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Synthesis Gas as Substrate for the Biological Production of Fuels and Chemicals

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ABSTRACT

Synthesis gas provides a simple substrate for the production of fuels and chemicals. Synthesis gas can be produced via established technologies from a variety of feedstocks including coal, wood, and agricultural and municipal wastes. The gasification is thermally efficient and results in complete conversion of the feedstock to fermentable substrate. *Clostridium ljungdahlii* grows on the synthesis gas components, carbon monoxide, hydrogen, and carbon dioxide. Production of acetic acid and ethanol accompanies growth with synthesis gas as sole source of energy and carbon. Rate and yield parameters are compared for *C. ljungdahlii* grown on carbon monoxide, or hydrogen with carbon dioxide.

Index Entries: Synthesis gas; ethanol; acetic acid; *Clostridium ljungdahlii*; autotrophic growth.

INTRODUCTION

Anaerobic bacteria capable of autotrophic growth on synthesis gas components offer an efficient route for fuel and chemical production from a variety of initial substrates. These bacteria catalyze a Fischer-Tropsch type reaction that occurs under mild conditions of temperature and pressure with the formation of specific products. *Clostridium ljungdahlii* was grown in batch culture with H_2 or CO as the growth substrate to determine the effect of substrate on fermentation parameters used in process design.

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THEORY

Synthesis gas, a mixture of carbon monoxide (CO), hydrogen (H_2), carbon dioxide (CO_2), methane, and other hydrocarbons with diluents and contaminants, such as nitrogen and hydrogen sulfide, is formed by the partial oxidation and pyrolysis of carbonaceous materials according to the equation:

$$C_aH_bO_c + \alpha\, O_2 + \beta\, H_2O \rightarrow \gamma\, CO + \delta\, H_2 + \epsilon\, CO_2 \qquad (1)$$

Gasification at 800°C or higher at near atmosphere pressure produces a syngas, rich in CO, H_2, and CO_2 (*1*), which is a suitable substrate for autotrophic acetogenic bacteria. The reaction conditions can be controlled by adjusting the ratio of oxygen to water in the feed. Reaction conditions can be chosen for most feedstocks to approach adiabatic conversion, thus preserving the energy content of the original resource.

Gasifier technology is applicable for any carbonaceous material, including biomass materials and such biologically recalcitrant materials as coal and petroleum wastes. Gasification of woody materials converts the complex carbon structure of cellulose, hemicellulose, and lignin to the simple synthesis gas components, allowing consumption by a single organism and thus simplifying the biological processing scheme. The gasifier equipment can be adapted to a variety or mixture of feedstocks allowing the inclusion of niche or seasonal resources in a highly flexible fuels and chemicals production process. Many of these resources will be waste products, such as municipal wastes or agricultural wastes like cereal straw or poultry litter, which pose intractable disposal problems. A further advantage of synthesis gas fermentation over the traditional digestion and saccharide fermentation of biomass materials is the potential expansion of the resource stream to include hydrocarbon materials such as polyethylene plastic and rubber.

Several anaerobic acetogenic bacteria have been found that grow autotrophically, synthesizing cell materials from simple inorganic substrates such as CO, H_2, CO_2, water, and mineral nutrients. These organisms derive energy for growth from the conversion of the reduced species, CO or H_2, to acetic acid by the acetogenic pathway proposed by Ljungdahl and Wood (*2,3*). The pathway was developed through studies of *Clostridium thermoaceticum* and *Clostridium thermoautotrophicum*. The pathway is illustrated in Fig. 1. Important characteristics of the pathway are the formation of the methyl moiety of acetate on tetrahydrofolate, transfer of the methyl group to carbon monoxide dehydrogenase (CODH) via a corrinoid enzyme, and a CODH-catalyzed condensation of the methyl group with a CO-derived carbonyl and coenzyme A to form acetyl-CoA, which is hydrolyzed to acetic acid. Net adenosine triphosphate (ATP) formation to supply energy for cell growth is zero for the pathway. ATP for autotrophic growth is

