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An innovative bioprocess for methane conversion to methanol using an efficient methane transfer chamber coupled with an airlift bioreactor

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ABSTRACT

Biosynthesis of methanol from methane as a direct method takes place at ambient temperature and pressure which causes a considerable reduction in process costs. In this study an efficient methane transfer chamber along with an external-loop airlift bioreactor were developed to dissolve methane and oxygen in the culture separately. Mass transfer coefficients for oxygen in the bioreactor and methane in the transfer chamber were obtained 97.2 h^{-1} and 70.8 h^{-1} respectively. Two strains of methanotroph bacteria (*AS1* and *AS2*) were also isolated from activated sludge. Factorial design of experiments for operational parameters showed a maximum productivity for *AS1* strain at 28 °C when using nitrate as nitrogen source. Batch runs in airlift bioreactor using the *AS1* strain and optimized operating parameters represented a peak of 1600 mg/L in methanol synthesis during the first 3:30 h without using inhibitor for methanol dehydrogenase (MDH) enzyme. Sequencing batch process, at the next step, was used to create intermediate lag phase and to increase the stability of microorganisms by forming flocs. The results of this study cleared that the designed system is safe and efficient to scale-up, and it can be considered as a potential alternative method in the production of methanol.

1. Introduction

Methane is one of the most abundant carbon feedstocks, energy source and the second most abundant greenhouse gas after CO_2. Methane has a negative impact on the atmosphere layer and global warming potential more than 20 times that of carbon dioxide (Park and Lee, 2013; Zakaria and Kamarudin, 2016). Due to being in gaseous format in ambient conditions, methane is expensive to store, transport and distribute (Yang et al., 2014). In order to address these issues it is desirable to convert the gas into easily handled liquid fuels and value-added chemicals such as methanol (Ge et al., 2014). Methane can be converted to methanol by thermochemical and biological methods. Thermochemical conversion and partial oxidation methods have always been expensive and less efficient because of their high C—H bond strength which needs high pressure and temperature as well as expensive chemical catalysts (Hwang et al., 2014; Patel et al., 2016b). Instead, biological conversion using methanotrophic bacteria is an efficient conversion because whole the process takes place within ambient pressure and temperature without requiring lots amount of energy to provide extreme operating conditions. The conversion rate and purity obtained by this method is high, and subsequently downstream processing costs will be reduced for refining the product (Ge et al., 2014; Schrader et al., 2009). *Methylosinus trychosporium OB3b* is a commonly used microorganism for methanol biosynthesis. For instance, two separate studies were conducted by Takeguchi et al. (1997) aiming to methanol accumulation using methanol dehydrogenase enzyme inhibitor and semi continuous process (Furuto et al., 1999; Takeguchi et al., 1997). In other study, Duan et al. (2011) applied a membrane silicon tubes inside the bioreactor

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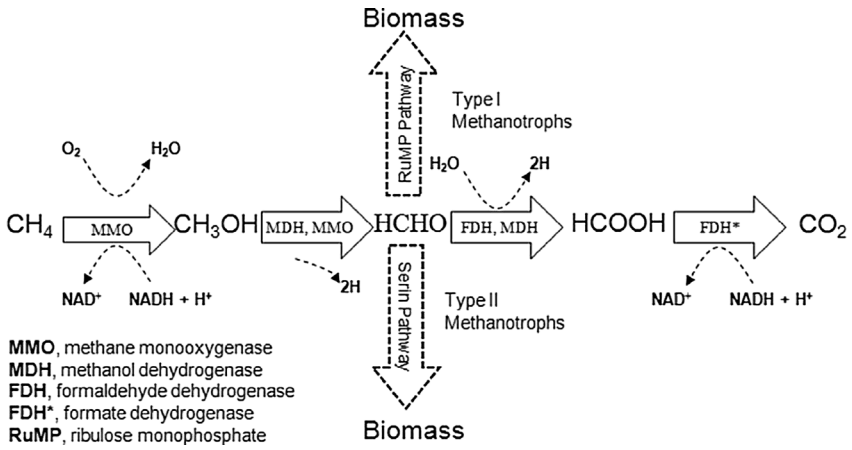

Fig. 1 – Metabolic pathways of Methanotroph bacteria along with involved metabolites and enzymes.

to separate methane and air aeration in order to improve the bioconversion rate, they succeeded to produce 950 mg L^{-1} methanol using a high cell density (17 g dry/L) microbial system (Duan et al., 2011). Kim et al. (2010) developed a compulsory circulation diffusion system in which methane/air mixture in equal ratio was collected in a rubber gas bag just after injecting to the bioreactor, producing almost 440 mg/L methanol in 16 h (Kim et al., 2010).

