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# Microbial Conversion of Synthesis Gas Components to Useful Fuels and Chemicals

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## ABSTRACT

Enriched culture techniques have been used to isolate microbial cultures exhibiting growth on synthesis gas components. Three rod-shaped, gram-positive cultures have been isolated from petroleum-contaminated soil, a cow manure–soil mixture, and sheep rumen fluid. Each culture exhibits growth on carbon monoxide as its primary carbon source, producing alcohols and acids in the fermentation medium. Quantities of up to 7.5, 0.58, and 0.25 g/L of acetate, ethanol, and methanol, respectively, have been produced in batch culture with lesser amounts of acetone, butyric, and propionic acid detected.

**Index Entries:** Synthesis gas; carbon monoxide; unicarbonotrophic growth; anaerobic microorganisms.

## INTRODUCTION

The combustion of carbonaceous materials (e.g., oil, coal, natural gas, and lignocellulosics) to produce energy results in the release of tremendous quantities of carbon dioxide—one of the predominant greenhouse gases *(1,2)*. However, these materials may be gasified under controlled conditions to produce synthesis gas. Synthesis gas consists predominantly of carbon monoxide (CO), carbon dioxide ($CO_2$), and hydrogen ($H_2$) with lesser amounts of sulfur and nitrogen compounds. Through subsequent processing, synthesis gas may be upgraded catalytically to valuable fuels and chemical feedstocks *(3,4)*.

One approach to upgrading synthesis gas employs microorganisms as biocatalysts. These microbes have a demonstrated ability to utilize synthesis gas components as their primary or sole carbon source and are known as unicarbonotrophs. An attractive feature of such a process is the ability, through gasification, to convert a variety of complex carbon materials to the molecularly simple components of synthesis gas—thus improving the process concerns for the microbial conversion step (e.g., no solids handling, enhanced mass transfer and reaction kinetics).

Several microorganisms have been identified as having the ability to convert synthesis gas components to liquid and gaseous products *(5–7)*. Sulfur gas poisoning (e.g., carbonyl sulfide and hydrogen sulfide), of particular concern when using

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chemical catalysts for upgrading synthesis gas, has received attention as a factor in the microbial conversion step. Syngas-converting microbes have demonstrated considerable sulfur tolerance. In some cases, cultures capable of sulfur gas removal may be used in sequential or cocultures, further enhancing the attractiveness of a microbial-conversion step *(8,9)*. The rich variety of microbial flora presents a strong likelihood that new species will be discovered with similar capabilities and that new metabolic products will emerge through continued research *(10)*.

This work presents preliminary results from a microbial screening program. Its purpose is to examine an array of natural inocula for microorganisms with a demonstrated ability to convert synthesis gas components to valuable products. Enriched cultures of three isolates have shown promising synthesis gas conversion rates with a variety of chemicals secreted in the fermentation medium.

## MATERIALS AND METHODS

### Isolation Medium

To isolate cultures from natural inocula selectively, a basal salts medium was prepared following the modified Hungate method *(11,12)* for working with anaerobic microbial cultures. The medium (constituents shown in Table 1) was selected for its suitability for meeting the nutritional requirements of a variety of anaerobic bacteria. Following medium preparation by boiling under a nitrogen ($N_2$)/carbon dioxide ($CO_2$) atmosphere, 50-mL aliquots were transferred to 150-mL (nominal volume) bottles via an automatic pipet flushed with the $N_2/CO_2$ mixture. The 150-mL bottles, rated for use at pressures near 4–5 atm, served as batch reactors for this stage of work. Once filled, bottles were closed with butyl rubber stoppers and aluminum crimp seals. Prior to inoculation, the growth medium was reduced with cysteine-hydrochloride and/or sodium sulfide (2.5 wt%, respectively) to provide a strictly anaerobic environment. Anticipating the likelihood of methane production, as with many anaerobic cultures growing in such environments, an agent to block methanogenesis chemically was added to initial inoculations. The chemical 2-bromoethanesulfonic acid (BESA) was chosen as a proven inhibitor to methane production *(6)*. It was added in a concentration of 0.01 g/50 mL of medium. Once an absence of methane production was noted, BESA was removed from the medium in subsequent transfers.