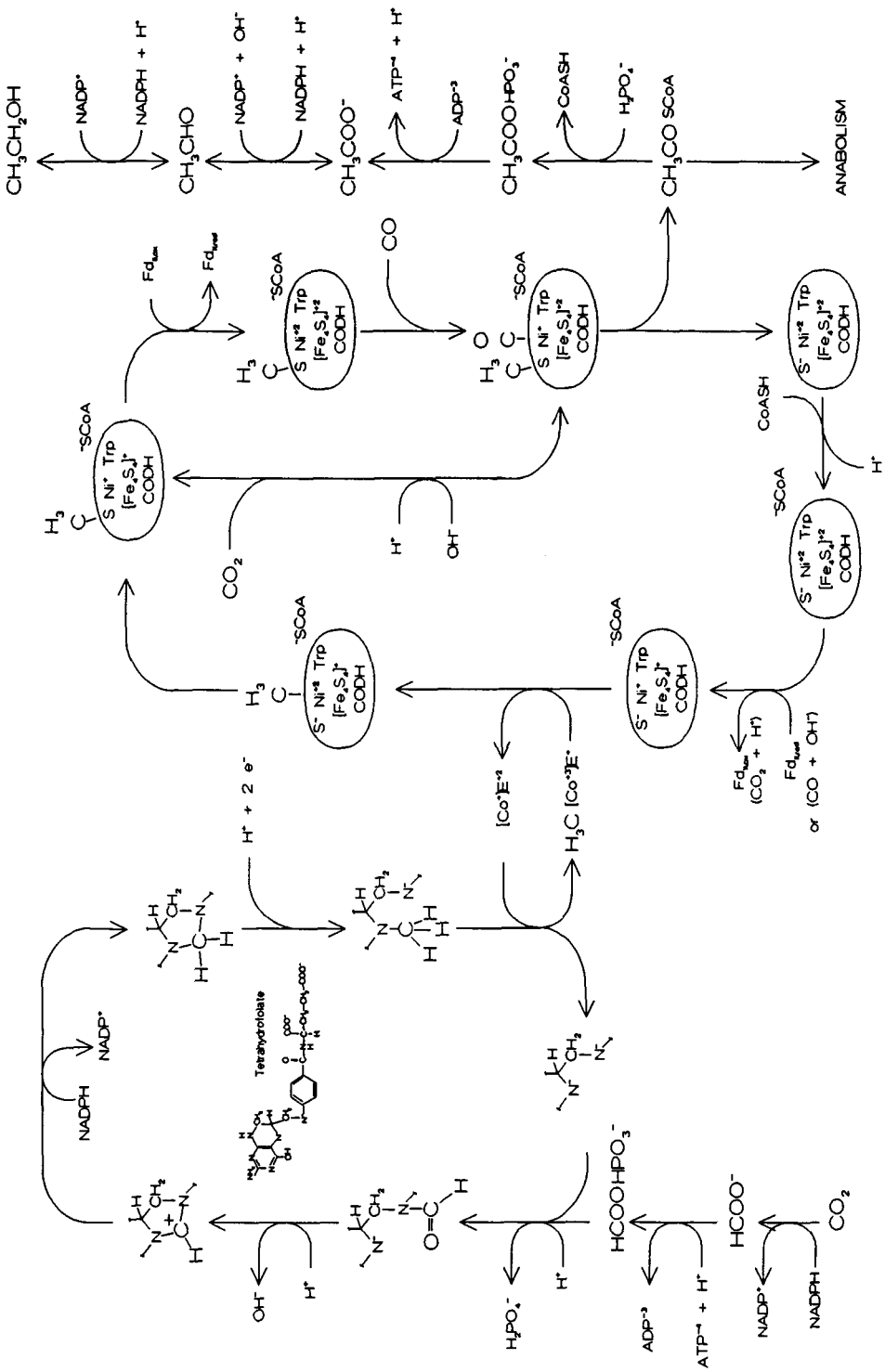

Fig. 1. Acetogenic pathway for autotrophic growth.

