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BIOLOGICAL PRODUCTION OF ETHANOL FROM COAL

UNIVERSITY OF ARKANSAS

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SUMMARY

A batch kinetic study involving Clostridium lungdahlii in a minimal medium was carried out in order to provide baseline data for the effects of nutrients on product ratio and kinetics. The use of this minimal medium containing vitamins, minerals, select amino acids and salts showed both a lower maximum specific growth rate and a lower maximum specific uptake rate than found when using a complex medium supplemented with 0.01% yeast extract. At the same time, the product ratio was improved slightly in favor of ethanol over acetate. Future experiments will measure the effects of ammonia and phosphate limitation on product ratio and process kinetics.

INTRODUCTION

Clostridium ljungdahlii, a strain isolated from animal waste in the University of Arkansas laboratories, converts CO, CO_2, and H_2 in synthesis gas to ethanol and acetate by the reactions:

$$6\ CO + 3\ H_2O \rightarrow C_2H_5OH + 4\ CO_2 \quad (1)$$

$$2\ CO_2 + 6\ H_2 \rightarrow C_2H_5OH + 3\ H_2O \quad (2)$$

$$4\ CO + 2\ H_2O \rightarrow CH_3COOH + 2\ CO_2 \quad (3)$$

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

Since acetate is produced in conjunction with growth and energy production by the bacterium, its production is favored over ethanol production, which requires energy for formation. In fact, batch studies without culture

manipulation showed a 20:1 ratio of acetate to ethanol. Various techniques have been employed in the University of Arkansas laboratories to significantly improve this ratio. The addition of low concentrations (30 ppm) of reducing agent to the liquid medium in batch culture resulted in the production of equimolar amounts of ethanol and acetate. The use of low concentrations (0.01%) of yeast extract and high partial pressures of H_2 in the gas phase favored ethanol as a product over acetate. Finally, a pH shift in the medium from 5.0 to 4.0 resulted in greater ethanol production in favor of acetate production.

There has also been significant success in improving the product ratio in favor of ethanol in continuous culture. Molar product ratios in the CSTR have been as high as 3 moles ethanol per mole of acetate by utilizing pH shift coupled with alternate medium constituents. Acetate has, in fact, been consumed in the CSTR instead of produced by employing a combination of pH shift, a low yeast extract concentration and the addition of benzyl viologen in a series of two CSTRs.

In addition to yeast extract, other nutritional factors such as phosphate or iron limitations in the medium have also been shown to favorably affect the production of alcohols in Clostridium acetobutylicum fermentations. Chemostat experiments with C. acetobutylicum grown in a phosphate-limited medium resulted in an increase in the butanol/acetone ratio from 2:1 to 3.8:1 when co-fermented on whey and lactate. When operated under iron-limited conditions, the ratio was improved from 2:1 to 8:1 (Bahl *et al.* 1986).

A minimal medium containing salts, vitamins, minerals and essential amino acids was developed to replace the complex medium, since the yeast extract

present in the complex medium contains a large array of nutritional compounds. Phosphate limitation, for example, may be studied by utilizing the minimal medium by first removing all phosphate from the medium and then replacing a portion of the phosphate. In this manner, incremental amounts of phosphate can be investigated for their effect on the product ratio and process kinetics.

The purpose of this report is to present kinetic information on the growth and uptake rate of C. ljungdahlii on CO, CO_2, and H_2 in the presence of the minimal medium. Kinetic information for C. ljungdahlii on a complex medium containing yeast extract is also presented for comparison purposes. This kinetic information obtained on minimal medium will form the baseline for future nutrient limitation studies involving ammonia, phosphate and other nutritional factors. Eventually, pH shift coupled with nutrient limitation and other factors will be tested in batch and continuous culture to assess the effects on process kinetics and product ratio.

