extract.

CWP was obtained from a cheese factory at Zamora (Spain). This substrate was used for evaluating the performance of the packed bed reactor. The chemical characteristics of CWP are presented in Table 1.

The H₂ producing microflora was generated from biosolid pellets obtained from the wastewater treatment plant of the city of León (Spain). Description of this plant is presented elsewhere.^[16] The solid content of these pellets was 93.6% of total solids (TS) and 61.6% of volatile solids (VS). In order to foster the adaptation of active H₂ producing bacteria, 10 g of pellets, 30 g of sucrose and tap water

were added to an Erlenmeyer flask until the working volume reached 500 mL. Batch fermentation was conducted in Erlenmeyers at mesophilic temperature (35 ± 1°C) for 32 h. Erlenmeyers were provided with magnetic stirrers. Samples were collected for microbial analysis at 24 and 32 h after the start-up of these systems. Subsequently, the culture was used as inoculum.

Erlenmeyer tests

Two Erlenmeyer flasks with a volume of 500 mL (working volume of 250 mL) were used as reactors. These Erlenmeyers were filled with PUF cubes with an average size of 3-cm side and porosity of 0.9 m³/m³. Inoculum was added until the working volume reached 250 mL. Reactors were operated under mesophilic regimen (35°C ± 1) in a semi-continuous mode during 50 d. Reactors operated at a hydraulic retention time (HRT) of 12.5 d (OLR of 1.6 g COD/L d) during the first 10 d. The HRT was subsequently reduced to a value of 2 d (OLR of 10.0 g COD/L d). After each feeding procedure the pH of the reactor was corrected to 5.5 units. Samples for microbial analysis were collected at the end of these experiments.

Tubular packed bed reactor

Figure 1 shows a schematic description of the reactor used in this study. This reactor was operated using SW as substrate in a first experimental stage and CWP in a second experimental period. The reactor was a column with a diameter of 92 mm, a height of 1700 mm and a working volume of 4 L (liquid volume). The packed bed reactor was maintained at mesophilic temperature (35 ± 1°C) by a water jacket. The feed was stored at 4°C in a feeding tank provided with a mechanical stirrer. The reactor was run on a semi-continuous mode of operation. The feeding

Table 1. Chemical characteristics of CWP.

Parameters	Units	CWP
TS	(g/L)	48.61 ± 0.02
VS	(g/L)	44.60 ± 0.04
COD	(g/L)	60.4 ± 1.6
pH	–	5.3
Alkalinity	(g/L)	0.92
KN	(g/L)	0.88
NH ₄ ⁺	(g/L)	0.08
Na ⁺	(mg/L)	291
Ca ⁺	(mg/L)	258
K ⁺	(mg/L)	1034
Cl ⁻	(mg/L)	1058
PO ₄ ³⁻	(mg/L)	<40
SO ₄ ³⁻	(mg/L)	104
NO ₃ ²⁻	(mg/L)	<20
Lactose	(g/L)	43.79 ± 0.09
Lactic acid	(g/L)	1.44 ± 0.01

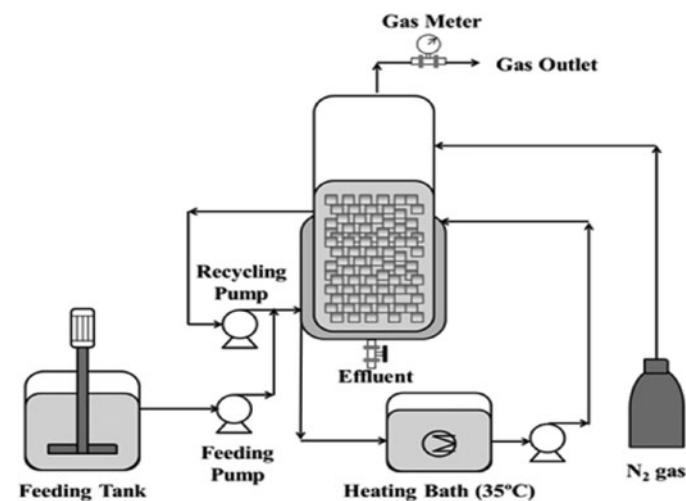


Fig. 1. Schematic description of the packed bed reactor for H₂ production.

