1 of 1

44-3
1

Report No. PETC-SYN 12-93

~~DRAFT~~

BIOCONVERSION OF COAL-DERIVED SYNTHESIS GAS TO LIQUID FUELS
Contract # DE-AC22-92PC92117

JUL 27 1993

Quarterly Technical Progress Report
April 1, 1993 - June 30, 1993

Contract Date: September 29, 1992
Anticipated Completion Date: September 28, 1992
Government Award for 1992-93: \$160,565

Program Manager
Dr. Malvina Farcasiu
Department of Energy
Pittsburgh Energy Technology Center

Contracting Officer's Representative (COR)
Dr. Shelby Rogers
DOE-PETC

Principal Investigators
Mahendra K. Jain
R. Mark Worden
Hans Grethlein

Project Personnel
Sudipta Chatterjee
Mike Guettler
John Vick Roy

Michigan Biotechnology Institute
3900 Collins Road
P.O. Box 27609
Lansing, MI. 48909
Tel.: (517) 336-4626
Fax : (517) 337-2122

July 16, 1993

~~DRAFT~~

44

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

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, *B.methylophilum* 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 syn gas.
- *Optimization of process conditions for fermentation of syn gas.
- *Evaluation of bioreactor configuration for improved mass transfer of syn gas.
- *Optimization of process conditions for reducing carbon and electron loss by H₂-CO₂ fermentation.

SUMMARY OF TECHNICAL PROGRESS

- *Isolation of CO utilizing anaerobic strains is in progress.
- *An increase in the ratio of butyrate to acetate has been obtained by the addition of hexadecane to syngas fermentations.
- * Celite, ion exchange resin, alumina and molecular sieves have been found suitable for immobilization of *B. methylophilum*.
- * The product profile for the fermentation of syngas by *B. methylophilum* is under investigation.
- * pH regulation of H₂ / CO₂ fermentation in the stationary phase, has resulted in a 5 fold increase in the ratio of butyrate to acetate produced.

TECHNICAL PROGRESS REPORT

1. Development of Superior Strains

Certain fluoroacetate resistant mutants *B. methylophilum* are expected to have a reduced acetate production which could contribute to an enhanced butyrate production. In order to obtain individual clones of fluoroacetate resistant *B. methylophilum* it is necessary to obtain well isolated colonies of *B. methylophilum* on whatever minimal medium is used. With CO as a substrate the minimal medium does not support the rapid development of individual colonies but plates containing 0.5% glucose produce colonies of adequate size in 36-48 hours.

To obtain resistant mutants, plates containing several concentrations of fluoroacetate are suffused with cells from actively growing cultures of *B. methylophilum*. With glucose as the substrate *B. methylophilum* generally is resistant to as much as 10 mg/ml fluoroacetate, although the growth of some clones are slowed by 4 mg/ml. Concentrations greater than 10 mg are being tested using glucose as a substrate, but since fluoroacetate is assimilated by the same route by which acetate is produced the amount of fluoroacetate assimilated could be very low when cells are producing large amounts of acetate from glucose. *B. methylophilum* is expected to assimilate acetate when it is grown on methanol or pyruvate and its sensitivity to fluoroacetate is also being plumbed using these substrates in place of glucose.

Among the sources enriched for new CO organisms are various marine sediments, chicken manure, cow manure, several composts, bog soil, municipal sewage sludge, a brewer's sludge, and a chemical manufacturer's sludge. None of these enrichments have produced growth on CO comparable to the CO growth of *B. methylophilum* and are

therefore somewhat disappointing. *Peptostreptococcus productus* is fast growing, frequently isolated CO utilizing strain. It is a benchmark of CO enrichment and it was anticipated that *P. productus* would be isolated from one or more of these sources. Since it has not yet been obtained, efforts to isolate CO strains are being renewed, enrichment designs are being reviewed, some modifications are being made to batch, continuous, and heterocontinuous enrichment systems.

2. Optimization of Process Conditions for Fermentation of Syn Gas

The solubility of gases such as CH₄, CO, and H₂ are reportedly higher in solvents such as hexane, cyclohexane and decane than in water (Chang, 1991). Based on this theory, 3 - 6% (by volume) of hexadecane was added to the fermentation media to study the effect of exogenous additions on syngas fermentation product formation. All experiments were carried out at 40 °C and in 60 ml serum vials containing 10 ml media. A 2.5 fold increase in the ratio of butyrate to acetate was observed over a period of 7 days in the presence of 3% hexadecane (Figure 1). Currently experiments are being carried out to elucidate the roles played by hexadecane in mass transfer and in cell metabolism.

The product profile for the fermentation of syn gas by *B.methylophilicum* is being evaluated in a batch system with continuous gas feed.

Figure 1. Effect of Addition of Hexadecane on Syngas Product Profile

3. Evaluation of Bioreactor Configuration for Improved Mass Transfer of Syn Gas.

Gas lift and trickle bed reactors using immobilized *B.methylophilicum* cells will be used to increase the mass transfer of syngas during fermentation. The growth of *B. methylophilicum* on matrices such as celite, activated carbon, ion exchange resins, molecular sieves, wood, and alumina was evaluated in order to test the possibility of cell immobilization by self-adhesion. For this purpose *B.methylophilicum* was grown in

serum vials in the presence of 9% (by weight) support, at 40 °C using syngas as substrate. The effect of support on cell immobilization was characterized by determining total protein synthesized and product formation, at regular time intervals.

The matrices were found to have very distinct effects on the product profile and total protein synthesized. Based on cell growth, the choice of support material was narrowed to celite, ion exchange resin, molecular sieves, and alumina. Future efforts will be directed at investigating the effect of support on cell viability, and product profile. The ultimate choice of the matrix of immobilization will be made after determining the fluidization velocities required to suspend the materials in a gas lift reactor.

4. Optimization of Process Conditions for Reducing Carbon and Electron Loss by H₂-CO₂ Fermentation.

The fermentation of H₂-CO₂ by *B. methylotrophicum* is designed to reduce the loss of carbon and electrons during the first stage of the two stage fermentation process. A strain of *B. methylotrophicum*, adapted on H₂-CO₂, is being used to convert the excess H₂-CO₂ to acids. Batch fermentation of H₂-CO₂ was carried out in a 2 L multigen fermentor at 40 °C and at 3 psi headspace pressure. Initially the fermentation was conducted at pH 7.0. On decreasing the fermentation pH from 7.0 to 5.8 - 6.1 in the stationary phase the ratio of butyrate to acetate was found to increase by a factor of 5 over 5 days (Figure 2).

Figure 2. Effect of pH Regulation on H₂-CO₂ Product Profile

REFERENCES / PUBLICATIONS

Chang, M.Y., (1991), Ph. D. Thesis, University of Pittsburgh, Pittsburgh, PA.

**DATE
FILMED**

10 / 13 / 93

END