ELIMINATION OF YEAST EXTRACT THROUGH ADAPTATION OF C. LJUNGDAHLII TO A "DEFINED MEDIUM"

Fermentation studies with C. ljungdahlii have been conducted in a basal medium containing primarily 0.01 mass % yeast extract, as well as B-vitamins and minerals. This level of yeast extract was chosen for batch experimentation after the evaluation of various concentrations of yeast extract and their effect on the level of ethanol produced and the ethanol/acetate molar ratio. Yeast extract is a complex medium constituent, consisting of vitamins, minerals, salts and amino acids. In addition, yeast extract is a very expensive medium component and causes the medium to be "undefined" in that the exact nutritional factors required by C. ljungdahlii for growth are unknown. Experiments were thus carried out in an attempt to

remove yeast extract from the medium after which the defined medium was evaluated for its influence on ethanol productivity.

The culture was gradually adapted to grow in a medium devoid of yeast extract following strategies similar to those employed by Savage and Drake (1985) with Clostridium thermoautotrophicum. The culture was sequentially transferred into medium containing decreasing concentrations of yeast extract (0.01, 0.005, 0.001, and 0 percent) and an enriched formulation in minerals, trace metals and B-vitamins shown in Table 1. To eliminate yeast extract ultimately, a solution of amino acids (Table 2) was added to the enriched medium as a yeast extract replacement. Next, the removal of the B-vitamins solution was attempted, but growth was prohibited. However, the culture was then successfully adapted to growth without the amino acid solution.

GROWTH AND UPTAKE KINETICS FOR C. LJUNGDAHLII

Complex Medium

Previous studies with C. ljungdahlii in complex medium containing 0.01% yeast extract showed that the standard Monod models for growth and CO uptake (Equations (5) and (6))

$$\mu = \frac{\mu_m P_{CO}^L}{K_p + P_{CO}^L} \tag{5}$$

and

$$q = \frac{q_m P_{CO}^L}{K_p' + P_{CO}^L} \tag{6}$$

reduced to zero order equations for $P_{CO} \leq 1.1$ atm. The specific growth rate, μ, may be shown as

$$\mu = \mu_m = 0.04\ h^{-1} \tag{7}$$

and the specific uptake rate, q, may be written as

$$q = q_m = 43.4 \text{ mmol CO/gcell}\cdot\text{h} \quad (8)$$

Equations (7) and (8) indicate that substrate inhibition by CO is negligible over the concentration (dissolved tension) range studied and that K_p and K_p' are small in comparison to P_{CO}^L. If these conditions exist, Equations (5) and (6) reduce to the zero order equations of Equations (7) and (8).

When the partial pressure range of CO was expanded further to 4.5 atm and a richer medium was employed, zero order behavior was still seen, although the values of μ_m and q_m were increased.

$$\mu_m = 0.079 \text{ h}^{-1} \quad (9)$$

$$q_m = 65.43 \text{ mmol CO/gcell}\cdot\text{h} \quad (10)$$

Thus, nutritional factors affect both growth and substrate uptake.

Minimal Medium

The effects of the minimal medium on process kinetics were also studied. The methods of kinetic analysis previously developed in the University of Arkansas laboratories were used in this mass transfer/kinetic analysis.

Five bottles (nominal volume of 1200 mL) were each filled with 200 mL of defined medium at pH 5.0 and with synthesis gas at approximately 7 psig. The culture medium was reduced with 0.5 g/L cysteine-HCl and inoculated with 10% by volume inoculum of C. ljungdahlii seed culture previously grown in defined medium. Figures 1-3 show experimental results for cell concentration, CO consumed and ethanol produced, respectively.

