PRODUCTION OF ADVANCED BIOBASED HYDROGEN ENRICHED METHANE FROM WASTE GLYCEROL IN A TWO-STAGE CONTINUOUS SYSTEM

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ABSTRACT

In the present work, a continuous process was developed for the production of advanced biobased hydrogen enriched methane, from crude glycerol in a two-stage reactor system. In the first step, biohydrogen production was studied, using attached mixed acidogenic consortia in an up-flow column bioreactor. Cylindrical ceramic beads with porosity corresponding to 600 m^2L^{-1} were used as attachment matrix of bacterial cells. The hydrogen yield and the substrate consumption were investigated for a hydraulic retention time of 24h, with feed pH values 6, 6.5 and 7 and a concentration of 20g/L. The effluent of the hydrogenic reactor was fed to a methanogenic continuous stirred reactor (CSTR) in which the effect of organic loading on the methane yield was studied. The gaseous phase of the reactors was mixed for the production of the final gasous biofuel (hythane). At glycerol concentration of 20 g/L, hydrogen was produced with a yield of 0.051, 0.070 and 0.094 L/g COD feed with feed pH values 6, 6.5 and 7 respectively. Additionally, methane was produced with a yield of 0.257 L/g COD feed (commercial glycerol in the feed), 0.283 L/g COD feed (crude glycerol in the feed) , 0.198, 0.242 and 0.273 L/g COD feed (effluents from the hydrogenogenic (1st stage), diluted with water to 5, 7.5 and 10 g COD / L) respectively.

INTRODUCTION

The replacement of natural gas in internal combustion engines by a blended gas of hydrogen 10–60% (v/v) with methane was shown to highly improve the combustion efficiency, decrease the fuel consumption, and reduce significantly the emissions of carbon monoxide, carbon dioxide, and nitrous oxides [1]. The production of this hydrogen enriched methane, sometimes called hythane® as it was trademarked by Hydrogen consultants Inc., USA [2], is mostly based on the catalytic conversion of natural gas [3], which is inherently an inefficient and unsustainable process. The challenge of producing low cost, sustainable, environmentally friendly and high-quality hydrogen enriched methane is a key factor that will allow it to attain its potential market position. A very promising approach embracing all the above requirements is the combined biological production of hydrogen and methane, which can be performed through a two-stage biological fermentation process through anaerobic dark fermentation of carbohydrate based wastes in the first-stage, and anaerobic digestion of the effluent in the second-stage.

Hydrogen-enriched methane, sometimes known as hythane®, offers a significant number of advantages. In the 1990's, it was demonstrated that the blending of hydrogen and methane led to a reduction of NOx and greenhouse gas emissions as well as to an overall improved combustion when compared with the combustion of methane. However, the challenge of producing low-cost, sustainable, environmentally friendly and high-quality hydrogen-enriched methane is a key factor that will allow it to attain its potential market position. A very promising approach, adressing all the above requirements, is the combined biological production of hydrogen and methane, which can be performed through a two-stage biological fermentation process. In the two-stage process, hydrogen is produced through anaerobic dark fermentation of carbohydrates in the first-stage, while the effluent of the first-stage reactor is converted to methane in the second-stage reactor [4-7]. However, in most of these studies published so far, the individual stages are not coupled, with control and mixing of the gaseous stream effluents, implying that the hydrogenogenic and methanogenic reactors have not been really integrated in a targeted producing process.

Previous results demonstrate that blending hydrogen with methane leads to a more environmentally friendly biofuel than methane alone. By using a two-stage process i.e. dark fermentation for hydrogen production (in the

1st stage) and subsequent anaerobic digestion of the 1st stage effluent for methane production (in the 2nd stage) in an integrated process could lead to an increase up to 37% of the overall conversion yield than in single-stage anaerobic digestion [8]. Taking advantage of the existing infrastructures of the biogas sector (production and distribution), along with the existing large-scale gas grid infrastructures, biohydrogen-enriched methane can be foreseen as a future highly sustainable alternative biofuel. To allow future development of this biofuel, efforts must be concentrated on finding solutions to guarantee a stable quality in its composition (10-15% H_2) and providing a wide range of renewable and sustainable substrates possibilities. In addition, in order to enhance the economic sustainability, efforts must also focus on flexibility of the process and maximization of the conversion of each potential feedstock.

