Fermentative hydrogen production from food – industry wastes in a CSTR-type reactor: The influence of Hydraulic Retention Time.

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ABSTRACT

This study focuses on the exploitation of food-industry wastes as a source for biohydrogen in a CSTR – type reactor, via fermentation. The substrate used was the aquatic suspension of a mixture of seven different out of date solid baby foods, coming from a big Greek food industry. Mesophilic (35ºC) fermentative hydrogen production was investigated at hydraulic retention times (HRTs) of 12, 8, 6 and 4 h. For the reactor start–up, indigenous microbial species were used as inoculum, contained in the substrate, incubated at 35ºC for 48 h. The experimental results obtained showed a long and stable reactor operation with high hydrogen content (50 - 60 %) in the gas phase. The optimum hydrogen production rate was observed for HRT = 8 h, being 5.63 ± 0.15 L / L reactor /d, while the yield of hydrogen produced was approximately 0.73 ± 0.03 mol H_2 / mol glucose consumed. When the HRT decreased to 6 h and 4 h, the hydrogen yield decreased to 0.56 ± 0.03 and 0.49 ± 0.02 mol H_2/ mol glucose consumed, respectively, implying that the reactor started to be kinetically limited. For all the HRTs the main metabolic products were acetic, butyric and propionic acids, constituting 57 – 66 % of the effluent Chemical Oxygen Demand (COD) concentration.

Keywords: fermentative hydrogen production, food industry wastes, HRT

INTRODUCTION

The increased world demand for energy as well as the decrease of fossil fuel deposits has necessitated a global search for alternative energy sources. Biohydrogen gas has attracted much interest as a promising alternative source of energy, due to its zero greenhouse emissions, compared to petroleum fuels [1]. The main advantage of biohydrogen is that it can be produced from renewable raw materials such as organic wastes, contributing to human-derived waste disposal and their recovery in an environmentally friendly manner [2].

A number of potentially suitable residual substrates have been evaluated for biohydrogen generation, through dark fermentation, so far [1-3]. Among these, the organic fraction of municipal solid wastes or food wastes represents a zero-cost, suitable source for hydrogen production, mainly due to its high carbohydrate content and its wide availability [4-6]. In the recent years, numerous studies have been published on fermentative hydrogen production of food waste, using pure [5,7,8] or mixed microbial cultures [9,10], using several reactor configurations, both batch [10,11] and continuous [12,13].

Hydrogen production via fermentation involves either facultative or strict anaerobic bacteria. The involved metabolic pathways can either be promoted or prohibited, depending on the prevailing operating conditions, which govern the production of specific volatile fatty acids (VFAs) and alcohols, including acetate, propionate, butyrate, lactate and ethanol. During carbohydrate fermentation towards acetate and butyrate, the production of 4 and 2 moles of molecular hydrogen per mole of glucose degraded, are produced respectively. However, propionate, ethanol and lactic acid can also be produced in mixed bacterial cultures, adversely affecting hydrogen production: propionate is a metabolite of a hydrogen-consuming pathway, while ethanol and lactic acid are associated with zero hydrogen production, when being the sole metabolic products [14]. Recently, it was found that hydrogen and butyrate could be produced from mixed substrates of acetic and lactic acids [15,16].

The aim of this study is to explore the possibility of producing biohydrogen, using a waste stream from a Greek food industry. The waste used was an aquatic suspension of a mixture of seven different out of date solid baby foods. Continuous mesophilic experiments in a stirred tank reactor were conducted to evaluate the fermentative hydrogen production from the waste, using mixed microbial cultures, at different hydraulic retention times (HRTs). It is well known that the HRT is an important parameter that influences the hydrogen production rate and operational stability of a hydrogen-producing reactor. The HRTs investigated during the working period of the acidogenic reactor were: 12, 8, 6 and 4 h.

