Biohydrogen Production from Renewable Organic Wastes

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Objectives

- Investigate different strategies for selective growth of hydrogen-producing bacteria (e.g., heat selection and pH control, hydraulic loading) in a mixed culture environment.
- Determine the biokinetics parameters of hydrogen-producing microorganisms.
- Identify and quantify hydrogen-producing bacterial population in a complex microbial community background using nucleic acid based technique - terminal restriction fragment length polymorphism (T-RFLP).
- Design and develop improved bioreactor system to favor the growth of hydrogen-producing bacteria.

Technical Barriers

This project addresses the following technical barriers from the Hydrogen Production section of the Hydrogen, Fuel Cells and Infrastructure Technologies Program Multi-Year R,D&D Plan:

- H. Fermentative Micro-organisms

Approach

- Develop batch scale and continuous reactor system to evaluate technical and practical feasibility of hydrogen production from synthetic and real waste streams.
- Study the effect of heat treatment of sludge on hydrogen production in a continuous experiment.
- Apply Monod equation to estimate kinetic constants for different types of waste streams.
- Apply nucleic acid based techniques for microbial community identification and quantification.
- Find the correlation between hydrogen yield and the *Clostridium* sp. in the continuous bioreactor.

Accomplishments

- Successfully enriched naturally available mixed seed (e.g. compost, soybean soils, anaerobic digester sludge, etc.) to microbial culture rich in hydrogen-producing bacteria.
- Accomplished a hydrogen yield of 2.2 mole/mole of sucrose by using mixed microbial culture with hydrogen conversion efficiency of 27.5%.
- Developed heat treatment strategy for selective growth of hydrogen producers.
- Evaluated the effect of preheat treatment of seed inoculum at 70-90^{o}C for 15-20 minutes followed by repeat heat treatment of settled sludge on hydrogen yield.
- Determined biokinetics parameters [e.g. specific growth rates (m), half saturation constant (Ks), growth yield (Y)] of hydrogen producers using different substrates.
- Identified and quantified the hydrogen-producing bacteria in a mixed culture environment using nucleic acid based technique known as terminal restriction fragment length polymorphism (T-RFLP).

Future Directions

- Evaluate different reactor configurations and control parameters to obtain higher hydrogen conversion efficiency using real waste streams, e.g. high fructose corn syrup, molasses, food waste, corn processing waste, etc.
- Develop pilot-scale hydrogen production demonstration project using real wastes.
- Continue identification and quantification of hydrogen-producing bacterial population in a complex microbial community background using nucleic acid based techniques.

Introduction

The environmental consequences of extensive use of fossil fuels have already begun to surface. The excessive use of fossil fuels is one of the primary causes of global warming and acid rain, which have started to affect the earth's climate, weather, vegetation and aquatic ecosystems (Hansen *et al*., 1981). Moreover, the U.S. relies heavily on imported petroleum from nations which are politically unstable. Due to global environment and national energy security considerations, a non-polluting and renewable energy source needs to be developed. Hydrogen is a clean energy source, producing water as its only by-product when it burns. Besides, hydrogen can be produced from renewable raw materials such as organic wastes. Therefore, hydrogen is a potential clean energy substitute for fossil fuels.

A new fermentation process that converts negative-value organic waste streams into hydrogen-rich gas has been developed by the Biotechnology Research Group at Iowa State University (Van Ginkel *et al*., 2001). The process employs mixed microbial cultures readily available in nature, such as compost, anaerobic digester sludge, soil, etc., to convert organic wastes into hydrogen-rich gas. An enriched culture of hydrogen-producing bacteria such as *Clostridia* was obtained by heat treatment, pH control and hydraulic retention time (HRT) control of the treatment system. The process not only generates environmentally clean energy - hydrogen, but also stabilizes the waste. Thus, the newly explored hydrogen fermentation technology could curtail the growing energy insecurity and eliminate the global and local pollution problems resulting from excessive use of fossil fuels. The biohydrogen fermentation technology could enhance the economic viability of many processes utilizing hydrogen as a fuel source or as a raw material.

Approach

A series of serum bottle tests were conducted to evaluate the environmental factors (e.g. pH and heat treatment) affecting biological hydrogen production and to investigate the feasibility of biohydrogen production from different substrates. Serum bottle tests were also conducted to determine the biokinetic parameters (e.g. specific growth rate, half velocity constant and growth yield) of hydrogen-producing bacteria for different substrates. Following the batch studies, completely mixed bioreactors were operated for continuous hydrogen production using anaerobically digested sludge that had been heat treated at 100°C for 15 minutes as microbial seed. The experimental set-up employed in this study is shown in Figure 1. Upon successful start-up, the operation of the bioreactor was optimized to maximize hydrogen production. The process