As is seen in Figure 1, the lag phase for cell growth ranged from approximately 100 to 150 hrs. This compares closely with lag phase times observed with fermentations in basal medium at similar synthesis gas pressures. Carbon monoxide consumption (Figure 2), though initiated at

different times (e.g. lag phases), progressed at similar rates and fell to zero concentration. When adjusted for the different lag phases, the time for complete conversion in each bottle was approximately 150 hrs; again, comparing closely with cultures grown in basal medium under similar operating conditions. Ethanol concentrations during the fermentation are given in mmoles in Figure 3. Peak levels of production for the five batch bottles ranged from 1.1 to 1.9 mmoles ethanol. As with the comparison made for the lag phase between this experiment and those using basal medium, these ethanol levels here are slightly higher than those obtained with C. ljungdahlii in basal medium. For cultures grown in basal medium at equivalent initial substrate levels, the peak ethanol levels varied from 0.6 to 1.1 mmol. However, these results are insufficient to attribute the slightly higher ethanol production solely to the change in medium composition.

The volumetric mass transfer coefficient (K_La/H) and the reaction kinetics parameters (μ_{max} and q_{max}) are presented in Figures 4-6. The mass transfer coefficient determined for this experiment (K_La/H = 5.40 mmol CO/atm·L·h) is lower than most values obtained previously in experiments with basal medium carried out under the same conditions. Similarly, the maximum specific growth (μ_{max}) and CO uptake (q_{max}) rates (0.020 h^{-1} and 21.95 mmol CO/gcell·h, respectively) are significantly lower than those calculated for C. ljungdahlii growing in basal medium. Apparently, nutrient limitation affected both cell growth and the ability to uptake substrate, while slightly improving the product ratio toward ethanol. Future experiment will evaluate the effects of nutrient limitation on product ratio and process kinetics.

REFERENCES

1. Bahl, H., M. Gottwald, A. Kuhn, V. Rale, W. Andersch, and G. Gottschalk (1986), *Appl. Environ. Microbiol* 52, 167.

2. Savage, M. D., and H. L. Drake (1986), *J. Bacteriol.* 165, 315.

Table 1

Enriched Medium Composition/100 ML Medium

5.0 ml mineral solution
0.5 ml trace minerals solution
2.0 ml B-vitamin solution
0.1 ml Resazurin solution (0.1%)
80 ml distilled water

Mineral Solution	g/L
$(NH_4)_2\ SO_4$	10.0
NH_4Cl	10.0
KH_2PO_4	136.0

B-vitamins	mg/L
Biotin	20
Folic Acid	20
Pyridoxal HCl	10
Lipoic A.(Thioctica)	60
Riboflavin	50
Thiamine HCl	50
Ca-D-Pantothenate	50
Cyanocobalamin	50
P-Aminobenzoic Acid	50
Nicotinic Acid	50

Trace Mineral Solution (g/L)	
Nitrilotriacetate	1.5
$MgSO_4 \cdot 7H_2O$	6.1
NaCl	1.5
$FeSO_4 \cdot 7H_2O$	0.1
$CoCl_2 \cdot 6H_2O$	0.1
$CaCl_2 \cdot 2H_2O$	0.1
$Zn\ Cl_2$	0.1
$CuCl_2 \cdot xH_2O$	0.01
$AlK(SO_4)_2 \cdot 12H_2O$	0.01
H_3BO_3	0.01
$Na_2\ MoO_4 \cdot 2H_2O$	0.01
$NiCl_2 \cdot 6H_2O$	0.05
Na_2SeO_3	0.0005
$MnSO_4 \cdot H_2O$	0.5

Table 2

Amino Acid Solution Composition

(16 mg/L of each)

valine	leucine
threonine	cysteine
arginine	glutamic acid
histidine	phenylalanine
methionine	serine
lysine	asparagine
	tryptophan

