

Impact of oleic acid on the fermentation of glucose and xylose mixtures to hydrogen and other byproducts

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ABSTRACT

Using low value lignocellulosic feedstocks to produce hydrogen (H₂) could be a more economically viable option in comparison to grains derived from agriculture crops. The glucose and xylose composition of lignocellulosic feedstocks is relatively high and can vary from 55–65% to 35–45%, respectively. Mixed anaerobic cultures which are suitable for fermentative H₂ production are capable of utilizing a variety of substrates. However, the H₂ yields from mixed anaerobic cultures are low because of the syntrophic association between H₂ producers and H₂ consumers. In this study, oleic acid (OA) was used to control the growth of H₂ consumers and hence, increase the H₂ yield. The H₂ yield was affected by changing the glucose to xylose ratio. In control cultures, the H₂ yield (1.6 ± 0.32 mol H₂ mol⁻¹ glucose) was low because the electron flux was diverted towards the formation of propionate and methane in addition to acetate and butyrate. In presence of 2000 mg L⁻¹ OA, 60–70% of the electron fluxes were diverted towards acetate and butyrate production and this resulted in a maximum H₂ yield of 2.84 ± 0.24 mol H₂ mol⁻¹ glucose. This study revealed that the addition of OA to the mixed anaerobic cultures is an effective method for diverting the electron fluxes to H₂ instead of CH₄. Based on a principal component analysis 1 (PCA 1), the control cultures were related; however, in case of the OA treated cultures, they were related in terms of PCA 1 and PCA 2.

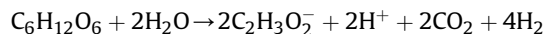
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1. Introduction

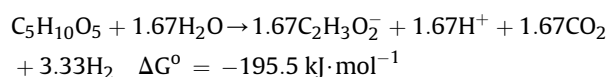
Producing hydrogen (H₂) from renewable carbon sources could alleviate many environmental, social and political issues associated with using fossil fuels. Hydrogen is a clean and environmentally friendly fuel. The annual demand for H₂ gas is rapidly increasing with a growth rate of approximately 10% [1]. Because H₂ is not directly available from natural resources, large quantities for commercial use are produced from the steam reforming of natural gas [2]. Alternative production routes using low cost renewable feedstock's are currently under investigation by many researchers. Anaerobic fermentation routes using mixed cultures have attracted the attention of many researchers because of the relatively mild conditions required to mediate the conversion reactions and the ability of the microorganisms to use chemicals derived from non-sterile agriculture residues [3].

Lignocellulosic residues include biomass derived from the agricultural as well as forestry sectors. In the United States (U.S.),

Canada and China, the amounts of agricultural and forest residues generated annually are shown in Table 1 [4–7]. Note the availability of lignocellulosic feedstock residues is unquestionable quite large for countries with huge landmass areas. The major components of lignocellulosic residues include cellulose (24–39%) and hemicelluloses (14–41%) [8]. These polymers can be hydrolyzed to hexoses and pentoses and subsequently converted into H₂ plus a series of carbon byproducts [9]. Assuming acetate is only the end product, glucose and xylose can be converted into H₂ with maximum theoretical yields of 4.0 mol H₂ mol⁻¹·glucose and 3.33 mol H₂ mol⁻¹·xylose (Equations (1) and (2)), respectively. If the fermentation proceeds via the butyrate pathway, the theoretical yields are 2.0 mol H₂ mol⁻¹·glucose and 1.67 mol H₂ mol⁻¹·xylose (Equations (3) and (4)).



$$\Delta G^\circ = -184.2 \text{ kJ} \cdot \text{mol}^{-1} \quad (1)$$



$$\Delta G^\circ = -195.5 \text{ kJ} \cdot \text{mol}^{-1} \quad (2)$$

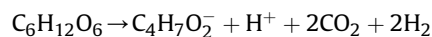
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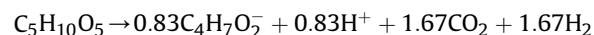
Table 1

Annual quantities of unused agricultural and forest residues inventory in the U.S., Canada and China.

| Country | Crop residues (million dry tonnes) | Forest residues (million dry tonnes) | Reference |
|---------|---------------------------------------|-----------------------------------------|-----------|
| US | 450 | 120 | [3] |
| Canada | 18 | 28 | [4,5] |
| China | 100 | 500 | [5,6] |



$$\Delta G^\circ = -257.1 \text{ kJ} \cdot \text{mol}^{-1} \quad (3)$$



$$\Delta G^\circ = -239.9 \text{ kJ} \cdot \text{mol}^{-1} \quad (4)$$

A variety of chemical and physical agents such as acid, heat, sonication, aeration and freeze/thaw and bromoethane sulfonate (BES) are utilized to inhibit hydrogen consuming microorganisms [9,10]. However, chemicals such as BES are not eco-friendly and they could impose major cost limitations in large scale applications [11]. In comparison, eco-friendly chemicals derived from agriculture crops such as long chain fatty acids (LCFAs) are effective inhibitors of H₂ consuming microorganisms at threshold levels [11]. During glucose fermentation, unsaturated LCFAs bearing 18 carbons have been reported to increase the H₂ production by inhibiting hydrogenotrophic methanogens [11]. To date evaluating the impact of LCFAs on mixed sugar fermentation has not been reported. Hence, the objective of this study is to assess the effect of oleic acid (OA) on H₂ production from mixed sugar (glucose and xylose) fermentation.

