EFFECT OF NH_3 AND H_2S ON THE BIOOXIDATION OF METHANE BY *Methylomicrobium album* ATCC 33003

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Introduction. Methane is one of the gases that cause the so called greenhouse effect (1). Biooxidation of gaseous emissions containing less than 5% v/v methane is an existing technology. One source of low concentration emission of methane is landfills (2). In this case are produced at the same time also other gases like NH_3 and H_2S, which could affect the activity of methanotrophic species present in the organic material.
The aim of this work was to evaluate the effect of different levels of NH_3 and H_2S on the ability of *M. album* ATCC 33003 to oxidize methane.

Methods. *M. album* was cultivated in 125 mL erlenmeyer flask stoppered with mininert valves (VICI, USA) having 25 mL of NMS medium (ATCC N°1306). Culture conditions were 30°C, pH 6.9 and shaking at 200 rpm. The gaseous components were added to the head space of flasks. All experiments started with 15% v/v of methane concentration. NH_3 was assayed at levels of 0, 50 and 500 ppm. H_2S was evaluated at 0, 0,1% and 1% (v/v).
Gases were measured with a Drager instrument (Xam 5600, Dräger, Germany) and biomass in a spectrophotometer at 600 nm (Jenway, USA) and converted to dry weight using a proper calibration curve.

Results. As shown in Fig. 1, 50 ppm of NH_3 practically does not affect the biooxidation capacity of *M. album* as compare with the biooxidation in absence of this particular gas. However, the 500 ppm level did affect the cell activity since methane biooxidation practically stop after 25 – 30 hours of cultivation time.

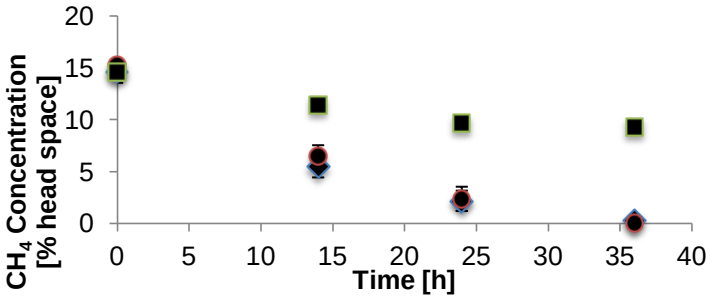

Fig.1 Variation of methane concentration in the head space of cultures of *M. album* ATCC 33003 growing under different NH_3 concentrations in the gas phase (♦ 0 ppm, ● 50 ppm, ■ 500 ppm) at 30°C and pH 6.9.

In Fig. 2 is observed the effect of H_2S on cell activity. In this case 0.1% of H_2S in the flask head space did not change the cell behavior as compare with the culture not containing this particular gas. The 1.0% level affected the cell behavior stopping methane biooxidation practically after 25 – 30 hours of cultivation time.

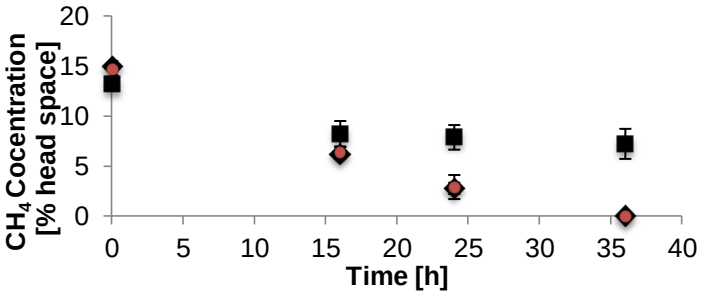

Fig.2 Variation of methane concentration in the head space of cultures of *M. album* ATCC 33003 growing under different H_2S concentrations in the gas phase (♦ 0 %, ● 0.1 %, ■ 1.0 %) at 30°C and pH 6.9.

In both cases is observed that initially the cell is able to biooxidize methane, but not completely as in the case of absence or the lowest concentration of NH_3 and H_2S evaluated. The reason of this behavior is not fully understood, but indicates that the inhibitory effect caused by these gases develops while cultivation proceeds.
The presence at inhibitory levels of the gases affects other parameters of the fermentation as shown in Table 1.

Table 1. Comparison of culture parameters in absence and presence of inhibitory levels of NH_3 and H_2S.

	Control	500 ppm NH_3	1.0% H_2S
Final biomass, g/L	0.55	0.18	0.26
Methane consumption, %	100	60	54

Conclusions. The highest concentrations of NH_3 and H_2S evaluated affect the methane biooxidation capacity of the methanotroph strain *M. album*. This situation could affect the effectiveness of a treatment *in situ* of a landfill by locally entrapment of either of these gases.

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