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Fermentative Hydrogen Production in Batch Experiments Using Molasses, Potato Processing Industry Wastewater and Chocolate Waste: Influence of Acidic Hydrolyzation

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In this study, the hydrogen production potential of molasses, wastewater from potato industry and chocolate waste as substrate was investigated. Results show that R_{max} (2.2 mLH_2/min) was obtained for molasses and chocolate. Hydrogen production rate was lower due to the complex structure of substrates. Therefore, we are interested in using hydrolyzate obtained from acid hydrolysis of substrates. In treated molasses and chocolate, H_{max} of 3190 and 6712 mL was obtained, increasing by 171 and 282 % as compared with the result of the untreated substrate, respectively. The net energy gain was calculated as negative for all substrates.

Keywords: Acidic-hydrolyzation, Biohydrogen production, Chocolate, Dark fermentation, Molasses, Potato processing wastewater.

INTRODUCTION

Hydrogen is a clean and efficient energy source. It possesses a high energy yield (286 kJ/mol). Hydrogen can be produced through various ways. Among various hydrogen production process, fermentative hydrogen production, although requiring a rather simple technology, can be quite complex in terms of optimization and repeatability, mainly due to the large number of the parameters that influence the process efficiency. Factors included inoculum, substrate, reactor type, nitrogen, phosphate, metal ion, temperature and pH influence the fermentative hydrogen production[1]

Fermentative hydrogen production is technically feasible to harvest hydrogen from carbohydrates in substrates by fermentative microbes. The following eqn. 1 simply describes the reaction. The conversion is not complete resulting in volatile fatty acids and alcohols as end products. Thus the feed stock-to-energy capture efficiency of dark fermentation is about 33 %. To improve hydrogen production by dark fermentation have been proposed some techniques such as feedstock pretreatment, nutrient augmentation and headspace pressure management[2].

$$C_6H_{12}O_6 + 2H_2O \longrightarrow 2CH_3COOH + 4H_2 + 2CO_2$$
$$\Delta G^\circ = -184.2 \text{ kJ} \quad (1)$$

One of the most critical issues for commercial application of biohydrogen is the high cost. High-carbohydrate wastewaters will be the most useful for industrial production of hydrogen. The maximum hydrogen production rate was governed by the complexity of carbohydrates in the substrate[3]. In dark fermentation, the abundance and composition of soluble metabolites can closely reflect the performance of hydrogen production[4]. The variety of carbohydrate during fermentation was similar to protein, but the amount of carbohydrate released and consumed after pretreatment and fermentation was a little lower than protein. The study conducted by Guo *et al.*[5] indicated that the organic matter in waste sludge used for hydrogen production was protein and then was carbohydrate.

Fermentative hydrogen production has been reported from a variety of waste materials; co-fermentation of municipal food waste and sewage sludge[6], mixture of municipal waste and slaughter house waste[7], waste sludge[5], cornstalk waste[8], corn stover[9], sugarcane bagasse[10], sweet sorghum extract[11], cheese whey[12], waste sludge[13], waste wheat[14], bagasse from sugar extraction processes[15], potato steam peels[16], soluble condensed molasses[17] and dairy manure[18].

To enhance the commercial viability of biohydrogen, a low-cost and high-conversion feedstock should be used. Among the possible condidates, starch is a suitable and cost-effective substrate for commercial biohydrogen production. The rate-limiting step for bioconversion of starch is usually the hydrolysis steps. A physicochemical or enzymatic pretreatment may be required to facilitate the H_2 production efficiency from starch[19]. Macromolecules must be cleaved into smaller molecules by pretreatment, then they can be taken into a cell and used for energy production. Acid hydrolysis is considered to be one of the most effective methods of solubilizing hemicellulose. The increase of acid concentration in the acid-hydrolyzing process could provide a strong or complete reaction for breaking down the chemical

bonds inside cellulose materials yielding the hydrolyzed products mainly sugars in the hydrolyzate[10].

Hydrolysis products contain not only soluble sugars but also a variety of by-products, such as phenol, furan and furfural compounds, adversely inhibiting the capability of hydrogen-producing bacteria to produce hydrogen. In addition, acid hydrolysis is commonly carried out at high temperature and corrodes equipment[20]. Although the higher HCl concentration was in favor of the hydrolyzation of substrate, but the high Cl^- anion concentration heavily inhibited the growth of hydrogen production bacteria[8,21].

