Hydrogen (H_2) Production with Carbohydrate Supplementation and pH Stress of *Escherichia coli*

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ABSTRACT

Escherichia coli cultures were grown in a microoxic environment to stimulate anaerobic fermentation and respiration. Cultures grown in potassium enriched Luria Broth (LBK) or minimal media (M63) were supplemented with glycerol (40 mM), sorbitol (20 mM), or potassium formate (20 mM). Using a Clark type Hydrogen Microsensor, the rate of hydrogen gas production during middle to late log phase of each culture was measured. *E. coli* W3110*ΔhybC* was grown in LBK buffered to pH 5.0, pH 6.0, pH 7.0, or pH 8.0 with appropriate buffering solutions (HOMOPIPES, MES, MOPS, and TAPS, respectively). These pH-adjusted cultures were similarly tested for rates of H_2 production. Production rates from all cultures were corrected for optical density (OD_{600}) of respective cultures using UV-VIS spectrometry. Potassium formate supplementation led to a dramatic increase in dihydrogen evolution (445±22 µM/Min formate vs. 40±4 µM/Min LBK). Metabolism of sorbitol lead to a higher rate of H_2 evolution. For the *E. coli* W3110*ΔhybC* strain, the rate of production was highest at pH 6.0 and 7.0

INTRODUCTION

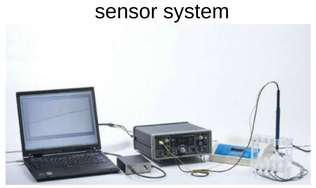
- Hydrogen gas is a sustainable alternative to a petrol based fuel economy.
- Despite being the most abundant element in the universe, H_2 is rarely found in our atmosphere.
- Hydrogen gas (H_2) is a clean burning fuel that combusts in the presence of O_2
- Gram for gram, hydrogen contains more energy than conventional fuels (142 MJ/kg in H_2 vs. 44.2 MJ/kg oil).
- Under fermentative conditions, *Escherichia coli* produces H_2 gas as a by-product.
- Biological production of H_2 is appealing because currently used methods of production, such as steam reformation ($CH_4 + H_2O \rightarrow CO + 3H_2$ *191.7 kJ/mol*) requires a large energy input.
- Non-virulent strains of *Escherichia coli* utilize several endogenous enzymes called hydrogenases that are capable of consuming ($H_2 \rightarrow 2H^+ + 2e^-$) or producing ($2H^+ + 2e^- \rightarrow H_2$) hydrogen. These enzymes serve several purposes to the bacteria, such as pH regulation.
- Currently, four bi-directional hydrogenases (*hya, hyb, hyc, hyp*) have been characterized in *E. coli*.
- Though it is a bi-directional enzyme, the third hydrogenase (*hyc*) has been demonstrated to preferentially catalyze the production of H_2.

MATERIALS AND METHODS

Strains and Constructs

Escherichia coli strain W3110 was used in the formation of mutation constructs deficient in one or more hydrogenase complexess. A mutant strain deficient in the consumption hydrogenase 2 (W3110 *ΔhybC*) was previously constructed by Dan Riggins (´12) through a transduction from the KEIO collection (*JWK2961*). The construction of a W3110 *ΔhypF* strain was completed by transduction from Keio strain JWK5443 (Khalid Eldahan ´10). This *hypF* was shown to be defect in all uptake and production hydrogenases.

Unisense© Hydrogen Micro-sensor system



Growth Conditions and H_2 Measurement

Microoxic cultures were grown overnight (16 hours) in 8 mL anaerobic tubes containing 8mL unbuffered Luria-Bertani broth supplemented with 100mM KCl. Serial dilution (1:100) into fresh LBK containing the appropriate carbohydrate and buffered to the desired experimental pH was done after the overnight growth period. Cultures were grown into mid-late log phase (OD_{600} 0.4 to 0.6) prior to measuring H_2 production rates. From each culture, a 2 mL sample was placed into a glass micro-respiration chamber (Unisense©) and sparged with nitrogen gas (100%) for 45 seconds. Cultures were mixed continuously with a magnetic stirrer set at 200 rpm and at 37°C while the hydrogen sensor was introduced. H_2 production of each culture was measured for 10 minutes after a 2-minute sensor equilibration period.

RESULTS

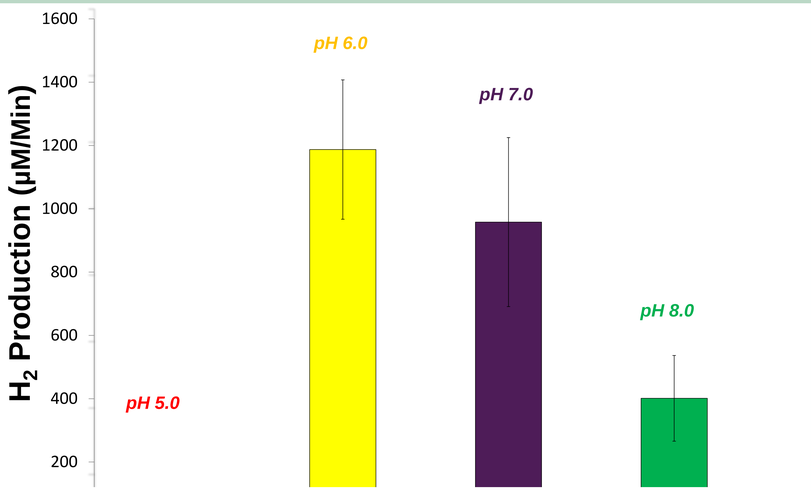


Figure 1. H_2 production of *E. coli* W3110 *ΔhybC* grown in Luria Broth with supplemental 100 mM KCl in place of NaCl (LBK). As specified, cultures were raised in LBK media buffered with the following buffers: HOMOPIPES (pH 5.0), MES (pH 6.0), MOPS (pH 7.0), and TAPS (pH 8.0). H_2 production values have been corrected for each culture's respective optical density at 600 nanometers.

