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# Syngas fermentation to biofuels: Effects of hydrogen partial pressure on hydrogenase efficiency

*Bradley E. Skidmore, Ryan A. Baker, Dila R. Banjade, Jason M. Bray, Douglas R. Tree, Randy S. Lewis**

*Department of Chemical Engineering, 350 CB, Brigham Young University, Provo, UT 84602, United States*

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ABSTRACT

Producing biofuels from gasified biomass (synthesis gas) via microbial fermentation is currently being pursued as one alternative in biofuels development. In synthesis gas fermentation, reducing equivalents from $H_2$ oxidation via hydrogenase is important towards directing more carbon towards product formation. In this work, kinetic studies of $H_2$ utilization via the *Clostridium P11* hydrogenase enzyme were performed to determine the most appropriate model to predict hydrogenase activity as a function of $H_2$ partial pressure. An important aspect of this work included the proper analysis of electron acceptors used in the kinetic studies. The $K_{H_2}$ model parameter governing the effect of $H_2$ partial pressure on activity was ~30 kPa (absolute), independent of the type and concentration of electron acceptor. The $K_{H_2}$ value indicates that $H_2$ partial pressures typically associated with syngas fermentation will result in compromised efficiency of the hydrogenase activity.



## 1. Introduction

Volatile fuel prices, diminishing world oil supply, and concern over increasing carbon dioxide emissions have created a desire to find alternatives to traditional oil-derived transportation fuels. Biofuels from renewable resources are among the most sought after alternatives. The most prevalent bio-derived fuel currently being used as a replacement for gasoline is ethanol. Virtually all of the commercial ethanol produced comes from the fermentation of simple sugars available from biomass such as corn, sugar cane, and sugar beets. However, due to the competition with food and the limited supply of feedstock, the development of biofuels from lignocellulosic materials is vigorously being pursued [1].

Two prevalent methods being explored for producing biofuels from lignocellulosic material include biological use of sugars obtained from hydrolysis of the biomass or use of syngas obtained via gasification of the biomass. Syngas is primarily composed of carbon monoxide (CO), hydrogen ($H_2$), and carbon dioxide ($CO_2$), although other impurities are present depending upon the biomass feedstock. Syngas can be converted to biofuels either by chemical catalysis (e.g. Fischer–Tropsch Synthesis) or microbial fermentation routes. The latter process has several advantages over metal catalysis

* *Corresponding author*. Tel.: +1 801 4227863; fax: +1 801 4220151.
E-mail address: randy.lewis@byu.edu (R.S. Lewis).
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that include better selectivity, lower processing temperatures, and fewer effects due to syngas impurities [2]. However, challenges still exist for microbial fermentation, including low cell density and low mass transfer rates for the gas [3]. Reports have noted the potential of 90–105 gallons of ethanol per ton of biomass for both syngas routes [4,5].

For the syngas/fermentation route, several microbial catalysts, primarily acetogenic bacteria, are capable of consuming syngas and producing useful end-products including alcohols and acids. Examples include *Clostridium ljungdahlii* [6], *Butyribacterium methylotrophicum* [3], *Clostridium autoethanogenum* [7], and *Clostridium carboxidivorans* [8]. Syngas fermentation usually occurs via the "Wood–Ljungdahl" pathway [9] where reducing equivalents are required in addition to the carbon that is incorporated into the backbone of the biofuel. As part of the overall pathway using several oxidation–reduction reactions, two converging pathways (one using $CO_2$ as the entering substrate and the other using either CO or $CO_2$ as the entering substrate) form a two-carbon acetyl CoA unit from which alcohol or acid products can be generated. Reducing equivalents needed for the metabolic process are obtained by either the oxidation of $H_2$ via the hydrogenase enzyme [10] or the oxidation of CO via the carbon monoxide dehydrogenase (CODH) enzyme. For industrial processes, it is preferred to obtain reducing equivalents from $H_2$, as this leaves the carbon in CO available for incorporation into the desired products. Processes that depend on CO oxidation to supply reducing equivalents greatly reduce the carbon-to-product yield.

