On Energy Sustainability of Dark Anaerobic Fermentation of Biohydrogen

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The only bio-H_2 production leads to a negative net energy balance, hence in this paper experimental tests in order to increase the energy recovery embedded in the substrate have been carried out. In order to try different strategies to recovery as much as possible the energy, we have realized several proofs of the concept tests on the following different approaches: i) Production of biogas on liquid metabolites; ii) Production of H_2 from sodium acetate by Microbial Electrolysis Cell (MEC) with cation exchange membrane (CEM). Both technologies demonstrate to be feasible and with the double positive effect: the energy recovery increment and the greater lowering of the organic wastewater load.

1. Introduction

During H_2-fermentation from glucose, only 1/3 of the energy available is converted into H_2, the others 2/3 remain occluded in the form of fatty acids, this leads to the fact that anaerobic H_2 technology is energetically un-sustainable (Ruggeri et al., 2010), despite we do not consider the energy cost of the pre-treatment which usually contains large quantities of recalcitrant compounds such as lignin and cellulose. In this paper we experimentally tested two different biotechnological routes to increase the energetic value of metabolic products of acetogenic fermentation at the end of H_2 production. The way to increase the overall energy of H_2 process is to extract the residual bio-energy embedded in the liquid metabolites mainly constituted from VFAs and alcohols. This energy can be produced in the form of methane, or directly as electricity, or as additional H_2 depending of technology used. Nowadays, several technology options are available, such of them with a sufficiently degree of technological maturation and other at infancy stage. The two stage anaerobic digestion (AD) consists in the separation of the natural ecology and metabolism of an anaerobic bacteria consortium in two distinct classes, optimizing the energy efficiency of the process: H_2 forming bacteria (HFB) and H_2 consuming bacteria (HCB). In the first stage acidic pH and short HRT conditions are chosen to produce H_2 and in the second stage neutral pH and higher HRT conditions are chosen to favorite methanogenesis with CH_4 production. Bio Electrochemically Assisted Microbial Reactor (BEAMR) or easily Microbial Electrolysis Cell (MEC) represents the newest approach for H_2 generation from biomass using bacteria: it permits a generation of hydrogen gas and other reduced products from organic electron donors. The terms

"electrochemically" and "assisted" are used in this process and therefore circumvent thermodynamic constraints that normally render the generation of compounds such as hydrogen unlikely (Clauwaert, 2009). MEC functions as a normal electrolysis cell but in this case the source of electrons is organic substrate and bacteria are the catalysts of the H_2 production. Examples of electron donors might be acetate and municipal or agro-industrial wastewater. The separation between anode and cathode is accomplished with an ion selective membrane. Ion selective membranes are used to obtain cathodic hydrogen gas that is almost pure. However, ion selective membranes give rise to higher ohmic voltage loss in the cell and a pH gradient over the membrane, resulting in a lower current production for a given applied voltage (Call et al., 2008).

2. Materials and Methods

2.1 Two stage process (H_2+CH_4)

A test at 35 °C was conducted to produce firstly H_2 from glucose (acidogenesis step) and after that biogas (methanogenesis step) from VFAs produced in the first step. The test was conducted in a stirred-batch reactor STR (Minifors HT, Switzerland), with 2 litres as working volume running under anaerobic conditions, obtained by sparging nitrogen gas at the beginning of the fermentation and with 100 rpm stirring operation. The initial glucose concentration was 60 g/l and the medium contained the macro and micro nutrient composition as reported in Fang et al. (2006). The seed microflora used as inoculum (10% w/w) was obtained from an anaerobic digested sludge, derived from the municipal wastewater treatment plant of Turin (SMAT S.p.A.). In the first step (acidogenesis) the inoculum was pre-treated with 1N HCl for 24 h till pH 3 in order to inhibit the methanogenic activity as reported by Chen et al. (2002) and Mu et al. (2006). For the second methanogenic step, an untreated inoculum was used. The gas evolution was constantly evaluated by a volumetric gas-counter (Milligas counter, Ritter) as well as pH, Redox Potential (ROP) and temperature which were logged through a data acquisition system (Iris, Infors HT). Gas composition was determined by off-line gas chromatographic analysis (Varian, CP 4900) taking gas sampling at different time interval. pH was measured by pH meter (Infors, AG Switzerland); Red-Ox Potential (ROP) was measured by Pt4805-DXK-S8/120 electrode (Mettler Toledo, Switzerland).

