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Effect of Temperature and Pressure on Growth and Methane Utilization by Several Methanotrophic Cultures

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ABSTRACT

Several methanotrophic microorganisms, i.e., *Methylococcus capsulatus* (Bath), *Methylomonas albus* (BG-8), *Methylosinus trichosporium* OB3b, and *Methylocystis parvus* (OBBP), were evaluated for growth and methane utilization. The effect of temperature was examined in the range of 25 to 45°C for growth and methane utilization. The temperature variations (25–35°C) had minimal effect on growth of *M. albus* and *M. parvus*. Methane consumption varied at different temperatures with a maximum of 0.67 mol%/h and 0.53 mol%/h. at 30 and 35°C, respectively, for *M. albus* and *M. parvus*. The growth and methane consumption was slower for *M. trichosporium* OB3b as a maximum methane consumption of 0.07 mol%/h was obtained at 25°C and growth was inhibited at 35°C. *M. capsulatus* grew the best at 37°C and growth was affected at higher temperature of 45°C. Of the different cultures examined, *M. albus* and *M. capsulatus* grew the best and were further evaluated for the effect of pressure in the range of 10–50 psi. The results obtained using *M. albus* demonstrated an enhancement in methane consumption rate by fourfold and final cell concentration by 40% at a pressure of 20 psi by injecting a methane/oxygen mixture, however further increase in the pressure up to 50 psi inhibited the growth. The inhibition was not seen with nitrogen incorporated mixture of oxygen and methane, which suggest that the high partial pressure of methane and/or oxygen are inhibitory for the growth of *M. albus*. *M. capsulatus* was more sensitive to pressure as evidenced by inhibition at the relatively low pressure of 10 psi.

Index Entries: *Methylococcus capsulatus*; *Methylomonas albus*; temperature; pressure.

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INTRODUCTION

Methanotrophic bacteria have in common the ability to utilize methane as a sole source of energy and as a major carbon source *(1)*. The methanotrophic bacteria that have been isolated and characterized to-date have all been Gram-negative, obligatory aerobic, and have intracytoplamic membranes *(2–4)*.

Methanotrophs are classified in several groups based on the metabolic pathway and other characteristics. Types I and X are phylogenetically related to the L-subgroup of the proteobateria *(5)*. Examples of these groups include *Methylomonas albus* and *Methylococcus capsulatus*, respectively. These bacteria utilize the RuMP pathway for formaldehyde assimilation, though other pathways do exist for their metabolism. Type II are also related to L-subgroup of the proteobacteria but uses the serine pathway for formaldehyde fixation. Examples of this group includes *Methylosinus trichosporium* OB 3b and *Methylocystis parvus*. Type X bacteria are able to grow at a higher temperature (> 40°C), however, Type II do not grow at temperatures above 40°C. Examples of Type II include *Methylosinus trichosporium* OB3. This is similar to Type I bacteria, except that it contains enzymes of the Calvin cycle and is capable of carbon dioxide fixation.

Based on this classification, the growth of different classes of methane-utilizing bacteria are dependent on temperature. Therefore, the goal of the present work was to determine the growth and methane utilization rate of different groups of methane-utilizing bacteria at different temperatures. The objective was also to determine the effect of pressure on growth of different organisms and to determine the levels for activation and inhibition of growth.

MATERIALS AND METHODS

Microorganisms

Cultures of *Methylococcus capsulatus* (Bath), *Methylomonas albus* (BG-8), *Methylocystis parvus* (OBBP), and *Methylosinus trichosporium* OB3b (ATCC 35070), was preserved in glycerol vials (15%) at − 80°C to minimize the genetic drift caused by repetitive transfers. Working cultures were maintained on nitrate mineral-salts medium (NMS) agar plates under a 1:3 methane/air atmosphere. Seed cultures were started in vials containing 50 mL of NMS medium, charged with methane/air, sealed with a rubber stopper and aluminum crimp, and incubated at 30°C. The composition of medium used in current investigations was the Higgin's nitrate mineral medium *(6)*.