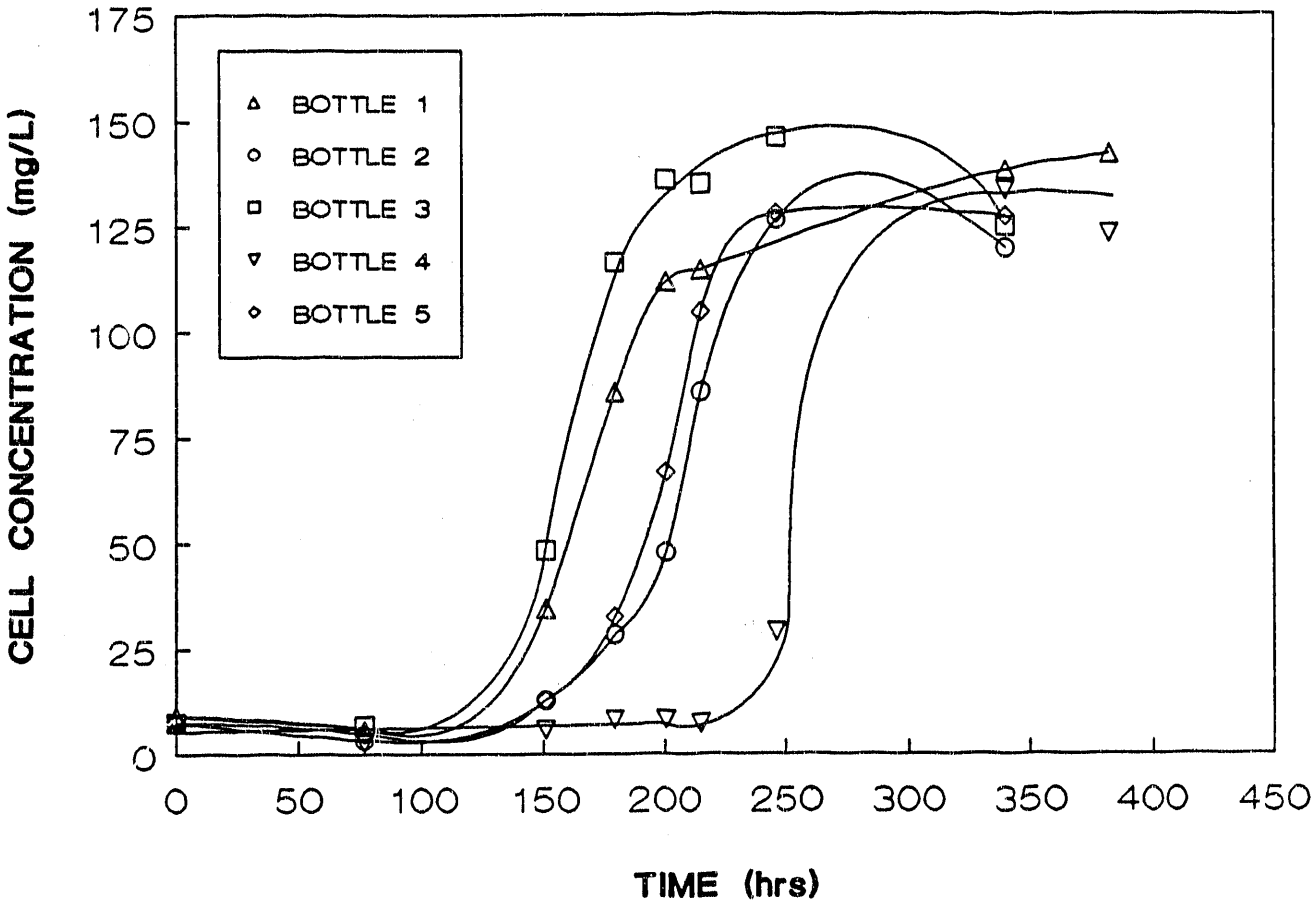

Figure 1. Cell concentration profile for *C. ljundahlii* in batch culture with a Defined Medium.