procedure was performed once per day by means of a peristaltic pump. In order to keep anaerobic conditions, the head of the reactor was purged with nitrogen gas during each withdrawing stage. Agitation was conducted by periodical recirculation of the liquid contained in the reactor by a peristaltic pump. The pH of the liquid inside the reactor was controlled in the range of 4.5–5.5 in order to avoid the growth of methanogens.^[17]

The column was packed with PUF cubes as support material. This reactor was inoculated with the liquor and PUF cubes obtained from Erlenmeyers flasks tests (previously described). Additional PUF cubes were necessary to complete the working volume of the reactor. A volume of 0.5 L of inoculum, which was previously prepared from biosolid pellets was also added to the reactor with the aim of increasing the initial amount of active microflora. Finally, a volume of 3 L of SW was added as substrate. The system worked in batch mode for 2 d and thereafter operated on a semi-continuous mode using SW as substrate. The reactor was initially operated with an HRT of 4 d (OLR 5 g COD/L d) and was gradually reduced to a value of 1 d. Variations in operating conditions were performed due to the worsening of the performance of the reactor on day 17 of operation.

Reinoculation was necessary on day 23. In this case, a volume of 2 L was removed from the packed bed reactor and was substituted by 1 L of inoculum and 1 L of the feeding solution (SW). The production of H₂ was recovered after 24 h and normal operation was resumed. Afterwards, the process was evaluated at an HRT of 2 d during 14 d.

In the next experimental phase, the feeding stream of the packed bed reactor was substituted by CWP. The reactor was operated in 4 periods (PI – PIV) as shown in Table 2. This table presents the description of operating conditions applied to the packed bed reactor. Dilution of CWP with tap water was performed to obtain the desired concentration of COD. The HRT was set at 2 d and OLR was reduced (from 18.8 to 6.3 g COD/L d). Finally, the HRT was reduced to 1 d (OLR of 12.5 g COD/L d). During period IV, alkaline solution was added to the feed to adjust the pH to 7.5 units. Samples for microbial analysis were collected at the end of periods I and III.

Biogas flow was measured using a water displacement device. Gas production results were normalized to a standard temperature (0°C) and pressure (760 mmHg). Furthermore, gas and liquid samples were collected for monitoring

Table 2. Operating conditions of packed bed reactor using SW and CWP as substrate.

Substrate	HRT (d)	OLR (g COD/L d)		
SW	2	10		
CWP	2	18.8 (PI)	12.5 (PII)	6.3 (PIII)
	1	12.5 (PIV)		

gas composition, TS, VS, COD, volatile fatty acids (VFA), ethanol, and lactic acid content.

Analytical techniques

Kjeldahl nitrogen (KN), TS, VS, COD, alkalinity, ammonium and pH were determined in accordance with Standard Methods.^[18] Chloride, phosphate, nitrate and sulphate were measured using capillary electrophoresis (Agilent G1600, Santa Clara, CA, USA). Inductively coupled plasma atomic emission spectrometer (ICP-AES) (Perkin Elmer Optima 2000 DV, Waltham, MA, USA) was used for determining calcium, potassium and sodium concentration.

Biogas composition was analyzed using a gas chromatograph (Varian CP 3800 GC, Santa Clara, CA, USA).^[6] Lactic acid content and lactose were quantified by high performance liquid chromatography (HPLC) equipped with a refractive index detector (RID) and a PL Hi-Plex H 8- μ m column from Varian. Sulphuric acid of 0.005 N was used as a mobile phase with a flow rate of 0.4 mL/min. Ethanol content was analyzed with a gas chromatograph (Varian CP 3800 GC) equipped with a fused silica capillary coated column Cp-Wax 57 CB (50 \times 0.25 mm \times 0.2 μ m) from Varian. The carrier gas was helium. The temperature of the injector and detector were both 250°C, and the temperature of the oven was initially set at 60°C for 2 min and thereafter increased to 180°C. Data obtained from reactor performance were analyzed by one way analysis of variance (ANOVA) using Origin 6.1 software (OriginLab Corp., Northampton, MA, USA).