Temperature and Pressure Studies

The temperature studies were conducted at 1 atm in serum bottles charged with different gases (CH_4, O_2, and N_2) at several different temperatures as shown in the results section. Pressure-effect experiments were

conducted with *M. albus* (at 30°C) and *M. capsulatus* (at 37°C) (Bath) at various (eight) pressures between 10 and 50 psi. Increased pressure was maintained both by using the gas substrate, a mixture of methane and air, and by using nitrogen gas in the presence of methane and air. This was done in an effort to determine the effects of total pressure, as well as partial pressure, of methane and air on the growth of methanotrophs. In all experiments, care was taken to maintain an approximately constant pressure by the addition of inert nitrogen.

ANALYSIS

Dry-Cell-Weight Estimation

The cell concentration was determined by a predetermined correlation between dry cell weight and optical density at 620 nm (Spectronic 20, Milton Roy, Rochester, NY). The fermentor broth was filtered through a 0.2-micron filter, washed twice with saline solution (0.85%), and dried overnight in the oven.

Gas Analysis

The concentration of methane, oxygen and nitrogen was determined using a Fisher gas partition model 1200 (Pittsburgh, PA) chromatograph equipped with a thermal-conductivity detector. Helium was used as the carrier gas at the flow rate of 30 mL/min. The column temperature was maintained at 50°C.

RESULTS

Selection of Microorganisms and Optimization of Temperature for Enhanced Utilization of Gaseous Substrates

In view of conducting research for co-oxidative products, several microorganisms were selected, and the effect of physical parameters on the mass transfer of methane was evaluated. The effect of temperature on growth and methane utilization was examined, on four selected mesophilic cultures. These cultures were *M. albus*, *M. parvus*, *M. capsulatus*, and *M. trichosporium*. At least three temperatures (25, 30, and 35°C) were evaluated. The results (Figs. 1–4) revealed that growth of *M. albus* and *M. Parvus* were not significantly affected by temperature variations. The maximum biocatalyst concentration of *M. albus* was in the range of 280 to 320 mg/L (dry weight) with a maximum at 30°C. This finding is in agreement with the literature values that showed a similar optimal of 30°C, though conducted at different composition of gas mixture. The methane content was reduced from 24 to 10% with a overall consumption rate of 0.22 mol%/h for this organism. During the initial period (16 h), the methane consumption of 0.67 mol/h was observed. The biocatalyst concentration for *M. parvus* was

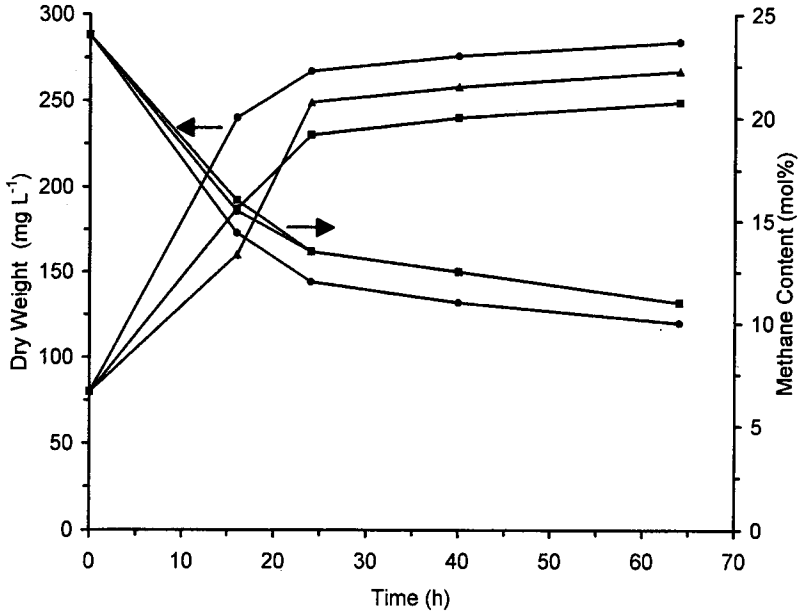

Fig. 1. The effect of temperature on the growth of *Methylomonas albus* (BG-8) (2:1 CH_4/O_2). (—▲—, 25; —●—, 30; —■—, 35).