Table 1
Heat of Combustion and Potential of Electrochemical Half Cell

Reduced species	ΔH_c^o, kJ/mol	$E^{o'}$, mV
CO	−282.99	−581 (Eq. [2])
H_2	−285.84	−420 (Eq. [3])

Table 2
Reaction Thermochemistry

Reaction	ΔH_f^o, kJ/mol rxn	$\Delta G^{o'}$, kJ/mol rxn	HHV conserved, %
Co → HAc (Eq. [4])	−262.2	−175.04	77.0
H_2 → HAc (Eq. [5])	−273.56	−94.92	76.2
CO → EtOH (Eq. [6])	−331.03	−224.75	80.5
H_2 → EtOH (Eq. [7])	−348.13	−104.57	79.7

HHV, higher heating value.

formed by an electron transport mechanism creating a transmembrane protonmotive force that drives an ATPase in the cell wall. CODH, in addition to its role in condensing acetyl-CoA, provides protons and electrons to the electron transport chain by the electrochemical half reaction for CO:

$$CO + H_2O \rightarrow CO_2 + 2H^+ + 2e^- \quad (2)$$

The hydrogen half reaction can supply energy for cell growth via a reaction catalyzed by hydrogenase:

$$H_2 \rightarrow 2H^+ + 2e^- \quad (3)$$

When hydrogen is the growth substrate, CODH provides the carbonyl for acetyl-CoA formation by the reverse of Eq. (2). The sum of Eq. (2) and the reverse of Eq. (3) is the water gas shift reaction used to correct the H_2:CO ratio for Fischer-Tropsch chemical synthesis.

The reactions of the pathway from CO to acetic acid, using Eq. (2) to provide the necessary reducing equivalents, sum to the overall reaction stoichiometry of Eq. (4):

$$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2 \quad (4)$$

When reducing equivalents are provided by H_2 the pathway sums to Eq. (5):

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \quad (5)$$

The stoichiometric coefficients are identical for the reduced species, CO and H_2. A review of the thermochemistry (*4,5,6*) of Eqs. (4) and (5), given in Tables 1 and 2, reveals similar molar heats of combustion for CO and

H_2, and similar enthalpy changes for the reactions. But the useful energy for cell growth, the free energy, $\Delta G^{o'}$, from the reaction of CO is nearly twice that available from the reaction of H_2.

Extension of the pathway to include conversion of acetic acid to ethanol is prompted by the growth associated production of ethanol by *C. ljungdahlii* (*7*). This extension is supported by the identification of the required enzymes in *Clostridium thermoaceticum* (*8*) and *Clostridium formicoaceticum* (*9*). The pathway reactions, from CO to ethanol, sum to the overall reaction of Eq. (6) and from H_2 to ethanol, to Eq. (7):

$$6\,CO + 3\,H_2O \rightarrow CH_3CH_2OH + 4\,CO_2 \quad (6)$$

$$6\,H_2 + 2\,CO_2 \rightarrow CH_3CH_2OH + 3\,H_2O \quad (7)$$

The thermochemistry of these reactions, as shown in Table 2, again shows similar heat of reaction and conversation of fuel value for CO and H_2. The free energy available for growth is more than twice for CO than for H_2.

Although the enthalpy values for the reactions of CO and H_2 through the acetogenic pathway to acetic acid and ethanol are nearly equal, the differences in the free energy yields from CO and H_2 should be significant in the kinetic and yield parameters of the synthesis gas fermentation. A significantly higher acetate yield per cell mass and a generally lower growth rate have been reported in defined medium for *C. thermoaceticum* and other acetogens on H_2 than on CO or glucose (*10*). The lower electrochemical potential of the CO half reaction may also introduce significant effects in the equilibrium of the electrochemical reactions of the pathway.

METHODS

Organism and Medium

Clostridium ljungdahlii, Strain PETC (ATCC 49587), was originally isolated from chicken waste in the University of Arkansas laboratories, and later identified and characterized by R. S. Tanner, University of Oklahoma, Department of Botany and Microbiology. Seed culture was from a Bioflo C30 fermenter (New Brunswick Scientific, Edison, NJ), operated with continuous feed of synthesis gas (20% H_2, 15% Ar, 55% CO, 10% CO_2) and medium defined in Table 3. The fermenter culture was grown under conditions yielding over 80% conversions of both H_2 and CO. Batch bottles with 50 mL of basal (*11,12*) defined medium (*see* Table 3) were inoculated with a 10% (v/v) inoculum from the fermenter and grown in a shaker incubator (New Brunswick Scientific) with mixed synthesis gas (24% H_2, 65% CO, 11% CO_2) at 37°C and 130 rpm. The substrate gas was replenished and the pH adjusted up to 4.5 with 0.5*M* NH_4OH to maintain the culture in logarithmic growth prior to inoculation.