2.2 Production of H_2 from sodium acetate by a MEC

The MEC was made of two Plexiglas frames (8x8x2 cm^3 per frame); the total reactor volume=0.256 L MEC is the sum of the total anodic (A) and cathodic (C) compartment. Two frames, A and C, were completely filled with granular graphite and connected to the external electric circuit with a graphite rod (5 mm diameter, Morgan, Belgium). A cation exchange membrane (CEM) (Ultrex CMI7000, Membranes International Inc.) was used to separate anode from cathode. The medium contained 6 g L^{-1} $Na_2HPO_4.2H_2O$, 3 g $L^{-1}$$KH_2PO_4$, 0.2 g L^{-1} $MgSO_4.7H_2O$, 0.1 g NH_4Cl, 0.0146 g L^{-1} $CaCl_2$ and trace elements as described in Clauwaert et al. (2009). 1 g of sodium acetate ($C_2H_3O_2$ Na) was fed in discontinuous mode to the anodic frame upon depletion. Peristaltic pumps (Watson Marlow) were used to circulate the anodic and cathodic liquid at 6 l d^{-1}. The external vessel of anodic compartment was inoculated with 10 mL anaerobic sludge taken from the anode of running microbial fuel cells in order to have a

conversion of acetate into H_2. Test was performed at room temperature (22 ± 2 °C). A power supply was used to obtain an applied voltage of 0.08 ± 0.02 mV. The current was measured by placing a 1.07 Ω resistor in the electrical circuit. A data acquisition unit (HP 34970A, Agilent, USA) was used to record the voltage difference and current every minute. The coulombic H_2 recovery was calculated as the ratio of current produced and theoretical current production from the substrate. The volume of gas was measured with water-replacement method, while the composition of gas produced, methane and carbon dioxide were analyzed with an Intersmat IGC 120 MB gas chromatograph; the qualitative hydrogen presence was determined by a H_2 sensor (OPUS, Zellweger Analytics, U.K.). Polarization curves were obtained with a potentiostat at a scan rate of 0.2 mV s^{-1} after an open circuit stabilization of 15 min. The ohmic cell resistance was determined with the current interrupt method (Clauwaert, 2009).

3. Results

3.1 Two stage process (H_2+CH_4)

In Figure 1 it is reported the test carried out on glucose to produce energy in the form of H_2 (from the first acidogenic stage) and methane from the following second methanogenic stage. Results show that after H_2 production the residual VFAs have been successfully used producing a suitable quantity of biogas, with approximately 80 % v/v of methane as mean value. From Figure 1(b) one can see that the pH after a controlled step increase from 5.2 to 5.8 and after that, pH was "naturally" increased towards neutral value. This strategy was adopted in order to permit to mixed microorganisms to gently adapt to the new environment. When the pH reaches the value 7.2 an inoculum constituted by mixed methanogens was added; the pH remains at this value for a long time (two weeks), which corresponds approximately to the length of methanogenesis lag phase. After about 11, 12 days of lag phase, gas evolution restarts and a few quantity of CH_4 was detected. In the following approximately 23, 24 d the concentration of CH_4 increases reaching 83 % before shutting down.

Table 1: Experimental energy obtained and efficiency of reaction involved in H_2 and CH_4 production from AD respect the energy embedded in 1 mol of glucose.