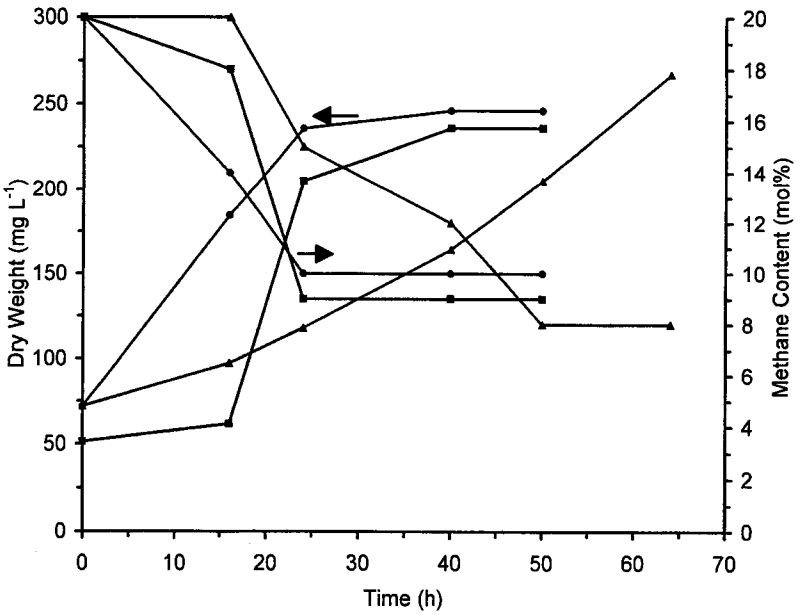

Fig. 2. The effect of temperature on growth of *Methylocystis parbus* (OBBP) (2:1 CH_4/O_2). (—▲—, 25; —●—, 37; —■—, 45).

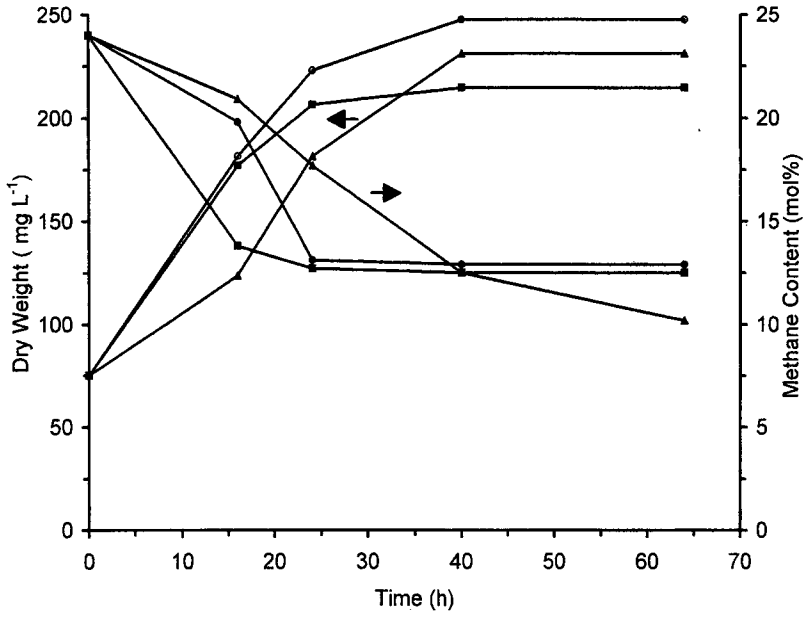

Fig. 3. The effect of temperature on growth of *Methylosinus trichosporium* (OB3b) (2:1 CH_4/O_2). (—▲—, 25; —●—, 30; —■—, 35).