The recent rapid growth of the biodiesel industry has generated a significant amount of glycerol as a byproduct. As a result, the price of glycerol is currently relatively low, making it an attractive starting material for the production of chemicals with higher values. Crude glycerol can be directly converted through microbial fermentation into various chemicals such as hydrogen. In the present study, a two-stage system for producing a gaseous mixture of hydrogen and methane, hythane, from glycerol was developed. In the first stage, biohydrogen production was achieved (the hydrogen fraction was approximately 41-45% in the gas phase) and the bioconversion efficiency and yields and the metabolic products were determined. In the second stage, originally the methane production was studied using commercial glycerol (as substrate), and compared to a previous study [9], and then crude glycerol was used as substrate (the methane fraction was approximately 70% in the gas phase).

MATERIALS AND EXPERIMENTAL STATUS

Hydrgen production in an Up-Flow Column Biorector (UFCB)

Continuous experiments were performed in a PVC up-flow, packed bed column bioreactor. The reactor was double-jacketted and temperature control (35±0.5 ^oC) was achieved via recirculation of warm water. For the immobilization of bacterial cells cylindrical ceramic beads with porosity corresponding to 600 m^2/L were used. The capacity of the reactor was 2.225 L, while the active volume after loading the ceramic beads, was 1.392 L. An acidogenic mixed culture derived from activated sludge, previously boiled for 20 min so as to inhibit methanogens [10-12], was used as inoculum. The bioreactor was operated in batch mode for 24h and was subsequently operated at a continuous mode with an HRT (Hydraulic Retention Time) of 36 h. Crude glycerol was kindly supplied by the biodiesel production company PETTAS SA, and had the following characteristics: purity 92.2±0.3%, pH 5.2 and COD 1.28±0.00 O_2/g waste. The basal medium used in all experiments was a synthetic solution 20 g/L glycerol which also supplemented with 0.75g/L yeast extract, phosphate buffer solution (K_2HPO_4 and KH_2PO_4) for PH control and trace elements (10ml/l). Trace element solution was prepared separately and was of the following composition (g/L): $CaCl_2.2H_2O$ 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, as well, 37.4, 72.3 and 71.9 K_2HPO_4 , 86.8, 44.8 και 38.4 KH_2PO_4 for pH control in the feed (PH=6, 6.5 and 7 respectively).

Fig. 1 UFCB (left) and CSTR (right)

Methane production in a Continuous Stirred Tank Reactor(CSTR)

For the needs of the experiment, a mesophilic (35^oC) CSTR-type anaerobic reactor was used. The capacity was 3 L and the reactor was started up with anaerobic biomass taken from a municipal sludge processing anaerobic digester. The reactor was cylindrical in shape, made of stainless steel and was continuously stirred. It was

externally surrounded by a jacket in which water circulated, maintaining water at 35^{o}C . There were three ports at the top of the digester: a) for feed addition, b) effluent removal (liquid and gaseous) and c) sampling.
The feed solution was kept refrigerated at 4^{o}C. The feeding medium consisted of 1.18 g/L $(NH_4)_2HPO_4$, 5.5 g/L $NaHCO_3$, 10 mL/L of the trace metal solution [13] and 0.3 g/L yeast extract and glycerol, initially commercial and subsequently crude, coming from the company PETTAS S.A.. In the sequel, the glycerol in the feed was replaced by a solution which was the mixture of the effluents from the hydrogenogenic reactor, diluted to a concentration of 5, 7.5 and 10 g COD / L.
During start-up, the reactor was filled with 3L of anaerobic sludge and remained for 24 h in batch mode. Then, the reactor was switched to continuous mode. The feeding of the bioreactor was intermittent and was done via a peristaltic pump, which was set to turn on briefly every 8 h, so that the mean hydraulic retention time (HRT) was 20 d.