### Natural Inocula Sources

Raw inocula were obtained from a variety of sources, including sewage sludge, crude petroleum-contaminated soil, manure–soil mixtures, and rumen and intestinal fluids from cows, sheep, and pigs. To optimize microbial viability and activity, contaminated soil samples were collected after several days of rainy weather and warm temperatures. Rumen and intestinal fluids were removed from freshly slaughtered animals at the university meat processing facility. All inocula were immediately transferred to reduced basal salts medium under a nitrogen atmosphere. After mixing with the medium, 5-mL samples were transferred via hypodermic syringe to medium in the 150-mL bottles followed by immediate gassing with a synthesis gas mixture to 10 psig. The mixture chosen for this study consisted of the following mole percentages: 13.2% $CO_2$, 22.5% $H_2$, and 64.3% CO. Gassed bottles were placed horizontally on a shaker/incubator at 37°C and 85 rpm.

Table 1
Basal Salts Medium for Isolating Anaerobic Cultures

| | Concentration, mg/L, unless specified otherwise |
|---|---|
| Pfennig minerals | |
| $KH_2PO_4$ | 500 |
| $MgCl_2 \cdot 6H_2O$ | 330 |
| NaCl | 400 |
| $NH_4Cl$ | 400 |
| $CaCl_2 \cdot 2H_2O$ | 50 |
| Pfennig trace metals | |
| $ZnSO_4 \cdot 7H_2O$ | 0.1 |
| $MnCl_2 \cdot 4H_2O$ | 0.03 |
| $H_3BO_3$ | 0.3 |
| $CoCl_2 \cdot 6H_2O$ | 0.2 |
| $CuCl_2 \cdot H_2O$ | 0.01 |
| $NiCl_2 \cdot 6H_2O$ | 0.02 |
| $Na_2MoO_4 \cdot 2H_2O$ | 0.03 |
| $FeCl_2 \cdot 4H_2O$ | 1.5 |
| $Na_2SeO_3$ | 0.01 |
| B-Vitamins | |
| Biotin | 0.1 |
| Folic acid | 0.1 |
| Pyridoxal-HCl | 0.05 |
| Lipoic acid | 0.3 |
| Riboflavin | 0.25 |
| Thiamine-HCl | 0.25 |
| Ca-D-pantothenate | 0.25 |
| Cyanocobalamin | 0.25 |
| *P*-aminobenzoic acid | 0.25 |
| Nicotinic acid | 0.25 |
| Supplements | |
| Yeast extract | 0.1% |
| $Na_2 \cdot 9H_2O$, 2.5% soln (w/w) | 2.0 mL |
| Cysteine-HCl, 2.5% soln (w/w) | 2.0 mL |
| Resazurin, 0.1% solution | 0.1 mL |

## Sampling and Analytical Procedures

Following the addition of synthesis gas, 50 mL of argon were added to each bottle with a gas-tight syringe. The addition of this biologically inactive, insoluble gas allowed the quantification of gas-phase components by the relation:

$$N_{CO} = [(\%CO)/(\%Ar)] * N_{Ar} \quad (1)$$

Similar determinations were made for carbon dioxide and hydrogen.

Gas samples of 150 µL were obtained periodically for analysis. Gas-phase analyses were made using a Hewlett Packard 5890 gas chromatograph equipped with a thermal conductivity detector (TCD) and a CarboPLOT 007 molecular sieve capillary column (25 m × 0.53 mm × 25 µm) using helium as the carrier gas. The injector, oven, and detector temperatures were 50, 80, and 110°C, respectively.

Table 2
Initial Inoculation for Isolating Anaerobic Cultures

| Culture ID | Time, d | pH | Co, mmol | $CO_3$, mmol | Comments |
|---|---|---|---|---|---|
| Petroleum–soil inoculum | 0 | 7.0 | 3.23 | 0.52 | |
| | 3 | 5.30 | 3.05 | 0.76 | |
| | 6 | 5.39 | 2.93 | 1.14 | |
| | 8 | 5.34 | 2.41 | 1.66 | Regassed |
| | 9 | — | 3.53 | 1.07 | |
| | 13 | 5.33 | 3.60 | 1.28 | Regassed |
| | 14 | — | 2.04 | 1.86 | |
| | 15 | 5.30 | 0.84 | 3.81 | |
| Cow–manure soil inoculum | 0 | 6.0 | 3.14 | 0.67 | |
| | 3 | 5.63 | 2.95 | 0.78 | |
| | 6 | 5.60 | 3.05 | 0.86 | |
| | 8 | 5.46 | 2.61 | 1.35 | Regassed |
| | 9 | — | 2.04 | 1.45 | |
| | 13 | 4.08 | 3.75 | 0.83 | Regassed |
| | 14 | — | 3.80 | 0.74 | |
| | 15 | 3.79 | 1.38 | 2.76 | |

