CHEMICAL ENGINEERING TRANSACTIONS, Volume 14, 2008
Edited by G. Marino, E. Bardone, A. Viglia
 ISBN 978-88-95608-02-0

Production Of Hydrogen By Wastewater Sludge Using Anaerobic Fermentation In A Pilot Scale Reactor

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Keywords: Bio-hydrogen production, sewage sludge, pilot scale reactor, kinetics

The aim of this study is to investigate the production of bio-hydrogen, via dark anaerobic fermentation in a pilot scale reactor under batch conditions, using a mixed microflora. As inoculum, anaerobic digested sludge, collected from municipal wastewater treatment plant, was used after being pre-treated with HCl 1N for 24h (pH 3) in order to inhibit the methanogenic bioactivity. As a source of carbon, sucrose at high concentrations (100 g/l) dissolved in a medium containing salts and micronutrients was employed. During fermentation temperature was maintained at room conditions (20°C), pH ranged from 7.2 to 5.2, due to the formation of Volatile Fatty Acids (VFA_s), that showed a butyrate-type fermentation. The reactor produced approximately 70 l of gas containing hydrogen (46%) and carbon dioxide (54%) with a global yield of 0.32 mol H_2/mol sucrose, whereas no H_2S or CH_4 was detected, suggesting that acid pretreatment plays an important role in the elimination of methanogenic bacteria.
Formation of VFA_s, sucrose concentration and biomass concentration in terms of CFU/ml of *Clostridium spp*. were reported at different times. The investigation of these parameters led to a kinetic study for the description of the dark anaerobic fermentation process, in order to obtain data for future experiments for the production of bio-hydrogen with a continuous stirred tank reactor (CSTR).

1. Introduction

Hydrogen is a clean and sustainable vector of energy, that can be produced with different technologies. One way to produce hydrogen is via dark anaerobic fermentation from carbohydrates following the acetate-butyrate pathways. The microrganisms mainly involved in the production of bio-H_2 belong to the Clostridial species as reported by Kim et al., 2006. *Clostridium spp*. are anaerobic, Gram positive and spore-forming bacteria, able to resist to shocks like heat treatment or acid-basic treatment. Moreover *Clostridium spp*. can achieve the highest H_2 yield per mole of glucose in dark anaerobic fermentation (1.61÷2.36 mol H_2/mol glucose) (Taguchi et al., 1995). Many researchers used pure culture of *Clostridium spp.* to enhance bio-hydrogen production (Chen et al., 2005; Liu et al., 2006) but with a cost of the process much higher if compared to other productive technologies.

In order to make bio-H_2 production more cost effective, mixed microflora, such as activated or digested sludge should be used at mesophilic temperatures (Lin et al., 2004; Zhang et al., 2006). During anaerobic digestion methanogenic or sulphate-reducing bacteria, normally present in anaerobic sludge, consume hydrogen produced by acidogenic bacteria. For the inhibition of methanogenic bioactivity many pre-treatments were proposed, for example acidification, basification, freezing and thawing or thermal shock (Mu et al., 2006; Mohan et al., 2007; Ting et al., 2007). In this work acid pre-treatment was performed for anaerobic digested sludge, demonstrating that is an effective method to avoid methanogenesis during fermentation.
This study also investigates how key parameters, like VFA_s and biomass, evolve during anaerobic fermentation in mesophilic conditions. A kinetic model of fermentation was developed for future work on CSTR reactors.

2. Materials And Methods

2.1 Reactor design

Dark anaerobic fermentation was carried out in a batch reactor of 35 l. The pressure inside the reactor can be set by regulating a relief valve; as soon as the pressure inside exceeds the backpressure, gas passes through the valve and is collected in another vessel of 2,5 l connected to a compressor which pressurizes the gas, ready then to be stored in gas sampling bags SKC, 232 series.
The reactor is also equipped with a mixer with a maximum speed of 30 rpm, and with a pH and temperature control system to study the influence of these parameters on the fermentative process. To create anaerobic conditions, the system is provided with a gas inlet at the bottom of the reactor, where inert gas, i.e. nitrogen, can be injected continuously.

