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**BIOCONVERSION OF COAL DERIVED SYNTHESIS GAS
TO LIQUID FUELS**

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Quarterly Technical Progress Report
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OBJECTIVES:

The overall objective of the project is to develop an integrated two stage fermentation process for conversion of coal-derived synthesis gas to a mixture of alcohols. This is achieved in two steps. In the first step, *Butyribacterium methylotrophicum* converts carbon monoxide (CO) to butyric and acetic acids. Subsequent fermentation of the acids by *Clostridium acetobutylicum* leads to the production of butanol and ethanol.

The tasks for this quarter were:

- Development / isolation of superior strains for fermentation of syngas.
- Optimization of process conditions for fermentation of syngas.
- Evaluation of bioreactor configuration for improved mass transfer of syngas.
- Development of a membrane-based pervaporation system
- Optimization of process conditions for reducing carbon and electron loss by H_2 - CO_2 fermentation.
- Synthesis gas fermentation in single-stage by co-culture.

SUMMARY OF TECHNICAL PROGRESS

- Isolation of CO utilizing anaerobic strains is in progress.
- The product profile for the fermentation of syngas by *B. methylotrophicum* has been investigated.
- Effect of carbon monoxide on growth of *C. acetobutylicum* has been determined.

TECHNICAL PROGRESS REPORT

2. Development of Superior Strains

We sought mutants of *B. methylotrophicum* (CO strain) that survive in the presence of FA, that lack one or more enzymes in the acetate specific pathway, and that are acetate minus. Mutants that have a diminished activity in this pathway should also have a survival advantage in the presence of FA. To obtain FA resistant mutants, PBBM plates containing 10-15 mg/l fluoroacetate were suffused with cells from actively growing cultures of *B. methylotrophicum*. After incubation these plates were used to inoculate 10 ml of PYG and allowed to grow for 48 to 72 h. Product profiles were determined using 1 ml samples that were acidified with 10 ml of 50% H_2SO_4 and centrifuged at 10,000 g. Many of the FA resistant clones that were obtained had product profiles significantly different from the parent CO strain of *B. methylotrophicum*. The amounts of acetate produced by the FA clones were reduced as much as 70% from the amount produced by the parent. The amount of butyrate produced was increased by as much as 100%. The gram ratio of butyrate to acetate ranged from 0.89 to 1.52,

up from 0.36 in the parent. Butanol, 0.3 g/l was produced by a FA resistant clone compared to a trace produced by the parent strain. The most dominant problem with the production of FA resistant strains of *B. methylotrophicum* is partial or complete phenotypic reversion to the parent type in the subculture of the original PYG tube. This reversion may be due to impure clones or genotypic reversion. The FA resistant clones maybe mixed with the parent strain that is growing or merely remaining viable in the background of the FA-glucose plates. Additional rounds of picking and streaking on FA-glucose plates may serve to purify resistant clones. The medium can to be made more inhibitory to the parent type and more selective of the resistant clones. *B. methylotrophicum* is expected to assimilate FA more readily when grown on pyruvate and recently cells have been grown with pyruvate instead of glucose to enhance the assimilation of FA. Ideally this substrate would also be used in place of glucose in FA plates, but pyruvate plates do not produce well isolated monoclonies, necessary to obtain FA resistant clones. Efforts are underway to enhance the growth on pyruvate-FA plates without defeating FA selectivity. Genotypic reversion can be overcome by screening for non-reverting clones.

3. Optimization of Process Conditions for Fermentation of Syngas

The product profile for the fermentation of syngas by *B. methylotrophicum* was evaluated in a batch system with continuous gas feed. Figure 1 gives the product and growth profiles.

5. Evaluation of Bioreactor Configuration for Improved Mass Transfer of Syngas.

Gas lift and trickle bed reactors using immobilized *B.methylotrophicum* cells will be used to maximize mass transfer.

Gas-Lift Fermentor Design and Operation

A diagram of the gas-lift fermentor is shown in Figure 2. The vessel and system are an LH 2L AirLift Fermentor and controls. Biomass is retained by gravity separation in the upper part of the fermentor to maximize solvent production. This system provides media and syngas recycle for optimum and substrate utilization. Gas circulation provides gas-liquid mass transfer, so a mechanical mixer is not required.