ANALYTICAL METHODS

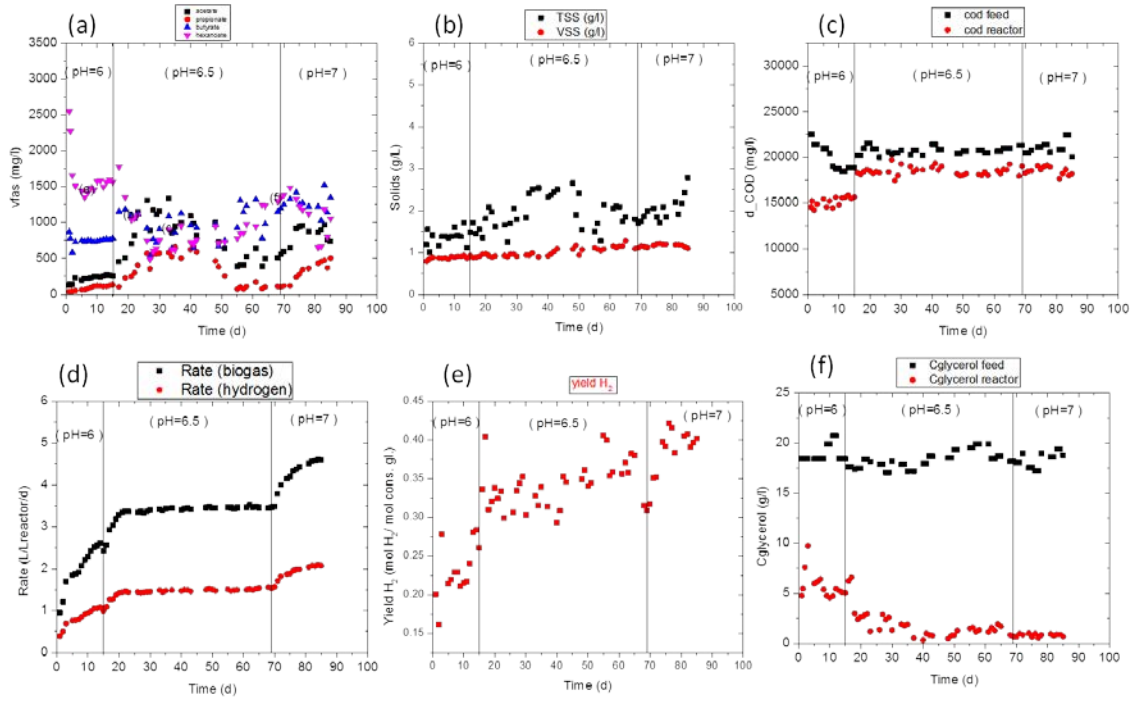
Total, volatile suspended solids (TSS, VSS) and dissolved COD (d-COD) were determined according to Standard Methods [14]. For the quantification of volatile fatty acids (VFAs: acetate, propionate, butyrate, valerate, isovalerate, isobutyrate and hexanoate) and alcohols (ethanol and butanol), acidified samples were analyzed by GC-FID (Varian CP-3800). Hydrogen and methane were quantified by GC-TCD (SRI 8610c) and glycerol and propanediol (PDO) were quantified by HPLC (DIONEX GP50) equipped with RI detector (Shodex RI-101).

RESULTS

- **Results for hydrogen production (1st stage)**

The yield of the continuous process for hydrogen production (1st stage) is shown in Figure 2 for three different periods, in terms of glycerol consumption, biohydrogen production, hydrogen molecular yield from the glycerol consumed, volatile fatty acids, total and volatiles suspended solids and dissolved COD.
The reactor was operated at an organic loading 20g crude glycerol /L, the same hydraulic retention time (HRT=24 h), but with different phosphate buffer solution in the feed (pH = 6, 6.5 and 7) during the three different periods. The performance of the reactor at steady state is presented in Tables 1 and 2. The glycerol consumption and the yield of biohydrogen from consumed glycerol had an increasing tendency as the pH increased in the feed.

Fig. 2 VFAs generation (a), TSS-VSS production (b), d-COD removal (c), gas production rates (d), molecular hydrogen yield (e) and glycerol consumption (f), throughout the operation of the reactor.