MATERIALS AND METHODS

Analytical methods

Determinations of Chemical Oxygen Demand (COD), total suspended solids (TSS) and volatile suspended solids (VSS) were carried out according to Standard Methods [18]. The measurement of carbohydrates was carried out according to Joseffson [19]. For the quantification of volatile fatty acids (VFAs) and ethanol, 1 mL of filtered sample acidified with 30 μL of 20% H_2SO_4 was analyzed with a gas chromatograph (VARIAN CP-30), equipped with a flame ionization detector and a capillary column (Agilent technologies, INC. 30 m × 0.53 mm). The oven was programmed from 105^{o}C to 160^{o}C at a rate of 15^{o}C/min,and subsequently to 235^{o}C (held for 3 min) at a rate of 20^{o}C/min for VFA analysis and from 60^{o}C (held for 1 min) to 230^{o}C (held for 0.5 min) at a rate of 45^{o}C/min for ethanol analysis. Helium was used as the carrier gas at 15 mL/min, the injector temperature was set at 175^{o}C and the detector at 225^{o}C and 200^{o}C, for VFA and ethanol determinations respectively. The concentration of lactic acid was measured with Megazyme D-/L-Lactic acid assay kits. The produced gas composition in hydrogen and methane was quantified with a gas chromatograph (SRI 8610c MG#1), equipped with a thermal conductivity detector and a packed column. The carrier gas was nitrogen for hydrogen measurements and helium for methane. The injector, column and detector temperatures were set at 90^{o}C, 35^{o}C and 100^{o}C, respectively. The volume of the produced gas was measured by the method of displacement of acidified water. The measurement of the pH was done using a HANNA (pH 211) pH-meter with a HANNA electrode (HI 1230).

Hydrogen production

A stainless steel, cylindrical CSTR–type reactor with a working volume of 0.4 L was operated under mesophilic conditions (35^{o}C). During start-up, the reactor was filled with the aquatic solution of the mixture of the seven solid baby foods at carbohydrates' concentration equal to 12.5 g/L and operated anaerobically at batch mode at 35^{o}C for 48 h, in order to activate the indigenous microbial species contained into the waste, as proposed by Antonopoulou et al.[19]. In the sequel, the reactor, was operated in a continuous mode at HRTs of 12, 8, 6 and 4 h. Feeding was programmed every 3h, with the stirring on, while the feed flask was kept at 4^{o}C.

The reactor was fed with an aquatic solution of the mixture of the solid wastes of 21.4 g substrate /L corresponding to a concentration of 12.5 g carbohydrates/L. It was supplemented with 5 g/L NaOH, 6.8 g/L KH_2PO_4, 2 g/L urea and 0.5 g/L yeast extract in order to maintain the culture pH at suitable levels for biohydrogen production [19]. It was also added a solution of trace metals containing (g/L): $(NH_4)_2HPO_4$ (7.21), $FeSO_4.7H_2O$ (0.7), $CaCl_2.2H_2O$ (22.5), NH_4CL (35.9), $MgCl_2.6H_2O$ (16.2), KCl (117), $MnCl_2.4H_2O$ (1.8), $CoCl_2.6H_2O$ (2.7), H_3BO_3 (0.51), $CuCl_2.6H_2O$ (0.24), $Na_2MoO_4.2H_2O$ (0.23), $ZnCl_2$ (0.19), $NiCl_2.6H_2O$ (0.2), H_2WO_4 (0.01). The main characteristics of the feed used in the present study are shown in table 1.

Gas and liquid samples were collected 10-15 min before feeding. The reactor performance (biogas production and compsosition in hydrogen, pH, carbohydrates, soluble COD, ethanol, lactate and VFAs concentration) was monitored on a daily basis. Gas samples were also analyzed for methane daily, in order to monitor whether methane production occured. Complete characterization was made when a steady state was reached. By steady state, it was meant that the variation of the monitoring parameters was less than 10 %.

Table 1 The main characteristics of the aquatic solution of the solid wastes, used as a feed of the reactor.

Characteristic	Value
pH	11.61 ± 0.44
TSS (g/L)	13.4 ± 1.05
VSS (g/L)	10.8 ± 0.42
Total Carbohydrates (g/L)	12.43 ± 0.73
Soluble Carbohydrates (g/L)	7.94 ± 0.35
Total COD (g/L)	21.30 ± 4.07
Soluble COD (g/L)	12.85 ± 1.90

RESULTS AND DISCUSSION

The acidogenic reactor was operated anaerobically for 54 days .As previously mentioned, the reactor initially was operated at an HRT = 12 h, (day1-20), and then the HRT was reduced to 8 h (day 21 – 39), 6 h (day 40-48) and finallyto 4 h (day 49–54), ensuring in each case that steady state was reached. It is also worth to mention that, during the operation of the hydrogen producing reactor, no methane was detected, as confirmed by methane measurements.

