

PRODUCTION OF BUTANOL AND ETHANOL
FROM SYNTHESIS GAS VIA FERMENTATION

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INTRODUCTION

Development of alternative liquid fuels based on raw materials other than petroleum would benefit the U.S. both economically, by reducing the trade deficit, and strategically, by reducing U.S. dependence on politically unstable oil exporters. The 1987 D.O.E. report entitled "Energy Security--a Report to the President" concluded that if present trends continue, the U.S. is likely to become far more dependent on the Persian Gulf for oil and that "The most promising technological opportunities for further reductions in oil consumption rest in the development of alternative fuel systems." Indirect coal liquefaction could provide such alternative fuels. In this process coal is first thermally converted to synthesis gas, consisting primarily of carbon monoxide (CO) and hydrogen (H₂). The gas is then catalytically reacted to form liquid fuels, such as mixed alcohols.

The catalytic gas-conversion step is subject to several processing problems. First, sulfur contaminants found in the synthesis gas, primarily hydrogen sulfide (H₂S) and carbonyl sulfide (COS), are potent catalyst poisons. The sulfur-gas content of coal-derived gases varies considerably, but is typically in the range of a few percent (TRW, 1980). These gases must be removed in energy-intensive purification steps that add significantly to the product costs, particularly for coals having a high sulfur content (Wilson et al., 1988). Second, catalytic processing of synthesis gas often requires strict CO/H₂ ratios to maintain a particular product mix, necessitating gas recompression and shift reaction conversion operations. Third, the gas-phase reactors operate at high temperatures and pressures. Operation under extreme conditions increases reactor costs and presents potential safety issues. Fourth, the product specificity of the catalysts is often poor, resulting in a broad product spectrum, decreased product yield, and increased separations difficulties. In some cases, hazardous or environmentally unacceptable by-products may also be produced.

Biological conversion of synthesis gas offers some key advantages over catalytic routes. Sulfur-gas removal will not be required. With sulfur-tolerant microorganisms, the H₂S and COS gases will pass through the process as inert components. Thus, the capital and operating costs associated with conventional gas separation and upgrading equipment could be avoided. Because the CO/H₂ ratio is not a key factor in biological conversion, gas-shift operations would be unnecessary. Also, biological reactions have evolved to operate efficiently at relatively low temperatures and pressures--at least an order of magnitude less than in conventional gas-phase catalysis. The milder processing conditions may reduce both operating and capital costs. Finally, biological catalysts are typically more specific than their inorganic counterparts. Consequently, few by-products result from synthesis gas fermentations, and no hazardous or xenobiotic fermentation by-products are expected.

The recent discovery of microbial pathways to directly convert CO to useful products such as butanol, ethanol, butyric acid and acetic acid provides significant new capabilities for biological processing of synthesis gas. The purpose of this paper is to summarize the novel pathways and fermentation patterns we have discovered in the anaerobe Butyribacterium methylotrophicum and to discuss their impact on the feasibility of producing butanol and ethanol by fermentation of synthesis gas.

ANAEROBIC BIOCONVERSION OF 1-CARBON COMPOUNDS

Numerous microorganisms are capable of metabolizing 1-carbon compounds such as CO, methanol, and CO₂. Those able to grow on 1-carbon compounds as the sole carbon and energy source are referred to as unicarbonotrophs. This trait is desirable for commercial synthesis gas bioconversions, because no additional organic feedstock would be required as a co-substrate. Anaerobic bioconversion is also preferable to aerobic, because in anaerobic fermentation no electrons are lost to molecular oxygen. Thus, the chemical energy content of the gas is efficiently conserved in the products. Two groups of unicarbonotrophic, anaerobic bacteria, acidogens and methanogens, can use 1-carbon compounds or acetate as energy sources. Acidogens convert 1-carbon compounds to fatty acids such as acetate and butyrate, while the methanogens produce methane (Zeikus, 1983).

