KINETICS OF GROWTH AND HYDROGEN UPTAKE
BY METHANOBACTERIUM FORMICICUM

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Fermentation kinetics for the growth and conversion of H₂ and CO₂ to CH₄ by M. formicicum were modeled by using Monod equations. The maximum specific growth rate and H₂ uptake rate, 𝜇_{max} and q_{max}, were found to be 0.053 h⁻¹ and 0.13 mol/h·g cell, respectively. The partial pressure of H₂ was found not to have a significant effect on either growth or H₂ utilization. The yield of CH₄ from H₂ was calculated as 0.27 mol/mol, which is within 7% of the theoretical value of 0.25.

INTRODUCTION

Methanobacterium formicicum, a slender, crooked, rod-shaped bacterium with blunt rounded ends, is capable of reducing CO₂ to CH₄ at mesophilic temperatures with H₂ or formate as electron donors (Boone and Mah, 1989). The bacterium is abundant in anaerobic sewage sludge digesters or anaerobic freshwater sediments, and is also present in low numbers in cattle rumen or as endosymbionts in anaerobic protozoa (Van Bruggen et al., 1984). M. formicicum and other methanogens have recently received attention for their ability to convert CO₂ and H₂ in coal-derived synthesis gas to CH₄. This process is described by the equation:

\[ \text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \quad <1> \]

This paper reports the results of a kinetic study aimed at determining CH₄ yields and kinetic parameters for rates of growth and H₂ uptake by M. formicicum by using the technique of Vega et al. (1989). Kinetic coefficients including the effect of substrate (H₂) are found and compared to the results of Shauer et al. (1982).

MATERIALS AND METHODS

Microorganism. Methanobacterium formicicum, ATCC 33274, was maintained in serum bottles containing methanogen medium [ATCC medium 1045 (Cote et al., 1984)] supplemented with NiCl₂ (Schonheit et al., 1979) under a H₂/CO₂ (75%/25%) atmosphere at a total pressure of 1 atm and 37°C. Seed cultures were prepared in the same manner, with shaking and regassing as needed for 5-10 min.

Equipment. Batch experiments were performed in 150 ml serum bottles sealed with butyl rubber stoppers and aluminum crimp seals. The fermentations were conducted in a New Brunswick
Scientific (New Brunswick, NJ) model G25 shaker incubator at 100 rpm and 37°C.

**Reactor Startup and Operation.** To begin an experiment, 75 ml of medium were added to the serum bottles under anaerobic conditions and autoclaved for 20 min at 2 atm. Prior to inoculation, the medium in each serum bottle was reduced with 1 ml of sodium sulfide (2.5%) and 1 ml of cysteine-HCl (2.5%). The bottles were then placed in the shaker incubator for 10-15 min at 37°C prior to inoculation with 5 ml of the seed culture. The proper gas phase was obtained by introducing H₂ and CO₂ through cotton filters and needle-tubing connectors into the bottles. Check valves connected to the outlet needle allowed various pressures to be achieved inside the bottles. In addition, pure H₂ was also added as needed. A well-measured volume of argon (20 ml) at ambient conditions was then introduced into each bottle to serve as a total pressure indicator. Once the experiment began, the reactors were sampled for gas phase composition, cell concentration and medium pH with time along the course of the fermentation.

**Analytical Methods.** Cell concentrations were estimated from turbidity measurements at 540 nm using a Milton Roy Co. (Rochester, NY) Spectronic 21 spectrophotometer. A linear calibration curve of cell concentration as a function of absorbance (X=395 ABS₅₄₀) was utilized in estimating cell concentrations from optical density readings.

The pH was held constant at 7.2 (±0.2 units) by the addition of CO₂ to the gas phase during sampling in conjunction with NaHCO₃ present in the liquid medium. The pH was monitored using a Corning (Medfield, MA) 140 pH meter.

The concentrations of the gas phase components were analyzed on a Perkin Elmer (Norwalk, CT) Sigma 300 gas chromatograph equipped with a thermal conductivity detector connected to a Perkin Elmer LCI-100 Laboratory Computing Integrator. A 1.8 m x 3 mm stainless steel column packed with Carbosphere (Alltech, Deerfield, IL), 60/80 mesh, was used for the analysis. The oven temperature was maintained at 135°C and both the injector and detector temperatures were 175°C. Helium was used as the carrier gas at a flow rate of 40 ml/min.

**Estimation of Kinetic Parameters.** The methods used in obtaining kinetic parameters for *M. formicicum* were outlined in detail previously for the production of acetate from CO by *Peptostreptococcus productus* (Vega et al., 1989). Briefly stated, cell and substrate concentration profiles were utilized first to calculate mass transfer coefficients for the batch bottles during the mass transfer limited region of the fermentation (where $p_a=0$). This was followed by the estimation of the liquid phase concentration ($p_i$) profiles for the balance of the fermentation. Initially, modified Monod models, accounting for possible substrate inhibition (Andrews, 1969), were then utilized in obtaining the kinetic parameters:

\[
\mu = \frac{\mu_{max} \cdot p_i}{K_p + p_i + (p_i)^2/W} \quad <2>
\]

\[
q = \frac{q_{max} \cdot p_i}{K_q + p_i + (p_i)^2/W} \quad <3>
\]

In Equations <2> and <3>, $\mu$ represents the specific growth rate, $q$ represents the specific uptake rate of H₂, $p_i$ is the dissolved H₂ tension in the liquid phase and $\mu_{max}$, $q_{max}$, $K_p$, $K_q$, $W$ and $W'$ are constants.

**RESULTS**

Typical cell, substrate and product concentration profiles for the conversion of H₂ and CO₂ to CH₄ by *M. formicicum* in batch culture are presented in Fig. 1-3.

![Fig. 1](image1.png)

**Fig. 1.** Effect of initial H₂ partial pressure on cell growth.

![Fig. 2](image2.png)

**Fig. 2.** Effect of initial H₂ partial pressure on H₂ consumption.