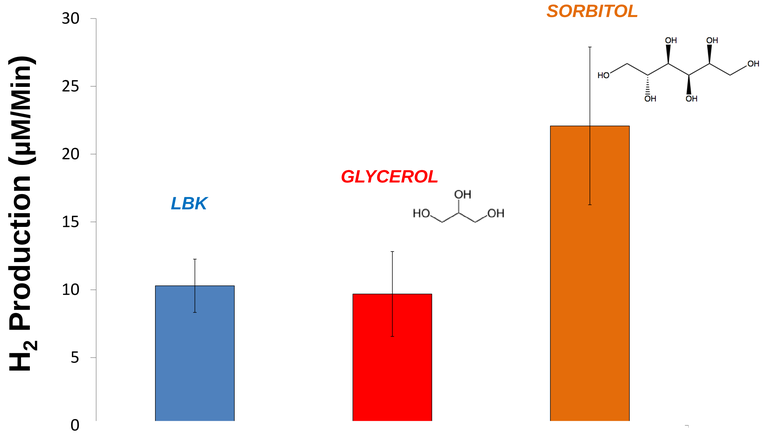


Fig. 2 H_2 production of *E. coli* W3110 grown in LBK with glycerol (40 mM) or sorbitol (20 mM) supplementation. Values were corrected for culture density (OD_{600}).

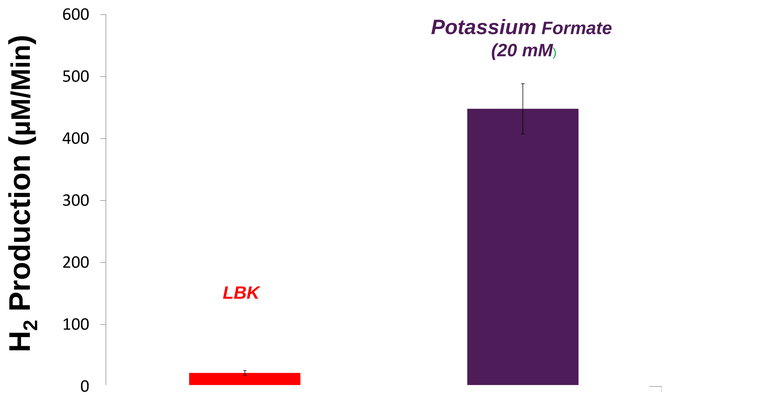


Fig. 3 H_2 production from *E. coli* W3110 cultures grown in LBK and LBK supplemented with Potassium Formate (20 mM). Production was higher when supplemented with formate (445±22 µM/Min formate vs. 40±4 µM/Min LBK). Values have been corrected for culture density (OD_{600}).

DISCUSSION

Figure 4. Metabolic schematic of hydrogen production mediated by the formate-hydrogen lyase system (FHL). Diagram adapted from Sawers *et. al* (2005).

- Formate supplementation was effective for increasing hydrogen production in *Escherichia coli* W3110. A 10-fold increase in H_2 evolution was observed with 20 mM potassium formate.
  - ➢ Formate is a precursor to H_2 synthesis (Figure 4).
- W3110 *ΔhybC* exhibited higher production rates at an acidic pH (pH 6.0).
  - ➢ This supports previous findings that *hyc* is important for acid-resistance.
- W3110 supplemented with sorbitol exhibited higher rates of hydrogen production than glycerol supplementation.
  - ➢ Because it is a by-product of biodiesel synthesis, use of glycerol as a fermentation substrate would be ideal in producing hydrogen gas. However, *E. coli* do not readily ferment glycerol in the anaerobic environment needed for hydrogenase function. Other sugar alcohols, like sorbitol, might be more suitable substrates.
- A next step in this investigation would be to supplement W3110 *ΔhybC* strains with formate and grow them at a lower pH. A more extensive screening of sugar alcohols might find a more efficacious substrate. Furthermore, exposure to weak permeant acids, such as benzoate, might provide further insights into pH dependence of hydrogen production through the FHL system.
- With a more thorough understanding of bacterial hydrogen production, we can engineer bacteria that can function for hydrogen fuel production on the industrial scale.

Exciting other work!

-Researchers have transplanted enzymes from cyanobacteria into *E. coli* to make strains to overproducing hydrogen (Maeda et. al. 2007).
-Other researchers have targeted and modified transcriptional factors in *E. coli* to get higher hydrogen yields (0.96 mol H_2/mol glucose vs. 0.54 mol H_2/mol glucose mol glucose in wild type *E. coli.*)(Zhanmin et. al. 2008).

REFERENCES

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