Microbial community analysis

DNAs from the cultures were extracted by using the PowerSoil™ DNA Isolation Kit (Mo Bio Laboratory Inc., Carlsbad, CA, USA). For DNA extraction and purification in this study, the 16S rDNA fragments were amplified by PCR (Polymerase Chain Reaction). The region corresponding to positions 357 and 518 in the 16S rDNA of *Escherichia coli* was PCR-amplified using the forward primer EUB357f (5'-CCTACGGGAGGCAGCAG-3') with a GC clamp (5'-CGCCCCGCCGCGCCCCGCGCCCCGCCGCCCCCGCCCC-3') at the 5' end to stabilize the melting behavior of the DNA fragments and the reverse primer UNIV518r (5'-ATTACCGCGGCTGCTGG-3').

PCR amplification was conducted in an automated thermal cycler (GeneAmp PCR System 9700, Applied Biosystem, Carlsbad, CA, USA). PCR products were electrophoresed on 1% (wt/vol) agarose gel in 1 \times TAE for 30 min for 80 V, and then checked with GelRed™ Nucleic Acid Gel Stain (Biotium Inc., Hayward, CA, USA) to confirm the amplification.

DGGE (Denaturing Gradient Gel Electrophoresis) was carried out using the Dcode™ Universal Mutation Detection System (BioRad, Hercules, CA, USA). PCR products were electrophoresed in 1 \times TAE buffer for 480 min at 70 V

and 60°C on polyacrylamide gel (7.5%) containing a linear gradient ranging from 40 to 60% denaturant. After electrophoresis, polyacrylamide gel was stained with GelRed™ Nucleic Acid Gel Stain (Bioutium) for 30 min, and then visualized on UV transilluminator. Most of the bands were excised from DGGE polyacrylamide gel for 16S rDNA sequencing. DNA fragments from the bands excised were PCR-amplified with the forward primer EUB357f without a GC clamp and the reverse primer UNIV518r. After PCR amplification, PCR products were purified using a Kit Nucleic Acid and Protein Purification Macherey-Nagel (Clontech, Mountain View, CA, USA). All the strands of the purified PCR products were sequenced with primers EUB357f by ABI PRISM Big Terminator Cycle Sequencing Kit and Amersham MegaBace DNA sequencer (GE Healthcare, Buckinghamshire, UK). Sequence data were analyzed with Chromas Lite 2.01 software (Ibis Bioscience, Carlsbad, CA, USA) and submitted to the non-redundant nucleotide database at GenBank using the BLAST program through the website (<http://www.ncbi.nlm.nih.gov/blast/>).

Results and discussion

Batch fermentation

Biogas produced after 32 h reached a maximum value of 978 mL (Fig. 2). The analysis of VFAs showed a content of 343 and 1776 mg COD/L of acetic acid and butyric acid, respectively. The results from DGGE analysis revealed that the genus *Clostridium* was dominant in the culture, specifically the species *Clostridium acetobutyricum* (at 24 h of culture) and *Clostridium butyricum* (at 32 h of culture). These *Clostridium* species are H₂ producers reported in several studies.^[19,20]

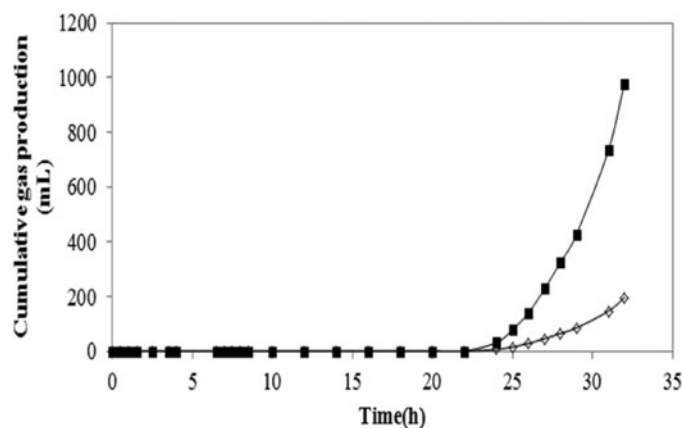


Fig. 2. Evolution of biogas and H₂ production during batch fermentation for activating H₂ producing microorganisms. H₂ production (—◇—), Biogas production (—○—), H₂ production (—■—).

