

## Conversion of syngas to ethanol via a new microbial species

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The development of renewable biofuels is a national priority motivated by both economic and environmental concerns, including reduction of greenhouse gas emissions, enhancement of domestic fuel supply and maintenance of rural economies. A multidisciplinary, multi-institutional team has been established in Oklahoma to apply a holistic approach to the conversion of low-cost biomass to ethanol.

The holistic approach involves the following steps:

- ?? Production, harvest and storage of agricultural crops from native grasslands. Switchgrass is currently the model crop.
- ?? Gasification of switchgrass using a fluidized-bed reactor to generate syngas ( $\text{CO}_2$ ,  $\text{CO}$ , and  $\text{H}_2$ ) and downstream processing of syngas to eliminate deleterious compounds; i.e. tar, ash, and other contaminants.
- ?? Microbial conversion of syngas to ethanol under anaerobic conditions in a bubble column bioreactor. Novel acetogens, identified by the research team, convert syngas to liquid products such as ethanol, butanol and acetate.
- ?? Economic analysis of the entire process from growth of the switchgrass to production of ethanol.

This work describes the initial studies of the microbial conversion of syngas components ( $\text{CO}$  and  $\text{CO}_2$ ) to ethanol using a recently isolated microbe (named P7) obtained from an agricultural lagoon. P7 is a novel clostridial species, based on 16S rDNA sequence analysis. The gram-positive, motile, rod-shaped bacterium grows on  $\text{CO}$ ,  $\text{CO}_2$ , and  $\text{H}_2$ . In addition to syngas components, the organism can also utilize sugars as carbon sources. The optimum pH for growth and ethanol production is in the range of 5 to 6.

Experiments were performed in a four-liter bubble column bioreactor made of plexiglass with a one-liter headspace. A fritted glass fiber disc with a pore size between 4 and 6 microns was assembled to the base of the column to disperse the feed gas to tiny bubbles. Liquid in the bubble column was recirculated for better mixing. A portion of the recycle stream was withdrawn as the product. The liquid feed was prepared in 20-liter glass tanks and fed to the bioreactor through a sterilization filter using a peristaltic pump. The feed gas flow rate was 200 ccm at 5 psig and consisted of  $\text{CO}$  (25%),  $\text{CO}_2$  (15%) and  $\text{N}_2$  (60%) blended from bottles. Hydrogen was not used in the initial study. The pH of the media was initially 5.75 and, as the reaction proceeded, was controlled at 5.2 using 1N NaOH. A pH controller was used to maintain the pH at the desired range. The reactor temperature was maintained at 37°C using a hot water jacket. Under sterile conditions the inoculum was transferred to the bioreactor and the cells were grown for at least 3 days in batch-mode. Following batch growth, a chemostat mode (continuous product and feed flow rates of 2 ml/min) was initiated. The cell concentration, inlet and outlet gas concentrations, and the exiting product concentrations (ethanol, butanol, and acetic acid) were measured at discrete time intervals.

Several continuous runs were performed lasting for over ten days. Initially, cell instability was observed following the first 5 to 7 days of operation. The instability was a result of the depletion of sodium sulfide in the liquid due to the stripping action of the gas phase and consumption by the cells. In later experiments, sodium sulfide was batch fed to maintain at least 0.1 ppm in the media, resulting in stable cell concentrations for as long as 30 days of operation (longer times were not assessed). Experiments

performed without CO<sub>2</sub> in the feed gases resulted in no cell growth even though a net CO<sub>2</sub> production was observed in the presence of CO/CO<sub>2</sub> feed gas.

The ability of P7 to produce butanol was observed and confirmed subsequently at the culture level. The ethanol concentration as measured by gas chromatography reached a steady state value of 0.2 wt%. The corresponding acetate concentration was less than 0.03 wt%. The butanol concentration reached a maximum value of 0.11 wt% but varied more significantly with time. Butyrate, an intermediate for the production of butanol, was not detected in measurable quantities. The net carbon recovery in the products and cells was determined by a carbon balance on the bioreactor. The overall carbon balance showed that greater than 90% of the consumed carbon was accounted for in ethanol, butanol, acetate, net CO<sub>2</sub> production and cell growth. The amounts of products stripped by the gas stream and that of dissolved gases in the product liquid were not initially considered for the carbon balance.

The experimental yield of products was around 25% (mole C in products produced/mole CO consumed) under steady state conditions. Theoretically, if ethanol was the sole product, 33% (molar carbon basis) of the CO could be converted to ethanol. The experimental yield of CO<sub>2</sub> was around 60% compared to the theoretical yield of 67% with ethanol as the sole product. At steady state, the apparent yields of ethanol, butanol, and acetate (mole C in products/mole CO consumed) were 0.15, 0.075 and 0.025 respectively. The yield of ethanol from CO as compared to acetate and butanol is higher by 6 and 2 times respectively, establishing a high level of product selectivity and specificity of P7. However, as much as 60% of CO was utilized for CO<sub>2</sub> production, as expected for growth on CO. By introducing hydrogen, the utilization of carbon for the production of ethanol can potentially be increased. Thus, P7 shows good potential for the conversion of syngas to ethanol.

Current work focuses on the utilization of hydrogen, the optimization of culture media, and improving ethanol concentrations in the bioreactor via increasing the cell concentration. Cell concentrations 10 to 20 times the current concentration have been observed in bioreactors. Thus, increasing the cell concentration 20-fold will likely increase the ethanol concentration 20-fold to acceptable levels (> 3 wt%) of distillation.