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A High Yield, Hydrogen Producing, Bacterial Community Enriched from Anaerobic Digester Leachate

Hang Zheng, Cathryn O'Sullivan, William Clarke, Biomass BioEnergy Group, School of Civil Engineering, University of Queensland, Australia

Raymond Zeng, Advanced Water Management Centre, University of Queensland

Mikel Duke, Institute of Sustainability and Innovation, Victoria University, Australia

1 Introduction

Hydrogen is a carbon-free energy source with high energy density (based on per unit of mass) [1]. Beyond that, it produces only water when combusted. In light of this, hydrogen is regarded as a promising energy carrier for the future.

The most widely used method for hydrogen production is the steam reforming [2], which reforms natural gas into hydrogen and carbon dioxide. This process needs extensive energy supply (electricity) and fossil fuels (natural gas), which makes it an environmentally unfriendly method. In order to make hydrogen a truly sustainable and carbon-free energy source, its production has to avoid the usage of fossil fuel based feedstocks.

In light of the above, fermentative hydrogen production from organic waste is regarded as the most feasible method for hydrogen production [3]. The theoretical maximum hydrogen yield from the fermentation of glucose is 4 mole-H₂/mole-glucose with acetate as the end product [4]. However, this yield has only been reported in a study using pure culture [5], which is not possible when working with real waste streams. To date, the reported hydrogen yields usually vary from 0.5-2.2 mole-H₂/mole-hexose by using mixed cultures [6]. Fermentation of glucose to other end products results in a lower theoretical yield, with butyrate yielding only 2 mole-H₂/mole-glucose whilst fermentation to ethanol yields no hydrogen [7].

The hydrogen yield can be improved by increasing the operating temperature. Several studies have shown that operation in thermophilic temperature ranges improves hydrogen yields relative to mesophilic temperatures due to higher reaction rates and decreased problems with contamination by methanogens [8, 9].

Therefore, thermophilic temperatures (65°C) were applied in this study to improve the hydrogen yield in batch experiments.

2 Materials and Method

2.1 Inoculums and substrate

Biological hydrogen production was assessed in 120 mL serum bottles with a working volume of 50 mL. An inoculum of hydrogen producing organisms was obtained by enriching an original culture from the leachate phase of an anaerobic digester treating mixed organic waste at a temperature of 47°C for biogas production. The culture was enriched through successive generations of batch cultures grown on glucose and media as described below.

Glucose (0.5 g/l) was used as substrate in all tests with nutrient solution containing minerals [10]. The solution was buffered with 4.1 g/l (20 mM) 3-Morpholinopropanesulfonic acid (MOPS) to pH 5.5.

2-Bromoethanosulfonate (BES, 20 mM) addition was used in the first 3 digestions (each time a reactor is started with fresh media and inoculated, it is considered a new digestion) in order to inhibit the growth of methanogens and associated CH₄ production. After the third transfer, BES addition was stopped completely. Erythromycin and Chloramphenicol (125 µg/ml for each) was used to inhibit the growth of bacterium.

2.2 Analysis

Hydrogen in the headspace of batch reactors was sampled by using a syringe with a pressure lock (SGE Analytical Science, Australia), and then analysed by gas chromatography (GC), which has been previously described elsewhere [11].

Liquid samples were taken after the gas sampling and filtrated with 0.22 µm filters (Milipore Corporation, USA). Glucose, formate, lactate and succinate were analyzed by using high performance liquid chromatography (HPLC). VFAs, and alcohols were analyzed by using GC, which has been previously described elsewhere [11].

3 Results and Discussion

An average yield of 2.49±0.09 mol-H₂/mol-glucose was obtained in the initial pH 5.5 batch experiments. The 3.12% error of the COD balance (Table 1) shows that the measurements were accurate.

Table 1: COD balance of initial pH 5.5 batch experiments. All units are in mg-COD except H₂ yield is in the unit of mol-H₂/mol-glucose. Carbon balances for all the batches were within 97% (data not shown). Lactate, formate, succinate, propionate and valerate were not detected in all the batches.

	Glucose Consumed	A.A.	B.A.	ETOH	BTOH	H ₂	Biomass	Balance	H ₂ yield
Average	-27.23	6.44	5.66	5.78	1.65	5.66	2.59	-1.04	2.49
Percentage (%)	-100	23.63	20.77	21.22	6.06	20.78	9.50	-3.84	
Stdev (n=5)	0	0.32	0.82	0.86	0.18	0.21	0.27	0.14	0.09

* Notations: A.A., acetic acid; B.A., butyric acid; ETOH, ethanol; BTOH, butanol

As shown in the Table 2, the measured hydrogen production was always higher than the yield associated with fermentation products by 30%. This suggested that the high hydrogen yields were associated with other path-ways which give a higher yield than the well known acetate fermentation path-way, or the continued oxidation of acetate to CO₂ and hydrogen in a process known as acetate oxidation.

Table 2: Summary of yield of acetate, butyrate, and hydrogen and in comparison with calculated hydrogen from experimental acetate and butyrate values.

	Measurements			Calculated H ₂ from			
	Acetate (mmol)	Butyrate (mmol)	H ₂ (mmol)	Acetate (mmol)	Butyrate (mmol)	Sub-total (mmol)	Delta H ₂ (Mea-Cal) (mmol)
Average	0.10	0.04	0.35	0.20	0.07	0.27	0.08
stdev (n=5)	0.005	0.008	0.01	0.01	0.02	0.02	0.02

Since the inoculums were from a methane producing digester, it is possible that the methanogens are still there to oxidize VFAs, e.g. acetate and butyrate. In order to confirm if the missing acetate and butyrate were due to the methanogens, a mixture of two anti-biotics were added to achieve final concentrations of 125 µg/ml of Erythromycin and 125 µg/ml of Chloramphenicol in the media. This should kill all bacterium and get rid of the effects of bacterium in the culture (Figure 1, top). No hydrogen or methane was produced in those batches. Analysis show that glucose was not consumed at all (data not shown).

