METHANE OXIDATION BY METHANOTROPHS FOR DENITRIFICATION

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Introduction. Methane-oxidising bacteria (methanotrophs) are unique in their ability to utilise methane as a sole carbon and energy source (1, 2). Methanotrophs have generated interest because of the environmental importance of methane oxidation, and their potential industrial and bioremedial applications (i.e. degradation in soil and groundwater of key pollutants such as trichloroethylene and nitrogen compounds) (2). Ammonia removal from wastewater is based on nitrification followed by denitrification. For denitrification the organic compounds present in the wastewater may serve as electron donors, however since they are quickly consumed, additional electron donors are frequently required. Methane by its properties (natural, abundant, and inexpensive) could be an alternative carbon source. Methanotrophs are enable to denitrify, but are also capable of producing a soluble organic compound that could be potentially used as electron donor for denitrification. The identity of this compound is still nowadays mysterious.
This work presents preliminary results in oxidising methane with methanotrophs in aerobic batch reactors for producing a soluble organic compound which will be used in a second step as carbon source in a denitrifying bioreactor.

Methodology. For the growth of methanotrophs, 1 g of soil inoculum was added to 25 ml of nitrogen mineral salts (NMS) liquid medium (1) in 125-ml bottles, which were sealed with a pharmaceutical septum. CH_4 (CH_4/air: 9/91, v/v) plus 2 ml CO_2 were injected into each vial. A potentiometric pressure sensor was used for measuring the relative pressure inside the vials. Since methane oxidation results in gas consumption, a pressure decrease is therefore considered to represent the biological methane oxidation activity. Turbidity was taken as presumptive evidence of growth of the methane utilizer. This was repeated several times through similar stages to enhance the enrichment process. CH_4 and CO_2 were measured by gas chromatography equipped with a TCD detector.

Results and Discussion. The gas consumption in terms of relative pressure inside the batch vessel, together with a blank experiment without CH_4, are presented in Fig. 1. On this curve, no latency phase, and a rapid consumption of gas before the stationary state, were observed. In Fig. 2, the uptake of CH_4, and CO_2 production are shown. The profiles of these elements are well correlated, with a regular increase during the first 60 hours and with a stabilisation at the end of the batch. The maximum rates obtained were 0.22 mmol CH_4/l.h, and 0.18 mmol CO_2/l.h. These curves demonstrate that methane oxidation occurs in batch culture getting CO_2 as final product (83% of the methane was oxidised in CO_2). The bacteria activity was observed to be optimal at 26°C. On the other hand, this activity was also improved when CO_2 was added to the gas phase at the beginning of the experiments, showing that methane oxidisers require CO_2 to allow growth initiation. This was in accordance to the literature (1).

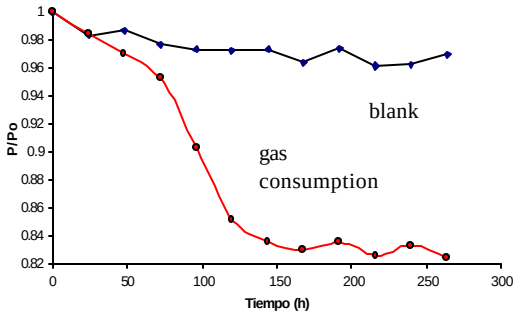

Fig.1 Evolution of gas (CH_4, CO_2, air) consumption in batch culture with methanotrophs

Conclusion. Additional experiments are currently been performed in continuous vessels for improving the rates of CH_4 oxidation, and studying the soluble organic compounds produced by methanotrophs. Such products will be validated as potential electron donors in denitrification.

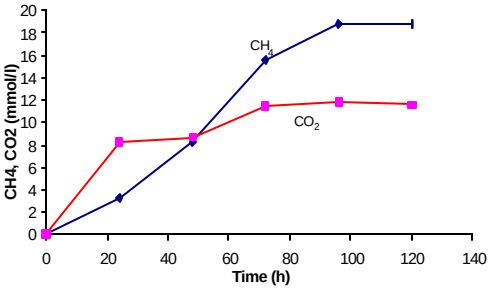

Fig. 2 Progression of uptake of CH_4, and CO_2 in batch culture

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References

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