2.2 Experimental procedure

An anaerobic digested sludge derived from the municipal wastewater treatment plant of Turin (S.M.A.T. Torino) was used as inoculum. In order to inhibit the methanogenic bioactivity the sludge was first treated for 24h with HCl 1N to obtain pH = 3. In fact, as reported by Chen et al. (2002) and Mu et al. (2006) no methane production could be observed after pre-treating the sludge at values of pH in a range of 3÷4 for 24h.
After this period of time the reactor was then fed with a medium containing inorganic salts, micronutrients and sucrose. Experimentation was carried out with a ratio between sludge and sucrose medium of 1:10 (v/v); in order to obtain a volume of 25 l inside the reactor, 2,5 l and 22,5 l of sludge and medium respectively were introduced in the reactor.
As a source of carbon, sucrose, at a concentration of 100 g/l, was dissolved in a medium consisting of (all in mg/l): $NaHCO_3$ 2679, NH_4Cl 5358, KH_2PO_4 536, K_2HPO_4 536, $CaCl_2$ 1072, $NiSO_4 \cdot 6H_2O$ 116, $MgSO_4 \cdot 7H_2O$ 686, $FeCl_3$ 43, $Na_2B_2O_7 \cdot 10H_2O$ 15, $Na_2MoO_4 \cdot 2H_2O$ 30, $ZnCl_2$ 49, $CoCl_2 \cdot 6H_2O$ 45, $CuCl_2 \cdot H_2O$ 21, $MnCl_2 \cdot 4H_2O$ 64, yeast extract 107 (Fang et al., 2006). The theoretical specific ratio C/N was fixed at 30 in order to guarantee a good nutritional supply to microrganisms.
Anaerobic conditions were created insufflating nitrogen inside the reactor, till no oxygen could be detected. Pressure inside the reactor was set at values of 20÷30 mbar,r,

while temperature remained at room conditions at about 20°C. To avoid biomass stratification the stirrer was set at the maximum speed of 30 rpm. During the process no pH and temperature regulation occurred.
The gas produced was constantly measured with a volumetric gascounter (Sacofgas S1ATG4), as well as pH, ORP and temperature which were logged through a data acquisition system. Gas composition was continuously evaluated online with a gas cromatograph (Varian, CP 4900), for the determination of hydrogen, carbon dioxide, methane, nitrogen, oxygen and hydrogen sulphide content.

2.3 Chemical and biological analysis

During fermentation different samples were taken at the following times: 0, 24, 46, 64, 72 and 91 h. Sucrose content of these samples was determined with an enzymatic kit (R-Biopharm, AG). Clostridial Plate Count was also performed according to Galli et al., 2003 and Laird et al., 2004, while the total ATP content was measured by light emission using the enzyme luciferase (Promega) and a luminometer (Junior LB9509, Berthold Technologies). After filtration of the samples on 45µ Whatman filter papers (40 grades) in a vacuum system (IRSA-CNR, 2006), VFA_s (Acetate, Butyrate, Propionate, Iso-butyrate) and Ethanol were determined using a gas cromatograph (EPA, 3810 and EPA 8260C).

3. Results

3.1 Gas production

Dark anaerobic fermentation took place in approximately 5 days producing a total gas amount of 66.9 l. Thanks to the acid pre-treatment no methane and hydrogen sulphide production was observed. Only hydrogen, carbon dioxide and nitrogen were detected, with small amounts of humidity. Table 1 shows gas production and composition during the process. The presence of nitrogen in the gas is related to the preliminary sparging of inert gas to create anaerobic conditions. In effect nitrogen content decreased during production, while hydrogen and carbon dioxide rose, due to the biological activity of *Clostridium spp*. In Fig. 1a total gas, hydrogen and carbon dioxide cumulative volumes, and the ratio between hydrogen and carbon dioxide from the beginning till the end of the experiment are reported. pH, without being regulated, constantly decreased from 7.2 to 5.2, as well as ORP reduced from -250 mV to -440 mV at t = 72h, slightly raising at the end of fermentation; Fig. 1b reports the trend of these two parameters.