The hydrogen production process is important to develop as a low energy consumption process. Therefore it is essential to generate high volumetric H_2 production at lower temperature[22]. To maximize net energy production, suitable cultures have to be employed with required nutrients; appropriate operating conditions have to be engineered to maximize electron flow towards hydrogen; energy input to the process has to be minimized and additional energy has to be recovered from the end products[2].

The objective of this research was to evaluate local sources of molasses, process wastewater from corn and potato processing industry and chocolate waste as substrate for their hydrogen production potential. After running untreated substrates, pretreatment experiments of substrates were conducted to evaluate whether pretreatment on the substrate would be necessary. The effect of the pretreatment on the subsequent fermentability of the resulting fractions was evaluated. The modified Gompertz equation as quantitive model to describe the hydrogen production from substrates was used. Also, the energy gain was calculated for untreated and treated substrates.

Gompertz equation in biohydrogen studies: Many kinetics models have been so far proposed to describe and evaluate the fermentative hydrogen production process. Kinetic models could be used to describe relationship among the principal state variables and to explain the behaviour of fermentation quantitatively. In addition, it can provide useful information for the analysis, design and operation of a fermentation process[23]. Biohydrogen researchers have used Gompertz equation to describe hydrogen production by dark fermentation. In this empirical approach, three model parameters lag time, H_2 production potential and H_2 production rate are adjusted to fit the Gompertz equation to experimentally measured hydrogen production data. Even though this curve-fitting approach yields high correlation coefficients between the observed and fitted hydrogen evolution data, the three model parameters determined by curve-fitting are restricted to specific experimental conditions and can not be used in a predictive mode. Gompertz equation can not account for any of the relevant process variables for predictive purposes[24]. The modified Gompertz equation was able to adequately describe the formation of various products such as butyrate and acetate for anaerobic hydrogen production by mixed cultures[23].

The hydrogen production could be described by the modified Gompertz model (eqn. 2)[18,25]:

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

where H is the cumulative hydrogen (mL), H_{max} is the maximum cumulative hydrogen (mL), r_{H_2} is the hydrogen production rate (mL/min), R_{maxH_2} is the maximum rate of hydrogen production (mL/min), λ is the lag time (min) and t is the fermentation time (min). Lag time is the time phase from the beginning of inoculation to the beginning of gas production.

By differentiating eqn. 2, the hydrogen production rate was expressed as:

$$r_{H_2} = R_{max,H_2}.\exp\left\{-\exp\left[\frac{R_{max,H_2}.e}{H_{max}}(\lambda - t)+1\right]+\left[\frac{R_{max,H_2}.e}{H_{max}}(\lambda - t)+1\right]+1\right\} \quad (3)$$

Evaluation of net energy gain: The theoretical net energy gain, E_n [kJ/g COD in feedstock] is defined as the total energy produced equivalent to the hydrogen volume generated, E_{df} [kJ], minus any heat energy required, E_f [kJ] to raise the reactor contents from ambient temperature [T_a] to the fermentation temperature [T_f]. In our study T_a was 25 °C and T_f was 37 °C. The following equations form in batch reactors[2]:

$$E_{df} = G_{\rho H_2}\,(LHV) \quad (4)$$

$$E_f = V_{\rho w} c_p\,(T_f - T_a) \quad (5)$$

where, G is the volume of hydrogen generated [L], ρH_2 is the density of gaseous hydrogen [8.9×10^{-5} kg/L], ρ_w is the density of water [1 kg/L], LHV is the lower heating value of hydrogen [120,000 kJ/kg], V is the liquid volume in the reactor [L], and c_p is the specific heat of water [4.2 kJ/kg °C]. Assuming negligible heat loss,

$$E_n = \frac{E_{df} - E_f}{VC} \quad (6)$$

where, C is the COD of the feedstock (g/L).

EXPERIMENTAL

Feeding: In this study, three different waste used as substrate for hydrogen production. These wastes were molasses, process wastewater from corn and potato processing industry (named as potato) and chocolate waste (named as chocolate).