The metabolism of homo-acetogens is characterized by synthesis of the intermediate acetyl-CoA from either heterotrophic or unicarbonotrophic modes of growth (Zeikus, 1983). Clostridium thermoaceticum generally ferments hexose or pentose to acetate (Fontaine, et al., 1942), but C. thermoaceticum type strain Fontaine can also grow readily on H₂/CO₂. It can also be adapted to grow on CO as an energy source. However, this organism is not able to utilize methanol (Kerby and Zeikus, 1983). Pentostreptococcus productus strain U-1 is also capable of growth and production of acetate on either CO or H₂/CO₂ (Lorowitz and Bryant, 1984). Similarly, Acetobacterium woodii ferments H₂/CO₂, glucose, lactate, formate (Balch, et al., 1977) and can grow on CO after adaptation (Kerby et al., 1983). This species also ferments methanol or cleaves and ferments the methoxyl moieties from a variety of aromatic acids (Balch, et al., 1977). Clostridium ljungdahli is able to co-metabolize CO and H₂ to form acetate and ethanol (Clausen and Gaddy, 1988).

Butyribacterium methylotrophicum is a unique microorganism capable of growing on multicarbon compounds (glucose, lactate, or pyruvate) as well as 1-carbon compounds (H₂/CO₂, formate or methanol) (Zeikus, et al., 1980; Lynd and Zeikus, 1983). A strain of B. methylotrophicum (designated the CO strain) was adapted to grow with a 12 h doubling time on 100% CO, producing acetate and minor amounts of butyrate (Datta, 1982; Lynd et al., 1982). Eubacterium limosum appears to be similar to B. methylotrophicum in general fermentation properties, including the ability to ferment methanol or CO (Sharak-Genthner and Bryant, 1982; Sharak-Genthner, et al., 1981). However, E. limosum requires adaptation and utilization of methanol and needs rumen fluid, yeast extract or acetate in the medium (Sharak-Genthner and Bryant, 1987). Moreover, it produces copious amounts of slime (Sharak-Genthner et al., 1981).

Recently, we have discovered unique metabolic properties of the CO strain of B. methylotrophicum that greatly enhance the prospect of commercially feasible synthesis gas fermentations. In extended batch fermentations, where 100% CO was continuously sparged as the sole carbon and energy source, pH was found to strongly influence the relative amounts of acetate and butyrate produced from CO. By decreasing the pH from 6.8 to 6.0, the fraction of electrons from CO going into butyrate was increased from 6% to 70% at the expense of acetate production (Worden et al., 1989). This finding was significant in that it demonstrated the synthesis of a 4-carbon organic acid from a 1-carbon, inorganic substrate. High levels of butyrate are desirable for the production of butanol in a two-stage

synthesis gas fermentation, as discussed below. In continuous-culture experiments using a 100% CO gas sparge, small quantities of butanol and ethanol were produced in addition to butyrate and acetate (Grethlein et al., in press). This discovery represented the first evidence of a direct microbial pathway for butanol production from CO.

In both batch and continuous-culture experiments with B. methylotrophicum, we have consistently observed a trend toward production of more reduced products (acids with longer chain lengths and alcohols) as the fermentation pH is reduced. This trend is evident in the steady-state fermentation carbon balances shown in Table 1 (Grethlein et al., in press). This pH effect provides a potential mechanism by which the product spectrum of CO fermentations may be manipulated to give either high acid or alcohol yields, depending on the fermentation objectives.

Although the pathways and stoichiometries obtained from the batch and continuous CO fermentations were promising, both the volumetric reactor productivities and the product concentrations were low. To increase these parameters, additional continuous-culture experiments were conducted using a microfiltration-based, cell recycle system. Details of the experimental system have been described elsewhere (Grethlein et al., manuscript submitted). Results to date have been encouraging. Five- to 20-fold increases in cell and product concentrations have been observed relative to continuous culture without cell recycle, and the system has been operated for more than 5 weeks at a time without significant membrane fouling.

SOLVENT PRODUCTION BY CLOSTRIDIUM ACETOBUTYLICUM

Acetone, butanol, and ethanol (ABE) were produced commercially via fermentation of glucose by Clostridium acetobutylicum from World War I until the 1950's. This batch fermentation follows a biphasic pattern (Weizmann, 1918). During the initial, acidogenic phase, growth is exponential, and organic acids (acetic and butyric) and H₂ are the primary products. After accumulation of a certain concentration of acids, the pH decreases to approximately 4.5, and the fermentation switches to the solventogenic phase, where the organic acids are reduced to solvents, the rate of H₂ gas production decreases, and cell growth ceases. Early attempts to induce C. acetobutylicum to take up acids for alcohol production indicated that a concentration of butyric acid higher than 2 g/L inhibited the solvent production (Soni et al., 1982). More recently, it has been shown that acids concentration in the first stage is a determinant factor for solvent production in the second stage of a two-stage continuous fermentation (Godin and Engasser, 1989).