Table 1. Total gas production and percentage composition

Runtime (h)	V (l)	H_2	CO_2	N_2
0	0.0	0.0	0.0	100.0
40.4	3.3	0.0	0.0	100.0
49.7	5.4	1.5	7.4	91.1
63.88	17.9	16.6	44.3	39.1
72.57	38.2	35.2	54.4	10.4
91.63	65.4	44.1	51.7	4.2
100.62	65.7	42.1	51.0	6.9

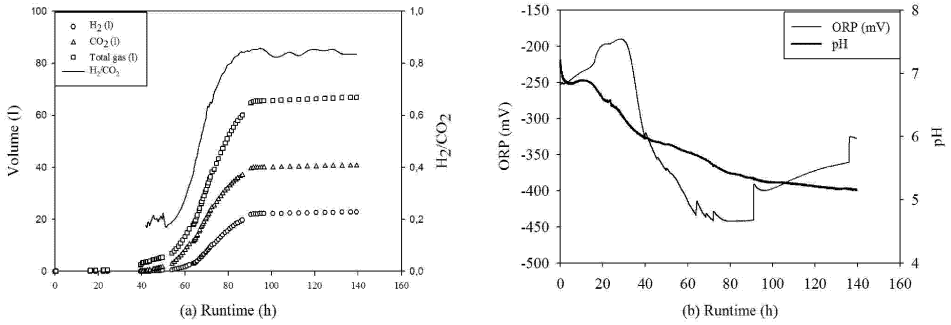

Fig. 1 – (a) Hydrogen, carbon dioxide , total gas production and hydrogen on carbon dioxide ratio; (b) pH and ORP

3.2 Chemical and biological analysis

Total sucrose decreased during fermentation from the starting concentration of 90 g/l till 51 g/l (Fig. 2a). In Fig. 2b the trend of bioluminescence proportional to the ATP level and the trend of biomass growth in terms of CFU/ml of *Clostridium spp.* are reported. The graph suggests that the maximum activity, in terms of viable colony count and chemiluminescence, was reached at t = 64h at the initial phase of hydrogen production.

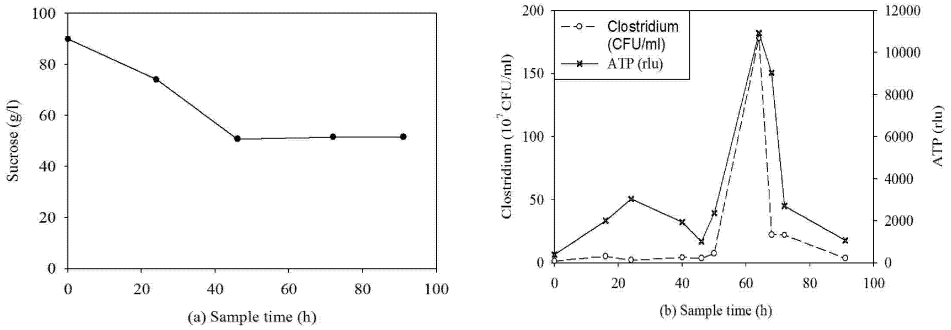

Fig. 2 – (a) Sucrose concentration; (b) Clostridial and ATP analysis

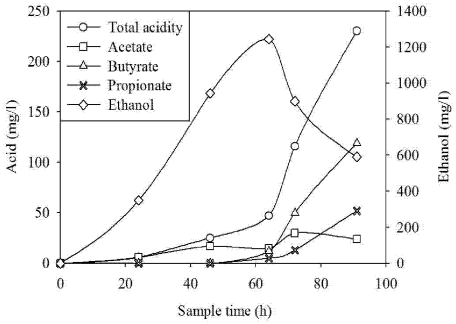

Fig. 3 –Volatile Fatty Acids (VFA_s)

Finally, Fig. 3 reports the volatile fatty acids (VFA_s) and ethanol produced along fermentation. The maximum concentrations of acetate, butyrate and propionate are 30, 120 and 52 mg/l respectively, while the total acidity reached its maximum at the end of fermentation at 230 mg/l. Besides the maximum concentration of ethanol was 1244 mg/l, observed at t = 64h at the initial phase of hydrogen production (Fig. 1a).

