Influence of Carbon Source Pre-Adaptation on Clostridium ljungdahlii Growth and Product Formation

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Abstract

Syngas fermentation is considered an alternate processing method for biofuel and biochemical production as part of thermochemical biomass conversion. Exposure of syngas fermenting microorganisms to sugars, either in the primary syngas fermentation or through pre-adaptation in the seed culture, has the potential to enhance overall fermentation performance and stress tolerance. In this rapid communication, Clostridium ljungdahlii was grown on different carbon sources including syngas only, syngas-fructose and fructose only to identify ideal pre-adaptation conditions for ethanol and acetate production from subsequent cultures grown in reactors containing syngas only or fructose-syngas substrates. In syngas only reactors, cultures pre-adapted to fructose had faster cell production rates (2X) and at least 83% higher ethanol and 16% higher acetate formation than cells pre-adapted on syngas or syngas-fructose. In syngas-fructose reactors, cultures did not show significant growth or acetate production differences under pre-adaptation treatments. Nevertheless, in these syngas-fructose reactors, cultures pre-adapted on syngas and syngas-fructose had nearly 20% higher ethanol production than those pre-adapted on fructose. Among pre-adaptation treatments, fructose had better results in syngas only reactors than syngas-fructose reactors. However, the presence of syngas in pre-adaptation cultures was better overall for ethanol production.

Keywords: Clostridium ljungdahlii; Synthesis gas fermentation; Pre-adaptation; Ethanol; Acetate; Fructose

Introduction

Conversion of synthesis gas to liquid fuels by biological catalysts has been suggested as a promising technology to achieve oil independence [1]. In this method, cellulosic biomass and other carbon sources (coal, industry and/or municipal waste, etc.) are converted to synthesis gas (syngas) by a well known process called gasification [2]. Syngas typically consists of carbon monoxide, hydrogen, carbon dioxide, methane, trace amounts of C3 hydrocarbons, water vapor, nitrogen and various contaminants including ash and tar [2]. This syngas is then fed to a microorganism which can utilize it as a carbon and energy source. The products of this fermentation could be alcohols (e.g. ethanol or butanol) and organic acids (e.g. acetate or butyrate) [3]. There have been a number of microorganisms isolated for use in this process, including C. ljungdahlii, C. autoethanogenum, C. carboxidivorans P7, C. ragsdalei, Alkalibaculum bachi and Butyribacterium methylotrophicum [4-7]. In addition, some companies are en route to commercializing their methods [8].

C. ljungdahlii, which has been studied in regard to synthesis gas fermentation [9,10], was one of the early microorganisms described to be able to ferment syngas components to ethanol and acetate via the Wood-Ljungdahl pathway. In addition to CO and H2, C. ljungdahlii can use sugars as a carbon source, with fructose being its preferred sugar for growth and product formation [11]. Growth of C. ljungdahlii solely on fructose results in faster growth and product formation rates compared to cultures provided only syngas [10,12,13]. Fermentation on sugars provides cells with increased reductant and energy to support primary metabolism as well as synthesis of enzymes that help cells manage environmental stresses, thereby enhancing overall culture productivity. On the other hand, primary syngas fermentations present challenges to cell metabolism as CO fixation is a reductant intensive process, and the ability to overcome growth inhibition resulting from substrate utilization and product formation is compromised [14]. Exposure of C. ljungdahlii to sugars, either in the primary syngas fermentation or through pre-adaptation in the seed culture, may enhance overall fermentation performance and stress tolerance.

In this rapid communication, the physiological advantage of growth on sugars is discussed in terms of C. ljungdahlii ability to acclimate and perform in syngas cultures, which has not been previously addressed in the syngas fermentation literature. Specifically pre-adaptation of cultures was explored to establish its significance on end-product formation (ethanol and acetate). C. ljungdahlii was grown on different carbon sources (syngas and fructose combinations) in a continuous gas feed, batch liquid system. Growth and product formation were evaluated for the fermentations that were initiated using cells pre-adapted to fructose, syngas, and mixed fructose-syngas substrates.

Materials and Methods

Organism and medium preparation

C. ljungdahlii (ATCC 55383) was obtained from the American Type Culture Collection. Cells were grown at 37°C on a modified Reinforced Clostridial Basal medium with additional salts, vitamins, and trace elements adopted from the ATCC 1754 PETC medium (RCM.NA.SVE) under N2 (pH 6.8) unless otherwise indicated [12]. Cultures provided with syngas (7.5 ml/min) were grown in a fermentation reactor presented in Figure 1A [13]. Bottled syngas used was an artificial mix of 50% N2, 20% CO, 20% CO2, and 10% H2. When added to...
medium in combination with syngas, fructose was used at 2.5 g/L. All cultures were inoculated at 5% v/v.

Inoculum preparation using different carbon sources

*C. ljungdahlii* was grown on RCM.NA.SVE (will now be referred to as medium). Cultures were initiated from a freezer stock (-80 ºC) and incubated (37ºC, 72 h) before 3 serial transfers every 24 h to achieve steady growth in medium with fructose (5 g/L). Active cells (472 mg dry cells/L) were transferred to carbon sources [medium-syngas, medium-syngas-fructose (2.5 g/L) or medium-fructose (5g/L)] to start seed cultures for fermentation studies. These seed cultures had an adaptation period of 18-24 hours. Fermentation reactors containing medium-syngas and medium-syngas-fructose at a working volume of 250 ml were inoculated with the three different seed cultures (472 mg dry cells/L in inoculum; 5% v/v addition). Medium seed culture combinations were grown in triplicate at 37 ºC without mechanical agitation Figure 1B. Cell growth was monitored and liquid and gas samples were taken every 6-8 h.