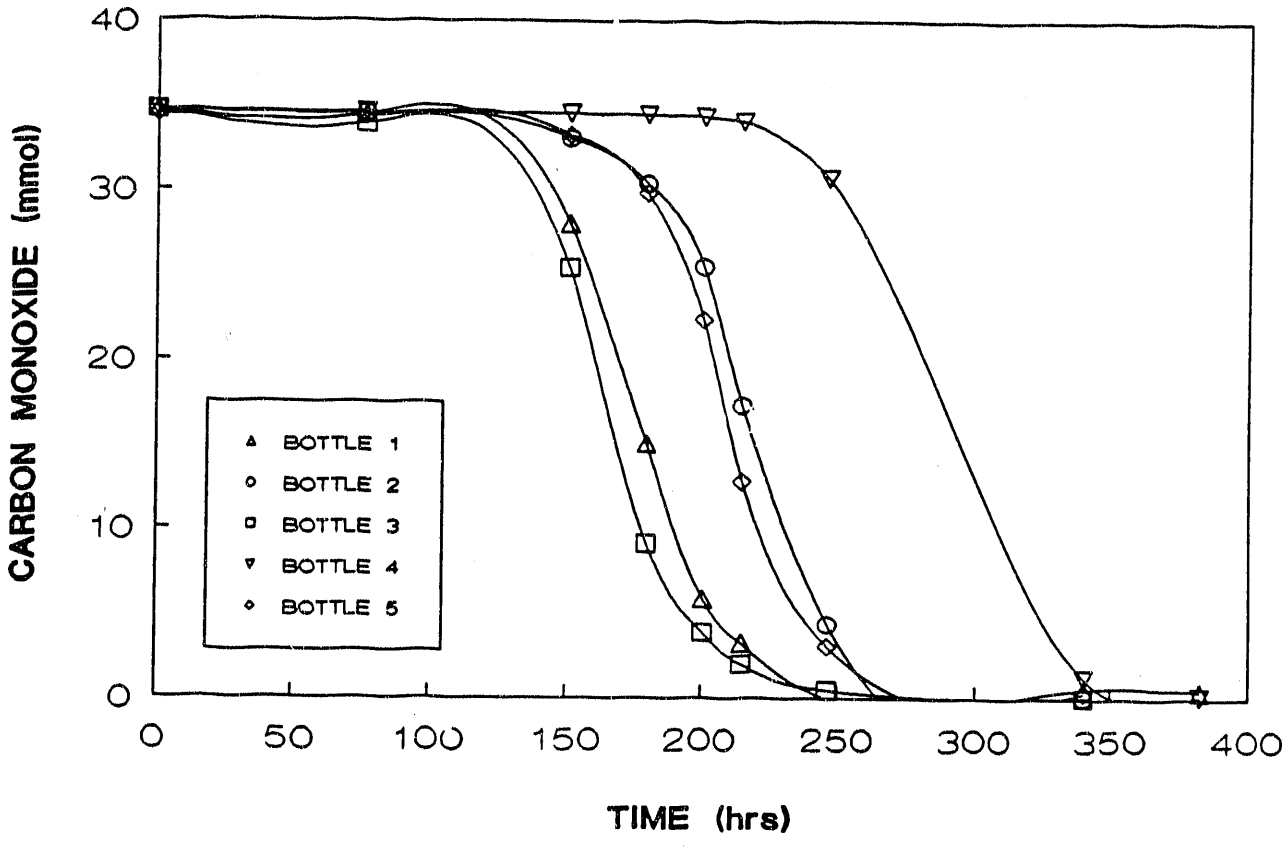

Figure 2. CO consumption profile for *C. ljungdahlii* in batch culture with a Defined Medium.

