

Introduction

Currently, approximately half of all hydrogen is produced using natural gas (1). Greener methods use wind, solar, and other sustainable energy sources to generate electricity for electrolysis. This, however, requires large amounts of water and energy, making it inefficient. Because of the known links between carbon dioxide and global warming, there has been an increasing interest in finding hydrogen production methods that produce little or no carbon emissions.

A promising and investigated method is the biological production of hydrogen through the degradation of carbon sources by anaerobic bacteria in a process called anaerobic fermentation (3). Fermentative hydrogen production is the anaerobic conversion of organic substrate to hydrogen by bacteria.

One possible organic substrate is glycerol. Glycerol is a by-product of biodiesel production. With the recent increase in biofuel manufacturing, crude glycerol is produced in large quantities as a waste product. Because glycerol has a highly reduced nature of carbon, there is potential for using it in fermentative metabolism (3).

Few microorganisms are able to metabolize glycerol fermentatively. Fermentative metabolism of glycerol has been studied in several species of the *Enterobacteriaceae* family, including *Citrobacter* (3). Research is currently being conducted to enhance fermentative hydrogen production to increase hydrogen yields for an energy efficient recovery from the substrate.

Objectives:

- Compare the fermentative process of *Citrobacter* using glycerol and glucose as substrates
- Investigate the amount and rate that hydrogen can be produced by *Citrobacter* when the substrate is varied.



Figure 1. Materials used for the fermentation bottles

Materials and Methods

A pre-culture media solution, called MGM, consisting of potassium chloride as a potassium source, ammonium chloride as a nitrogen source, a phosphate buffer, and a mineral and vitamin solution was used. Glass bottles with stoppers and aluminum seals were used to insure the bacteria were growing in an anaerobic environment (Fig. 1). The fermentation bottles were filled with 60 mL of MGM solution. Four replicates of each substrate were tested. Each bottle was purged with nitrogen to remove oxygen, then autoclaved to prevent contamination. The bottles, excluding the controls, were inoculated with *Citrobacter*. These bottles were then placed in a heated (30°C) shaker (150 rpm) compartment. At time increments of 24 hours, samples were taken from each bottle in a clean hood. At each sampling, excess pressure was removed and the headspace gas composition and pH of the bacteria/media solution were measured.

Results

For *Citrobacter* in MGM and glycerol, the hydrogen content peaked four days after inoculation, then remained constant (Fig. 2). For MGM, glycerol, and sodium carbonate, the bacteria growth was more gradual with the maximum hydrogen content at 11 days (Fig. 3). For MGM, glycerol, and sodium bicarbonate the maximum hydrogen production occurred after 11 days (Fig. 4). *Citrobacter* produces carbon dioxide in addition to hydrogen, approximately 4% of the headspace for glycerol and 6.5% for both sodium carbonate and bicarbonate (Figs. 2-4). As more hydrogen is produced, the solution becomes more acidic (Fig. 5). Compared to the pH changes of glycerol fermentation, the pH of glucose fermentation becomes acidic more quickly (Fig. 6).

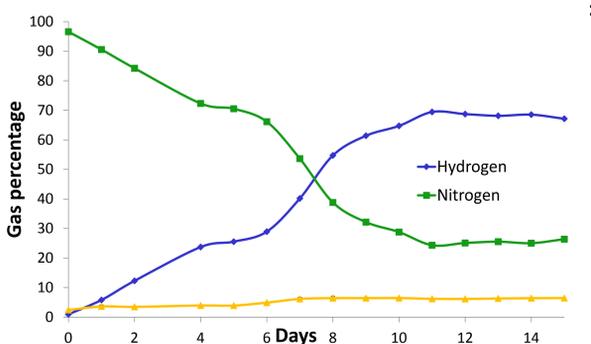


Figure 3. Headspace composition measurements for *Citrobacter* using MGM medium, glycerol, and sodium carbonate.