The molassees used as substrate was collected from local sugar refining industry. The molasses, containing a high concentration of carbohydrates including sugars, was diluted by water to a certain concentration and minimum 45 % (w/w) of molasses corresponded to readily fermentative sugars. Potato processing wastewater contains high concentrations of biodegradable components such as starch, in addition to high concentrations of COD (5500 mg/L), total solid (5000 mg/L) and total kjeldahl nitrogen (250 mg/L). Aquous solution of chocolate expired self life was chosen as the model of carbohydrate-rich waste water from chocolate production plant. Chocolate waste contains carbohydrate 61 % (w/w).

Seed sludge and pretreatment methods: The use of anaerobic microflora to provide seeds cultures for hydrogen-producing microflora is an attractive option. The seed anaerobic sludge was collected from Anaerobic Wastewater Treatment Plants (at acidification phase) of PAKMAYA which produces yeast from molasses (Kocaeli/Turkey). The total solid content

of the sludge was 27510 mg/L (in dry weight). The volatile suspended solid (VSS) concentration of the sludge was 10600 mg/L. The pretreatment of the seed sludge was performed by heating the sludge at 100 °C for 60 min to inhibit the activity of hydrogen consumers and to harvest hydrogen-producing spore forming anaerobes. The seed sludge used without acclimated with substrate.

Pretreatment methods of substrates: In the experimental work, substrates diluted with distilled water were used. COD of diluted substrates are 5150, 5260 and 17450 mg/L for molasses, potato and chocolate, respectively. Substrates were pretreated by hydrolyzation for 60 min at pH 2, 100 °C. After pretreatment, pH of substrates was adjusted to 5.5 with NaOH. All substrates were used in batch experiments without any nutrient and trace element addition.

Experimental procedure: Two series of batch experiments were conducted to determine: (1) the hydrogen production potential of different substrates and (2) the effect of pretreatment of substrate on hydrogen production potential. Since all operation conditions in the reactors were set in the ranges most favorable, therefore, the pretreatment of substrates process becomes the sole factor determining the hydrogen production.

The batch experiments were performed in a 7L bioreactor equipped with an ADI 1030 system controller and BioXpert 2 data-acquisition software (Applikon Biotechnology, The Netherlands). The batch reactor filled with 3.3 L mixtures, comprising the inoculum (0.3 L) and the substrate solution (3 L). pH was monitored online (AppliSens, The Netherlands) pH values of mixtures was adjusted to 5.5 with HCl and NaOH solution with a peristaltic pump during fermentation. Reactor was filled with nitrogen gas to remove oxygen, create an anaerobic environment. At each time interval, the biogas produced was instantly released from the headspace, creating no overpressure and continuously measured by a flow meter appropriate (Bronkhorst) for small volumes after cooling. The H_2 content of the biogas was monitored on-line with a H_2 sensor (BCP-H_2, Blue Sens). Temperature in the reactor was controlled using a platinum probe Pt 100. Temperature was automatically maintained at the level of 37 °C using an electric jacket and was recorded on-line. Stirrring velocity was maintained at 100 rpm. Mixing was performed with two Rushton turbines. Additional sensor was connected to the reactor for measuring the redox potential. The transmitter for oxidation reduction potential (ORP) was connected to a computer for on-line data acquisition. Nitrogen was used as carrier gas with a flow-rate of 100 mL/min to carry to H_2 analyzer of biogas. Also, ORP reduced by nitrogen flow in fermentation processes.

RESULTS AND DISCUSSION

The profiles of cumulative hydrogen production for molasses, potato and chocolate were described by the modified Gompertz equation shown as eqn. 1 (Fig. 1). Hydrogen produced after a lag phase and cumulative yield increased with time before reaching the maximum.