Continuous cultures of C. acetobutylicum may be maintained in either the acidogenic or the solventogenic phase by manipulating the fermentation conditions. A generally observed trend is that higher pH values are associated with acid production, and lower pH values favor solvent production (Kim and Zeikus, 1985). Meyer et al. (1986) found that CO gasing led to continuous butyrate uptake and solvent production without the production of acetone. Extremely high specific butyrate uptake rates and butanol production rates were measured during this CO challenge. These trends were attributed to altered electron flow arising from CO inhibition of production hydrogenase activity. During acidogenesis, excess electrons are typically eliminated by H₂ formation. Because this reaction was inhibited by CO, electron flow to other products had to be increased. Acetone formation does not consume electrons, so the cellular control mechanisms shut off carbon flow to acetone, and increased carbon flow to electron-consuming alcohol production.

ENERGETICS OF ACID AND ALCOHOL PRODUCTION FROM CO

The ΔG° values for production of acids and alcohols from CO, shown in Table 2, were calculated using published free energy of combustion data (Roels, 1983).

The values for butyrate and acetate production are approximately the same, and only slightly higher than those for butanol and ethanol production. All four reactions are sufficiently exergonic to drive ATP synthesis. However, it is not currently known whether metabolic mechanisms exist in B. methylotrophicum for net ATP synthesis during direct conversion of CO to alcohols. Acetyl CoA and butyryl CoA are thought to be intermediate branch points from which either acids or alcohols may be produced. Acid production generates ATP but consumes no electrons, whereas alcohol production consumes electrons but produces no ATP. Consequently, the cells can replenish energy reserves via acid production and eliminate excess electrons via alcohol production. If no net ATP is generated via alcohol formation, then alcohols must be produced either in the absence of growth or concurrently with acid formation. Further research is needed to elucidate the mechanisms for energy flow within B. methylotrophicum under these fermentation conditions.

PROPOSED PROCESSES FOR SYNTHESIS GAS BIOCONVERSION TO BUTANOL AND ETHANOL

Single-stage Fermentation Process

A proposed single-stage fermentation process is shown in Figure 1. In this process, CO and H₂ would be fed to a bioreactor containing B. methylotrophicum, in which the fermentation conditions were optimized for solventogenesis. Our previous results indicate that growth and alcohol production are metabolically uncoupled, and that a reduction in pH may be used to trigger solventogenesis. Thus, the fermenter could be operated batchwise, with a pH shift at the onset of the stationary phase (Worden et al., 1989). Alternatively, the fermenter could be operated continuously, using pH oscillations to allow alternate periods of alcohol production and cell regeneration. In either case, the product stream would be sent to a separation unit for selective removal of the alcohols, and the acids would be recycled to the reactor for further conversion. Either cell recycle or cell immobilization would be used to maintain high reactor cell densities.

This single-stage process appears to be technologically feasible, based on preliminary fermentations using 100% CO as the gaseous feedstock. At a pH of 5.5, transient butanol concentrations as high as 2.7 g/L have been achieved in continuous culture using cell recycle (Grethlein et al., manuscript submitted). By comparison, butanol concentrations from commercial ABE fermentations were approximately 16 g/L. Thus, even before process and strain optimization, CO fermentation yields are within an order of magnitude of the maximum butanol concentration produced by C. acetobutylicum from glucose.

Ideally, the reducing equivalents needed to convert acetic and butyric acids to the respective alcohols would come from H₂ in the synthesis gas. B. methylotrophicum is known to take up and metabolize H₂ and CO₂ to form primarily acetate (Lynd and Zeikus, 1983). However, the effects of factors such as the presence of CO and fermentation pH on this reaction are unknown. Carbon monoxide is a potent inhibitor of production hydrogenase in C. acetobutylicum (Kim et al., 1984), but its effect on the uptake hydrogenase of B. methylotrophicum is not known. Fermentation experiments using a mixture of H₂ and CO are currently underway in our laboratory to determine these effects. Even if hydrogen uptake is inhibited by CO, it may be possible to substitute other metabolizable electron donors, such as glucose. This substitution should not greatly affect the process economics, because only a small amount of the electron donor would be required. On a combustion energy basis, 83% of the butanol energy content would come from CO, and only 17% would come from the secondary electron donor.