In general, polypropylene diol (PDO) was the major soluble metabolite produced in all cases, as shown before [15]. In terms of VFAs produced, butyrate and/or hexanoate were the dominant ones. Traces of propionate and valerate were also detected, as well as small amounts of ethanol. The theoretical production of H_2 from glycerol during acidogenesis is 2 mol /mol of butyrate and 3 mol/mol of acetate, whereas during ethanol production

1mol/mol of ethanol produced [16,17] and, also, a maximum of 3 mol H_2 can be produced per mol of glycerol when acetate is the single fermentation end product.[18]. The measured values for each period are 0.26 moles H_2/mol glycerol, 0.36 moles H_2/mol glycerol and 0.43 moles H_2/mol glycerol for pH=6, 6.5 and 7 respectively in the feed.

Table 1. Performance results of the up-flow continuous column bioreactor at steady state for various pH values in the feed.

C Glycerol in Feed	HRT : 24 h 20 g/L		
	pH=6	pH=6.5	pH=7
pH	5.3±0	5.8±0.0	6.1±0.0
VSS , g/L	0.9±0.0	1.1±0.1	1.2±0.0
TSS, g/L	1.4±0.2	1.8±0.3	2.2±0.3
Glycerol $_{reactor}$, g/L	5.0±0.3	1.3±0.4	0.8±0.1
% Glycerol removed	74.0	91.7	95.6
V H_2, L/d	1.37±0.11	2.10±0.04	2.86±0.05
Butyrate, g/L	0.76±0.02	1.12±0.18	1.25±0.17
Acetate, g/L	0.26±0.02	0.48±0.09	0.85±0.09
Hexanoate, g/L	1.54±0.05	1.06±0.22	0.91±0.24
PDO, g/L	3.6±0.2	5.7±0.9	7.0±0.2
Ethanol, mg/L	0.56±0.05	0.15±0.02	0.20±0.02
Yield H_2, mol/mol glyc consumed	0.24±0.03	0.36±0.03	0.40±0.08

Table 2. COD balances of the up-flow continuous column bioreactor at steady states.

C Glycerol in Feed	HRT : 24 h 20 g/L		
	pH=6	pH=6.5	pH=7
d-COD feed, g/L	18.78±0.21	20.85±0.3	21.20±1.01
d-COD reactor, g/L	15.38±0.50	18.45±0.43	18.28±0.47
% COD consumed	18.1%	11.5%	13.6%
COD H_2, g/d	0.9±0.1	1.3±0.0	1.8±0.0
COD VFAs, g/L	5.76±0.16	5.71±0.69	6.93±1.07
COD reactor, g/L	6.1±0.4	1.6±0.5	1.0±0.1
COD PDO, g/L	6.1±0.4	9.6±1.6	11.9±0.4
COD Ethanol, g/L	1.18±0.09	0.31±0.04	0.42±0.03

