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Influence of biomass density and food to microorganisms ratio on the mixed culture type I methanotrophs enriched from activated sludge

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ABSTRACT

Methanotrophic based process can be the remedy to offset the wastewater treatment facilities increasing energy requirements due to methanotroph's unique ability to integrate methane assimilation with multiple biotechnological applications like biological nitrogen removal and methanol production. Regardless of the methanotrophic process end product, the challenge to maintain stable microbial growth in the methanotrophs cultivation bioreactor at higher cell densities is one of the major obstacles facing the process upscaling. Therefore, a series of consecutive batch tests were performed to attentively investigate the biomass density influence on type I methanotrophs bacterial growth. In addition, food to microorganisms (F/M), carbon to nitrogen (C/N) and nitrogen to microorganisms (N/M) ratio effect on the microbial activity was studied for the first time. It was clarified that the F/M ratio is the most influencing factor on the microbial growth at higher biomass densities rather than the biomass density increase, whereas C/N and N/M ratio change, while using nitrate as the nitrogen source, does not influence methanotrophs microbial growth. These study results would facilitate the scaling up of methanotrophic based biotechnology by identifying that F/M ratio as the key parameter that influences methanotrophs cultivation at high biomass densities.

Introduction

Due to the ongoing population growth, wastewater treatment facilities (WWTFs) are required to handle higher wastewater volumes which are associated with higher energy requirements. Therefore, there is a great interest in resources recovery from waste streams to offset its treatment operational cost and energy inputs. Methane in the form of biogas is commonly produced throughout the anaerobic digestion of the wastewater collected sludge (Tchobanoglous et al., 2003). However, enormous energy input is required to store and transport methane to be used as a commodity. Therefore, most of the WWTFs were induced to flare the produced biomethane or use it only for heating purposes in the winter time (US EPA, 2011). This can be referred to multiple reasons. (1) The existence of gaseous impurities such H_2S, CO_2, moisture, and siloxane which require cost and time intensive pre-treatment (Tchobanoglous et al., 2003). (2) The low handling and storage capacity due to its gaseous nature and low boiling temperature under ambient conditions, hence, methane is typically distributed/stored after being pressurized or liquefied in a cost intensive process (Ge et al., 2014). (3) In comparison with methanol as an example of the end products, methane has a very low volumetric energy yield (40 MJ/L), whereas, methanol volumetric energy yield is equal to 18×10^3 MJ/L (Hwang et al., 2014). Hence, it is more desirable to convert methane into more transportable and

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storable commodities such as methanol and bioplastics (Strong et al., 2015). Unfortunately, significant energy input is required to break the carbon–hydrogen (C–H) bond in the methane molecule (Lieberman and Rosenzweig, 2004). However, methane can be utilized biologically under ambient temperature and atmospheric pressure (Conrado and Gonzalez, 2014).

Two distinct clusters of microorganisms can activate the methane stable C–H bond which are ammonia oxidizing bacteria (AOBs) and methane oxidizing bacteria (methanotrophs) (Fei et al., 2014; Hanson and Hanson, 1996; Kalyuzhnaya et al., 2015). AOBs expressed relatively low methane uptake capacity due to the competition between methane and ammonia on the AMO (Taher and Chandran, 2013). On the other hand, methanotrophs have attracted the attention due to their distinguish capability to assimilate methane. Furthermore, methanotrophs can integrate methane assimilation with multiple biotechnological application methanol, single cell proteins, biopolymers, and ectoine production (Strong et al., 2015). Collectively, the utilization of methanotrophs in recovering resources from biogas produced throughout waste streams is a prominent research area, especially, in the enhancement of its productivity and overcoming the challenges from the perspective of biotechnology and bioreactor engineering.

As previously mentioned, methanotrophs rely on the methane to obtain their cellular carbon and energy (Hanson and Hanson, 1996). Methanotrophs are phylogenetically grouped into three distinct types; type I (*Gamma* subdivision of *Proteobacteria* phylum), type II (*Alpha* subdivision of *Proteobacteria* phylum), and type III (*Verrucomicrobia* phylum) (Semrau et al., 2010). In comparison with other types, type I methanotrophs are more beneficial to be employed in biotechnological application. Type I expressed higher methane affinity, growth rates, and energy efficiency (Bowman, 2006; Karthikeyan et al., 2015; Whittenbury et al., 1970). Thus, type I methanotrophs usually dominate mixed cultures under nutrients sufficient conditions (Henckel et al., 2000; López et al., 2014).