Table 3
Medium Composition (in mg/L)

	CSTR Medium	Basal Defined Medium
Pfennig's minerals		
KH_2PO_4	—	500
$MgCl_2 \cdot 6H_2O$	375	330
NaCl	150	400
NH_4Cl	—	400
$CaCl_2 \cdot 2H_20$	150	50
Pfennig's trace metals		
$ZnSO_4 \cdot 7H_2O$	0.75	0.1
$MnCl_2 \cdot 4H_2O$	0.225	0.03
H_3BO_3	2.25	0.3
$CoCl_2 \cdot 6H_2O$	1.5	0.2
$CuCl_2 \cdot H_2O$	0.075	0.01
$NiCl_2 \cdot 6H_2O$	0.15	0.02
$Na_2MoO_4 \cdot 2H_2O$	0.225	0.03
$FeCl_2 \cdot 4H_2O$	11.25	1.5
Na_2SeO_3	0.075	0.01
B-vitamins		
Biotin	0.040	0.033
Folic acid	0.005	0.033
Pyridoxal-HCl	0.0025	0.017
Lipoic acid	0.015	0.1
Riboflavin	0.0125	0.083
Thiamine-HCl	0.01	0.083
Ca-D-Pantothenate	0.155	0.083
Cyanocobalamin	0.0125	0.083
P-Aminobenzoic acid	0.0125	0.083
Nicotinic acid	0.0125	0.083
Other		
$(NH_4)_2HPO_4$	1500	—
H_3PO_4	1125	—
KCl	112.5	—

Equipment and Procedures

Medium preparation was carried out under a nitrogen atmosphere as described by Hungate (*13*), and Ljungdahl and Wiegel (*14*). Batch experiments were carried out in serum stoppered bottles with working volumes of 158 mL (Wheaton Scientific, Millville, NJ) using 50 mL of basal defined medium. Bottles were steam sterilized at 121°C for 20 min. Each bottle was flushed with 200 kPa (15 psig) substrate gas through 22-gage syringe needles for 2.0 min and pressured to 200 kPa. The substrate gas was either

H_2/CO_2 (75/25%) or CO/CO_2 (80/20%). Methane, 50 mL at ambient conditions, was added to all bottles as a reference inert, and 50 mL of CO_2 was added to the H_2 bottles to assure an adequate supply of carbon for growth. Bottles were reduced with 1 mL of 2.5% cysteine $HCl \cdot H_2O$ in 0.2*N* NaOH and shaken overnight prior to inoculation with 20% (v/v) seed culture. All experiments were performed in the shaker incubator at 37°C and an agitation rate of 125–140 rpm. The pH was maintained near 4.5 by the addition of 0.5*M* NH_4OH when the samples were taken.

Analytical Procedures

Cell concentrations (in mg/L) were determined by comparing optical density readings at 580 nm in a Bausch and Lomb (Milton Roy Company, Rochester, NY) Spectronic 21 spectrophotometer with a standard calibration curve. Gas compositions were obtained by gas chromatography (Model: Sigma 300, Perkin-Elmer, Norwalk, CT) with a 1.8 m × 3 mm Carbosphere (Alltech, Deerfield, IL) 60/80 mesh, column at 100°C. Liquid analyses were performed by gas chromatography (Model: HP 5890 Series II with HP Chemstation data processing software, Hewlett-Packard, Avondale, PA) on previously acidified samples in a 1.5 m × 3 mm column packed with Porapak QS (Alltech), 100/200 mesh, at 190°C. 1-Propanol was used as the internal standard for liquid analysis.