While this was an expected result, a surprising result was achieved when 2-Bromothanosulfonate (BES, 20mM) was applied to the serum bottles to inhibit possible methanogens that has survived through the enrichments. Tiny amount of hydrogen has been produced on the 1st day but no more hydrogen or methane was produced after the 1st day in those batches (Figure 1, bottom). Further analysis show that glucose was not consumed at all (data not shown).

After washing away the inhibitors, hydrogen yield was again retrieved (Figure 1). This result suggests that the enriched culture contains bacterium and possibly methanogens. This indicates that both bacterium and the archaea are needed to give a high hydrogen yield in the glucose fermentation. The presence of the two groups of microorganisms might improve the energy efficiency through the interspecies cooperation, which made the acetate oxidation possible. Further work is needed to explore the mechanisms.

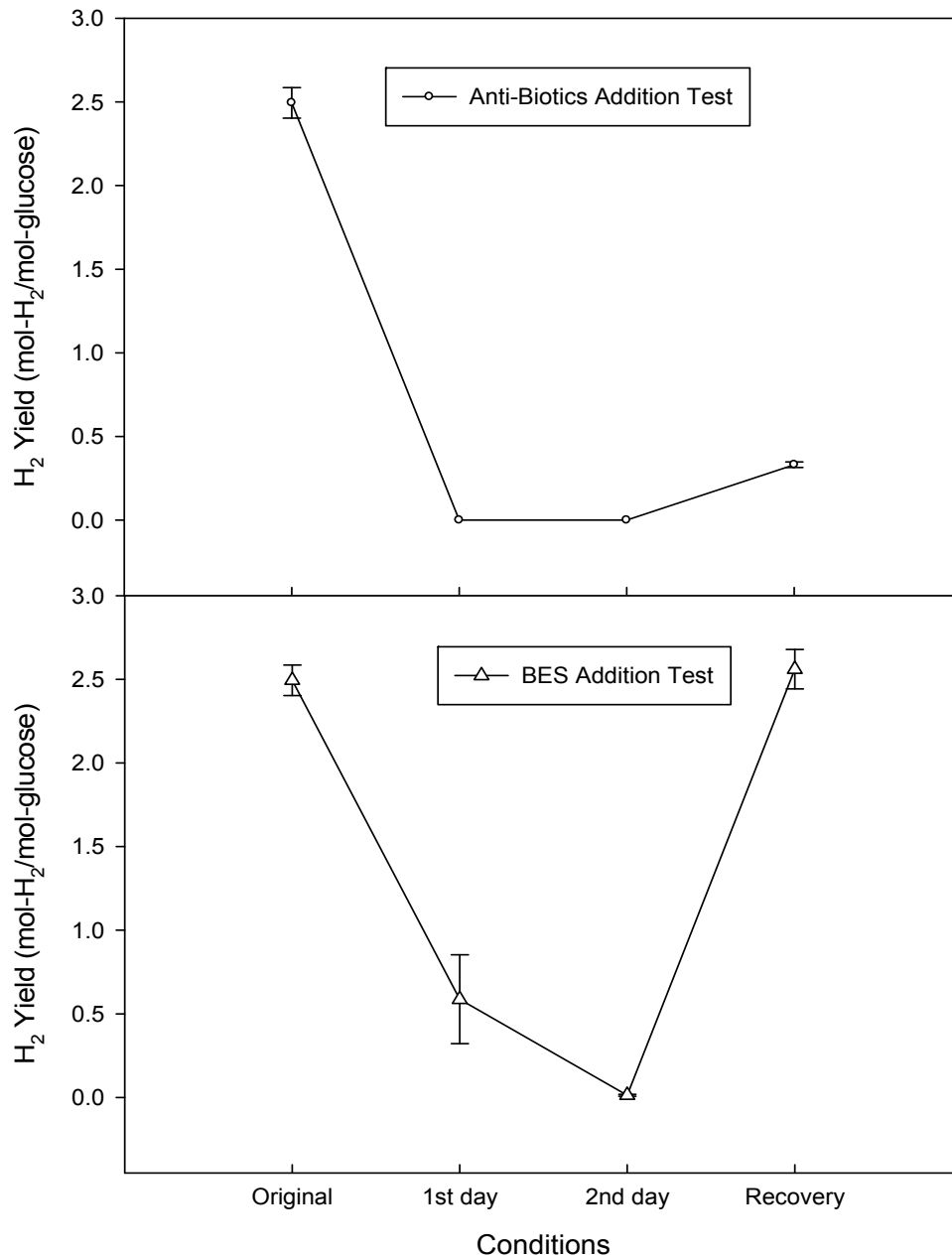


Figure 1: Hydrogen yields of batch experiments after application of antibiotics (top) and BES (bottom).

4 Conclusion

A culture has been enriched for fermentative hydrogen production. The hydrogen yield achieved was 2.49 mol-H₂/mol-glucose.

The applications of inhibitors for bacterium and methanogens showed that the enriched culture contains bacterium and methanogens and it needs the presence of both the two to give the high hydrogen yield from degradation of glucose.

In all, this study suggests that high hydrogen yields can be achieved if favourable conditions are provided.

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