Erlenmeyer tests

Figure 3 shows the profiles obtained for the production of biogas and H₂. The gas production initially observed was intermittent. This behavior was probably due to the high content of organic compounds in the inoculum. Feeding was stopped on day 8, and then, for 5 days, the pH of the systems was controlled in the range of 4.5–5.5 units. Afterwards, feeding was resumed at an OLR of 10.0 g COD/L d. The performance of the system remained unsteady with a fluctuating production of biogas as it may be observed from Figure 3. H₂ and CO₂ were the main gaseous products. The highest HY attained was 135.9 L H₂/kg COD. Instability might be explained by the absence of stirring in the system that favored the accumulation of dissolved H₂. High partial pressures were likely to occur (not measured), due to the high biogas accumulation observed in the headspace of the Erlenmeyer flasks.

High H₂ partial pressure is one of the causes of decreasing HY. When H₂ production follows the Pyruvate formate lyase (PFL) enzyme system, the production of this gas can continue as long as the partial pressure is less than 30 kPa, whereas from nicotinamide adenine dinucleotide-reduced form (NADH), this pressure must be less than 60 Pa.^[21] It has been reported that stirring clearly improves H₂ production in comparison with static fermentation experiments.^[22] Although microflora was submitted to an adverse environment, H₂ production was maintained for over a period of 30 days.

Signals of biomass accumulation could be visible on the surface of the support material. However, microbial analysis at the end of this experiment revealed the dominance of *Sporolactobacillus* sp. These microorganisms have been reported to decrease HY due to inhibitory effects caused by the excreted bacteriocins.^[23]

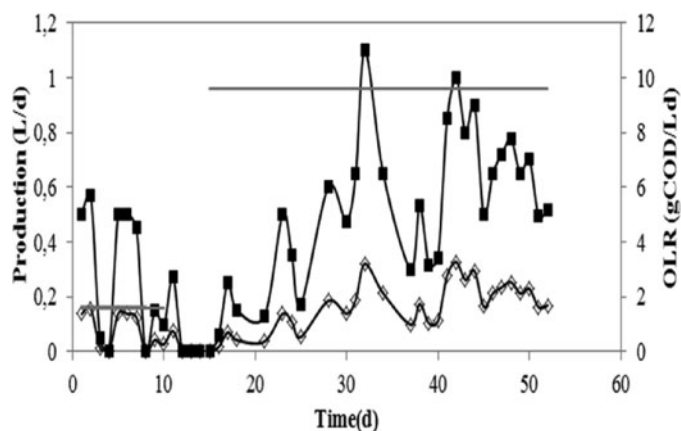


Fig. 3. Evolution of biogas production, H₂ production and OLR applied during Erlenmeyer flask tests. H₂ production (—◇—), Biogas production (—■—), OLR (—).

Tubular packed bed reactor: Synthetic wastewater

H₂ and CO₂ were the main gaseous products obtained and CH₄ was always under the detection limit (1%). The reactor was characterized by an initial low production of biogas as consequence of the characteristics of the inoculum used (Fig. 4). The strategy of modifying operating conditions by a decrease in HRT from 4 to 1 d in a period of 10 d did not result in a better performance. The maximum value of biogas produced was 2.0 L/d with H₂ content of 35.1%, which was obtained on day 11 of operation. An increase in OLR to 20 g COD/L d resulted in a severe reduction in H₂ production.

Total VFA concentrations were initially low (Fig. 5) and the pattern of acid production followed a decreasing trend (values in the range 622.0–312.9 mg COD/L) with acetate being the main component. Moreover, after reaching an HRT of 1 d, the production of H₂ decreased immediately. This result may be a consequence of the operating conditions applied to the system. Biomass was not properly fixed and was washed out from the reactor.