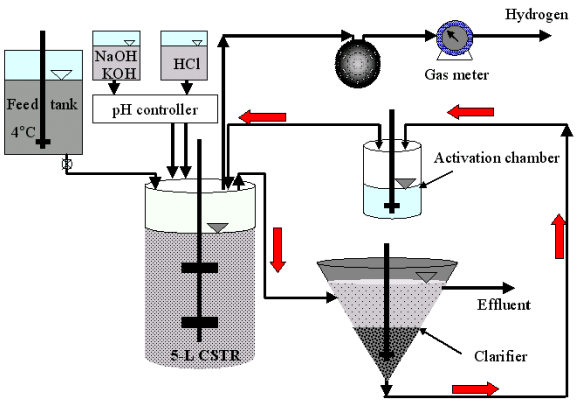

Figure 1. Experimental Setup of Continuous Bioreactor

optimization included determination of optimum operating pH, combination of temperature and duration of heating of settled biomass, frequency of heat treatment, chemical oxygen demand (COD) loading rate and HRT.

A nucleic acid based technique – DNA fingerprinting called terminal restriction fragment length polymorphism (T-RFLP) – was used to identify the abundant populations in a complex microbial community background. This method involves the extraction of DNA from the biomass samples and the amplification of the 16S ribosomal DNA (16S rDNA) gene using the polymerase chain reaction (PCR) with appropriate primers. The forward primer was labeled with fluorescein. The fluorescently labeled PCR products were digested with restriction enzymes *HaeIII, MspI*, and *RsaI*. The fluorescently labeled terminal restriction fragments obtained in this manner were separated by gel electrophoresis.

Results

A series of batch test results showed that a pH of 5.5 was optimum for hydrogen production without any detection of methane as evident from the highest hydrogen conversion efficiency as indicated in Figure 2. This pH was therefore selected for the continuous phase testing. Batch tests also showed that heat treatment of seed inocula at 70-90^{o}C for 15-20 minutes enhanced the hydrogen production by more than five times with respect to control (without heat treatment). Such an increase was likely due to more favorable conditions to spore-forming hydrogen producers by reducing nonspore-forming hydrogen consumers.

Based on biokinetic studies, the specific growth rates (μ) of hydrogen-producing bacteria were found to be 0.10 hr^{-1}, 0.176 hr^{-1} and 0.215hr^{-1} respectively for sucrose, non-fat dried milk (NFDM) and food waste (produce + deli). These values were significantly higher than that for hydrogen-oxidizing bacteria (0.055 hr^{-1}) [Rittmann and McCarty, 2001]. This suggests that a hydrogen-producing bioreactor could be operated at much shorter HRT than the conventional methanogenic reactor. Thus, HRT could be one of the important factors to select the predominance of hydrogen producers in anaerobic reactor. The biohydrogen production rates at various initial substrate concentrations for different substrates are shown in Figure 3.