Two-Stage Fermentation Process

The flowsheet of a proposed 2-stage fermentation process is shown in Figure 2. In the first, acidogenic stage, CO would be converted to primarily butyric

and acetic acids using an acidogenic culture of B. methylotrophicum. Results to date suggest that B. methylotrophicum is quite well suited for this application. Using fermentation conditions appropriate for acidogenesis, butyrate and acetate concentrations of 4 g/L and 8 g/L, respectively, have been achieved in steady-state, continuous operation with cell-recycle (Grethlein et al., manuscript submitted). Future strain development efforts should increase these values even further. The acids produced in the first stage would then be combined with H₂ from the synthesis gas (or some other electron donor) in the second stage to yield butanol and ethanol. Two alternative biocatalysts are currently under investigation for use in the second stage: C. acetobutylicum and B. methylotrophicum. C. acetobutylicum has been studied much more extensively and thus has better characterized enzymes and metabolic-regulation mechanisms. It is capable of producing high concentrations of mixed solvents when grown on glucose, and can also take up externally added acids and H₂ for solvent production. When grown in a glucose-limited chemostat in the presence of CO, H₂ and acetone formation are inhibited, while butyrate uptake and butanol production are increased to extremely high levels (Meyer et al., 1986). These effects are beneficial, since production of H₂ and acetone reduces alcohol yields. Thus, it may be possible to use residual CO from the first stage to improve alcohol yields in the second stage.

The solventogenic capability of B. methylotrophicum was discovered in our laboratory only recently (Grethlein et al., in press), and it has not yet been well characterized. Results to date have included transient butanol concentrations as high as 2.7 g/L in continuous operation using cell recycle. Ongoing investigations include characterization of enzyme-regulation mechanisms, and capacity for H₂ and acid assimilation.

As indicated in Figure 2, bioreactors for both stages will utilize either cell recycle or immobilization to maintain high cell concentrations. The product stream from the solventogenic bioreactor will be stripped of alcohols in a separation unit and then recycled to the first stage.

FUTURE PROSPECTS FOR BIOCONVERSION OF SYNTHESIS GAS TO BUTANOL AND ETHANOL

Two basic problems with the classic ABE fermentation using C. acetobutylicum were identified in a report to the Office of Technology Assessment entitled "Biological Production of Liquid Fuels and Chemical Feedstocks" (Humphrey and Nolan, 1979): the formation of multiple products, requiring additional recovery steps, and the low solvent yields (34 g/100 g fermentable sugar). Our results to date indicate that these problems may not be as severe for the unique synthesis gas fermentation processes proposed here, as described below.

Acetone production by B. methylotrophicum has never been observed. It is currently unknown whether the enzymes necessary for acetone production from acetoacetyl CoA (e.g., phosphate acetoacetyltransferase, acetoacetate kinase and acetoacetate decarboxylase) are lacking in B. methylotrophicum, or whether one or more of these enzymes may be under strong regulation by CO. In the proposed two-stage process, where C. acetobutylicum is used in the solventogenic stage, the presence of CO has been shown to block acetone production while enhancing butanol production to the highest levels ever reported (Meyer et al., 1986). Thus, it appears possible to avoid undesirable acetone formation.

Despite extensive strain-development and optimization efforts, ABE fermentations using C. acetobutylicum are presently unable to achieve high electron recovery in butanol and ethanol. An improved process for ABE production from corn starch using an efficient, asporogenous mutant and stillage recycle was recently described by Marlatt and Datta (1986). In this process, only 63% of the electrons from the glucose ends up in the alcohols, and 31% ends up in H₂ and acetone. For our proposed process using C. acetobutylicum in the solventogenic bioreactor, the presence of CO may block or reduce unwanted production of H₂ and

acetone, maximizing electron capture in alcohols. Less is known about the electron-capture efficiency of B. methylotrophicum. Using carbon and electron balances (Erickson and Oner, 1983), we have estimated that in recent continuous fermentation experiments using cell recycle, as much as 44% of the electrons from CO has been transferred to butanol (Grethlein et al., manuscript submitted). The butanol concentration measured during this time was 2.7 g/L. However, because these data were not measured at steady-state, they should only be taken as suggestive that high electron capture efficiency in alcohols appears possible with B. methylotrophicum.