RESULTS

Substrate (H_2 or CO) consumption is presented for six otherwise identical experiments in Fig. 2. Each bottle initially contained about 7.6 mmol of substrate. The consumption curves were consistent for both substrates, with the initial consumption rate of H_2/CO_2 leading that of CO/CO_2 by a factor of 1.5. Substrate consumption rates were nearly equal in the late fermentation, but the specific uptake of substrate, moles of substrate consumed per gram of cells per hour, was more than twice as fast for H_2 than for CO (*see* Table 4). Cell growth, presented in Fig. 3, in stark contrast to to substrate uptake was much faster for CO than H_2. The medium nutrient limitation of 350–400 mg of cells/L was not approached on the initial substrate charge, but the fermentation appeared to be mass transfer limited late in the fermentation. Specific growth rates for H_2 were only half those for CO (*see* Table 4), with doubling times of 21.3 and 12.6 h, respectively.

Product formation, presented in Fig. 4, mirrored substrate uptake with the CO grown culture lagging the H_2 culture. The ratio of ethanol to acetic acid produced from H_2 was half that from CO. However, ethanol production was significiant on both substrates, particularly for these substrate limited batch reactors. The specific production rate of the H_2 culture given in Table 4, was more than 2.5 times that for CO.

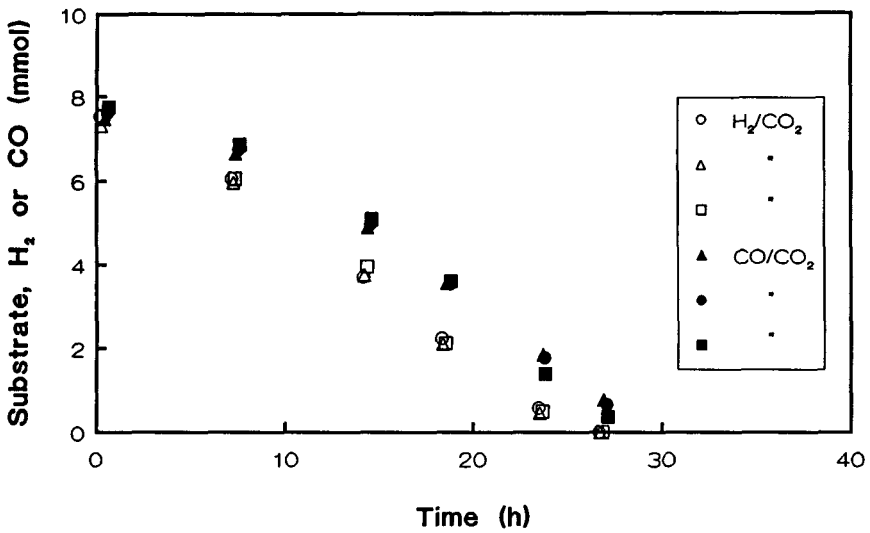

Fig. 2. H_2 or CO consumption by *C. ljungdahlii* in batch culture.

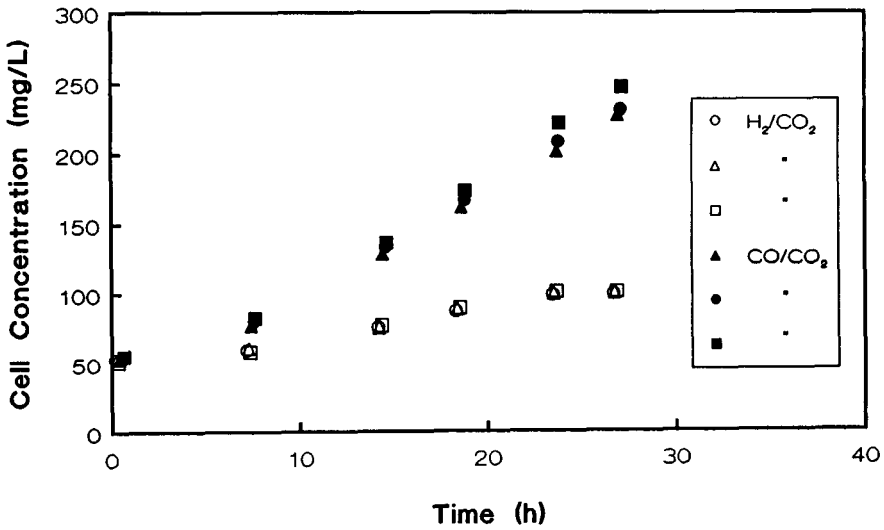

Fig. 3. Cell growth on H_2 or CO for *C. ljungdahlii* in batch culture.