Sterile, fresh media is metered into the system with a Cole-Parmer Model 7520-25 peristaltic pump. Fresh syngas is controlled by a Cole-Parmer Model N062-01 rotameter and is filter-sterilized. The vessel consists of a tapered glass tube with a gas sparger at the base. The broth circulates up from the base, concurrently with the gas, and is removed by a draw tube at the top of the vessel to maintain a constant level. Broth is recirculated by a peristaltic pump and a product bleed is maintained to match the rate of fresh feed. Syngas is also recirculated by a peristaltic pump and a bleed is maintained through a rotameter and sterile filter

to match the rate of fresh syngas added. pH is controlled with an LH Model 505 controller and pumps and an Ingold DPAS pH electrode. Temperature is controlled by circulating water through the fermentor jacket using a VWR Model 1145 temperature bath. Sample ports are provided in the media and gas recycle lines for sterile sample withdrawal via syringe.

6. Synthesis Gas Fermentation in Single Stage by Co-Culture

Experiments have been designed to determine the possibility of growing a co-culture of *B. methylotrophicum* and *C. acetobutylicum*. Figure 3 reflects the effect of CO on the growth rate of *C. acetobutylicum*. The percentage of CO was varied from 66 to 20%. The higher level represents the concentration of CO in synthesis gas (65%). Information regarding the effects of 0-10% CO on the growth of *C. acetobutylicum* is available in literature (Kim et al., 1984). Hence, 20% was chosen as the lower limit. The trend in decreasing growth rate with increasing levels of CO reflects the same pattern of inhibition as reported (Kim et al., 1984).

In a separate set of experiments, the influence of CO on the butyrate uptake potentials of 'solventogenic' *C. acetobutylicum* is being investigated. It has been postulated that H₂ metabolism by *C. acetobutylicum* can be directed towards solvent production by the addition CO to the cell culture (Kim et al., 1984). However, it still remains to be determined whether the conversion of exogenous butyrate to butanol can be regulated in a similar fashion by CO.

7. Development of a Membrane-Based Pervaporation System for Continuous Removal of Alcohols from Fermentation Broth.

Pervaporation Solvent Recovery System Design and Operation

A diagram of a CSTR fermentor coupled with pervaporative solvent recovery is shown in Figure 4. Biomass is retained in the system by the same pervaporation membrane that removes the product solvents. Since pervaporation removes only volatile components, the unconverted organic acids remain for recycle to the fermentor. Gas-liquid mass transfer is provided by mechanical agitation with a Rushton-style turbine.

Sterile, fresh media is metered into the system with a Cole-Parmer Model 7520-25 peristaltic pump. Fresh syngas is controlled by a Cole-Parmer Model N062-01 rotameter and is filter-sterilized. The fermentor is a New Brunswick Scientific 2L "Multigen" unit. Broth is recirculated by a peristaltic pump and a product bleed matches the rate of fresh feed. Syngas is also recirculated by a peristaltic pump and a bleed is maintained through a rotameter and sterile filter to match the rate of fresh syngas added. pH is controlled with an LH Model 505 controller and pumps and an Ingold DPAS pH electrode. Temperature is controlled by circulating water through the fermentor jacket using a VWR Model 1145

temperature bath. Sample ports are provided in the fermentor vessel, media recycle line, and gas recycle line for sterile sample withdrawal via syringe.

The media recycle flows through a New Brunswick Scientific "Megaflow" membrane filter holder in which a sheet of Zenon Environmental, Inc. ethanol/butanol-selective membrane is held. The volatiles pass through the membrane and into a sweep-gas flow to be condensed and recovered. A Fisher "Maxima" DSA vacuum pump provides the driving force for volatiles to permeate through the membrane. A sample port is provided in the line between the dry ice trap and the vacuum pump for the analysis of non-condensable species (H_2S , H_2 , CO , CO_2).

PRESENTATIONS / PUBLICATIONS

1. Chatterjee, S., Vick Roy, J., Grethlein, H., Worden, R.M., and Jain, M.K. 1993. Anaerobic fermentation of sulfur containing coal derived synthesis gas to liquid fuels. Presented at the Fourth International Symposium on Biological Processing of Fossil Fuels. September, 1993, Alghero, Italy.

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1. Kim, B. H., Bellows, P., Datta, R., and Zeikus, J. G. 1984. Appl. Environ. Microbiology, 48(4):764.

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Figure 1. Product and Growth Profiles for Fermentation of Synthesis Gas by *B. Methylophilicum*

Fig. 2. SYNGAS GAS-LIFT FERMENTOR

Figure 3 Effect of Carbon Monoxide on Growth of *C. acetobutylicum*

Fig. 4 SYNGAS PERVAPORATION SOLVENT RECOVERY SYSTEM

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