Substrate and end product analysis

Headspace gases were analyzed by gas chromatography (GC) on a Carbosieve S-II, 100/120 mesh stainless steel column using a thermal conductivity detector (Shimadzu GC-17A). Ethanol and acetate concentrations were determined in acidified samples by GC (Shimadzu LC20) as follows: liquid samples (600 μL) were centrifuged (10 min, 18,400 x g, 25°C). Supernatants were filtered through a 0.22 μm syringe filter into a crimp vial and analyzed on a HPX-87H column (65ºC) using a refractive index detector. The eluent (0.6 ml/min) was sulfuric acid (5 mM).

Statistical analysis

Cell growth and liquid product analysis from different pre-adaptation sources were evaluated using the General Linear Model (GLM) in SAS® Version 9.1 (SAS Inc., Cary, NC, USA). Assessment of statistical significance for pre-adaptation on different carbon sources and different production growth substrates was set at P < 0.05.

Results and Discussion

Growth of *C. ljungdahlii* and product formation in syngas reactors

*C. ljungdahlii* cultures were pre-adapted on three different carbon source combinations (syngas, syngas-fructose or fructose). Subsequently, these cells were transferred to fermentation reactors with either syngas or syngas-fructose medium. In syngas only reactors, cells pre-adapted on fructose produced the highest cell densities reaching maximum growth after 24 h Figure 2A. This was unexpected given that these cells would have to switch from fructose to syngas fermentation. Syngas and syngas-fructose pre-adapted cells reached statistically significant maximum densities after 40 and 56 h, respectively. Cells pre-adapted on syngas-fructose showed the longest lag-time, contributing to increased time to reach peak growth, where fructose only cultures had cell production rates of 10.4 mg/L/h, while syngas and syngas-fructose cultures both had cell production rates of 5 mg/L/h (calculated during exponential growth phase). Interestingly, after 56 h cell density was statistically similar independent of pre-adaptation medium, possibly due to product inhibition or depletion of an essential nutrient in the cultures pre-adapted to fructose.

Ethanol production in syngas reactors was significantly higher (P < 0.05) when cells were pre-adapted on fructose, reaching a maximum of 2.2 mM at 64 h of growth Figure 3A and seemed to be non-growth-associated since these cells reached stationary phase after 24 h. Cells pre-adapted on syngas also reached maximum ethanol production after 64 h, while cells pre-adapted on syngas-fructose reached a maximum after 48 h. Although acetate is considered to be a growth associated product, it was continuously accumulated during the growth phase as well as after the stationary phase was reached Figure 3C. Cells pre-adapted on fructose produced significantly higher acetate concentrations than cells pre-adapted on syngas and syngas-fructose after 56 h (P < 0.05) with a maximum of 34.3 mM. Again, in syngas reactors, cells pre-adapted to syngas-fructose show a lag time in acetate production when compared to fructose or syngas pre-adapted cells but reached comparable concentrations by the end of the experiment. Despite final cell concentrations reached, cells pre-adapted on fructose demonstrated increased productivity and rate of cell, ethanol and acetate production. If culture conditions were modified to limit growth inhibition, cells pre-adapted on fructose would provide even greater conversion efficiency in syngas fermentations, especially those cultured beyond 24 h.

Growth and product formation in syngas-fructose reactors

Significant growth differences were not observed among the three pre-adaptation sources in syngas-fructose reactors Figure 2B. These cultures did not show a lag-time and reached maximum cell density after 24 h. Cell production rates were approximately 43 mg/L/h for syngas and fructose pre-adapted cultures and 37 mg/L/h for syngas-fructose.
pre-adapted cultures (calculated during exponential growth phase). Cells growing in syngas-fructose reactors had higher cell densities (P < 0.05) than cells growing in syngas reactors (787 mg/L vs. 332 mg/L), independent of pre-adaptation source.

Ethanol concentration in syngas-fructose reactors was statistically higher in cultures pre-adapted to syngas only and syngas-fructose than those pre-adapted on fructose Figure 3B. Pre-adaptation had no effect on acetate production as all cultures produced statistically the same amount of acetate Figure 3D. Independent of pre-adaptation, both ethanol and acetate production seem to be growth associated as production decreases when cells reach stationary phase. It has been previously suggested that ethanol production occurs in non-growth conditions [10]. We observe this trend in syngas only medium but not when fruc-
Based on this study, it was concluded that for syngas-fructose reactors, pre-adaptation is not necessary as the overall fermentation was not affected, but in syngas only reactors a fructose pre-adaptation will be beneficial as it increases cell numbers and liquid products. The growth history of *C. ljungdahlii*, and likely other autotrophic bacteria, as it relates to metabolic capacity (i.e. bioenergetics, pathway induction) is important to cell performance in syngas reactor systems.

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