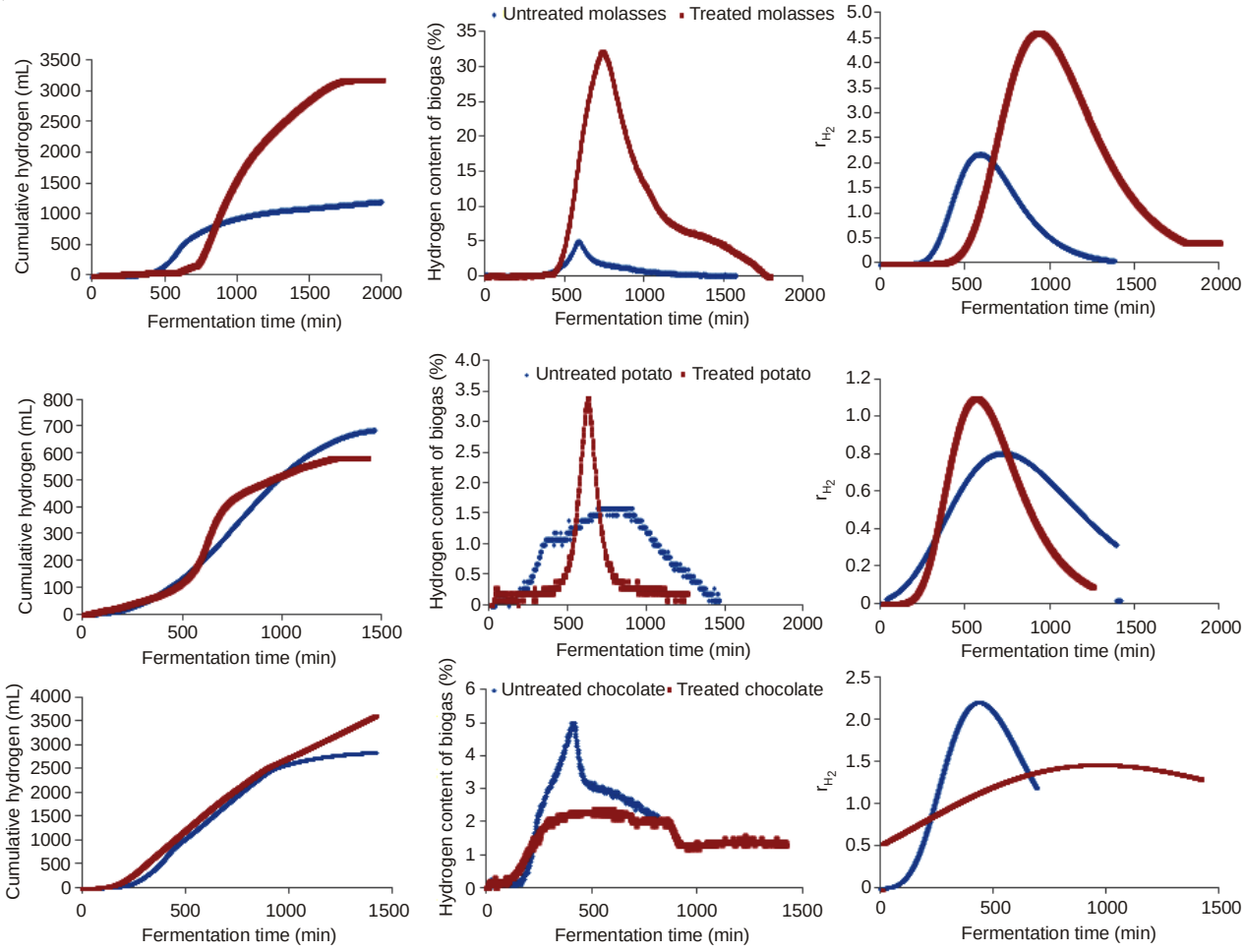

Fig. 1. Hydrogen production *versus* fermentation time for untreated and treated substrates

TABLE-1
BIOHYDROGEN PRODUCTION FROM UNTREATED AND TREATED SUBSTRATES

Substrate	H_{max} (mL)	R_{max} (mL H_2/min)	λ (min)	R^2	COD removal (%)
Molasses					
Untreated	1176	2.2	409.3	0.997815	37.5
Treated	3190	4.6	671	0.997703	30
Potato					
Untreated	646.8	0.8	338.3	0.999463	12
Treated	583	1.1	364.5	0.991092	13
Chocolate					
Untreated	1756	2.2	492.4	0.998855	31
Treated	6712	1.5	266.7	0.997918	30

In order to determine the hydrogen production potential of untreated and treated substrates, we compared Gompertz coefficient. Cumulative hydrogen production and R_{max} were used as comparison criteria. For all substartes, Gompertz coefficients were given in Table-1. eqn. 1 adequately described biohydrogen production showing regression coefficients (R^2) above 0.99. Overall hydrogen gas conversions were 0.39 L H_2/L wastewater for molasses, 0.22 L H_2/L wastewater for the potato wastewater and 0.59 L H_2/L wastewater for chocolate. Ginkel *et al*.[26] obtained as 2.1-2.8 L H_2/L wastewater for the potato wastewater[26]. The highest hydrogen production rate (2.2 mLH_2/min (0.96 L/d L)) was obtained for molasses and chocolate within untreated substrates (Table-1). Some authors had reported hydrogen production potential from experiments using molasses. Aceves-Lara *et al*.[27] obtained the best hydrogen production 15.3 LH_2/d L at pH 5.5 and hydraulic retention time of 6 h using molasses in continuous stirred tank reactor. These variations in hydrogen production were in low part due to the soluble sugar in the composition of the substrate.