Table 4
Rate Parameters for Fermentation of CO and H_2 by *C. ljungdahlii*

Substrate	Sp. uptake rate, mol/g h	Sp. growth rate, g/g h	Sp. prod'n rate, mol/g h	EtOH/HAc, mol/mol
CO	0.0385	0.0600	0.0060	0.658
H_2	0.0795	0.0326	0.0159	0.297

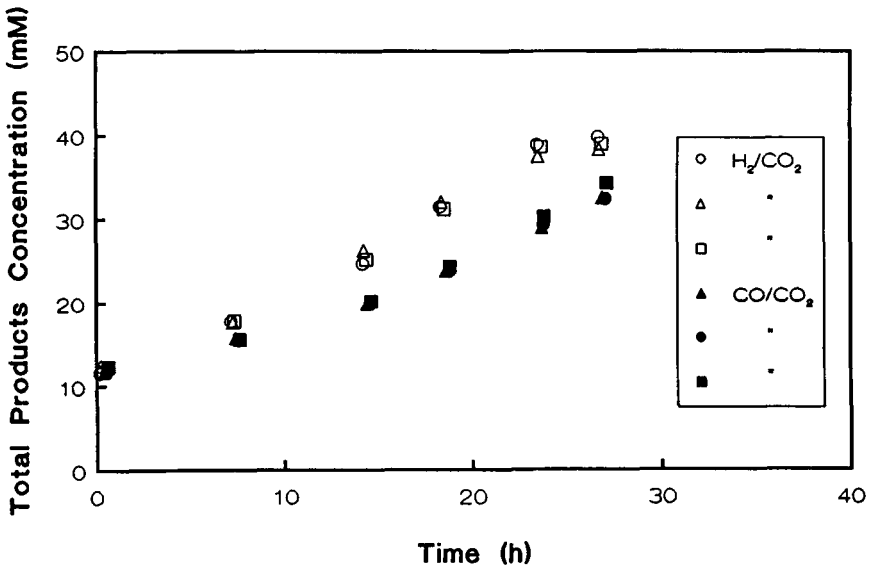

Fig. 4. Ethanol and acetic acid production by *C. ljungdahlii* on H_2 or CO in batch culture.

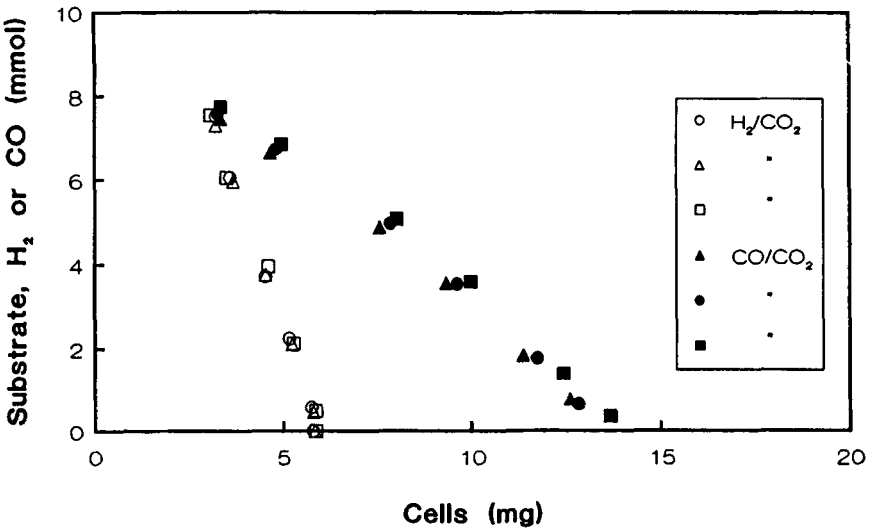

Fig. 5. Cell growth on H_2 or CO for *C. ljungdahlii* in batch culture.

The consumption of substrate gas during cell growth is depicted in Fig. 5. The H_2 culture produced much less cell mass despite consuming an equal molar amount of substrate. The yield of cells from substrate, $Y_{X/S}$ in Table 5, found as the reciprocal of the slope in Fig. 5, was only 27% of that obtained from CO.