In figure 1, the content of hydrogen in the gas phase during the experimental period is presented. A long and stable operation with high hydrogen content in the gas phase was observed, as the biogas hydrogen content lied between 50 and 60%. These results are similar with those reported by Sivagurunathan et al. [13], who investigated high-rate fermentative hydrogen production, using beverage wastewater in an immobilized cell reactor.

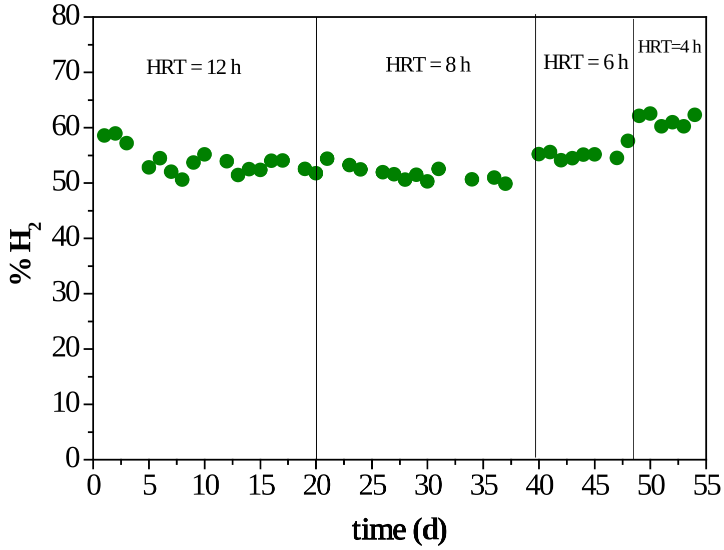

Fig.1 Hydrogen content in gas phase of the reactor.

In figure 2, the biogas and hydrogen production rates at all HRTs, are presented. As anticipated, production rates increased with an HRT decrease. The higher hydrogen production rate was observed for HRT = 4 h and it was 4.3 ± 0.1 L H_2/d, corresponding to a hydrogen yield of 85.7 ± 1.7 L H_2/kg waste.

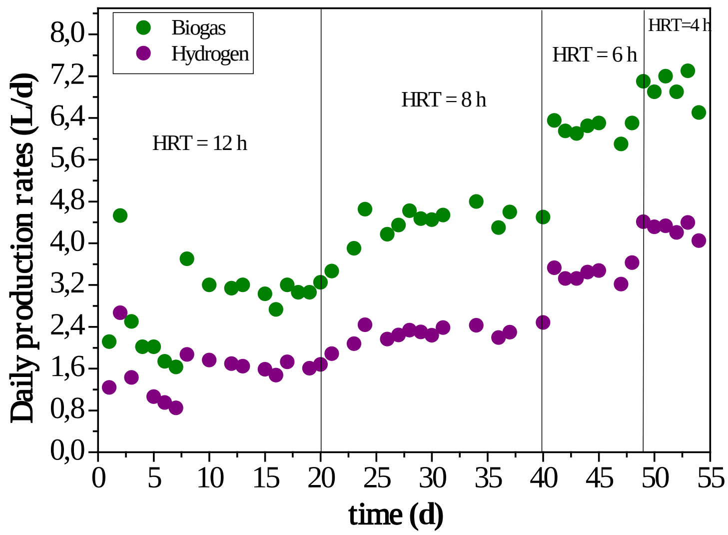

Fig. 2 Biogas and hydrogen production rates of the acidogenic reactor.

In figure 3, the concentration of the non-consumed carbohydrates expressed as glucose equivalents is presented. For the first two HRT values (12 and 8 h), the carbohydrates' consumption was almost complete (96.7 ± 0.7 % and 95.0 ± 1.4 % respectively). At the HRT of 6 h, a slight increase in their concentration was observed (1.1 ± 0.3 g/L) while at the HRT of 4 h a significant carbohydrate residue was observed (3.2 ± 0.4 g/L), indicating that the reactor started to be kinetically limited for low HRTs.