Reinoculation was performed on day 23. The inoculum used was prepared from biosolid pellets as previously described in materials and methods section. The reactor was operated at higher HRTs. As it can be seen from Figure 4, the performance of the reactor rapidly improved and biogas production was recovered. From day 36 onwards, the system was evaluated at an HRT of 2 days (OLR 10 g COD/L d). Although at these conditions the HY was increased to 48.4 L H₂/kg COD, this value was lower when compared to the maximum observed in the previous immobilized system and those reported in literature for FHP. Keskin et al.^[24] employed an immobilized reactor packed with ceramic balls. These authors achieved an HY of 189 mL H₂/g sucrose using a solution of 10 g sucrose/L and an HRT of 24 h, while Chang et al.^[25] reported an HY of 42–130 mL H₂ /g COD using sucrose at a concentration of

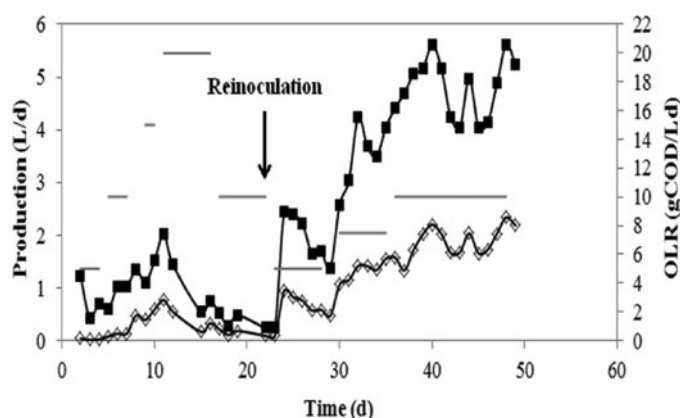


Fig. 4. Biogas production, H₂ production and OLR applied during fermentation experiments in a packed bed reactor employing SW as substrate. H₂ production (—○—), Biogas production (—■—), OLR (—).

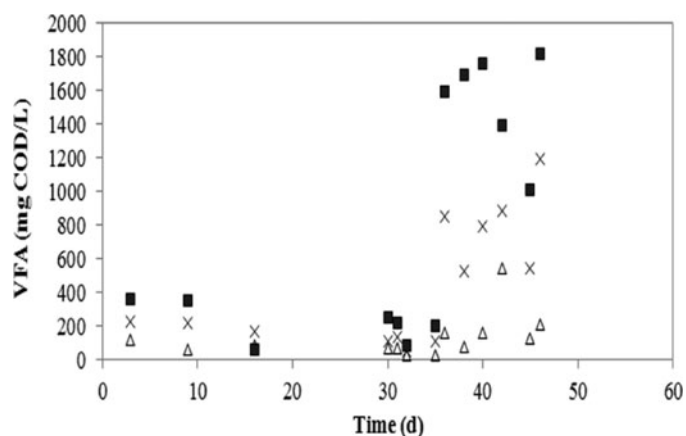


Fig. 5. Concentration of VFAs during fermentation experiments in a packed bed reactor employing SW as substrate. Acetic acid (×), Propionic acid (Δ), Butyric acid (■).

20 g COD/L in a fixed-bed reactor packed with expanded clay and activated carbon.

From Figure 5, it can be observed that H₂ production was accompanied by an increase in acid concentration. The main intermediaries were acetic and butyric acids. Propionic acid was also present although in much lower concentration. Several authors have proposed the ratio of butyric to acetic acid (Bu/Ac) as an indicator of substrate metabolism and predictor of HY.^[26,27] During these experiments (Bu/Ac) ratio was in the range of 0.7–0.9. Previously, during batch fermentation with sucrose, the presence of *Clostridium butyricum* was detected combined with a Bu/Ac ratio of 2.0. The reduction of Bu/Ac ratio might be associated to the presence of other fermenters in the reactor that lead to shifts in metabolic pathways and as a consequence decrease the volume of H₂ produced.

Tubular packed bed reactor: Cheese whey permeate

Biogas and H₂ production are presented in Figure 6. Parameters for evaluating the performance of the reactor are summarized in Table 3. H₂ content in the gas phase was between 20 and 27% and CO₂ was the main gaseous product detected (73–80%). No CH₄ was observed, indicating the absence of methanogens. The reduction applied in OLR resulted in a decrease in daily production of H₂. However, HY was not affected when OLR was reduced from 18.8 to 12.5 g COD/L d.