The unique metabolic capabilities for CO conversion found in B. methylotrophicum indicate its potential for production of butanol and ethanol from synthesis gas in either a 1- or 2-stage fermentation system. In addition, the CO-induced enhancement of acid uptake and butanol production in C. acetobutylicum make this species well-suited for assimilation of H₂ and acids in the second, solventogenic stage. Research to better understand the complex metabolic regulation patterns of these two species is continuing in our laboratory. Successful manipulation of carbon and electron flow in these cultures could make bioconversion of synthesis gas to butanol and ethanol a commercial reality.

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REFERENCES

- Balch, W. E., S. Schoberth, R. S. Tanner and R. S. Wolfe. (1977) *Int. J. of Syst. Bacteriol.* 27:355.
- Clausen, E. C. and J. L. Gaddy (1988) Proc. of Indirect Liquefaction Contractors Review Meeting for U.S. D.O.E., Pittsburgh Energy Technology Center, 639-670.
- Datta, R. (1982) *Biotech. and Bioeng. Symp.* 11:521-532.
- Erickson, L. E. and M. D. Oner. (1983) *Ann. N.Y. Acad. Sci.* 413:99.
- Fontaine, F. E., W. H. Peterson, E. McCoy, E. Johnson and M. T. Titter. (1942) *J. Bacteriol.* 43:704.
- Godin, C. and J. M. Engasser. (1989) *Biotechnol. Lett.* 12:903-906.
- Grethlein, A. J. (1989) M. S. Thesis, Dept. of Chemical Engineering, Michigan State University, East Lansing, Michigan.
- Grethlein, A. J., R. M. Worden, M. K. Jain and R. Datta. 1990. *Appl. Biochem. Biotechnol.* 24/25 (in press).
- Humphrey, A. E. and E. J. Nolan (1979) Biological Production of Liquid Fuels and Chemical Feedstocks, Report to U.S. Office of Technology Assessment, 5-7.
- Kerby, R., W. Niemczura and J. G. Zeikus. (1983) *J. Bacteriol.* 155:1208-1218.
- Kerby, R. and J. G. Zeikus. (1983) *Curr. Microbiol.* 8:27-30.
- Kim, B. H., P. Bellows, R. Datta and J. G. Zeikus. (1984) *Appl. Environ. Microbiol.* 48, 4,764-770.
- Kim, B. H. and J. G. Zeikus. (1985) *Dev. Ind. Microbiol.* 26:549-556.
- Lorowitz, W. H. and M. P. Bryant. (1984) *Appl. Environ. Microbiol.* 47:961-964.
- Lynd, L. H., R. Kerby and J. G. Zeikus. (1982) *J. Bacteriol.* 149:255-263.
- Lynd, L. H. and J. G. Zeikus. (1983) *J. Bacteriol.* 153:1415-1423.
- Marlatt, J. A. and R. Datta. (1986) *Biotechnol. Progress*, 2,1, 23-28.
- Meyer, C. L., J. W. Roos and E. T. Papoutsakis. (1986) *Appl. Microbiol. Biotechnol.* 24:159-167.
- Roels, J.A. (1983) Energetics and Kinetics in Biotechnology, Elsevier, New York, 40.
- Sharak-Genthner, B. R., C. L. Davis and M. P. Bryant. (1981) *Appl. Environ. Microbiol.* 42:12-19.
- Sharak-Genthner, B. R. and M. P. Bryant. (1982). *Appl. Environ. Microbiol.*, 43:70-74.
- Sharak-Genthner, B. R. and M. P. Bryant. (1987) *Appl. Environ. Microbiol.*, 53:471.
- Soni, B. K., K. Das and T. K. Ghose. 1982. *Biotechnol. Lett.* 4:19-22.

TRW (1980) Development Status of Key Emerging Gasification Systems, TRW Report, Sept., 1980.

Weizmann, C. (1918) U. S. Patent #1,315,585.

Wilson, J. S., J. Halow and M. R. Ghate. (1988) Chemtech. Feb. 1988, 123-128.

Worden, R. M., A. J. Grethlein, J. G. Zeikus and R. Datta. (1989) Appl. Biochem. Biotech. 20/21:687-698.

Zeikus, G. Z. (1983) Adv. Microb. Physiol. 24:215-297.

Zeikus, J. G. (1980) Ann. Rev. Microbiol. 34:423-464.

Zeikus, J. G., L. H. Lynd, T. E. Thompson, J. A. Krzycki, P. J. Weimer and P. Hegge. 1980. Curr. Microbiol. 3:381-386.