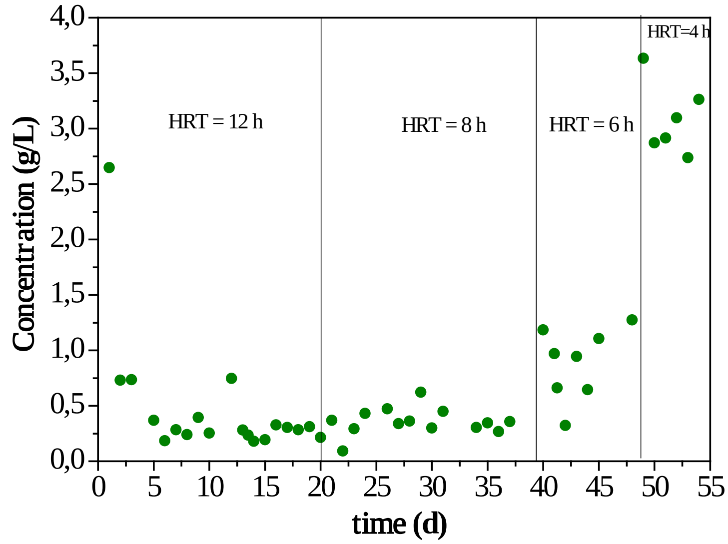

Fig. 3 The concentrations of the non-consumed carbohydrates during the operational period of the reactor.

The concentrations of the main metabolic products measured during the operational period of the hydrogen producing reactor are presented in figure 4. Iso-butyric, iso-valeric and valeric acid as well as ethanol were not detected, while propionic acid was produced in small quantities. The low concentrations of propionic acid indicate an efficient hydrogen production process, as the formation of propionate consumes hydrogen [13,14]. As observed, the distribution of the metabolic products was different at the various HRT values. At the HRT of 12 h, the dominant metabolic products were both acetic and butyric acids with a concentration of 2.9 ± 0.1 g/L and 3.2 ± 0.5 g/L respectively. For the HRT of 8 h, butyric acidproduction prevailed (4.7 ± 0.2 g/L), while the concentration of acetic acid decreased to 2.0 ± 0.2 g/L. Lactic acid started being produced with a concentration of 0.8 ± 0.2 g/L. At the HRT of 6 h, both butyric and lactic acid dominated, with concentrations of 3.5 ± 0.2 g/L and 2.9 ± 0.4 g/L respectively, and finally at the HRT of 4 h, lactic acid prevailed as the dominant metabolic product (3.0 ± 0.1 g/L). The soluble metabolites' profile obtained from the present study indicates a mixed-type fermentation of the food industry wastes used. The same metabolic products (acetic, butyric, propionic and lactic acid) were observed by Lee et al. [20], who investigated hydrogen production in a CSTR reactor using glucose as a substrate.

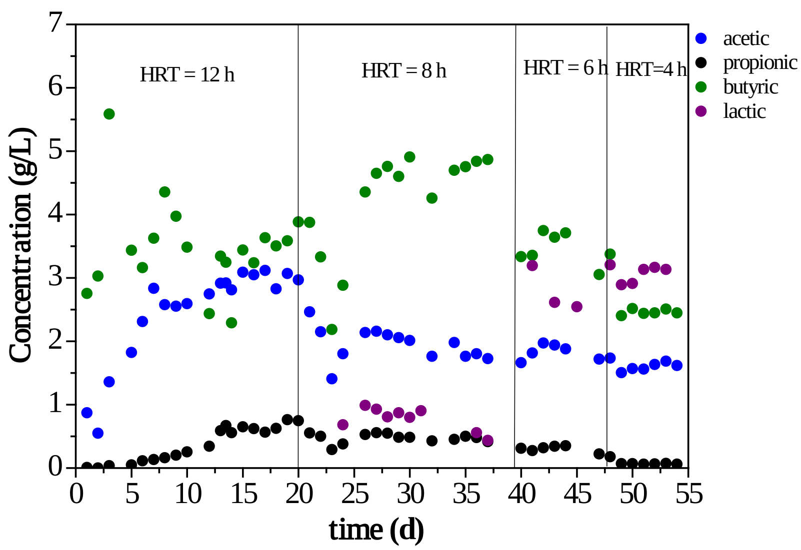

Fig. 4 The concentrations of the main metabolic products produced.

In table 2, the main characteristics of the four steady states achieved are shown, while in table 3 the main yields and rates are given. The pH of the reactor lied between 5.5- 5.7. This pH range is consistent with Tawfik's et al. [21] results, who reported a pH range 5.2 – 5.5 as the optimal for bio-hydrogen production from municipal food waste using an anaerobic baffled reactor. In the present study, the higher hydrogen yield was observed for a pH of 5.7. This pH value is slightly higher than that obtained by Antonopoulou et al. [19] who studied fermentative hydrogen production from sweet sorghum extract, under different pH values. Although the aforementioned studies report that the optimum pH values for fermentative hydrogen production lies between 5-5.7, different values have also been reported as optimum for fermentation. This, could be attributed to different kinds of inocula used, different substrates or reactor types and sometimes, to the difference in the pH range studied [22]. At the HRT of 12 h, the measured soluble COD was equal to 68% of the theoretical soluble COD, that corresponds to the metabolic products and the non-consumed carbohydrates, indicating that there were some other metabolic products, such as alcohols that there were not detected.

