International Biodeterioration & Biodegradation xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Behavior of type II methanotrophic bacteria enriched from activated sludge process while utilizing ammonium as a nitrogen source

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ARTICLE INFO

Keywords:
Methanotrophs
Activated sludge
Biogas
Ammonium
Food to microorganisms ratio
Methane to oxygen ratio

ABSTRACT

Methanotrophs are considered one of the most important biological sinks for methane emissions mitigation. Additionally, they can be employed in various environmental biotechnologies for resources recovery from waste streams. Type II methanotrophs attracted several researchers for their unique ability for biopolymers accumulation, however, maintaining a mixed culture dominated by these microorganisms remains a challenge for long-term operation. To establish an ammonium-driven selection, this study shows the behavior of type II methanotrophs mixed culture enriched from activated sludge when subjected to different ammonium and copper concentrations under different operational conditions through a series of batch experiments. Ammonium concentrations above 20 mmol declined the growth rate and the biomass yield by 75% and 50% respectively. Adjusting the food to microorganisms ratio (F/M) and the Nitrogen to microorganisms ratio (N/M) doubled the growth rate of the enriched culture and mitigated ammonium inhibitory effects showing their great potential to be used as selection parameters for type II without losing cellular growth. Cultures grown in copper free medium were more tolerant to higher ammonium concentration. On the other hand, cultures supplemented with a copper concentration of 10 μm achieved the highest growth rates under the adjusted conditions of C/N, F/M and N/M ratios.

1. Introduction

Recently, excessive effort is targeted towards methane emissions mitigation. Atmospheric methane concentration has already reached 1.75 ppm with an anticipated concentration of 4 ppm in 2050 if no actions are taken to address this problem (Francisco José Fernández, 2005). The main problem associated with methane emissions is the ability of methane molecules to absorb 20 times more heat than the carbon dioxide therefore; minimizing methane emissions will have 20–60 times impact on the global warming phenomena (Strong et al., 2015). Anthropogenic methane sources resulting from human activities represent about 70% of global methane emissions and wastewater treatment facilities represents 20% of these anthropogenic emissions (Kirschke et al., 2013). Methane produced from waste treatment facilities i.e. biogas can be employed in various biotechnological applications rather than being flared to the atmosphere.

Many wastewater treatment plants use the produced biogas during anaerobic treatment processes for heat and electricity generation. However, the cost and efficiency of converting the biogas into heat and electricity is mammoth and the regenerating process is significantly low. Nevertheless, the recent discoveries in the biological utilization of biogas using methanotrophs gave the wastewater treatment plant a new perspective in methane mitigation while recovering value added products such as biopolymers, methanol and lipids or participation in other processes as bioremediation and denitrification (Strong et al., 2015; Zhu et al., 2016).

Methanotrophic bacteria have the ability to utilize single-carbon (C1) compounds (i.e. methanol or methane) as a carbon and energy source (Hanson and Hanson, 1996). Methanotrophs oxidize methane through a series of interlinked reactions into carbon dioxide with methanol, formaldehyde and formate as intermediates to fulfil their energetic and cellular requirements. As shown in Fig. 1a, The first step of this reaction is the oxidation of methane into methanol using their unique methane monooxygenase (MMO) enzyme in which a reducing equivalent break the O_2 bond and one oxygen atom is reduced to H_2O while the other is incorporated in the methanol conversion. MMO is found in two forms; the soluble form (sMMO) found in the cell cytoplasm and the particulate one (pMMO) located in their intracytoplasmic membrane (ICM) (Chistoserdova and Lidstrom, 2013). The expression of either enzyme is mainly regulated by the copper concentration in the growing medium. The sMMO is expressed in copper free medium or at very low concentration while the pMMO requires copper in order to be

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https://doi.org/10.1016/j.ibiod.2018.03.010
Received 15 October 2017; Received in revised form 24 February 2018; Accepted 21 March 2018