3.3 Kinetics study

On the basis of results reported in figures 1a and 2a two regression curves for hydrogen production and sucrose consumption were built. For both diagrams a modified Gompertz equation suitable for the description of the microbial growth in a batch culture was used. The hydrogen production was described with the modified model presented by Lay et al., 1999

$$H = H_{\max} \times \exp\left\{-\exp\left[\frac{R_{\max,H_2} \times e}{H_{\max}}(\lambda - t) + 1\right]\right\} \quad (1)$$

where H represents the cumulative volume of hydrogen produced (l) at the time t, $R_{max,H2}$ is the maximum value of hydrogen production rate (l/h) whereas λ is time of lag phase (h). By differentiating eq. (1) hydrogen production rate r_{H2} was calculated as

$$r_{H_2} = R_{\max,H_2} \times \exp\left\{-\exp\left[\frac{R_{\max,H_2} \times e}{H_{\max}}(\lambda - t) + 1\right] + \left[\frac{R_{\max,H_2} \times e}{H_{\max}}(\lambda - t) + 1\right] + 1\right\} \quad (2)$$

On the other side sucrose consumption could be represented by the modified Gompertz model proposed by Fan et al., 2004

$$S_0 - S = S_{\max} \times \exp\left\{-\exp\left[\frac{R_{\max,S} \times e}{S_{\max}}(\lambda - t) + 1\right]\right\} \quad (3)$$

where S_0 is the initial concentration of sucrose at t = 0h (90 g/l), S is the actual concentration inside the reactor and S_{max} is the maximum sucrose consumption. By differentiating the eq. (3), the rate of substrate consumption r_S is given by

$$r_S = R_{\max,S} \times \exp\left\{-\exp\left[\frac{R_{\max,S} \times e}{S_{\max}}(\lambda - t) + 1\right] + \left[\frac{R_{\max,S} \times e}{S_{\max}}(\lambda - t) + 1\right] + 1\right\} \quad (4)$$

All parameters in the equations were estimated by minimizing the sum square of errors between experimental data and model prediction. Equations 1 and 3 are reported in figure 4a and 4b.

The trend of hydrogen production rate is traced as a function of time as shown in Fig. 5. The exponential phase of hydrogen production occurred in the range of t = 60÷100h and the maximum production rate was found at about t = 70h when sucrose concentration was 51 g/l (fig. 4a).

The following section describes the use of kinetic results in order to evaluate retention time (HRT) in a continuous plant to reach optimal conditions as discussed above with a high initial sucrose concentration. By choosing the optimal concentration of sucrose at which the reactor should work for maximizing hydrogen productivity, for an established value of incoming sucrose concentration S_0, retention time τ could be easily evaluated by steady-state balance, assuming an ideal behaviour inside the reactor CSTR:

$$\tau = \frac{V}{Q} = \frac{S_0 - S_{SS}}{r_S} \tag{5}$$

where Q represents the flow (l/h) inside the reactor, S_0 and S_{SS} are respectively the inlet and optimal sucrose concentrations, V is the volume of the reactor and r_S is the sucrose consumption rate at S_{SS} value.

By fixing an initial sucrose concentration, retention time that maximizes hydrogen production could be easily found with equation (5). Nevertheless, in order to effectively obtain hydrogen, retention time must be compared with biomass growth time in order to avoid problems connected with the so called "wash out" phenomena. In this sense many reviews in literature report values of maximum specific growth rate μ_{max} in the range of 0.08÷0.125 h^{-1} (Chen et al., 2001; Mu et al., 2007). Fixing the initial sucrose concentration in the range of 60÷70 g/l, the dilution rate $D = \tau^{-1}$, calculated according to the eq. (5), varies in the range of 0.011÷0.025 h^{-1} which is much smaller than μ_{max}, well distant from wash out conditions.

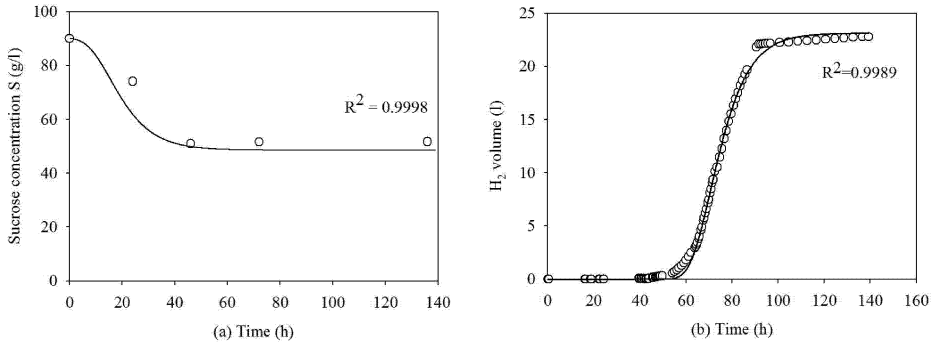

Fig. 4 – (a) Regression curves for sucrose consumption and (b) hydrogen production

