The Effect of Carbon Loading and Fatty Acid Concentration on the Production of Biohydrogen by Anaerobic Fermentation of Agricultural Waste Feedstocks

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Introduction

- The long range goal of our research is to develop a Bio-reactor capable of fermenting agricultural feedstock's by mixed microflora found in livestock manure to efficiently produce H_2 as an energy/fuel source.
- Hydrogen is a clean energy source and combustion of hydrogen produces only water as a by-product, making it a nonpolluting, carbon-free alternative energy source.
- Farms in the US produce 1.5 billion tons of animal waste annually.
- Animal waste contain undigested carbohydrates as well as other potential sources of hydrogen.
- Cellulosic Energy sources such as Switchgrass have also shown great potential as Energy crops.
- Direct fermentation of carbohydrate feedstock's by microorganisms is one of a number of potential technologies for producing renewable hydrogen and avoiding environmental pollution.
- The Production of hydrogen by anaerobic fermentation has great potential for reducing farm waste and producing energy from undigested carbohydrates in manure and cellulosic feedstocks.
- Animal waste also contains Volatile Fatty Acids(VFA) which are known to inhibit the hydrogen production in anaerobic fermentation.
- In this experiment we demonstrates the effect of Volatile Fatty Acids (VFA) and Carbon Loading on the production of Hydrogen by mixed culture anaerobic fermentation.

Composition of Switchgrass

Dry matter	94%
Cellulose	42%
Hemicellulose	32.5%
Acid Detergent Lignin (ADL)	6.5%
Ash	0.65%

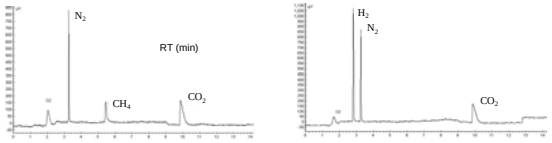
Experimental Methods

- Seed sludge used in this study contained 20g of dried (40ºC for 3 days) cow dung sludge, mixed with 40 ml of water and 40 ml of nutrient solution.
- The nutrient solution contained the following inorganics (mg/L) for bacterial growth: 5240 of NH_4HCO_3, 125 of K_2HPO_4, 15 of $MgCl_2.6H_2O$, 25 of $FeSO_4.7H_2O$, 5 of $CuSO_4.5H_2O$, 0.125 of $CoCl_2.5H_20$ and 5600 of $NaHCO_3$. The nutrient solution was purged with N_2 for few minutes. This mixture had pH 7.0 and two types of seed culture were prepared: 1)Heat treated (100ºC for 30 min) 2) non-treated (virgin).
- Switchgrass (SG) samples were collected from Clemson University's Pee Dee Research and Education center, Florence, SC. Dry SG was ground by 1093 Cyclotec Sample Mill, Foss Inc., Sweden.
- The fermentation was carried out in 60 ml serum bottle and 10 ml head space vial with glucose and SG, respectively.
- The SG (8%) was pretreated with 1% H_2SO4 or 1% NaOH at 121ºC for 30 min. When the temperature of the pretreated suspension reached room temperature it was neutralized by the addition of acid or base.
- One set of experiment was carried out using 4 ml of pretreated SG suspension and 2 ml of diluted virgin or heat treated seed culture in 10 ml head space vial. Another set of experiment was carried out using 2%, 4% and 6% glucose with or without VFA (acetate, propionate and butyrate) mixture plus 20ml heat treated seed culture. The vial was purged with N_2 gas, capped and incubated anaerobically at 40ºC for 3 days.
- Hydrogen concentrations were measured by gas chromatography (CP-3800, Varian Inc, USA).
- Experimental data was analyzed with SPSS 12.0 and graphs were generated with Mathematica 6.

Acid Pretreatment

Fig. 1a: 1% H_2SO_4 pretreated switchgrass + virgin seed culture pH 7.0

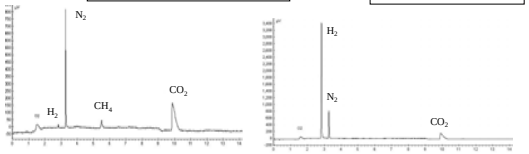
Fig. 1b: 1% H_2SO_4 pretreated switchgrass + heat treated seed culture pH 7.0



Alkaline pretreatment

Fig. 2a: 1% NaOH pretreated switchgrass + virgin seed culture pH 7.0

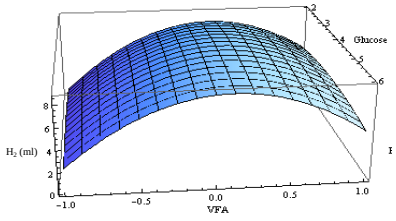
Fig. 2b: 1% NaOH pretreated switchgrass + heat treated seed culture pH 7.0



Results and Discussion

Cow dung has a mixed microflora composed of cellulolytic, proteolytic, amylolytic, acetogenic, sulfate reducing bacteria and methanogens. Switchgrass (SG) (*Panicum virgatum*) contains cellulose, hemicellulose and lignin. Lignin in lignocellulosic biomass is known to create obstacles during enzymatic hydrolysis and microbial activity. Therefore, pretreatment is required to either partially remove or break up lignin structure, thereby, enzymes can diffuse into the cellulose polymer and degrade it into monomeric fermentable sugars. Degradation of pretreated SG by cow dung produced CO_2 and CH_4 (Fig. 1a and Fig. 2a). Due to the interspecies H_2 transfer, we did not see any H_2 present. When inoculated with heat treated seed culture, we found H_2 produced and the disappearance of CH_4 in the chromatograms (Fig. 1b and Fig. 2b). This is probably due to non-spore forming methanogen not surviving exposure to high temperature. This is supported by previous results [1]. Production of H_2 from 2% and 4% glucose solutions were nearly the same whereas it was 2.2 times higher with 6% glucose as compared with 2% or 4% glucose (Table 1) which is similar with the previous result [2]. H_2 production increased as the volatile acid (VFA) concentration was increased from the control to the mid-level mixture concentrations (acetate 10mM, propionate 2.5mM and butyrate 2.5mM). High levels of VFA's also decreased H_2 production mixture. (Fig. 3). Optimum H_2 production was observed with 6% glucose at mid-level VFA concentrations.

Fig 3. Effect of VFA and glucose on H_2 production



$$Y_{H2} = 2.395x_1 - 4.850x_1^2 + 1.579x_2 - 0.174x_2^2 - 0..208x_1x_2 + 4.905$$

Table 1

Run	Code	Real values				
	X1	X1 (mM)			X2 (%)	Y (ml)
	VFA	Acetic acid	Propionic acid	Butyric acid	Glucose	Hydrogen
1	-1	0	0	0	2	1.068
2	0	10	2.5	2.5	2	6.741
3	1	20	5	5	2	1.716
4	-1	0	0	0	4	1.222
5	0	10	2.5	2.5	4	7.899
6	1	20	5	5	4	6.472
7	-1	0	0	0	6	2.373
8	0	10	2.5	2.5	6	8.633
9	1	20	5	5	6	3.6

Future Plan

- Continue experiments to optimize factors important to H_2 production from Switchgrass & Animal Waste.
- Scale-up: Lab (10-60ml) → Pilot (1-100L) → Production (100L to 200 m^3).

Conclusion

- Virgin seed culture produce CH_4 and CO_2 from dilute acid or base treated Switchgrass.
- Heat treated seed culture produce H_2 and CO_2 instead of CH_4.
- H_2 production depends on carbon loading.
- Low concentration of VFA increased H_2 production.

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