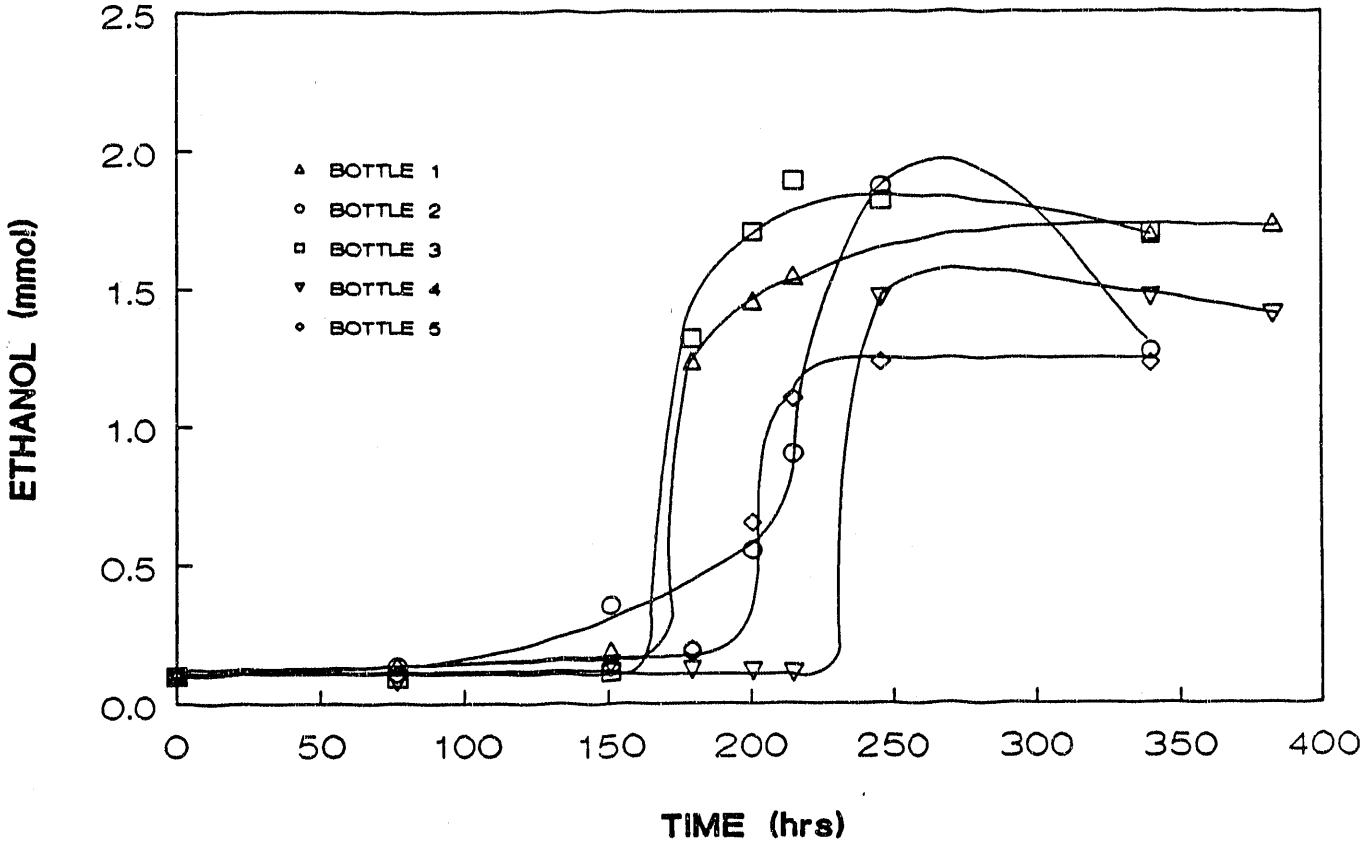

Figure 3. Ethanol production profile for *C. ljundahlii* in batch culture with a Defined Medium.