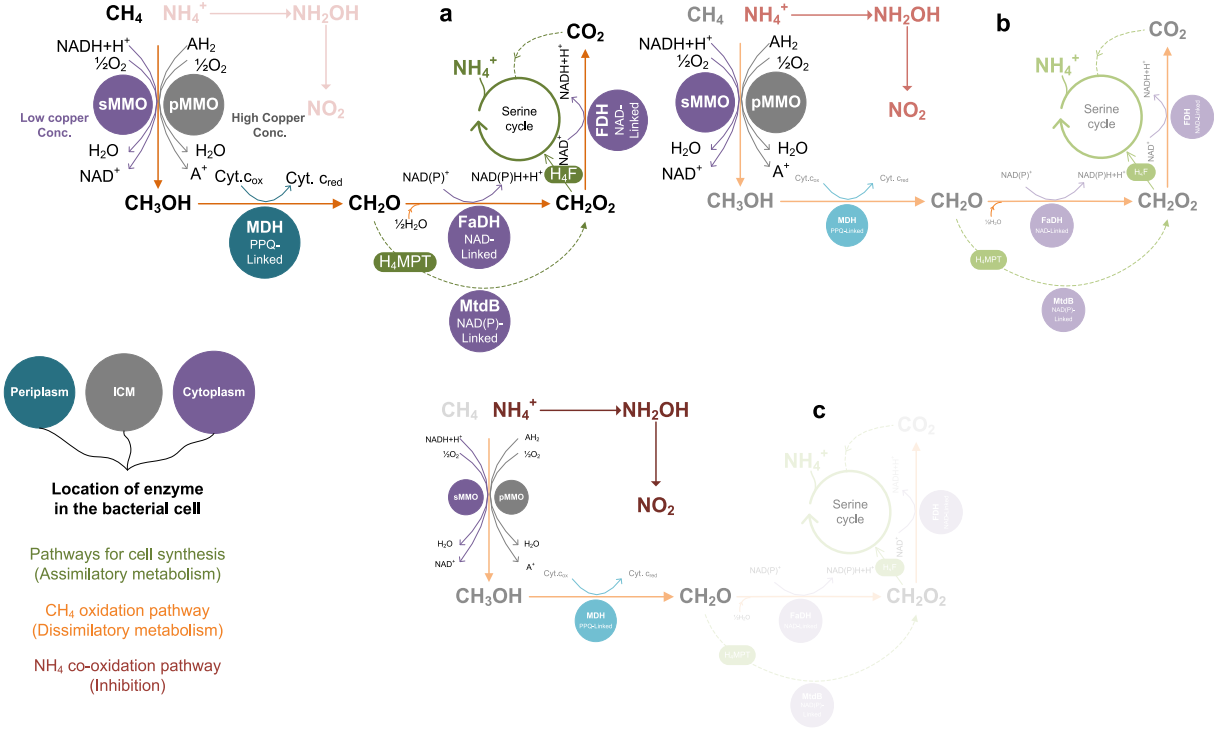

Fig. 1. Methane oxidation pathway for type II methanotrophs and the effect of ammonium co-oxidation on methane metabolism a) ammonium is used for cell synthesis b) competition between methane and ammonium for MMO c) inhibition by the by-products resulting from ammonium co-oxidation.

activated (Semrau et al., 2010). The sMMO enzyme has attracted many researchers due to its ability to co-metabolize a wide range of alkanes, alkenes, halogenated aliphatics, monoaromatics, diaromatics and alicyclic compounds which can be utilized in different bioremediation processes (Karthikeyan et al., 2015; Strong et al., 2015).

Secondly, methanol is oxidized to formaldehyde using the quinoprotein methanol dehydrogenase (MDH) located in the periplasm of the gram negative methanotrophs (Smith et al., 2010). Due to its toxicity, formaldehyde is rapidly converted to formate where two systems have been suggested for formaldehyde to formate oxidation. Firstly, oxidation is catalyzed by formaldehyde dehydrogenase (FaDH) which is either NAD-linked or quinoprotein and cytochrome-linked enzyme (Karthikeyan et al., 2015). The second suggestion is the tetrahydromethanopterin (H_4MPT)-linked formaldehyde oxidation pathway. Within H_4MPT pathway, MtdB enzyme reduces $NAD(P)^+$ and produces NAD(P)H (Kalyuzhnaya et al., 2015). The last step is the production of CO_2 through the NAD-dependent enzyme formate dehydrogenase (FDH) (Strong et al., 2015). The last three steps are considered to be the major source for the reducing equivalents required for the simultaneous methane metabolism (Hanson and Hanson, 1996).

Aerobic methanotrophs are mainly classified into two main types according to their carbon assimilation pathway. Type I methanotrophs undergo the ribulose monophosphate (RuMP) pathway while type II methanotrophs use the Serine pathway (Hanson and Hanson, 1996). Type I methanotrophs are characterized by their higher growth rates, higher efficiency in methane oxidation and can incorporate 5–15% of the generated CO_2 into cellular biomass. On the other hand, type II methanotrophs can form a slower but more stable communities (Henckel et al., 2000; Chi et al., 2012), incorporate upto 50% of CO_2 in cellular biomass and their unique ability for biopolymers accumulation under unbalanced conditions (Bowman, 2006; Karthikeyan et al., 2015). These biopolymers can be considered a green alternative for resources-consuming plastics due to their similarity in physical and chemical properties (Byrom, 1993; Raza et al., 2018) with the ability of different microorganisms to degrade them at their end-life minimizing their impact to the environment compared to petroleum based plastics (Muenmee et al., 2015, 2016).

Due to the ability of type II methanotrophs in biopolymers accumulation and possession of sMMO enzyme, Cultivation of type II methanotrophs gained a great interest in the last few years. However, the majority of the conducted research studies were mainly focused on pure cultures experiments to study the factors affecting different type II strains growth including nitrogen source type and concentration, phosphorus and copper concentration, pH, temperature and methane to oxygen ratio (Bowman and Sayler, 1994; Grosse et al., 1999; Takeguchi and Okura, 2000; Rostkowski et al., 2013).

In order to scale-up the use of type II methanotrophs and integrate it into wastewater treatment plant, enrichment of type II methanotrophs in mixed cultures from different seed sources such as activated sludge may be beneficial in eliminating the sterilization requirements for running pure cultures bioreactors. Moreover, the accompanying microorganisms could help in eliminating some toxic metabolites leading to an improvement in the growth conditions (Chidambarampadmavathy et al., 2015). However, maintaining a stable community dominated by type II methanotrophs remains one of the major challenges towards the sustainability of a methanotrophic bioreactor (Pieja et al., 2011; Criddle and Sundstrom, 2015). In addition, methods for targeting type II using pH, dilution, methane limitation and copper concentration were not reliable on long-term basis. On the other hand, applying nitrogen gas as the sole nitrogen source for type II selection resulted in the slowest growth rate among all other nitrogen sources i.e. ammonium and nitrate (Rostkowski et al., 2013) due to the sensitivity of the nitrogen fixing enzymes towards oxygen which are mainly found in type II methanotrophs. It is noteworthy to mention that the elimination of copper was assumed to select type II by activating the sMMO enzyme, which is found only in type II methanotrophs, however, it was recently discovered that type I methanotrophs can express this enzyme (van der Ha et al., 2013; Cantera et al., 2016). Moreover, some sMMO lacking methanotrophs (i.e. *Methylomicrobium* and *Methylobacter*) can survive and grow under very low copper concentration (Semrau et al., 2010; Li et al., 2014).

Ammonium as a nitrogen source can be used as a main selection