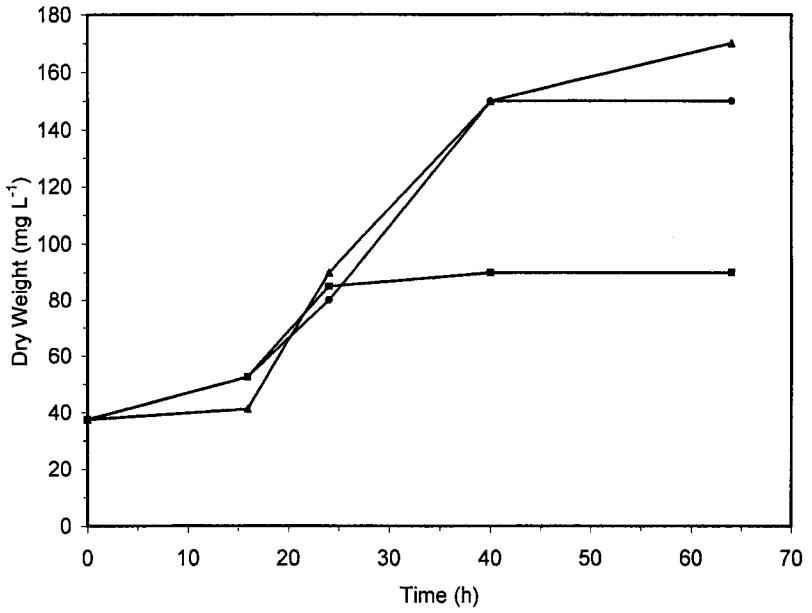

Fig. 4. The effect of temperature on growth of *Methylococcus capsulatus* (Foster and Davis) (2:1 CH_4/O_2). (—▲—, 25; —●—, 30; —■—, 35).

Table 1
Baseline Kinetic Data for Methanotrophs Grown at Optimal Temperature

Cultures	Temperature °C	Growth Rate mg/h	Methane Consumption Rate mg/mol%/h	Cell Yield mg/mol%CH_4
M. albus	30	4.4	0.31	20
M. parvus	30	3.9	0.35	20.7
M. trichosporium	25	1.8	0.05	n.d
M. capsulatus	37	6.2	0.62	24.6

in the range of 260 to 300 mg/L with a maximum of 300 mg/L at 30°C. The methane consumption rate was 0.22 mol%/h at 30°C. During the initial period (16 h), the maximum methane consumption rate of 0.53 mol%/h was observed. The growth of *M. trichosporium* was the same at both 25 and 30°C, but was inhibited at 35°C. The biocatalyst concentration for *M. trichosporium* was in the range of 67 to 127 mg/L with a maximum of 127 mg/L at 25°C. *M. capsulatus* (Foster and Davis) showed a biocatalyst concentration in the range of 235–260 mg/L with a maximum of 260 mg/L at 25°C. The methane consumption rate of 0.275 mol%/h was maximum at 45°C. The effect of temperature is summarized in Table 1. The biocatalyst concentration of 6.2 mg/L was highest for *M. capsulatus*. These results indicate that the maximum growth and methane consumption were achieved with *M. albus* and *M. capsulatus;* therefore, *M. albus* and *M. capsulatus* were selected subsequently for pressure studies.

Effect of Pressure

The purpose of the pressure experiments was to enhance the mass transfer of methane for enhancing the growth. The pressure experiments were conducted with two of the better growing cultures of *M. albus* and *M. capsulatus*. The objective was to determine the growth under control conditions. The reactor were pressurized by increasing the partial pressure of methane and oxygen (2:1). Next, to prove that there was inhibition caused by increased partial pressure of oxygen, the CH_4/O_2 was left constant but pressure was increased with an inert gas like nitrogen. The results (Fig. 5) demonstrated that up to 20 psi, the growth was enhanced as compared to the control. At the high pressure of 50 psi (CH_4/O_2 mixture), a marked inhibition was observed.

The results summarized in Table 2 revealed a significant enhancement in growth rate (40%) and methane consumption rate (up to 20 psi) beyond which a significant inhibition was observed. The reactor first flushed with methane/air and then brought to the desired pressure point with nitrogen did not show the inhibition. These studies indicated that a higher pressure (>25 psi) of methane/oxygen was inhibitory for the growth of *M. albus*. The

Table 2
Effect of Pressure on Growth and Methane Consumption Rates using *M. albus*

Test Pressure psig	Growth Rate mg/h	Methane Consumption Rate mol% CH_4/h
10	10	0.21
15	10	0.25
20	11	0.35
25	9	0.22
30	5	0.04
35	4	0.04
40	2	0.09
45	2	0.11
50	1	0.08

[a] The vessel were pressurized with CH_4/O_2 mixture in the head space.