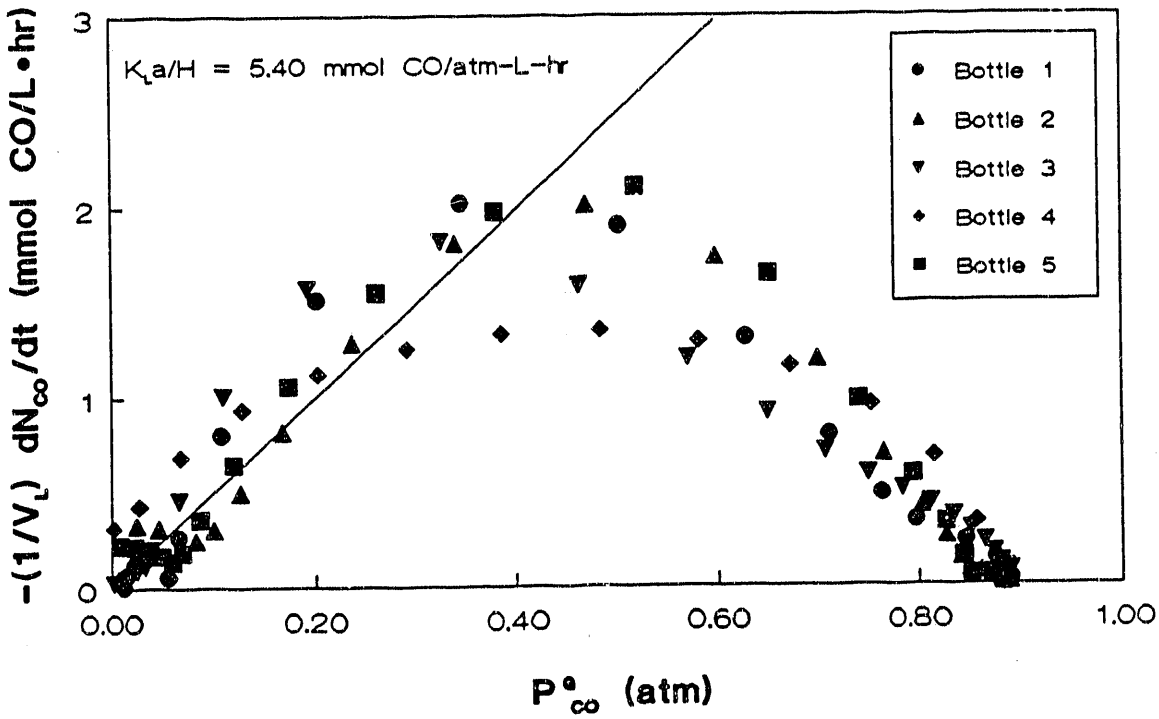

Figure 4. The volumetric uptake rate of CO as a function of the gas phase partial pressure in defined medium.