The predominant metabolites were butyric and acetic acids (Fig. 7). In general, the production of acetic and butyric acids also favors the production of H₂, although the presence of acetic acid may also be related to the consumption of H₂, which consequently leads to lower H₂ yields.^[28] Furthermore, small proportions of solvents were found in the reactor during the experiment. The concentration of ethanol reached values in a range from 48 to 1168 mg COD/L and 1181 to 1611 mg COD/L during the first

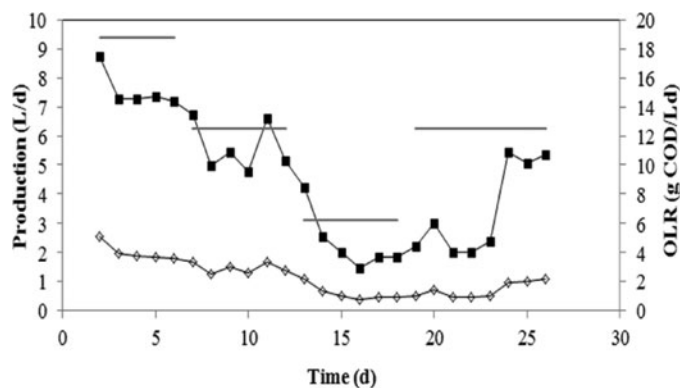


Fig. 6. Biogas production, H₂ production and OLR applied during fermentation experiments in a packed bed reactor employing CWP as substrate. H₂ production (—◇—), Biogas production (—■—), OLR (—).

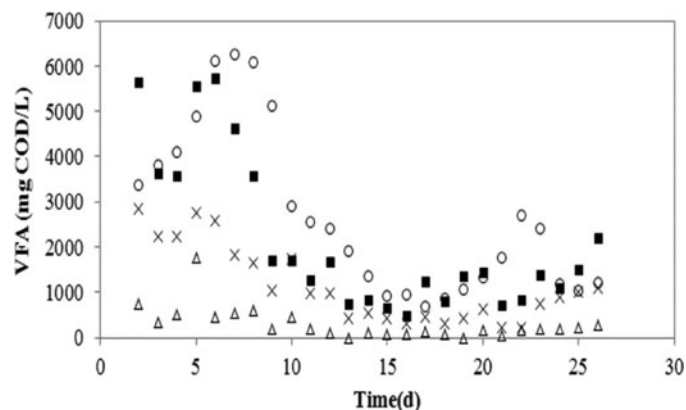


Fig. 7. Concentration of VFAs during fermentation experiments employing CWP as substrate. Acetic acid (×), Propionic acid (Δ), Butyric acid (■), Lactic acid (○).

and second period of operation, respectively. Afterwards, ethanol was no longer detected. Solvent fermentation is associated with the early steps of sporulation of *Clostridium*. In response to hostile conditions, such as oxygen, heat, acids, or bases, the physiology of *Clostridium* may change from vegetative cells to endospores.^[29] According to this, it might be assumed that the decrease in OLR favored an environment for *Clostridium* population to exist as vegetative cells, therefore decreasing the solvent production.

It was also observed that at the lowest OLR tested, the HY obtained was reduced. This reduction was followed by a decrease in the content of VFAs. The decrease in OLR also caused a decrease in the content of lactic acid (Fig. 7). These results are in agreement with those reported by Kim et al.^[30] These authors reported a dependence of H₂ production on substrate concentration, with a lower HY being reported with the decrease in substrate concentration.

The metabolic products as well as the volume of H₂ gas produced depend on the dominant microbial species contained in the reactor and on the metabolic pathway they followed under the prevailing conditions. The presence of other co-existing microorganisms might hinder H₂ production by acting as H₂ consumers or substrate competitors.^[31]

Samples taken from the reactor at the end of periods I and III were analyzed by DGGE. The presence of *Sporolactobacillus sp.* and microorganisms of the genus *Prevotella* were detected in the culture obtained from both samples. Members of the genera *Prevotella* were found in other

H₂ producing reactors,^[8,10] although these organisms have been reported in literature with no fermentative capacity for producing H₂.