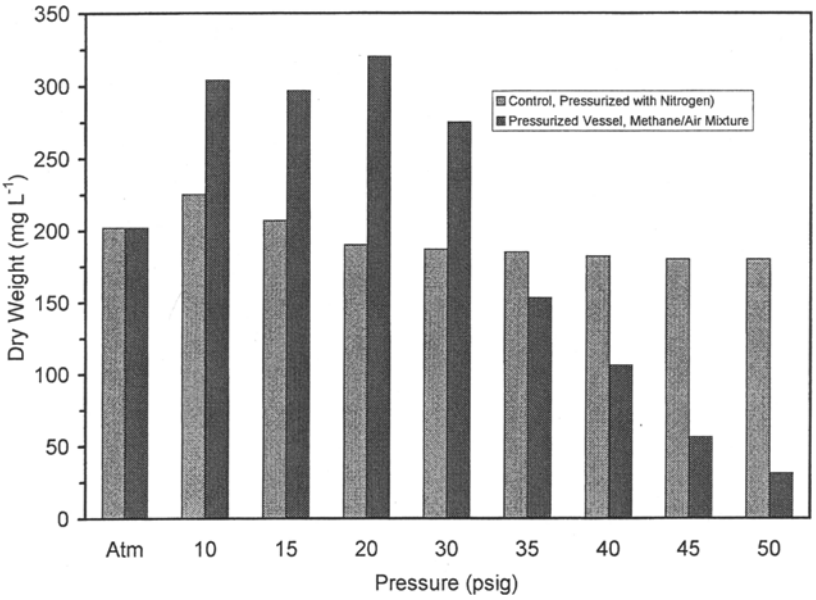

Fig. 5. Comparison of *Methylomonas albus* growth at various pressures (pressurized with CH_4/O_2 2:1).

effect of pressure on growth rate and methane consumption rates suggested maximum growth of *M. albus* was achieved at 20 psi. The maximum methane consumption of 0.35 mol%/CH_4/h was achieved at this pressure.

Experiments were also conducted to determine the effect of pressure on growth of *M. capsulatus*. The results shown indicated (Figs. 6 and 7) no

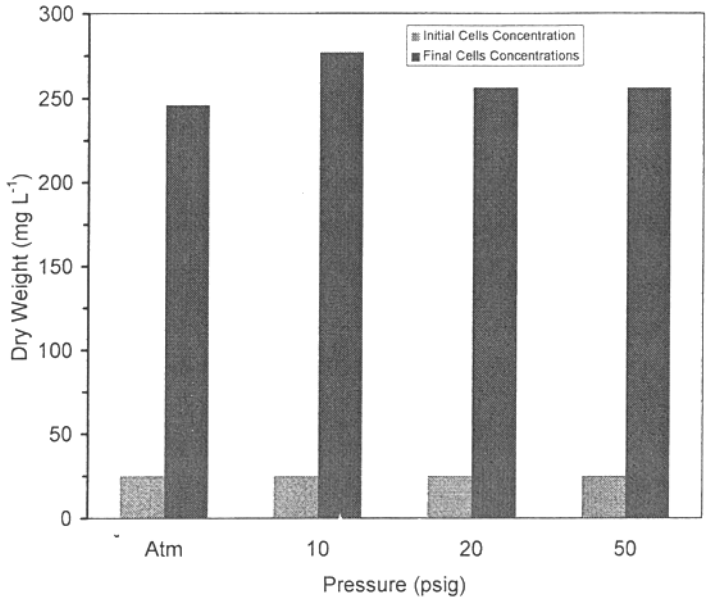

Fig. 6. Comparison of *Methylococcus capsulatus* (Bath) at various pressures (pressurized with CH_4/O_2 2:1).