Since syngas compositions can vary depending upon the gasification process [11] and the use of hydrogenase as a generator of reducing equivalents is important for maximizing the carbon-to-product yield, it is important to assess the efficiency of hydrogenase in the presence of various $H_2$ partial pressures. $H_2$ partial pressure may vary within a reactor according to the $H_2$ feed gas composition and the utilization rate of $H_2$. The rate at which reducing equivalents are produced by hydrogenase in a biological process depends both upon the total number and type of hydrogenase enzymes present in cells and the efficiency with which hydrogenase performs its metabolic function. Efficiency defined here refers to the actual rate at which reducing equivalents are generated relative to the maximum possible rate at which they may be generated. Genetic engineering may eventually be used to over-express the number of enzymes in a cell [12], but changing the number of enzymes present does not improve the efficiency at which each enzyme operates.

Three enzymes in the "Wood–Ljungdahl" pathway (hydrogenase, formate dehydrogenase (FDH), and CODH) use the primary gases of the syngas feed stream ($H_2$, $CO_2$, and CO) as their substrates and can have their efficiencies affected by the partial pressures to which the enzymes are exposed. This work explores the effect of $H_2$ partial pressure on the hydrogenase efficiency and develops a kinetic model to predict the efficiency. Development of an efficiency model allows greater insight on how varying $H_2$ partial pressures resulting from differing feedstocks, various gasification processes, and reduction of $H_2$ during the reaction process can affect the enzyme efficiency of generating electrons during syngas fermentation for the production of biofuels.

## 2. Materials and methods

### 2.1. Microorganism

A novel clostridial species denoted as P11, which can utilize syngas for ethanol production, was kindly provided by Ralph Tanner of the University of Oklahoma following isolation from an agricultural lagoon. P11 was passaged in 30 mL media (described below) three times using strict anoxic techniques in the presence of 40% CO, 30% $H_2$, and 30% $CO_2$. During the exponential growth phase, a 10% inoculum obtained from the third passage was added to 100 mL of media contained in a 250 mL capped bottle. The headspace was purged with 40% CO, 30% $H_2$, and 30% $CO_2$ at 221 kPa (note: all pressures reported in text are absolute values) and the gases were replaced on nearly a daily basis. This latter bottle was used to obtain cell samples for the hydrogenase assay. Cell samples were taken during the stationary phase of cell growth. All studies were at 37 °C.

### 2.2. Media

The media contained per liter: 25 mL mineral stock solution, 10 mL calcium stock solution, 10 mL trace metals stock solution, 10 mL vitamin stock solution, 10 g morpholinoethanesulfonic acid (MES) buffer, 0.5 g yeast extract, and 10 mL cysteine-sulfide solution (composed of 40 g/L L-cysteine and 40 g/L sodium sulfide nonahydrate). Resazurin, a redox potential indicator, was added (10 drops) to indicate the presence or absence of oxygen. The mineral stock solution contained (per liter) 20 g magnesium sulfate heptahydrate, 10 g potassium chloride, and 10 g potassium phosphate monobasic. The calcium stock solution contained (per liter) 10 g calcium chloride dihydrate. The trace metals stock solution contained (per liter) 2 g nitrilotriacetic acid (adjusted to pH 6.0 using potassium hydroxide), 1 g manganese (II) sulfate, 0.2 g cobalt (II) chloride hexahydrate, 0.2 g nickel (II) chloride hexahydrate, 0.1 g anhydrous sodium selenate, 0.8 g ammonium iron(II) sulfate hexahydrate, 1 g 99% zinc sulfate heptahydrate, 0.02 g sodium molybdate dihydrate, and 0.2 g sodium tungstate dihydrate. The vitamin stock solution contained (per liter) 0.005 g p-(4)-aminobenzoic acid, 0.002 g D-biotin, 0.005 g D-pantothenic acid hemicalcium salt, 0.002 g folic acid, 0.01 g sodium 2-mercaptoethanesulfonate (MESNA), 0.005 g nicotinic acid, 0.01 g pyridoxine hydrochloride, 0.005 g riboflavin, 0.005 g thiamine hydrochloride, 0.005 g thioctic acid, and 0.005 g vitamin B-12. Following mixing, the media pH was adjusted to 6.0 using a 5 N potassium hydroxide solution. All chemicals were purchased from Sigma Chemical Company, St. Louis, MO. When needed for cell use, media was purged of $O_2$ using strict anoxic techniques following which the cysteine-sulfide solution noted above was added and the media was autoclaved at 121 °C for 15 min.

### 2.3. Hydrogenase assay solutions

An electron acceptor solution and a cell solution were prepared for each hydrogenase assay in separate 13 mL Hungate test tubes. The electron acceptor solution was composed of