Table 5
Yield Parameters for Fermentation of CO and H_2 by *C. ljungdahlii*

Substrate	$Y_{X/S}$, g/mol	$Y_{P/S,\ exp}$, mol/mol	$Y_{P/S,\ th}$, mol/mol	$Y_{P/X}$, mol/g
CO	1.378	0.156	0.217	0.113
H_2	0.373	0.200	0.231	0.533

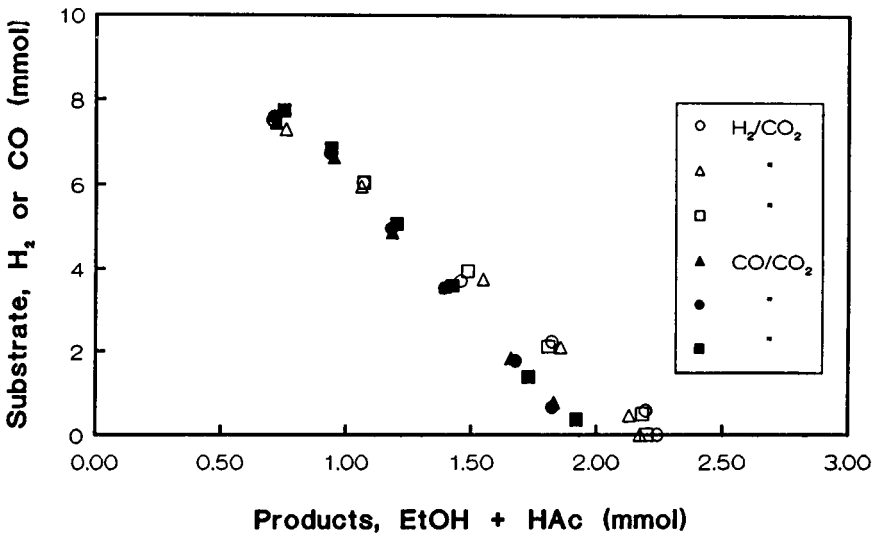

Fig. 6. Ethanol and acetic acid production on H_2 or CO by *C. ljungdahlii* in batch culture.

The consumption of substrate as ethanol and acetic acid were produced is shown in Fig. 6. The H_2 culture formed more product from the initial substrate than did the culture grown on CO. The experimental yield of products from substrate, $Y_{P/S,exp}$ in Table 5, which is the reciprocal of the slope from Fig. 6, is 28% greater for H_2 grown *C. ljungdahlii* than for the same culture grown on CO. The ethanol to acetic acid ratio was used with the stoichiometry of Eqs. (4)–(7) to calculate a theoretical yield of products from substrate, $Y_{P/S,th}$, also shown in Table 5. The difference between $Y_{P/S}$ from theory and from experiment is two to four times the amount of cell carbon formed per mole of substrate and reflects the additional requirements for energy and reducing equivalents for anabolic synthesis of cell material. This was about 12% of substrate consumed for CO and 6% for H_2.

The formation of products with cell growth is presented in Fig. 7. The slopes of these linear plots are the growth related yield of products from cells, $Y_{P/X}$ in Table 5. Product formation under the conditions of this experiment was nearly completely growth related, hence the linearity of the plots. The culture grown on H_2 produced 33% more ethanol and acetic acid than the culture grown on CO, but only 27% of the cell mass. Thus, the yield of products per cell from H_2 was 4.7 times that from CO.