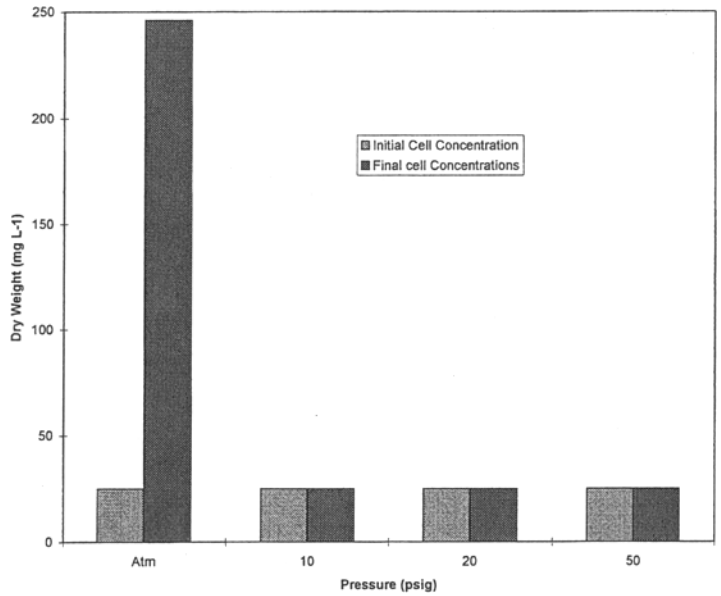

Fig. 7. Comparison of *Methylococcus capsulatus* (Bath) at various pressures (pressurized with nitrogen).

inhibition in reactors pressurized with nitrogen. The growth of *M. capsulatus* was more sensitive to pressure, as a significant inhibition was observed at the relatively low pressure of 10 psi (Fig. 6). However, pressurizing with nitrogen resulted in a better growth, confirming again the inhibition of methane/air (Fig. 7).

The results summarized in Table 2 revealed a significant enhancement in growth rate for *M. albus* (40%) and methane consumption rate (up to 20 psi) beyond which a significant inhibition was observed. The reactor first flushed with methane/air and then brought to the desired pressure point with nitrogen did not show the inhibition of microorganisms. These studies indicated that a higher pressure (>25 psi) of methane/oxygen was inhibitory for the growth of *M. albus*. The effect of pressure on growth rate and methane consumption rates suggested that maximum growth of *M. albus* was achieved at 20 psi. The maximum methane consumption of 0.35 mol%/ CH_4/h was achieved at this pressure.

From the results it is quite evident that different groups of bacterial have different temperatures optimal for growth and methane utilization. The methane utilization was maximum in the initial period of incubation for all the organisms examined. *M. capsulatus* showed higher biocatalyst growth rate (6.2 mg/L/h) as well as methane yield. The higher growth could be attributed possibly to the higher optimal temperature of growth. On the contrary, the growth rate for *M. trichosporium* OB3B was low. This can once again be explained by the fact that optimal temperature of growth is 30°C (*7*). These finding suggests that biocatalyst growth rates for methane-utilizing bacteria are dependent on temperatures. The sensitivity of *M. albus* and *M. capsulatus* was different as the later showed inhibition at 10 psi reactor pressure. These facts suggests that different methane-utilizing organisms are sensitive to increased partial pressure of oxygen or methane.

Pressure studies conducted with *M. albus* and *M. trichosporium* OB3B demonstrated that enhanced pressure up to 20 psig increases the growth rate. Pressure higher than this with methane/oxygen has resulted in reduction of biocatalyst growth. This reduction in growth could be attributed to the interaction of growth substrate (methane or oxygen) with biocatalyst. The inhibition was not seen when the reactor was pressurized with inert gas (nitrogen).

CONCLUSIONS

Several conclusions can be drawn from these studies as described below:

1. The methane-consumption rate for all the organisms examined was higher in the initial period of growth (< 16 h).
2. *M. capsulatus* showed the highest growth rate and *M. trichosporium* OB3B showed the lowest rates. These rate differences could be ex-

plained on the basis of higher (37°C) and lower optimal growth temperatures (30°C) for these microorganisms.

3. Enhancing the pressure with growth substrate has enhanced the biocatalyst concentration up to 20 psi, beyond which a inhibition of growth of *M. albus* was observed. *M. capsulatus* was more sensitive to growth substrates as seen by growth inhibition at the lower pressure of 10 psi.
4. Neither of the organism tested showed inhibition when inert gas was used for pressure, suggesting that these organisms are sensitive to presence of higher levels of growth substrate (methane or oxygen).

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