Effect of pretreatment to substrate: After running untreated substrates, pretreatment experiments of substrates were conducted to evaluate whether pretreatment on the substrate would be necessary. Due to the complex structure of substrate, hydrolysate of substrate are a very attractive raw material for the production of hydrogen. Therefore, we are interested in using hydrolyzate obtained from acid hydrolysis of substrates.

The H_2 production results coming from untreated and treated substrates are compared in Table-1. Results indicated that the highest hydrogen production rate was obtained in treated molasses, R_{max}, the maximum hydrogen production rate increased from 2.2 to 4.6 mL/min by pretreatment. Fermentation of treated molasses test showed that it is necessary to pretreatment in order to achieve a satisfactory performance of fermentation. Slightly higher hydrogen yields were obtained in treated potato experiment.

We deduced that an increase in the hydrogen production possibly was due to an increase in the soluble sugar in the hydrolysate composition of the acid pretreated substrate.

For the molasses and potato, the hydrogen content of biogas peaked at around 5 and 1.6 %, respectively. This values increased to 32.2 and 3.4 % by pretreatment. However, the peak value decreased from 5 to 2.4 % for chocolate (Fig. 1).

As seen from Table-1, the lag phase was prolonged with application of a pretreatment for molasses and potato. After pretreatment of chocolate, the lag-phase time decreases from 492.4 to 266.7 min. The highest R_{max} was obtained from pretreated molasses, the highest cumulative hydrogen was obtained from pretreated chocolate.

Fermentative hydrogen production process does not significantly reduce the organic content of the feed. Usually, chemical oxygen demand (COD) removal is below 20 % during hydrogen production process. This can be removed in a subsequent anaerobic digestion step with the conversion of organic content to methane[28]. In this study, COD removal is below 37 % (Table-1). So it should be considered further anaerobic treatment for methane production of effluent from hydrogen producing.

Evaluation of net energy gain: E_f value was calculated as 166.3 kJ in all experiments. According to Table-2, the net energy gain (E_n) is negative for substrate. For molasses and chocolate, pretreatment step increases E_n value, but stil remains negative. The fermentation temperature has to be less than present temperature for the net energy gain to be positive.

TABLE-2
NET ENERGY GAIN FOR UNTREATED AND TREATED SUBSTRATES

Substrate	E_{df} (kJ)	E_n (kJ/g)
Molasses		
Untreated	12.56	-9.05
Treated	34.07	-7.78
Potato		
Untreated	6.91	-9.18
Treated	6.23	-9.22
Chocolate		
Untreated	18.75	-2.56
Treated	71.68	-1.64

Conclusion

In this study, we demonstrated fermentation to hydrogen of wastes in a batch bioreactor inoculated with a microbial consortium obtained from acidification stage of anaerobic plants. We also investigated the effects of the pretreatment on the subsequent fermentability of the resulting fractions. The energy gain was calculated for untreated and treated substrates. The following conclusions may be drawn:

In pretreatment of molasses and chocolate, H_{max}, the maximum cumulative hydrogen of 3190 and 6712 mL was obtained, increasing by 171 and 282 % as compared with the result of the un-pretreated, respectively. Acid hydrolysis treatment supported hydrogen fermentation since the microbes are able to hydrolyze the oligomeric sugars. Results show that the

maximum H_2 production rates of untreated chocolate and molasses (2.2 mL/min) were better than that untreated potato, whereas the maximum H_2 production rate of treated molasses (4.6 mL/min) was better than that treated potato and chocolate.

The net energy analysis revealed a negative energy gain at experiment conditions. To improve the net energy gain, fermentation temperature can be reduce or effluent of bioreactor can be evaluate for methane production.

Economic wise, we prefer that any nutrient solution and fermentation medium were not used in dark fermentation. The hydrogen yield can be increase by addition of external nutrients to the fermentation medium.

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