Two-milliliter liquid samples were removed for analysis. Liquid samples were acidified with 3% (w/w) hydrochloride acid (HCl) and mixed with isopropanol (i-P) as an internal standard once i-P was determined not to be a liquid product. Samples were analyzed by gas chromatography using an HP 5890 equipped with a flame ionization detector (FID) and an HP-FFAP capillary column (30 m × 0.53 mm × 1.0 μm) with helium as a carrier gas. The injector and detector were operated at 200°C. The oven was operated with a program initiated at 45°C ramped to 150°C over 5 min.

## RESULTS AND DISCUSSION

Initially, raw inocula were added to basal medium at various initial pH values ranging from 5 to 7. Gas and liquid samples were obtained several times weekly. Prior work *(13,14)* indicated that a relatively slow adaptation to growth on synthesis gas would be required over a period of weeks or months. However, a rapid onset of CO depletion occurred with several inocula sources, including the petroleum–soil source, the cow manure–soil source, and to a lesser extent the sheep rumen fluid inoculum. Over a period of 2 mo, no other inocula exhibited significant synthesis gas consumption and were subsequently discarded. Representative data for the petroleum–soil (PS) and cow–soil (CS) inocula are shown in Table 2. The data are reported at total millimoles of gaseous substrate (CO and $CO_2$) present. The cultures were regassed several times during this experiment. With each bottle of PS inoculum, the pH moderated from its initial range of 5–7 to approx 5.3 within 72 h, and remained relatively constant throughout the 15-d period of measurement. Likewise, all CS inocula initially approached a pH near 5.5. A gradual downward trend in pH was observed to be near 3.8 accompanying higher concentrations of acetate—compared to relative amounts observed with the PS inocula (data not shown). Acetate concentration showed a continued

Table 3
Parent Culture Conditions for pH Study

| Culture ID | Age, d | pH | Acetate, g/L | Ethanol, g/L | Methanol, g/L | Comments |
|---|---|---|---|---|---|---|
| Petroleum–soil inoculum PS3-1 | 7 | 6.16 | 3.15 | 0.287 | 0.105 | Rod-shaped gram + |
| Cow manuer–soil inoculum CS1-1 | 7 | 6.27 | 4.55 | 0.322 | 0.112 | Rod-shaped gram + |
| Sheep rumen Fluid SR4-1 | 7 | 6.50 | 2.91 | 0.469 | — | Rod-shaped gram + |

increase in the CS bottles to an ultimate concentration between 1.5 and 2.0 g/L. Within 48 h of inoculation, acetate levels in the PS bottles reached and remained at approx 0.5 g/L. A third inoculum, sheep rumen fluid (SR), exhibited a similar CO consumption and production of liquids, but to a lesser extent than with the PS and CS inocula described earlier in this paragraph.

In all bottles, a variety of liquid products were observed. Although acetate was the predominant product, propionate, butyrate, methanol, and ethanol were also observed. An increase in $CO_2$ production was noted in all bottles, as well. No significant amounts of methane were produced with any inocula during this initial experiment. Therefore, BESA was removed from subsequent transfers—a decision revealing interesting consequences with the petroleum–soil culture in later transfers.

A second transfer (to triplicate bottles) exhibited a relative increase in the rate of CO consumption for the PS inocula (data not shown). Within 48 h, all bottles were essentially depleted of CO, requiring regassing. At day 5, after two regassings, the PS bottles had accumulated an average of 7.5 g/L acetate and 0.36 g/L ethanol with lesser amounts of methanol, propionate, and butyrate. Identification of trace amounts of acetone were tentatively detected in some bottles containing the PS inoculum. The CS bottles exhibited a slower rate of CO depletion in comparison to its parent inoculum. Acetate accumulated to an average of 4.0 g/L in each of the triplicate bottles in 5 d. The ethanol and methanol concentrations at this time averaged 0.37 and 0.10 g/L, respectively.