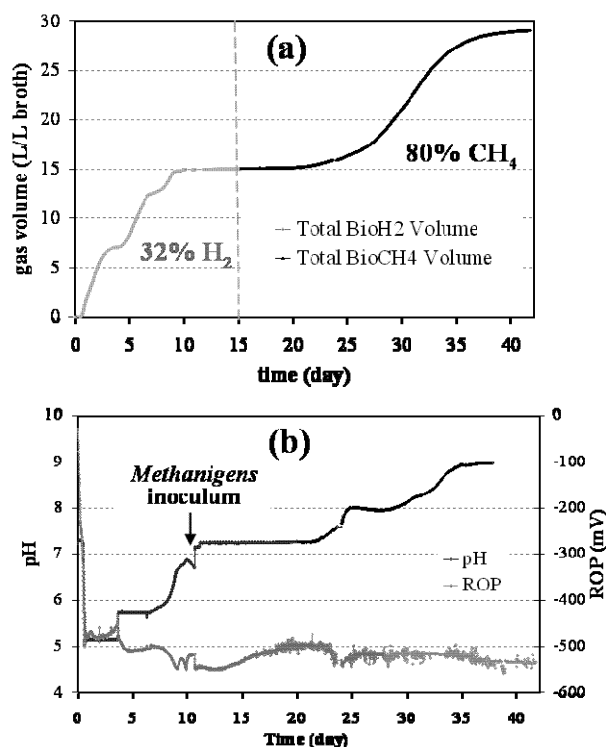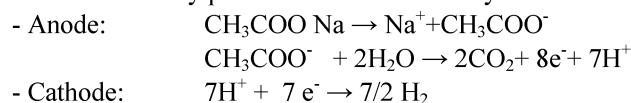| Experimental Energy obtained from two-stage	Energy yield (kJ/mol glucose)			
	H_2	CH_4	Total	Comparison
Energy content in glucose (Low heat value)	-	-	2872	100%
First step-Experimental H_2 production	328.1	-	328.1	11.4%
Second step-Experimental CH_4 production	-	1253.8	1253.8	43.7%
Two-step (H_2+CH_4) process: $C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2+4CO_2+2\ CH_4$	328.1	1253.8	1581.1	55.1%

Figure 1: Time curve evolution of glucose fermentation (initial concentration 60 g/l): (a) cumulative gas production H_2 and CH_4, (b) pH and ROP evolution.

Red-Ox Potential (ROP) in the methane lag phase slowly increases towards less reductive values while during methane evolution it remained always constant at very reductive values (about -520 mV). The energy efficiency of H_2 production by AD is very low (11.4 %), because parts of the initial energy contained in the carbon source is converted into VFAs. The second methanogenic stage greatly increases the overall energy efficiency till 55.1%. From the experimental calculations results that two-stage AD instead of standard AD for biohydrogen production, gives a higher yield of 55.1 % against 11.47 %, as reported in Table I.

3.2 Production of H_2 from acetate by a MEC

Sodium acetate was oxidized in the bioanode by microorganisms while H_2 was electrochemically produced in the cathode by the following half reactions:

- Anode: $CH_3COO\ Na \rightarrow Na^+ + CH_3COO^-$

    $CH_3COO^- + 2H_2O \rightarrow 2CO_2 + 8e^- + 7H^+$

- Cathode: $7H^+ + 7\ e^- \rightarrow 7/2\ H_2$

From Figure 2 one can see that after 2 days of lag-phase the moles of electrons (Coulombs) sharply increase, cumulative H_2 production follows the same shape. This is due to the directly proportionality between Coulombs (mol e^-) and hydrogen.
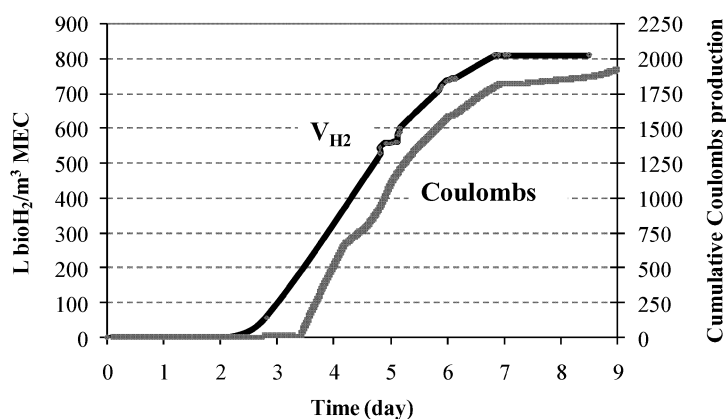
Figure 2: Cumulative chemical H₂ and Coulombs production in MEC test fed-batch with sodium acetate.