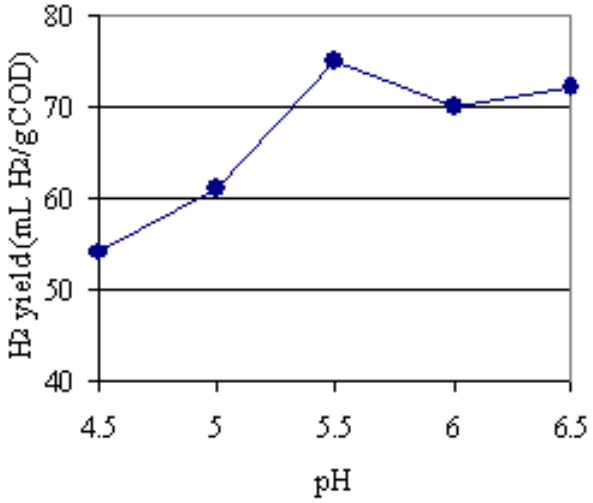

Figure 2. Effect of Ph on Hydrogen Production in Batch Studies

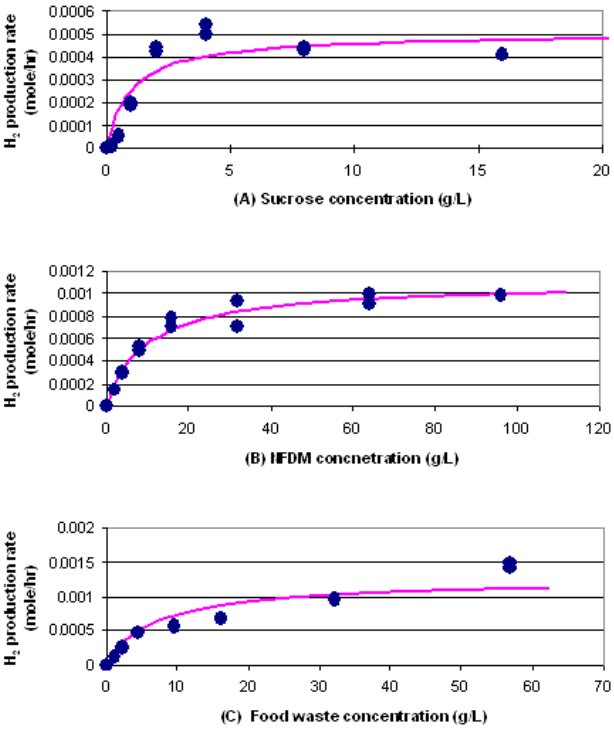

Figure 3. Biohydrogen Production Rates at Various Initial Substrate Concentrations (A) Sucrose; (B) Non-Fat Dry Milk; (C) Food Waste

A continuous bioreactor was operated for about 140 days at a substrate concentration of 20 g/L (sucrose) in a semi-batch feed at an HRT of 24 hr. The seed sludge was preheat-treated at 100°C for 15 minutes followed by a series of heat treatment at 70°C for 20 minutes on day 29 (Phase I), day 113 and day 129 (Phase III). The result is presented in Figure 4. In Phase I, heat treatment was applied to only one-third of total biomass and the reactor showed no significant improvement in hydrogen yield. In Phase III, when all of the biomass was heat treated, the hydrogen production rates increased from 5.0 L/day to 8.3 and 13.0 L/day, respectively on day 113 and day 129. The corresponding hydrogen yields increased from 0.85 mole H_2/mole sucrose to 1.42 and 2.2 mole H_2/mole sucrose, respectively on day 112 and day 130. Thus, the repeated heat treatment was effective in selecting hydrogen producers and activating spore germination.

The microbial community analysis conducted at University of Illinois Urbana-Champaign during the experimental run time is presented in Figure 5. The studies showed that the highest level of H_2 was observed when the populations of *Clostridium* clusters I and II were active in Phases I and III. Thus, repeat heat treatment of all biomass was necessary to achieve sustainable hydrogen production. *Clostridia* are obligate anaerobic acidogenic bacteria that can form spores (endospores) to protect themselves against unfavorable environmental conditions, such as high temperature; however, when the favorable conditions return, they can geminate and become vegetative cells.

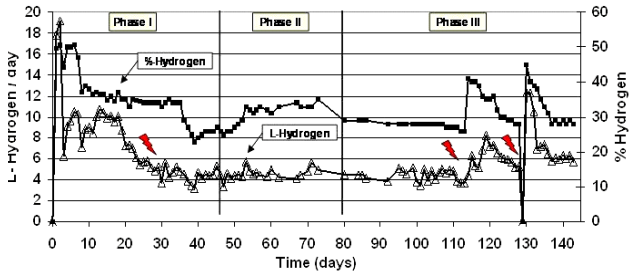

Figure 4. Biohydrogen Production Rate With Periodic Heat Treatment Bioreactor

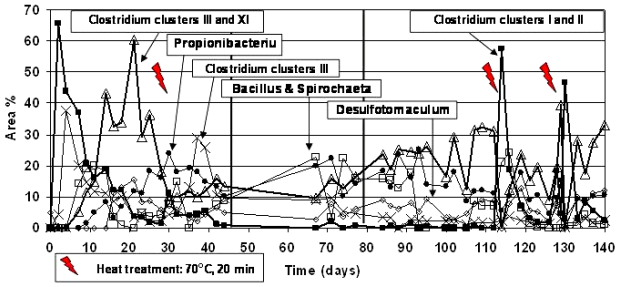

Figure 5. Dominant Microbial Populations in the Reactor as Indicated by T-RFLP Analysis With Restriction Enzyme Msei

A two-stage reactor with the first stage for hydrogen production and the second stage for culturing hydrogen producers is currently being investigated in our laboratory.

Conclusions

- An operational pH of 5.5 was shown to be optimal for hydrogen production.
- Hydrogen-producing bacteria have specific growth rates 2 to 4 times higher than the hydrogen-oxidizing methanogens.
- Both initial heat treatment of the seed inoculum and repeat heat treatments of the biomass during the reactor operation promoted hydrogen production by eliminating non-spore forming hydrogen-consuming microorganisms and by activating spore germination.
- Sustainable hydrogen production was possible with pH control and repeat heat treatment of settled sludge at 70°C for 20 minutes.
- Terminal restriction fragment length polymorphism (T-RFLP) analysis showed that *Clostridium* clusters I and II and *Bacillus* species were dominant populations in the bioreactors.

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3. Simmons J J, Padmasiri S, Duangmanee T, Sung S and Raskin L. Microbial study during hydrogen fermentation in continuous flow bioreactors. *Annual Meeting of the Society for Industrial Microbiology*, August 10-14, 2003, Minneapolis, MN.