Aside from the most typical studies on *Methylosinus* and *Methylococcus* strains, some novel methanotrophs have also recently been isolated from extreme environments for methanol biosynthesis (Bowman, 2011; Siljanen et al., 2011). For example, Sheets et al. (2016) could isolate a new potential strain (14B) from solid-state anaerobic digestate to produce methanol up to 430 mg/L within 48 h (Sheets et al., 2016). Similarly, another methanotroph named *SAD2* was isolated from a hydrogen sulfide (H_2S)-rich anaerobic digester by Zhang et al. (2016), producing nearly 350 mg/L methanol in 48 h.

As Fig. 1 shows schematically, all aerobic methanotrophs share a general pathway, through which CH_4 is finally oxidized to CO_2. Methanol is the first intermediate metabolite which is synthesized by methane monooxygenase (MMO) enzyme (Chowdhury and Dick, 2013; Hanson and Hanson, 1996). There are two forms of MMO enzymes which oxidize methane to methanol in methanotrophic bacteria depending on copper concentration in the medium: soluble (sMMO) and particulate (pMMO). Soluble MMO (sMMO) is synthesized in the cell cytoplasm whenever the copper concentration is limited, whereas pMMO is attached to the membranes.

Besides all attempts to increase methanol concentration, the biological method is still facing with some challenges due to the difficulty of the methanol accumulation in the presence of the remainder methanol dehydrogenase (MDH) enzyme activity, and the need for reducing equivalents for MMO activity (Patel et al., 2016a). Furthermore, the scale-up barriers of the process including low-efficient gas–liquid mass transfer rate and the safety risks of methane and air mixture have yet to be solved.

In this work a new bioprocess including a scalable methane transfer chamber connected to a bench-scale airlift bioreactor were designed to provide high concentrations of dissolved methane and oxygen according to their stoichiometric ratios for the bacterial consumption. A hydraulic transfer chamber was designed to dissolve methane properly into the broth culture using headspace gas absorption mechanism. Then, the medium culture containing dissolved methane was easily conducted to the external-loop airlift bioreactor under a deep aeration bioprocess. The couple of methane transfer chamber and airlift bioreactor not only regulated the oxygen and methane dissolution rates for higher methanol accumulations, but it also reduced the risk of the explosive contact between oxygen and methane gases using a separated gas uptake mechanisms.

Two strains of methanotroph bacteria (*AS1* and *AS2*) were isolated firstly from anaerobic activated sludge and the influence of stain type, temperature, nitrogen source and methane-to-air ratio (v/v) on methanol production were examined by using a factorial experimental design technique in a lab-scale experiments. Then, the performance of the bench-scale innovative bioprocess system was evaluated for methanol production by using selected strain and operating conditions obtained from the lab-scale experiments.

2. Materials and methods

2.1. *Isolation and purification of Methanotroph bacteria*

Two strains of methanotroph bacteria were separated and enriched from activated anaerobic sludge. NMS (nitrate mineral salts) containing in (g L^{-1}) $Ca(NO_3)\cdot 7H_2O$ (0.025); $CuSO_4\cdot 5H_2O$ (0.004); $FeSO_4\cdot 7H_2O$ (0.014); KH_2PO_4 (1.6); $MgSO_4\cdot 7H_2O$ (0.080); $NaNO_3$ (1.180); Na_2HPO_4 (1.160); and $ZnSO_4\cdot 7H_2O$ (0.034) and trace element solution (0.05% (v/v)) were used as growth medium. The initial pH of the NMS medium was between 6.7 and 7. In order to isolate methanotroph bacteria, 2 g anaerobic activated sludge (4% w/v) was inoculated into 250 mL flask including 50 mL cultivation medium without any carbon source. A mixture of air and methane in equal volume ratio (50/50 (v/v)) with flow rate of 200 cm^3/min was blown (10 min per day) into the flask for one week. The sealed flask was incubated at 30 °C on a rotary shaker at 200 rpm. Within the second week, 10 mL of the contents of the flask was inoculated into the 500 mL flask containing 100 mL NMS medium by repeating cultivation conditions exactly the same as the first week in the shaker. Since the methane was the sole carbon source accessible in the medium, only microorganisms were able to grow which could metabolize methane firstly. At the next stage, isolation process of methanotrophs on the solid state culture was carried out for four weeks. Firstly, 1–2 mL of the broth medium was added into solid culture plates including 2%–3% (v/v) methanol as carbon source. Secondly, the species in several colonies were transferred by streaking on new solid culture plates without methanol. Plates were aerated by methane/air equal mixture (50/50 (v/v)) at the desiccators for 1–2 weeks to figure out unique colonies of methanotrophs. The process of screening distinct strains was repeated for several times for every fast-growing colony by streaking them on a solid-state growth medium and by using pure methane (99.99%) as the sole carbon source. As a result, two particular methanotroph isolates were turned out in pale pink and off-white colors named *AS1* and *AS2* respectively.