## pH Effects on CO Consumption and Product Concentrations

The PS, CS, and SR isolates were transferred successively at 5–7 d intervals to enrich the adaptive species. After four successive transfers, an experiment was conducted to examine the initial pH effects on gas consumption and the relative amounts of liquid products obtained in the fermentation broth. Seed inocula for each isolate were selected from bottles that had shown a rapid depletion of CO with time and an accompanying high level of liquid products (when compared to other bottles of the same inoculum source). Table 3 provides the conditions for the source inocula used in this experiment. Each of these cultures was initiated at pH 7.0 and regassed five times over 7 d. Preliminary observations indicate an abundance of rod-shaped, gram-positive bacteria in each culture.

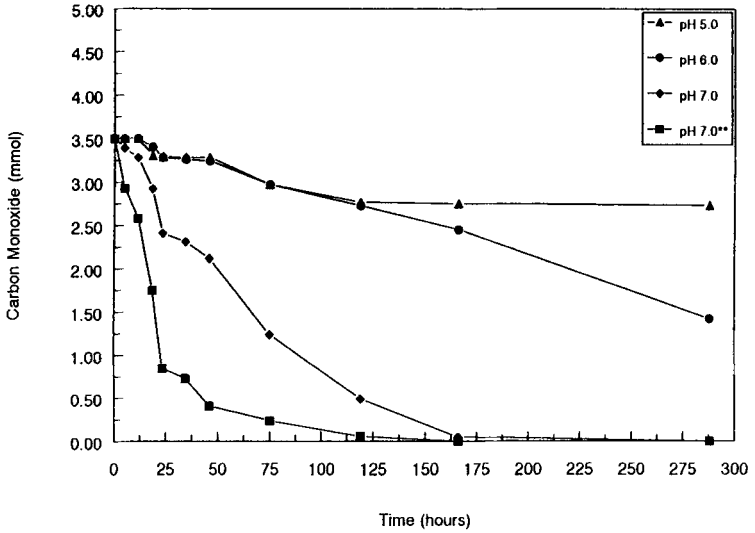


Fig. 1. Petroleum/soil isolate CO consumption at initial pH values. -▲- pH 5.0, -●- pH 6.0, -◆- pH 7.0, -■- pH 7.0 (added reducing agent *[see text]*).

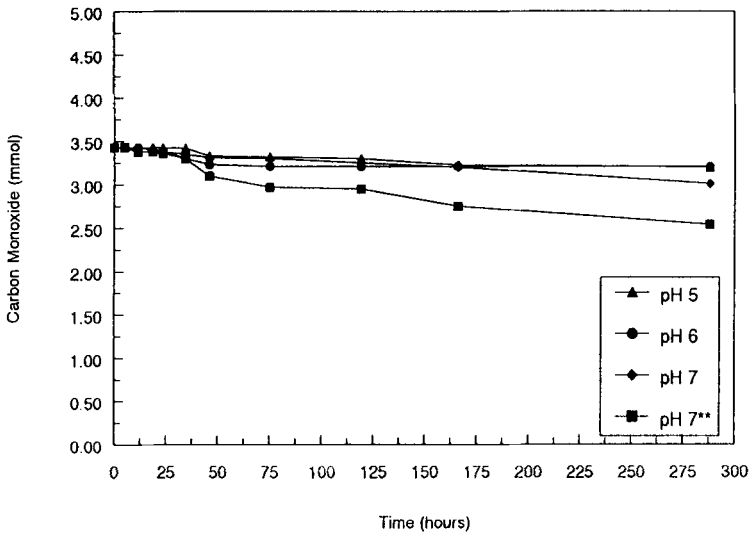


Fig. 2. Cow manure/soil isolate CO consumption at initial pH values. -▲- pH 5.0, -●- pH 6.0, -◆- pH 7.0, -■- pH 7.0 (added reducing agent *[see text]*).