The coulombic H₂ recovery expresses the recovery of electrons as current generated over the amount of electrons dosed as sodium acetate (Cook and Schlegel, 2002), which is 84.4 %. To calculate the amount of electrons recovery we used the recorded current $I(t)$ generated vs. time. Sodium acetate was oxidized in the bioanode by microorganisms while H₂ was electrochemically produced in the cathode. The ratio $\Sigma I(t)/96485 C mol^{-1}$ gives the mol of electron recovery. Other efficiencies of the process are indicated in Table 2. The high coulombic H₂ recovery based on the amount of substrate consumed demonstrates that sodium acetate is a good substrate like electrons donor also at ambient temperature. The cation exchange membrane results useful to achieve a high flux of protons H⁺ from anode to cathode. MECs with a CEM resulted in production of relative pure hydrogen gas in the cathode (Rozendal et al., 2007) and they avoid the direct cross over conversion of the organic substrate by aerobic microorganisms, which lowers the coulombic efficiency (Liu and Logan, 2004). CEM however cause a higher ohmic cell resistance and the build-up of a pH gradient across the membrane (Clauwaert and Verstraete, 2008; Clauwaert, 2009 and Rozendal et al., 2006) that brings to alkalinization effect that becomes more dominant in poorly buffered solution. Here, we have found that when this membrane is omitted from the MEC, methanogenesis can easily become dominant (results not reported) and we have a mixture of gas composed from the carbon dioxide, hydrogen gas and methane.

Table 2: Experimental yield and efficiencies of the microbial electrolysis cell process

Efficiencies of the Microbial electrolysis cell	
Y_{H2}	2.18 mol $_{H2}$/mol $_{Sodium\ acetate}$ or 5.31 g $_{H2}$/g $_{SodiumAcetate}$
$\eta_{H2} = Y_{H2}/Y_{theoretical}$	54.45 %
Theoretic V_{H2}	0.29 L
V_{H2} obtained	0.21L
$\eta_{vol\ H2} = V_{H2,obtained}/V_{H2,theoretic}$	70.69%

4. Conclusion

In this paper we have presented two different biological routes to further increase successfully the overall balance of bioenergy in the bio-hydrogen technology production: the conventional anaerobic digestion and a novel microbial electrolysis cells fed with the volatile fatty acids and sodium acetate respectively, after the first step of H_2 production by AD. Furthermore, other H_2 can be extract from a VFA as acetic acid from a MEC working at ambient temperature. Therefore, a second biogas step and a MEC process could be used as a way to use residual fatty acids considering that the net energy balance of the only biological H_2 production via dark-fermentation is not always positive.

References

Call D. and Logan B.E., 2008, Hydrogen production in a single chamber microbial electrolysis cell lacking a membrane, Environment Science Technology 42, 3401-3406.

Chen C.C, Lin C.Y. and Lin M.C, 2002, Acid-base enrichment enhances anaerobic hydrogen production process, Applied Microbiology Biotechnology 58, 224-228.

Cook A. M. and Schlegel H. G., 1978, Metabolite Concentrations in Alcaligenes eutrophus H 16 and a mutant defective in Poly-βHydroxybutyrate synthesis, Archives of Microbiology 119, 231-235.

Clauwaert P. and Verstraete W., 2008, Methanogenesis in membraneless microbial electrolysis cells, Applied Microbiology Biotechnology 82, 829-836.

Clauwaert P., 2009, Electrodes as electron donors for microbial reduction processes, PhD Thesis, Ghent University, Belgium.

H.P.F. Fang, C. Li and T. Zhang, Acidophilic biohydrogen production from rice slurry, 2006, International Journal of Hydrogen Energy 31, 683-692.

Liu H. and Logan B.E., 2004, Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane, Environmental Science and Technology 38, 4040-4046.

Mu Y., Yu H.Q. and. Wang G, 2006, Evaluation of three methods for enriching H_2-producing cultures from anaerobic sludge, Enzyme and Microbial Technology 40(4), 947-953.

Rozendal R.A., Hamelers H.V.M. and Buisman C.J.N., Effects of membrane cation transport on pH and microbial fuel cell performance, 2006, Environmental Science and Technology 40, 5206-5211.

Rozendal R.A., Hamelers H.V.M., Molenkamp R.J. and Buisman J.N., 2007, Performance of single chamber biocatalyzed electrolysis with different types of ion exchange membranes, Water Research 41, 1984-1994.

Ruggeri B., Tommasi T. and Sassi G., 2010, Energy balance of dark anaerobic fermentation as a tool for sustainability analysis, International Journal of Hydrogen Energy, 35, 10202-10211.