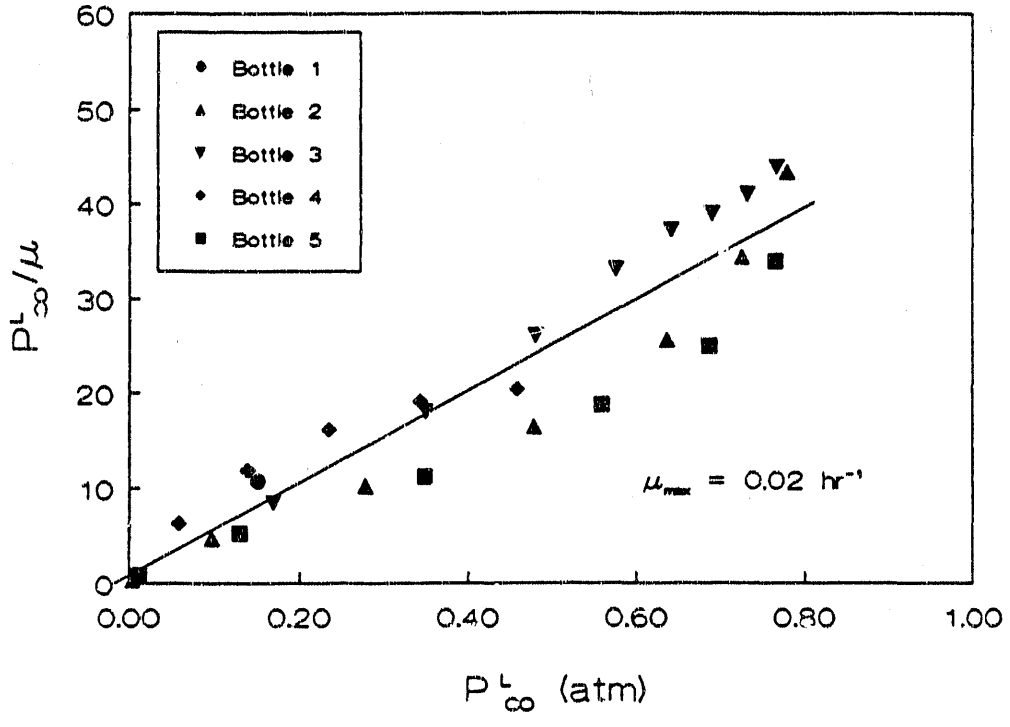

Figure 5. Monod model for the rate of cell growth (μ_{max}) in defined medium.

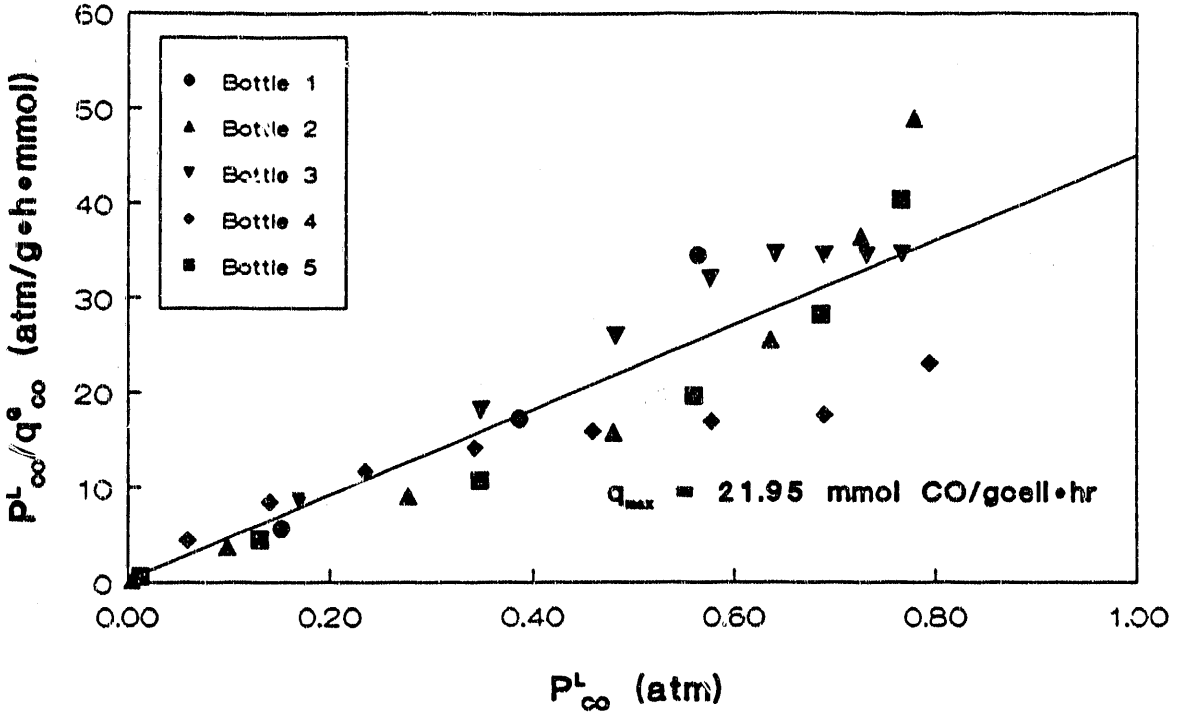

Figure 6. The partial pressure of CO (P^L_{CO}) over the specific uptake rate of CO (q^o_{CO}) plotted as a function of P^L_{CO} for defined medium.

DISCLAIMER

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