Each bottle contained 50 mL of medium initially at a pH of 5.0, 6.0, or 7.0. All bottles were reduced with 0.5 mL of 2.5% (w/w) cysteine-HCl. In addition, one set of bottles at pH 7.0 received an added amount of sodium sulfide (0.5 mL of 2.5 wt%). This combination of reducing agents provided for a lower redox potential than cysteine-HCl alone—a condition often favoring growth of obligate anaerobes *(15)*.

Results for CO consumption with time are shown in Figs. 1–3. Individual data points are connected for visual clarity. It is clearly seen that, at increasingly lower initial pH values, the rate and extent of CO consumption declined dramatically. The distinction is particularly marked with the cultures PS3-1 and SR4-1. A similar trend

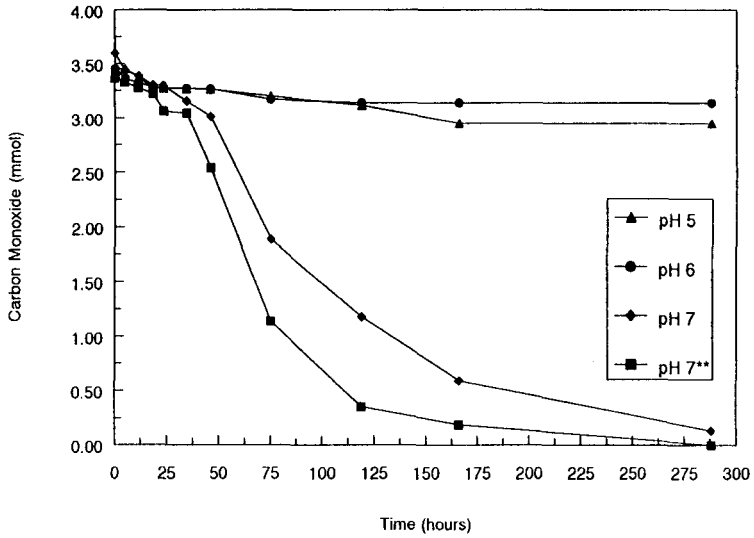


Fig. 3. Sheep rumen isolate CO consumption at initial pH values. -▲- pH 5.0, -●- pH 6.0, -◆- pH 7.0, -■- pH 7.0 (added reducing agent *[see text]*).

was observed for isolate CS1-1, though it generally displayed a much slower rate of CO depletion (with incomplete utilization for any of the bottles over the observed time period). Bottles containing the CS1-1 isolate also produced relatively small amounts of alcohols and acids (data not shown). The PS3-1 and SR4-1 isolates initiated at pH 7.0 consumed essentially all CO over the 12-d observation period.

Data for the three primary liquid products (acetate, ethanol, and methanol) are shown for the PS3-1 and SR4-1 isolates in Figs. 4 and 5. Only data for the bottles containing the added reducing agent are depicted. These bottles produced significantly higher quantities of acetate, methanol, and ethanol than those reduced with only cysteine-HCl.

With the PS3-1 isolate, the acetate concentration rose to 5.3 g/L within 24 h, peaking at 6.0 g/L followed by a gradual decrease in concentration. The rate of methanol production closely followed acetate rising to a concentration of 0.14 g/L before quickly dropping to zero. During the production of acetate and methanol, the ethanol concentration dropped slightly from an initial value of 0.042 g/L and then slowly rose to an ultimate concentration of 0.116 g/L.

Initially, the SR4-1 production rates of all three major liquid products were closely matched. After 24 h, product concentrations peaked at 4.85, 0.581, and 0.25 g/L for acetate, ethanol, and methanol, respectively. Although the methanol concentration dropped to zero (also seen in the PS3-1 isolate), both acetate and ethanol showed decreases and again rose to ultimate concentrations of 6.38 and 0.26 g/L, respectively.

The liquid products for the remaining bottles containing only cysteine-HCl were significantly lower with concentrations reaching only 1.0, 0.15, and 0.05 g/L (average values) for acetate, ethanol, and methanol.

In all bottles, $CO_2$ was produced with liquid products. However, the two bottles containing PS3-1 at pH 7.0 exhibited consumption of $CO_2$ with a concomitant production of methane once CO was depleted. Methane production was detected at 166 h, rising to ultimate levels of 2.51 and 2.11 mmol by the completion of the experiment at 288 h.