2. Materials and methods

2.1. Inoculum source

Experiments were conducted using a mixed anaerobic inoculum which treated an effluent from a food processing industry (Cornwall, Ont., Canada). The culture was maintained in a 4-L semi-continuous reactor (designated as Reactor A) at 37 °C and fed 5000 mg L⁻¹ sugar (2500 mg L⁻¹ xylose plus 2500 mg L⁻¹ glucose) (Spectrum Chemicals, CA) every 6–7 days. The volatile fatty acids (VFAs) concentration and the amount of gas produced were used to determine when all the glucose plus xylose and byproducts were consumed (every 5–6 days). Inoculum from Reactor A (approximately 18,000 mg L⁻¹ volatile suspended solids (VSS)) was diluted with basal medium to achieve 5000 mg L⁻¹ VSS in a second 4-L semi-continuous reactor (designated as Reactor B). Reactor B (fed 5000 mg L⁻¹ sugar (2500 mg L⁻¹ xylose plus 2500 mg L⁻¹ glucose)) served as the inoculum source for all experiments. Both reactors were maintained at 37 °C. The composition of the basal medium used for dilution was adapted from Alost et al. [12] had a pH of 8.2–8.0 and contained the following constituents (mg L⁻¹ of distilled water): NaHCO₃, 6000; NH₄HCO₃, 70; KCl, 25; K₂HPO₄, 14; (NH₄)₂SO₄, 10; yeast extract, 10; MgCl₂·4H₂O, 9; FeCl₂·4H₂O, 2; resazurin, 1; EDTA, 1; MnCl₂·4H₂O, 0.5; CoCl₂·6H₂O, 0.15; Na₂SeO₃, 0.1; (NH₄)₆MoO₇·4H₂O, 0.09; ZnCl₂, 0.05; H₃BO₃, 0.05; NiCl₂·6H₂O, 0.05; and CuCl₂·2H₂O, 0.03.

2.2. Experimental design

Experiments were designed to evaluate the effect of OA on H₂ production from mixed sugars (glucose and xylose) which were fermented at an initial pH of 5.5 using cultures maintained at

37 ± 1 °C. The study was conducted under batch conditions with individual sugars and varying ratios of glucose to xylose (Table 2). The reaction mixture consisted of 2000 mg L⁻¹ VSS, 2000 mg L⁻¹ OA (Sigma–Aldrich, CA) plus 5000 mg L⁻¹ sugar. Control cultures were prepared with no OA and only glucose or xylose. Control experiments were also conducted using cultures fed with OA and without glucose and xylose in order to assess H₂ production from OA degradation. In all the conditions examined, the controls and cultures containing OA were prepared in triplicate.

2.3. Hydrogen production studies using oleic acid

The experimental methods adapted for this work were described previously by Alost et al. [12] and Lalman and Bagley [13]. The batch reactors (160 mL serum bottles) were prepared in a COY® anaerobic chamber under a 70–75% N₂/20–25% CO₂ atmosphere containing approximately 1% hydrogen. The bottles were sealed with Teflon-lined silicone rubber septa and aluminum caps after adding the culture plus basal medium (a total volume of 50 mL). Next, 20 mL of gas from the anaerobic chamber was injected into the headspace in order to avoid the formation of a negative pressure during sampling. Prior to feeding the substrates, the bottles were agitated for 24 h to remove H₂ which was added to the headspace from the anaerobic chamber. Several control experiments were conducted to establish the 24 h agitation period. Depending on the condition examined, varying volumes of each substrate (sugar plus OA), equivalent to the volume of liquid removed, were added to the serum bottles to maintain a total volume of 50 mL. An orbital shaker (Lab Line Instruments Model 3520, Iowa) was used to agitate the bottles at 200 rpm, 37 ± 1 °C and pH 5.5 over the duration of the study. Sugar mixtures (glucose and xylose) were added to the cultures on Day 0 and again on Day 4 (Table 2). After the first injection of sugars (5000 mg L⁻¹), headspace and liquid samples were withdrawn at specified intervals over 4 days and analyzed for H₂, methane, VFAs, alcohols and sugars. In all the bottles receiving OA and in the controls (only sugars), 5000 mg L⁻¹ sugars were injected again on Day 4 after sparging the headspace with N₂ (99.99%, Praxair Inc.) for 3 min. Following Day 4, headspace and liquid samples were removed at specified intervals and for H₂, CH₄, VFAs and alcohols over a 3 day period.

An LCFA stock solution (50,000 mg L⁻¹) was prepared by melting OA *au bain-marie* in hot NaOH [14]. To provide initial OA levels of 2000 mg L⁻¹, 2 mL of the 50,000 mg L⁻¹ saponified stock solution was added to the serum bottles. The glucose stock solution was prepared with a 100,000 mg L⁻¹ concentration.

2.4. Analytical methods

VFAs, alcohols, sugars and gas analyses were conducted in accordance with Ray et al. [11]. The detection limit incorporating

Table 2Experimental design for H₂ production with different glucose and xylose ratios in presence and absence of oleic acid.

| Expt. No | Oleic acid (mg L ⁻¹) | Glucose (mg L ⁻¹) | Xylose (mg L ⁻¹) | Glucose:Xylose ratio |
|----------|-------------------------------------|----------------------------------|---------------------------------|-------------------------|
| 1 | 0 | 0 | 5000 | 0.00:1.00 |
| 2 | 0 | 5000 | 0 | 1.00:0.00 |
| 3 | 2000 | 5000 | 0 | 1.00:0.00 |
| 4 | 2000 | 3750 | 1250 | 0.75:0.25 |
| 5 | 2000 | 2500 | 2500 | 0.50:0.50 |
| 6 | 2000 | 1250 | 3750 | 0.25:0.75 |
| 7 | 2000 | 0 | 5000 | 0.00:1.00 |

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