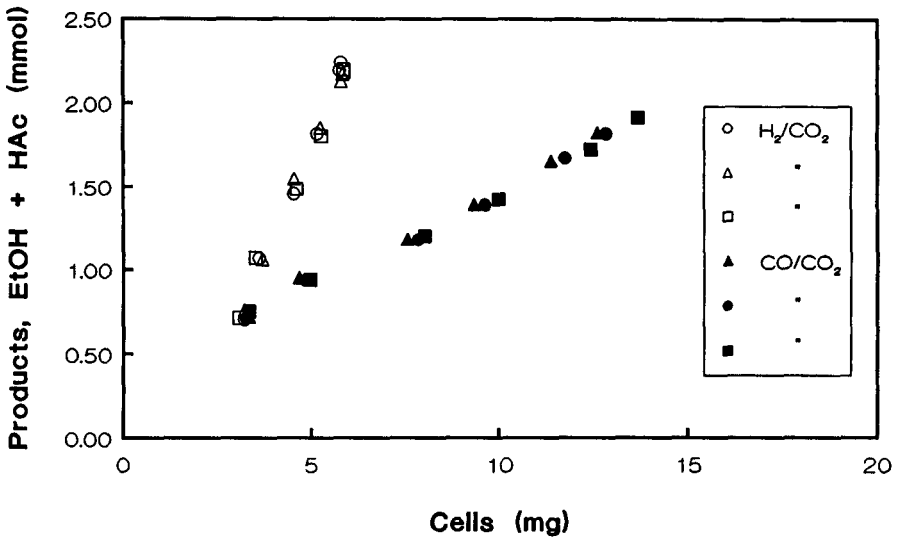

Fig. 7. Ethanol and acetic acid production from cell growth for *C. ljungdahlii* in batch culture with H_2 or CO.

DISCUSSION

The rate-limiting step for the growth of *C. ljungdahlii* on synthesis gas can be in the mass transfer of substrate to the cell, in the oxidation of substrate to provide reducing equivalents, in the pathway reactions used for energy generation or in the anabolic synthesis of cell materials. In this experiment, the initial substrate pressures, solubilities (1/H is 1.3 and 1.5 $\times$ 10^{-5} mole fraction/atm for H_2 and CO, respectively; where H is the Henry's law constant (*5*)), and liquid phase diffusivities were similar for both substrates, so differences should be attributable to reaction thermodynamics or kinetics at the cellular level. The cultures grown on either substrate became mass transfer limited at practically identical rates, about 0.34 mmol/h. The mass transfer controlling resistance was in the liquid film of the gas–liquid interface. The specific uptake of substrate, mol substrate/g cells h, was for H_2 twice that for CO. The effective activity of hydrogenase per cell, under the reaction conditions, was higher than that of CODH. The higher uptake of H_2 was not a mass transfer effect, but reflects the ability of each cell to process the substrate.

The specific growth rate, g cells/g cells h, of H_2 grown culture was half that for cultures grown on CO. Energy derived from the pathway reactions of Fig. 1 is required for the anabolic reactions of cell synthesis and the rate of culture growth is limited by the rate of energy generation. The reactions of the pathway are electrochemical in nature; reactant and product concentrations determine reaction equilibrium, direction, and rate. The higher free energy of the CO reactions is manifested in lower electrochemical potential within the cell and a higher electrical component

of the proton-motive force. This results in a higher yield of ATP from the protons traversing the ATPase.

The products, ethanol and acetic acid, are by-products of the energy-generating reactions used to support anabolic synthesis. The higher rates of product formation from H_2 are the result of faster substrate uptake and lower energy evolution than in the CO reactions.

The yield of cells from substrate is related to the free energy, $\Delta G^{o'}$. For the reactions of CO at the standard conditions of reactant and product concentrations (1.0 atm for gases and 1.0*M* for aqueous solutes), the free energy is twice that obtained from the corresponding reaction from H_2. The free energy change for a reaction is a strong function of concentration of reactants and products. CO_2, as a reactant in the reactions of H_2 and a product in the CO reactions, will reduce the useful energy from H_2 and increase that from CO when it is present in less than the standard concentration. In this way the actual free energy and cell yield from CO might be increased to four times compared to H_2.

The efficiency of the extraction of useful energy from H_2 is less than that from CO, so less of the processed substrate is diverted to anabolic synthesis of cell material. In these experiments 87% of the theoretical yield of products was obtained from H_2 compared to only 72% from CO. These losses reflect the diversion of acetyl-CoA to anabolism and diversion of additional energy for the anabolic synthesis.

CONCLUSIONS

CO is the preferred substrate for cell growth. Despite concern about inhibition, CO provides a higher specific growth rate and is conducive of ethanol production. H_2 is far superior for the formation of products, ethanol and acetic acid. H_2 provides a higher specific production rate and increased efficiency of substrate conversion to products and nutrient utilization. Fermentation of mixed substrates with balanced consumption of H_2 and CO should give intermediate yields, and may result in complementary rate effects.

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