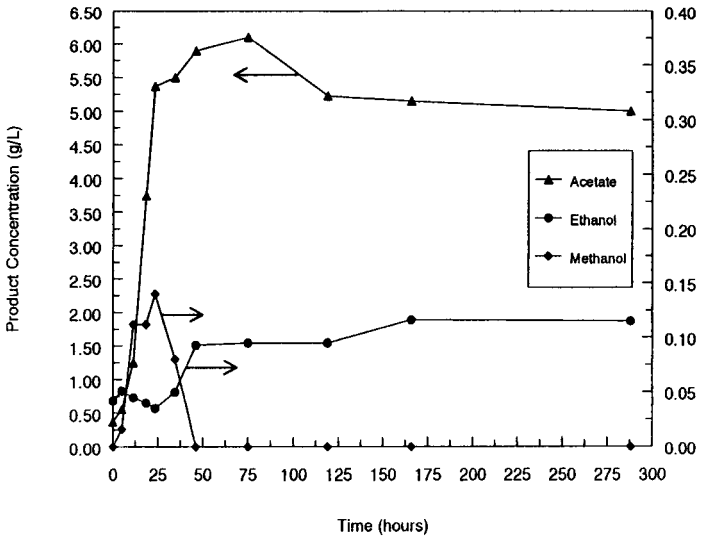


Fig. 4. Petroleum/soil isolate (PS3-1) liquid products at pH 7.0.* -▲- Acetate, -●- ethanol, -◆- methanol (added reducing agent *[see text]*).

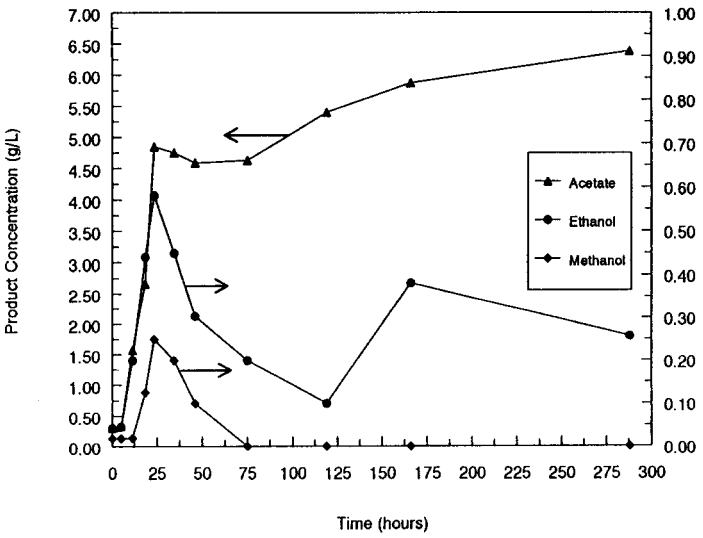


Fig. 5. Sheep rumen isolate (SR4-1) liquid products at pH 7.0.* -▲- Acetate, -●- ethanol, -◆- methanol (added reducing agent *[see text]*).

## CONCLUSIONS

Three rod-shaped, gram-positive cultures have been isolated and enriched in basal salts medium—each demonstrating growth on synthesis gas components as their primary carbon source. A variety of liquids were produced (predominantly acetate, ethanol, and methanol). These liquid products serve as valuable fuels or chemical feedstocks. Quantities of up to 7.5, 0.58, and 0.25 g/L of acetate, ethanol, and methanol, respectively, were produced in various batch cultures. Traces of acetone were noted in the petroleum–soil isolate, as well. Future studies will focus on fermentation condi-

tions that maximize the production of selected products. The potential for producing alcohols at the expense of acetate will be investigated. When compared to parallel work conducted by Barik et al. *(6)*, the cultures isolated in this study exhibited similar rates of CO consumption, but at significantly earlier stages of culture transfer (e.g., in just two to three transfers over a couple of weeks). Of particular interest is the strong growth and consumption of CO by the petroleum–soil isolate.

Further characterization is needed to identify the strain(s) present in each culture and to describe the growth characteristics of individual species in the event of identifying a new strain or species. Optimization of the growth medium (e.g., removal of yeast extract–an expensive medium constituent) is necessary to improve process economics.

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