Type I methanotrophs, similar to other types, oxidize methane terminally to carbon dioxide throughout consecutive intermediate oxidation steps (Hanson and Hanson, 1996; Kalyuzhnaya et al., 2015). Owing to the possession of methane monooxygenase (MMO) enzyme, the cells can oxidize methane into methanol as the first oxidation step. No methanol is accumulated as it rapidly oxidized to formaldehyde in a reaction stimulated by methanol dehydrogenase enzyme. Catalyzed by formaldehyde and formate dehydrogenase, part of the formaldehyde is converted into formate which is subsequently oxidized into carbon dioxide. The remaining part of formaldehyde is utilized throughout the ribulose monophosphate (RuMP) pathway for cell replication (Chistoserdova and Lidstrom, 2013). Throughout the last three steps, electrons are produced to be manipulated in producing Adenosine triphosphate (ATP) to provide the cells with needed energy (Madigan et al., 2015).

Diversified factors are affecting methanotrophs microbial activity such as nitrogen source and copper concentration. However, the challenge to maintain stable microbial growth at higher cell densities is one of the major obstacles facing the process upscaling. It was reported that high cell densities are associated with poor microbial growth (Han et al., 2009; López et al., 2014). This was referred to the limited gas diffusion from the gas phase to the aqueous phase (Strong et al., 2016). In order to overcome such obstacle, the addition of methane vectors (5% paraffin oil) to enhance methane solubility was proposed (Han et al., 2009). Furthermore, different bioreactor configurations were developed and relatively higher microbial activity was achieved (Helm et al., 2008; Wendlandt et al., 2001, 2005). However, the previous studies were performed under different operational conditions such as methane loading rate and initial biomass density. Moreover, biomass density change is typically associated with the change in other conditions such as the methane to microorganisms ratio. Throughout this study, the biomass density effect has been explored independently. Furthermore, the influence of other parameters associated with biomass density change including food to microorganisms (F/M), carbon to nitrogen (C/N), and nitrogen to microorganisms (N/M) ratios on the microbial activity were assessed.

1. Materials and methods

1.1. Chemicals and operational conditions

Throughout all of the incubations, modified nitrate mineral salts medium (Mod-NMS) was used as the growing medium. Mod-NMS has been modified from the NMS described in (Bowman, 2006) and its composition is as following (in g/m^3): $NaNO_3$, 3400; $MgSO_4{\cdot}7H_2O$, 1000; $CaCl_2{\cdot}6H_2O$, 200; KH_2PO_4, 272; K_2HPO_4, 610; Ferric EDTA, 4. 1 mL/L; $CuSO_4{\cdot}5H_2O$, 5. In addition, trace elements solution is used with concentration of 1 mL/L. it contains the following (in mg/L): Disodium EDTA, 500; $ZnSO_4.7H_2O$, 10; $MnCl_2{\cdot}4H_2O$, 3; H_3BO_3, 30; $Na_2MoO_4{\cdot}2H_2O$, 3; $FeSO_4{\cdot}7H_2O$, 200; $NiCl_2{\cdot}6H_2O$, 2; $CoCl_2{\cdot}6H_2O$, 20. Nitrate and copper concentrations were modified based on growth optimization previously performed. Gases (oxygen, methane, and helium) of more than 99% pureness were used (Praxair Technology, Inc., Danbury, CT, USA).

Unless otherwise stated, all incubations were performed in 250-mL sealed serum bottles capped with rubber stoppers. Specific biomass densities were suspended in 50 mL of Mod-NMS as the growth medium. The headspace was filled with methane and oxygen with 1:1 as molar ratio after being evacuated for 5 min. The mixing speed was controlled using MaxQ™ 4000 Benchtop Orbital Shakers (Thermo Fisher Scientific Inc., Waltham, MA, USA) at 165 r/min. Whereas, the bottles were running at room temperature ranging from 23 to 27°C. Incubations time ranged from 30 to 35 hr.

1.2. Inoculum and methanotrophs type I enrichment

Filtrated waste activated sludge was suspended, after being centrifuged, in the Mod-NMS. The sludge was obtained from Humber wastewater treatment plant (Toronto, Canada). The initial biomass density was equal to 0.5 ± 0.07 optical density at 600 nm (OD_{600}). The gaseous headspace was replenished on daily basis. Whereas, the biomass was transferred into fresh medium three times a week in which cultures were centrifuged (4200 ×*g*) for 20 min and re-suspended into the fresh Mod-NMS medium. After the first transfer, the methane consumption was observed. After 12 to 15 days, gaseous uptake and bacterial growth became stable in the four seeded bottles and the

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