

Short Communication

Oxygen uptake kinetics Of *Pseudomonas* sp. strain VB grown under limiting concentration of 2-methoxyethanol

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Oxygen uptake and 2-methoxyethanol oxidation kinetics of 2-methoxyethanol degrading bacterium *Pseudomonas* sp. strain VB were studied. The bacterium was grown under oxygen saturating conditions with limiting concentrations of 2-methoxyethanol. *Pseudomonas* sp. strain VB was found to utilize one mole of oxygen per one mole of 2-methoxyethanol as opposed to four moles of oxygen required for total oxidative metabolism. This indicates that intermediate metabolites are involved during the degradation of 2-methoxyethanol under aerobic conditions.

Key words: Oxygen uptake, 2-methoxyethanol, *Pseudomonas* sp. strain VB biodegradation.

INTRODUCTION

2-methoxyethanol is a member of the organic compounds called glycols. It comprises one unit of ethylene oxide, with a C-O-C ether linkage terminating with an hydroxyl bond (-OH) at one end. The ether linkage is the most common and unifying structural feature which confers on both biological and xenobiotic compounds a high degree of resistance to biological mineralization (Tidswell et al., 1996). Its diverse uses makes the substance ubiquitous as an environmental xenobiotic which has considerable implications within the global biosphere.

The microbial mixed cultural degradation of 2-methoxyethanol under anaerobic conditions has been reported (Tanaka et al., 1986; Tanaka and Pfennig, 1988). A novel bacterium *Pseudomonas* sp. strain VB able to degrade 2-methoxyethanol under aerobic conditions was isolated from anaerobic sewage sludge (Ekhaise, 2002). The bacterium utilizes 2-methoxyethanol as sole source of carbon and energy.

Oxygen metabolism of aerobic microorganisms can be studied using an oxygen electrode. Enzymes are readily studied using a Clarke oxygen electrode provided oxygen is involved in the reaction. Glucose oxidase, D-amino oxidase and catalase are examples of enzymes whose

properties can be studied in this way (Wilson and Walker, 1994).

The goal of this study was to determine oxygen uptake kinetics of the newly isolated bacterium, *Pseudomonas* sp. strain VB in relation to substrate concentration.

MATERIALS AND METHODS

Chemicals

All the chemicals used were of analytical grade ($\geq 99.9\%$ pure). 2-methoxyethanol was obtained from Fluka (Buchs, Switzerland).

Organism and culture conditions

An overnight culture of *Pseudomonas* sp. strain VB was used to inoculate 10 L of mineral salt medium containing 126.7 mmol of 2-methoxyethanol. The culture was aerated by bubbling air through it and incubated at 30°C. The growth was monitored turbidometrically at 436 nm with a spectrophotometer (LKB Biochrom Ultrospec 4050, Cambridge, England). The cells were harvested after 24 h of incubation.

Measurement of oxygen uptake and substrate oxidation kinetics

The determination of oxygen uptake rate was performed polarographically at room temperature using a Clarke-type oxygen electrode (Model 10 Ranke Brothers, Birmingham, Great Britain). An overnight culture of *Pseudomonas* sp. strain VB was centrifuged, washed twice and re-suspended in 1 ml of fresh phosphate buffer (50 mM, pH 7.2). From this cell suspension, 100

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μl was added to 3 ml of saturated oxygen-phosphate buffer. After 10 min, the oxygen consumption by the intact cells was measured and this constituted the endogenous respiratory rate. The following limiting concentrations of 2-methoxyethanol 42.2, 84.5 and 105.5 nmol were added to the cultures respectively and after 10 min, oxygen consumption rate was measured. This is the exogenous respiratory rate. The oxygen consumption rate at the expense of the substrate was determined by subtracting the value for endogenous respiratory rate from exogenous respiratory rate.

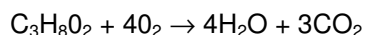
Table 1. Oxygen consumption rate with limiting 2-methoxyethanol concentration.

2-ME (nmol ml ⁻¹)	O ₂ uptake (nmol ml ⁻¹)	O ₂ /2-ME	2-ME/O ₂
Control ¹	143.3	-	-
42.2	35.5	0.84/1	1.2/1
84.5	80.2	1.0/1	1.0/1
105.5	95.4	0.9/1	1.1/1

¹Control was determined under endogenous rate without substrate.

RESULTS AND DISCUSSION

The stoichiometry of 2-methoxyethanol with *Pseudomonas* sp. strain VB as shown in Table 1 was calculated to be in the ratio of 1:1. *Pseudomonas* sp. strain VB utilizes 1 mol oxygen per 1 mol 2-methoxyethanol as compared to the theoretically predicted ratio of 4 mol oxygen per 1 mol 2-methoxyethanol for the complete oxidation of 2-methoxyethanol under aerobic conditions. Total oxidation of 2-methoxyethanol is as follows:



The substrate oxidation curves obeyed Michaelis-Menten kinetics (not shown). The rate of oxygen consumption (velocity rate) was plotted against varying concentrations of 2-methoxyethanol. The double reciprocal according to Lineweaver-Burk was used to determine the K_m and V_{\max} values of 11.526 mM and 33.856 mM, respectively.

The measurement of the initial rate of an enzyme-catalyzed reaction is fundamental to a complete understanding of the mechanism by which the enzyme works (Wilson and Walker, 1994), as well as the estimation of the activity of an enzyme in a biological sample. This is influenced by many factors, among which are substrate concentration, enzyme concentration, pH, temperature and the presence of catalyst. For the majority of enzymes, substrate dependent rate of reaction varies hyperbolically with substrate concentration. At low substrate concentrations in such cases approximately first order kinetics are observed, but at high substrate concentrations saturation zero-order kinetics exists and

the initial rate is dependent on substrate concentration as expressed by the Michaelis–Menten reaction.

Stoichiometrically, *Pseudomonas* sp. strain VB utilizes one mole of oxygen per one mole of 2-methoxyethanol as compared to four moles of oxygen per one mole of 2-methoxyethanol required for the total oxidative metabolism (Table 1). This finding therefore supports a hypothesis in which the degradative pathway is not a one-step reaction mechanism and this indicate that intermediate metabolites are involved during the biodegradation of 2-methoxyethanol in *Pseudomonas* sp. Strain VB under aerobic conditions.

Competition only for oxygen is probably rare under natural conditions since limiting amounts of the electron donors also influence the performance at low oxygen availability (Bodiler, and Laambroek, 1997). From the 2-methoxyethanol oxidation kinetics of *Pseudomonas* sp. strain VB, it is evident that the bacterium uses oxygen as a good electron donor. For single enzymes, the K_m and V_{\max} values range from 9.5×10^{-5} to 2.5×10^{-2} μM (Voet and Voet, 1990). The values obtained in this study are so high that, multiple enzymes may be involved in the degradation processes. Since the magnitude of K_m value varies widely with the identity of the enzyme and the substrate, it can be concluded that the bacterium possessed a high substrate utilization capacity with low catalytic efficiency.

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