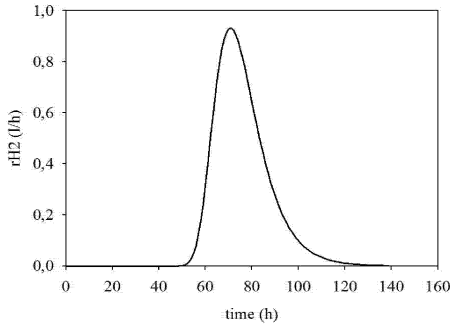

Fig. 5 – Hydrogen production rate r_{H2}

4. Discussion and conclusions

This experiment proved that it is possible to produce biohydrogen in a pilot scale reactor using digested sludge as inoculum and sucrose as a substrate, without enriching the culture with clostridial species. The experiment confirmed that acid pre-treatment is a valid method to inhibit methanogenic bioactivity, since no methane nor hydrogen sulphide was observed in gas produced.

After a lag phase of 48h, necessary for the bacteria sporulation, as suggested by Hawkes et al. (2002), hydrogen production began with a maximum rate of 0.94 l/h at t = 74h, resulting in a final volume of hydrogen produced of 23 l on a total volume (H_2 + CO_2) of 66.9 l. At the end of production, considering only the contributions of hydrogen and carbon dioxide, a concentration of 46% in H_2 was reached, equivalent to a hydrogen/carbon dioxide ratio of 0.86. Sucrose concentration reduced from 90 g/l to 51 g/l, with a final yield in hydrogen production of 0.32 mol H_2/mol sucrose. This value, obtained working at 20°C and with mixed microflora, is lower than the values that could be found by many researchers (Taguchi et al., 1995) working at higher temperatures (30÷35°C) and in some cases with pure cultures. Total Clostridium plate count showed a maximum of $1.8x10^9$ CFU/ml at t = 64h, when ATP reached its maximum of 11000 rlu; pH decreased constantly from 7.2 to 5.2, while ORP diminished showing a minimum of -440 mV at t = 72h, in correspondence of the maximum hydrogen production rate. This value indicates that a reducing atmosphere required for hydrogen producing bacteria was established, while at the same time the reduction of pH is connected with the formation of volatile fatty acids.

Fang et al., 2002, found that predominant bacteria present in a mixed culture enriched by acid pre-treatment belong to species in the genera *Clostridium*, *Enterobacter* and *Citrobacter*. Some species, i.e. *C. butyricum* and *C. tyrobutyricum*, produce butyrate and acetate as their main fermentation byproducts, whereas other species, like *C. acetobutyricum* follow the homoacetogenic pathway.

In this study the major volatile fatty acids produced are essentially acetate and butyrate, according to the following reactions

$$C_{12}H_{22}O_{11} + 5\ H_2O \rightarrow 4\ CH_3COOH + 4\ CO_2 + 8\ H_2 \quad (6)$$

$$C_{12}H_{22}O_{11} + H_2O \rightarrow 4\ CH_3CH_2CH_2COOH + 4\ CO_2 + 4\ H_2 \quad (7)$$

This suggests that mixed microflora observes a typical butyrate-type fermentation as main metabolic pathway, even though small amounts of propionic acid and acetate could be detected along the process, as a result of other types of fermentations, such as propionate-type or homoacetogenic. Observing Fig. 3 it is clear that reaction (6) and (7) are only a rough approximation of reality, so that biochemical pathways need to be analyzed in more detailed experimental tests. The study of the kinetics of hydrogen production and sucrose consumption through the modified Gompertz equations led to the definition of the optimal conditions (initial sucrose concentration S_0 and retention time τ) for working in a continuous mode. As a first consideration an optimal sucrose concentration for maximizing hydrogen productivity was found at a value of 51 g/l. Further on, a relationship between S_0 and τ was outlined, so that for initial values of

sucrose of 60÷70 g/l, a dilution rate $D = \tau^{-1}$ of 0.011÷0.025 h^{-1} is needed to obtain the maximum H_2 production rate preventing at the same time wash out. In any case all of the above considerations are valid for high sucrose concentrations experimentally tested (i.e. S > 50 g/l).

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