In addition, the presence of *Sporolactobacillus sp.* was also found during the fermentation of sucrose in the previous assay carried out in Erlenmeyers Flasks. Despite the fact that H₂ production did not cease during the experiment, H₂ producers were not identified in any of the two samples taken. This is explained by the size of their population which was below the limit of detection by DGGE technique. During the operation of the reactor, an enrichment of non-hydrogen producers took place that competed for substrate up-take and negatively affected the prevalence of H₂ producers. The persistence of a mixed microbial population with a low proportion of hydrogen-producing bacteria could explain the lower yield (Table 3) obtained when compared with the maximum theoretical expected value (8 mol H₂/mol lactose).

Similar results were reported by Perna et al.^[32] These authors performed experiments in an up-flow anaerobic packed bed reactor treating cheese whey. They obtained a maximum HY of 1.1 mol H₂/mol lactose. However, they also reported a mixed population with non-hydrogen-producing organisms and a low proportion of H₂ producing fermenters when performing microbial analysis. On the other hand, Davila-Vazquez et al.,^[33] when studying the fermentative production of H₂ with cheese whey as substrate, reported the presence of *Clostridium sp.* as the main

Table 3. Performance of the packed bed reactor during experiments feeding with CWP as substrate.

Period	I	II	III	IV
HRT(d)	2	2	2	1
OLR(g COD/L d)	18.8	12.5	6.3	12.5
H ₂ content (%)	25.7 ± 0.8	26.3 ± 1.0	25.7 ± 1.2	19.4 ± 1.4
HY(L H ₂ /kg COD _{feed})	24.8 ± 3.8	25.0 ± 3.1	18.1 ± 3.2	18.2 ± 1.0
HY(mol H ₂ /mol lactose)	0.5 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1

organism and the HYs attained were in the range of 2.1–2.8 mol H₂/mol lactose. In this line, to improve the performance, it will be necessary to regularly modify operational conditions to reduce the dominance of non-hydrogen producing microorganisms. Among these alternatives, repeated heat shock has been claimed as one of the most effective ways to avoid competition and maintain a successful operation.^[34] However, an evaluation of the cost of energy involved under continuous operation should be performed prior to consider this alternative as a viable option.

Finally, the effect of increasing the OLR (by a reduction in HRT) over H₂ production was investigated. In this case, the concentration of COD in the influent was kept constant (12.5 g COD/L) but HRT was reduced to 1 day. During 3 days under these conditions, it was not observed an increase in H₂ production, while lactic acid concentration increased to reach a maximum value of 2688 mg COD/L. Due to the high content in lactic acid presented in the liquor, it was evaluated the effect of increasing the pH of the feeding solution based on results of Zhu et al.^[35] These authors worked under batch conditions and demonstrated that H₂ production of feedstocks with limited alkalinity improved significantly when buffer addition is applied in order to attain a feeding solution with a pH value greater than 6.0. A higher initial pH could be sufficient to rapidly buffer acid production and allow an increase in hydrogen production, thus reducing the inhibitory effect over hydrogen producers. Based on this, it was decided to increase the alkalinity of the feeding solution to obtain an initial pH of 7.5 units. As a result, an increase in the production H₂ was observed along with an increase in VFA concentration during this stage, while the concentration of lactic acid decreased indicating a possible shift of the metabolic pathway. HY was able to recover to the value previously obtained at an HRT of 2 days.

Conclusion

The production of H₂ from SW and CWP was studied using a packed bed reactor with PUF as support material. Under semi-continuous operation the maximum HY achieved was 48.4 L H₂ /kg COD when using SW as substrate at an HRT of 2 d. When using CWP, the effect of varying operating conditions was tested by reducing the OLR of the reactor. The decrease in OLR from 18.8 to 6.3 g COD/L d resulted in a significant drop in HY. The experimental results indicated the presence of non-H₂ producers that might have competed for substrate and negatively affected the prevalence of H₂ producers. Despite of this, H₂ production was detected throughout the experiment. It was observed that the H₂ production was favored whenever feeding was interrupted, higher OLR was applied, and the pH of the feeding solution was set at 7.5 units. Moreover, operational conditions and possible pretreatments should be further evaluated in order to enhance H₂ productivity.

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