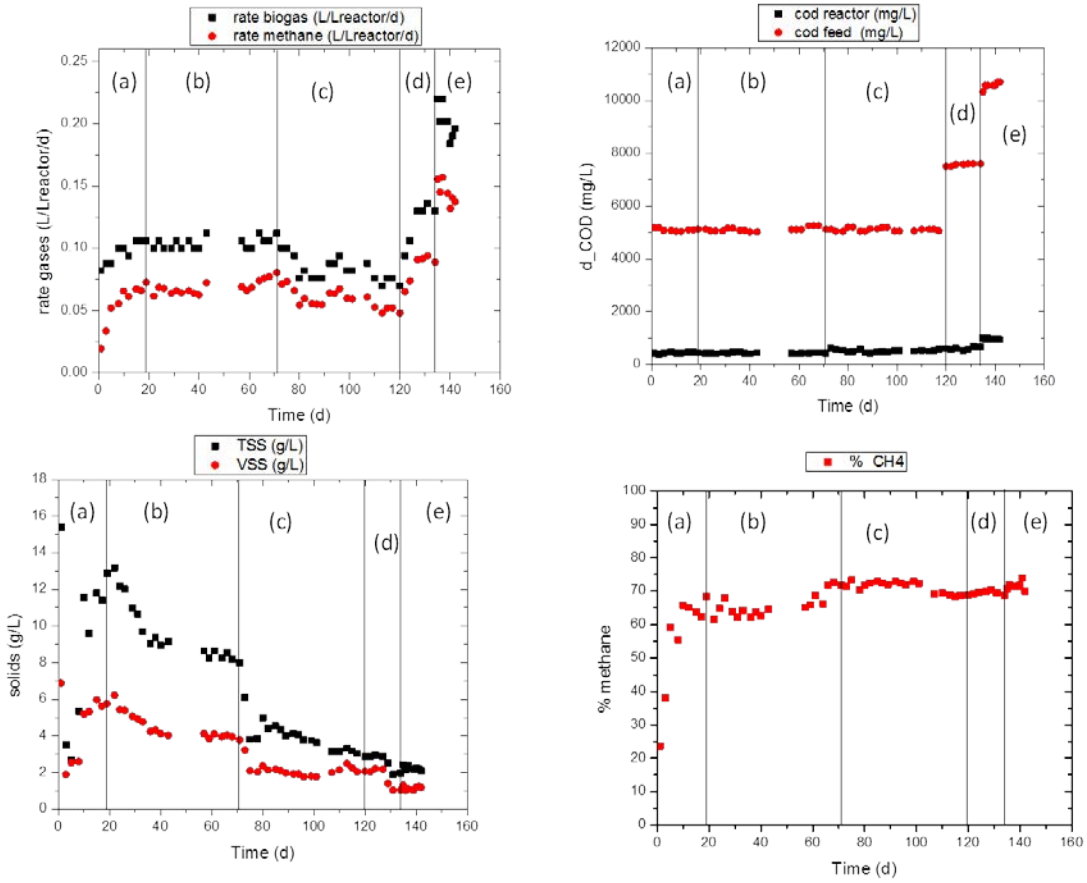
Results for methane production (2nd stage)

The performance of the methanogenic reactor (2nd stage) is shown in Figure 3 for three different periods. The biogas and methane production, the percentage concentration (%), the total and volatile suspended solids and soluble COD are presented.

The CSTR-type reactor was initially operated at an organic loading of 5 g commercial glycerol / L (first period) and then at 5 g crude glycerol /L (second period) . In the third period, the reactor was fed, with effluents from the hydrogenogenic (1st stage), diluted with water to 5 g COD / L, to 7.5 g COD / L and then to 10 g COD / L.

It should be noted that the experimental data for all of the periods showed that the concentrations of volatile fatty acids, glycerol, propanediol, and ethanol were not detectable, in contrast to the hydrogenogenic reactor. In the future the dilution of the hydrogenogenic reactor will be diluted less and less aiming at feeding to the methanogenic reactor the undiluted effluent from the hydrogenogenic reactor. This may require a higher retention time, in order to avoid digester instability.

Fig. 3 Rate of biogas and CH_4 production **(1)**, d-COD in the feed of the reactor **(2)**, TSS-VSS concentrations **(3)**, percentage of CH_4 (%) **(4)** for (a) organic loading 5g commercial glycerol /L, (b) 5g crude glycerol /L, (c) mixed effluent from the hydrogenic reactor diluted to 5 g/L, (d) mixed effluent from the hydrogenic reactor diluted to 7.5 g/L, (e) mixed effluent from the hydrogenic reactor diluted to 10 g/L



CONCLUSIONS

The present study dealt with the development of a two-stage anaerobic system for hydrogen and methane production (with the ultimate aim of producing hythane) from crude glycerol, a by-product of the biodiesel industry. A continuous process was investigated for the fermentative hydrogen production from crude glycerol using an up-flow column bioreactor proved to be very efficient. The effect of pH in the feed was studied. It was shown that when operated at high pH (7) the bioreactor hydrogen yield was significantly higher. Methane production in a CSTR from (a) commercial glycerol, (b) crude glycerol and (c) hydrogenogenic reactor effluent was also studied..

The results were also very quite satisfactory for methane production (~70% methane content). It was seen that the amount of methane generated was approximately the same in the first two periods and there was a decrease by 35% when the feed was switched to hydrogenogenic reactor effluent (diluted with water to 5 g COD / L) and an increase by 17.5 % when the effluent in the feed was diluted to 7.5 g COD / L and by 70.66% when the effluent in the feed was diluted to 10 g COD / L.

