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# An innovative membrane bioreactor for methane biohydroxylation

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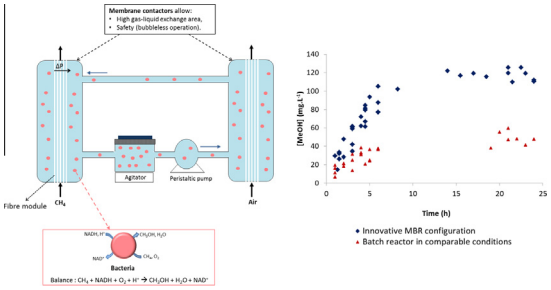
## HIGHLIGHTS

- Methane biohydroxylation was done for the first time in a new MBR configuration.
- This new MBR couples two macroporous membrane contactors.
- This MBR exhibits a 2-fold enhanced mass transfer compared to a batch reactor.
- This MBR avoids gas bubbles and dangerous gas mixtures during operation.
- The obtained productivity is 35-fold higher than the only other MBR reported.

## GRAPHICAL ABSTRACT




An innovative configuration of membrane bioreactor (MBR) for methane hydroxylation

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## ABSTRACT

In this study, a membrane bioreactor (MBR) was developed for efficient, safe microbial methane hydroxylation with *Methylosinus trichosporium* OB3b. This innovative MBR, which couples a bioreactor with two gas/liquid macroporous membrane contactors supplying the two gaseous substrates (methane and oxygen) was operated in fed-batch mode. The feasibility and the reproducibility of this new biohydroxylation process were first demonstrated. The mass transfer within this MBR was twice that observed in a batch reactor in similar conditions. The productivity reached with this MBR was 75 ± 25 mg methanol (g dry cell)$^{-1}$ h$^{-1}$. Compared to the literature, this value is 35 times higher than that obtained with the only other fed-batch membrane bioreactor reported, which was run with dense membranes, and is comparable to those obtained with bioreactors fed by bubble-spargers. However, in the latter case, an explosive gas mixture can be formed, a problem that is avoided with the MBR.



## 1. Introduction

Methane constitutes a large carbon feedstock that can potentially be used for the production of valuable oxygenated liquid products such as methanol or formic acid (Caballero and Perez, 2013). Developing processes that allow the selective hydroxylation of methane, i.e., its conversion into methanol, is thus of great interest. The high stability of its C–H bonds makes methane remarkably inert, however, and despite recent progress, its chemical activation remains unselective and highly energy consuming (Alvarez-Galvan et al., 2011; Mansouri et al., 2013). Microbial methane hydroxylation by aerobic methanotrophic bacteria has shown promise in this domain as this bioconversion is specific and takes place in mild physiological conditions. The conversion of methane into methanol is catalyzed by an MMO (Methane Mono-Oxygenase) in presence of oxygen according to the balance equation (Green and Dalton, 1989):

$$CH_4 + NADH + O_2 + H^+ \rightarrow CH_3OH + H_2O + NAD^+ \quad (1)$$

Isolated MMOs still exhibit limited activity and stability (Balasubramanian et al., 2010; Ito et al., 2014), which explains why whole-cell biocatalysts are currently preferred for methane biohydroxylation. The non-pathogenic bacterium *Methylosinus*

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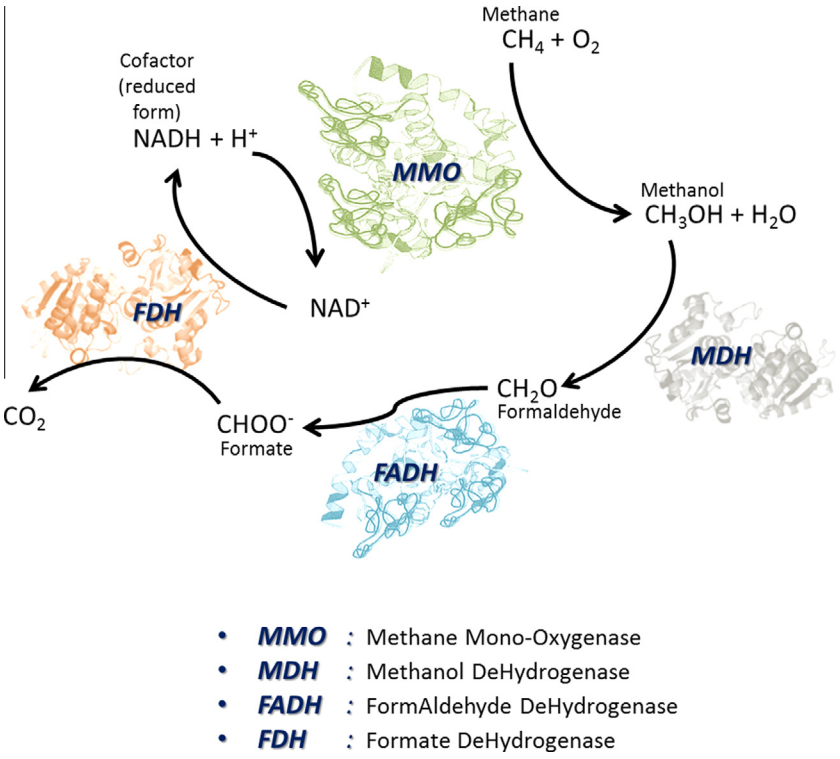