Table 2 The main characteristics of the four steady states of the hydrogen- producing reactor.

Characteristic	**Value**			
	HRT= 12 h	HRT= 8 h	HRT= 6 h	HRT= 4 h
pH	5.72 ± 0.07	5.68 ± 0.05	5.46 ± 0.08	5.48 ± 0.07
TSS (g/L)	7.82 ± 0.40	6.45 ± 0.28	6.61 ± 0.25	7.88 ± 0.54
VSS (g/L)	6.72 ± 0.45	5.34 ± 0.18	5.45 ± 0.11	6.60 ± 0.42
Content in hydrogen (%)	52.43 ± 1.03	50.95 ± 0.75	55.70 ± 1.34	61.47 ± 1.06
Acetic acid (g/L)	2.94 ± 0.12	2.00 ± 0.17	1.83 ± 0.10	1.57 ± 0.05
Butyric acid (g/L)	3.19 ± 0.52	4.71 ± 0.19	3.46 ± 0.16	2.45 ± 0.05
Propionic acid (g/L)	0.63 ± 0.08	0.50 ± 0.05	0.26 ± 0.08	0.07 ± 0.00
Lactic acid (g/L)	-	0.77 ± 0.20	2.89 ± 0.36	3.03 ± 0.14
Soluble COD measured (g/L)	15.59 ± 0.69	17.10 ± 0.30	15.60 ± 0.48	16.98 ± 0.36
Soluble COD theoretical (g/L)	10.58	12.95	13.09	12.79
% Soluble COD (theoretical/measured)	68	76	82	76

According to tables 2 and 3, the maximum hydrogen production yield was obtained for HRT 8 h and it was equal to 0.73 ± 0.03 mol H_2/mol consumed carbohydrates.The main metabolic product during this HRT value was butyric acid, verifying that the production of butyric acid enhances bio-hydrogen production [4,19]. This yield is similar to the respective one obtained by Antonopoulou et al. [23] who investigated dark fermentation of sweet sorghum extract in a CSTR type reactor, under different initial substrate values. Moreover, it can be seen that the maximum production rate was observed at the HRT of 4 h and it was equal to 10.79 ± 0.21 L H_2/L reactor/d. This rate is 3.7 times higher than the respective one achieved in the study of Venetsaneas et al. [24] who investigated fermentative hydrogen production of cheese whey in a CSTR type reactor, using indigenous microbial species.

Table 3 The main yields and rates of the hydrogenogenic reactor during the steady states.

Characteristic	Value			
	HRT= 12 h	HRT= 8 h	HRT= 6 h	HRT= 4 h
L H_2/L reactor/d	4.13 ± 0.14	5.63 ± 0.15	8.72 ± 0.32	10.79 ± 0.21
L H_2/ kg waste	96.27 ± 3.36	87.60 ± 2.40	101.75 ± 3.71	83.94 ± 1.63
L H_2/ kg VS waste	98.24 ± 3.43	89.40 ± 2.45	103.84 ± 3.79	85.67 ± 1.66
mol H_2/mol consumed carbohydrates	0.71 ± 0.03	0.73 ± 0.03	0.56 ± 0.03	0.49 ± 0.02

CONCLUSIONS

In this study, the influence of the hydraulic retention time was investigated during fermentative hydrogen production of food industry wastes in a CSTR–type reactor. The reactor was fed with an aquatic solution of seven solid baby foods in an initial carbohydrates' concentration of 12.5 g/L. The reactor was operated at HRTs of 12, 8, 6 and 4 h, using indigenous microbial species. The maximum hydrogen production yield was obtained for the HRT of 8 h and was 0.73 ± 0.03 mol H_2/mol consumed carbohydrates, while butyric acid was the dominant metabolic product. In conclusion, the results of the present study demonstrated that solid wastes of food industry can be exploited for hydrogen production in continuous bioreactors, in a viable and effective process.

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