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REFERENCES

[1]. Alavandi, S., Agrawal, A.: Experimental study of combustion of hydrogen-syngas/methane fuel mixtures in a porous burner. Int. J. Hydrogen Energy 33, 1407–1415 (2008).

[2]. Gattrell, M., Gupta, N., Co, A.: Electrochemical reduction of CO2 to hydrocarbons to store renewable electrical energy and upgrade biogas. Energy Convers. Manage. 48, 1255–1265 (2007)
[3]. Pandey, R., Ratnani, K., Ahmed, S., Williams, J.: Direct conversion of methane to hythane. US Patent 5516967, (1996)
[4]. Antonopoulou, G., Stamatelatou, K., Venetsaneas, N., Kornaros, M., Lyberatos, G.: Biohydrogen and methane production from cheese whey in a two-stage anaerobic process. Ind. Eng. Chem. Res. 47, 5227–5233 (2008)
[5]. Koutrouli, E.C., Kalfas, H., Gavala, H.N., Skiadas, I.V., Stamatelatou, K., Lyberatos, G.: Hydrogen and methane production through two-stage mesophilic anaerobic digestion of olive pulp Bioresour. Technol. 100, 3718–3723 (2009)
[6]. Cavinato, C., Bolzonella, D., Fatone, F., Cecchi, F., Pavan, P.: Optimization of two-phase thermophilic anaerobic digestion of biowaste for hydrogen and methane production through reject water recirculation. Bioresour. Technol. 102, 8605–8611 (2011)
[7]. Cavinato, C., Giuliano, A., Bolzonella, D., Pavan, P., Cecchi, F.: Bio-hythane production from food waste by dark fermentation coupled with anaerobic digestion process: A long term pilot scale experience. Int. J. Hydrogen Energy 37, 11549-11555 (2011).
[8]. Massanet-Nicolau, J., Dinsdale, R., Guwy, A., Shipley, G.: Bioresour. Technol 129, 561-567 (2013)
[9]. Vlassis, T., Antonopoulou, G., Stamatelatou, K., Lyberatos, G.: Anaerobic treatment of glycerol for methane and hydrogen production. Global NEST Journal, 149-156 (2012)
[10]. Chen, C.C., Lin, C.Y., Lin, M.C.: Acid-base enrichment enhances anaerobic hydrogen production process. Appl. Microbiol. Biotechnol. 58, 224–228 (2002)
[11]. Chang, J.-S., Lee, K.-S., Lin, P.-J.: Biohydrogen production with fixed-bed bioreactors. Int. J. Hydrog. Energy 27, 1167–1174 (2002)
[12]. Ntaikou, I., Antonopoulou, G., Lyberatos, G.: Biohydrogen Production from Biomass and Wastes via Dark Fermentation: A Review. Waste Biomass Valor, 1:21–39 (2010)
[13]. Skiadas, I.V., Lyberatos, G.: The Periodic anaerobic baffled reactor. Water Science and Technology 38, 401-408 (1998)
[14]. APHA, AWWA & WEF: Standard Methods for the Examination of Water and Wastewater. 19th Ed.. Washington, D.C. (1995)
[15]. Han J, Lee D, Cho J, Lee J, Kim S.: Hydrogen production from biodiesel byproduct by immobilized Enterobacter aerogenes. Bioprocess Biosyst Eng. 35, 151-157 (2012)
[16]. Akutsu, Y., Lee, D.-Y., Li, Y.-Y., Noike, T.: Hydrogen production potentials and fermentative characteristics of various substrates with different heat-pretreated natural microflora Int. J. Hydr. Energy 34, 5365-5372 (2009)
[17]. Ito, T., Nakashimada, Y., Senba, K., Matsui, T., Nishio, N.: Hydrogen and ethanol production from glycerol-containing wastes discharged after biodiesel manufacturing process J. Bios. Bioeng. 100, 260-265 (2005)
[18]. Maru, B.T., Bielen, A. A. M., Kengen, S. W. M., Constantí, M., Medina, F.: Biohydrogen production from glycerol using Thermotoga spp.Energy Procedia 29, 300–307 (2012)