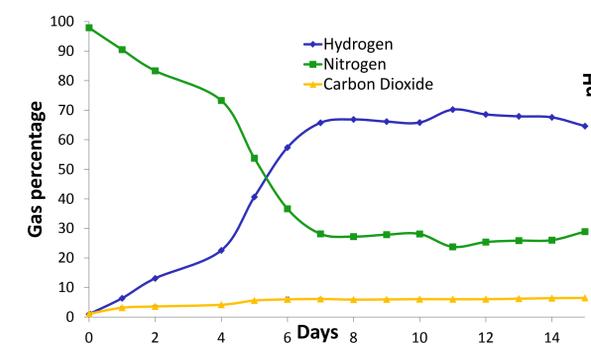


Figure 4. Headspace composition measurements for *Citrobacter* using MGM medium, glycerol, and sodium bicarbonate.

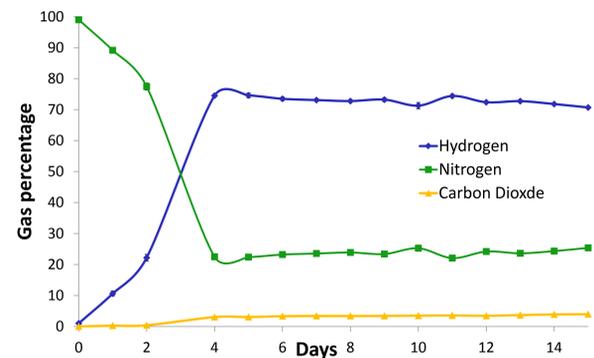


Figure 2. Headspace composition measurements for *Citrobacter* using MGM medium glycerol.

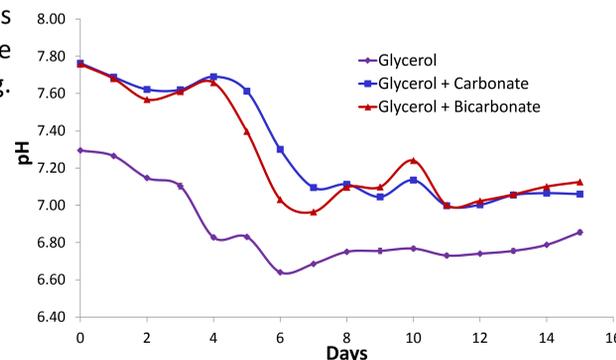


Figure 5. pH change over time for *Citrobacter* in MGM medium, glycerol, and varying additives

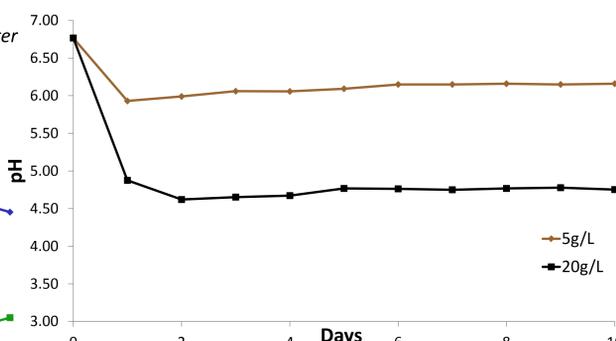


Figure 6. pH change over time for *Citrobacter* in MGM medium and glucose (5 g/L and 20 g/L)

Discussion

Citrobacter in a solution of MGM with glucose as the substrate produced 75% to 79% hydrogen while *Citrobacter* with a substrate of glycerol produced 68% to 74% hydrogen. The substrate with sodium bicarbonate produced the least amount of hydrogen and the most amount of carbon dioxide suggesting that it is not the ideal substrate. The maximum hydrogen headspace composition for both glucose and glycerol substrates were similar, however, the time it took to reach that percentage was different. When *Citrobacter* had a substrate of glucose, it approached its maximum hydrogen content between 1–2 days. When using glycerol as a substrate the hydrogen production and pH changes were more gradual. The sudden drop of pH in glucose fermentation might prevent further utilization of the substrate. This suggests that compared to glucose, glycerol is a better substrate for hydrogen production.

Future research:

- Use different concentrations of glycerol (ex. 5 g/L, 10 g/L, 20 g/L)
- Take HPLC measurements at each sampling interval
- Measure headspace gas composition and pH of bacteria/media solution at shorter intervals

References

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- Rabaey, Korneel, Peter Girguis, and Lars K. Nielsen. "Metabolic and practical considerations on microbial electrosynthesis." *Current Opinion in Biotechnology* 10 Jan. 2011: 1-7.
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Acknowledgements