Table 1. Influence of pH on Steady-State Fermentation Stoichiometry of Butyribacterium methylotrophicum (Source: Grethlein et al., in press)

pH	Fermentation Stoichiometry*
6.8	$4\text{CO} \longrightarrow 2.09 \text{CO}_2 + 0.63 \text{Ac} + 0.043 \text{Bu} + 0.027 \text{EtOH} + 0.43 \text{Cells}$
6.5	$4\text{CO} \longrightarrow 2.13 \text{CO}_2 + 0.56 \text{Ac} + 0.082 \text{Bu} + 0.026 \text{EtOH} + 0.37 \text{Cells}$
6.0	$4\text{CO} \longrightarrow 2.27 \text{CO}_2 + 0.30 \text{Ac} + 0.161 \text{Bu} + 0.032 \text{EtOH} + 0.029 \text{BuOH} + 0.31 \text{Cells}$

Table 2. Standard Free Energies of Reaction for CO Bioconversion

Reaction Stoichiometry*	ΔG° (kcal/gmole CO)
$10 \text{CO} + 4 \text{H}_2\text{O} \longrightarrow \text{Bu} + 6 \text{CO}_2$	- 9.7
$4 \text{CO} + 2 \text{H}_2\text{O} \longrightarrow \text{Ac} + 2 \text{CO}_2$	- 9.8
$12 \text{CO} + 5 \text{H}_2\text{O} \longrightarrow \text{BuOH} + 8 \text{CO}_2$	- 9.0
$6 \text{CO} + 3 \text{H}_2\text{O} \longrightarrow \text{EtOH} + 4 \text{CO}_2$	- 8.1

*Ac, Bu, EtOH, and BuOH stand for acetic acid, butyric acid, ethanol, and butanol, respectively. The coefficient on cells represents the number of moles of carbon contained in the cells mass.

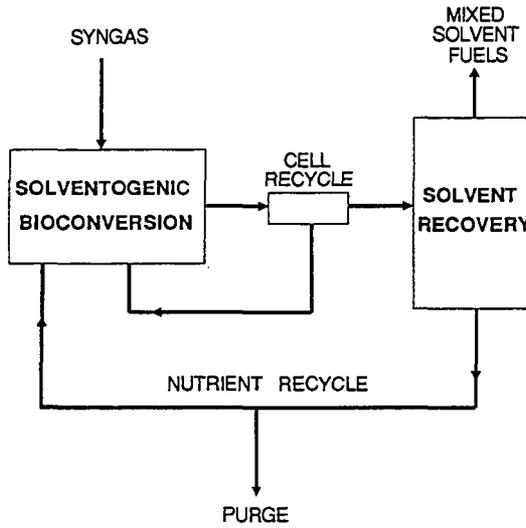


Figure 1. One-Stage Fermentation Process for Synthesis-Gas Bioconversion

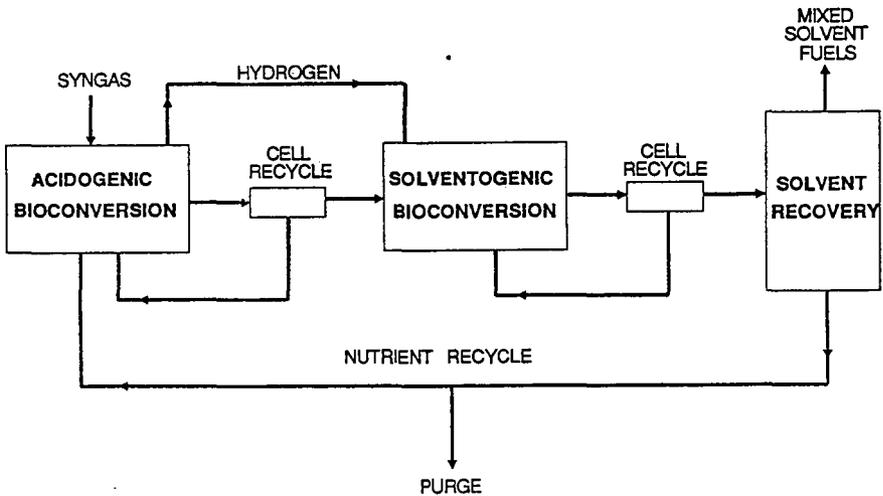


Figure 2. Two-Stage Fermentation Process for Synthesis-Gas Bioconversion