**Fig. 1.** Metabolic pathway used by methanotrophic bacteria for methane oxidation.

*trichosporium* OB3b has notably been identified as an efficient biocatalyst of this bioreaction (Duan et al., 2011; Kim et al., 2010; Lee et al., 2004; Mehta et al., 1991).

The metabolic pathway used by aerobic methanotrophic bacteria for methane assimilation is described in Fig. 1 (Ayala and Torres, 2004; Rojo, 2009; Tabata and Okura, 2008). As depicted, methane oxidation in whole cells does not stop at methanol but continues up to carbon dioxide, which points out the need to break the methanotrophic pathway after its first step. This problem can be solved by inhibiting the methanol dehydrogenase (MDH) activity with, for instance, the addition of phosphates and NaCl, and regenerating the NADH cofactor required for the hydroxylation reaction with the addition of sodium formate, which plays the role of electron donor (Duan et al., 2011; Kim et al., 2010; Lee et al., 2004; Mehta et al., 1991).

From the literature, it appears that the implementation of microbial methane hydroxylation in a bioreactor raises problems in terms of mass transfer efficiency and safety linked to the gaseous nature of the substrates (methane and oxygen). Regarding the mass transfer between the gas phases and the liquid containing the bacteria, it should be enhanced to avoid any substrate limitation. From the safety viewpoint, the risk lies in the fact that the mixture of the two gaseous substrates could be explosive: methane in air is indeed explosive between 5% v/v (LEL: Lower Explosive Limit) and 15% v/v (UEL: Upper Explosive Limit). To the best of our knowledge, batch bioreactors (Duan et al., 2011; Kim et al., 2010; Lee et al., 2004), fed-batch bioreactors (Kim et al., 2010; Lee et al., 2004; Markowska and Michalkiewicz, 2009) and a continuous bioreactor (Mehta et al., 1991) have been implemented to perform methane biohydroxylation.

In the case of batch reactors (Duan et al., 2011; Kim et al., 2010; Lee et al., 2004), the headspace is commonly filled with a substrate mixture containing a large methane excess to avoid explosion. In such reactors, the effective mass transfer area, which corresponds to the physical contact surface between gas and liquid, is relatively small and may limit the transfer.

This notably explains why continuously sparging the gaseous substrates directly into the bacterial suspension constitutes a usual way of feeding to prevent any substrate depletion (Kim et al., 2010; Lee et al., 2004; Markowska and Michalkiewicz, 2009). However, the use of bubble-spargers results in the formation of gas bubbles that may produce an explosive gas mixture.

To avoid this drawback, Duan et al. (2011) used dense silicone tubes to supply the substrates independently without any bubbles. However, the dense nature of the membranes used limited transport through the membrane to diffusion and, finally, was very penalizing for the mass transfer in comparison to spargers (Kim et al., 2010; Lee et al., 2004; Markowska and Michalkiewicz, 2009).

Membrane contactors, which involve macroporous membranes, are known to have a large surface area and have shown their ability to enhance gas/liquid mass transfer (Sanchez Marcano and Tsotsis, 2004). In particular, Coutte et al. (2010) demonstrated that membrane contactors with macroporous membranes could be used to efficiently oxygenate a bioreactor without forming any gas bubbles in the reactor.

In the present study, methane biohydroxylation was implemented for the first time in an innovative membrane bioreactor (MBR) configuration based on the coupling of a bioreactor and two macroporous membrane contactors (each contactor being fed separately with methane or air). In this MBR, the bacterial suspension circulates in a closed loop in the shell side of the contactors, thus allowing continuous feeding of the gaseous substrates into the reaction medium. In this work, the bacterium *M. trichosporium* OB3b was chosen as the biocatalyst and methane hydroxylation tests were first conducted in batch bioreactors to choose the reaction medium and study the hydroxylation performance in different conditions (biocatalyst concentration and the ratio of the gas headspace volume to the liquid volume). In a second part,