JOURNAL OF ENVIRONMENTAL SCIENCES XX (2017) XXX–XXX

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jes

JES JOURNAL OF ENVIRONMENTAL SCIENCES www.jesc.ac.cn

Enrichments of methanotrophic–heterotrophic cultures with high poly-β-hydroxybutyrate (PHB) accumulation capacities

Tingting Zhang, Xiaowei Wang, Jiti Zhou, Yu Zhang*

Key Laboratory of Industrial Ecology and Environmental Engineering, School of Environmental Science and Technology, Dalian University of Technology, Dalian 116024, China. E-mail: zhangshaotin.g@163.com

ARTICLE INFO

Article history:
Received 2 December 2016
Revised 9 March 2017
Accepted 13 March 2017
Available online xxxx

Keywords:
Methanotroph
Activated sludge
PHB
N_2-fixation
Methane monooxygenase

ABSTRACT

Methanotrophic–heterotrophic communities were selectively enriched from sewage sludge to obtain a mixed culture with high levels of poly-β-hydroxybutyrate (PHB) accumulation capacity from methane. Methane was used as the carbon source, N_2 as sole nitrogen source, and oxygen and Cu content were varied. Copper proved essential for PHB synthesis. All cultures enriched with Cu could accumulate high content of PHB (43.2%–45.9%), while only small amounts of PHB were accumulated by cultures enriched without Cu (11.9%–17.5%). Batch assays revealed that communities grown with Cu and a higher O_2 content synthesized more PHB, which had a wider optimal CH_4:O_2 range and produced a high PHB content (48.7%) even though in the presence of N_2. In all methanotrophic–heterotrophic communities, both methanotrophic and heterotrophic populations showed the ability to accumulate PHB. Although methane was added as the sole carbon source, heterotrophs dominated with abundances between 77.2% and 85.6%. All methanotrophs detected belonged to type II genera, which formed stable communities with heterotrophs of different PHB production capacities.

Introduction

Polyhydroxyalkanoates (PHA), an intracellular carbon and energy reserve material produced by many different bacteria, is a potential substitute for traditional plastics with the advantage of biodegradability and biocompatibility. Poly-β-hydroxybutyrate (PHB) is the most abundant PHA (Strong et al., 2016). To reduce PHB production cost, the combined use of activated sludge and waste organic carbon has become one of the focuses in the PHB biosynthesis field (Jiang et al., 2009). Many efforts have been made toward the study of using wastes generated from agriculture-based industries or waste activated sludge fermentation liquid as renewable carbon sources (Akaraonye et al., 2010; Cai et al., 2009; Lee et al., 2014; Zhang et al., 2014). Recently, the production of bioplastics from CH_4 is also receiving increasing research attention (Strong et al., 2016). Abundant CH_4 is discharged during fossil fuels extraction and organic waste anaerobic degradation process (Karthikeyan et al., 2015a; Rostkowski et al., 2012). Gas mixtures containing methane less than 30% are not allowed to be employed in industrial processes, resulting in immense waste of resources (Li et al., 2013). It is estimated by Listewnik et al. (2007) that the cost of PHB could be reduced by approximately 30%–35% with the use of waste CH_4 as feedstock. Moreover, methane could be regenerated after PHB-based productions being discarded and degraded in landfills. Methane could be used as sole carbon source and energy source by methanotrophs, which are mainly composed of two groups: type I (γ-proteobacteria) and type II (α-proteobacteria). It has been reported that PHB production capacity is restricted to type II methanotrophs, while type I genera

* *Corresponding author*. E-mail: zjitidlut@163.com (Jiti Zhou).

http://dx.doi.org/10.1016/j.jes.2017.03.016
1001-0742/© 2017 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Published by Elsevier B.V.

produce exopolysaccharides as a carbon reserve material (Strong et al., 2016; Pieja et al., 2011).

Under appropriate selective pressures, mixed cultures capable of high levels of PHB production can be enriched and maintained. In nature, methanotrophs always coexist with other microorganisms, such as algae and heterotrophic bacteria (Ho et al., 2014). For methanotrophic–heterotrophic communities, the predominance of type II genera might favor their PHB production capacity. However, type I cultures grow fast and predominate under conditions generally recommended for methanotrophic cultivation (Hanson and Hanson, 1996). It has been reported that only 2.5%–8.5% PHB was accumulated by methanotrophic–heterotrophic consortium continuously cultivated in liquid medium with nitrogen source (Karthikeyan et al., 2015b). Meanwhile, methane-driven microbial consortium intermittently subjected to N limitation also has been reported to only accumulate PHB at low level (López et al., 2014). Nitrogen fixation capabilities are broadly distributed among methanotrophs, but more common in the type II genera (Auman et al., 2001). It is well known that nitrogenase activity is sensitive to oxygen. However, the maximal oxygen concentration permitted by type II genera growing on N_2 is higher than the one for type I organisms. Additionally, type I genera grew significantly slowly on N_2 compared with type II methanotrophs (Murrell and Dalton, 1983). The provision of N_2 as the sole nitrogen source might provide an effective selective pressure for enriching methanotrophic–heterotrophic communities dominated by type II methanotrophs. Moreover, methanotrophic–heterotrophic communities enriched at different oxygen concentrations might have different microbial community structures and show distinct PHB production capacities.

A wide variety of heterotrophs have the ability to accumulate PHB (Keshavarz and Roy, 2010). In methanotrophic–heterotrophic communities, methanotrophs form the basis of a microbial food chain by supplying by-products and metabolites derived from methane oxidation to other heterotrophs (Ho et al., 2014). In return, these heterotrophs can stimulate the growth of methanotrophs by removing toxic metabolites (methanol or formaldehyde) or providing essential metabolites (such as cobalamin) (van der Ha et al., 2013). The oxidation of methane is initiated by methane monooxygenase (MMO). MMO can be expressed in two forms: soluble MMO (sMMO) within the cytoplasm and particulate MMO (pMMO) within intracellular membrane. The expression of sMMO and pMMO is regulated by the concentration of Cu. sMMO is only expressed under Cu starvation conditions, while pMMO is only present with sufficient Cu (Murrell et al., 2000). Previous reports suggest that more type II genera than type I organisms can produce sMMO (Auman et al., 2000, 2001). It has been reported that type II methanotrophs outcompeted type I methanotrophs under Cu-limiting conditions (Graham et al., 1993; Hanson and Hanson, 1996). It is probable that the presence of sMMO could favor type II over type I methanotrophs. However, on the other hand, it has been suggested that pMMO has a higher affinity for methane than sMMO (Shah et al., 1996). Hence, the expression of pMMO might accelerate the conversion of methane to other organic compounds that are accessible to heterotrophs, resulting in higher growth rate and higher PHB production capacity of methanotrophic–heterotrophic communities. Therefore, the effect of MMO is unpredictable.

Thus, the PHB production capacities of methanotrophic–heterotrophic communities enriched under N_2-fixing conditions at different oxygen partial pressures with the expression of different forms of MMO were determined in this research, aiming at seeking an optimal selection pressure to enrich a methane-driven microbial consortium with excellent PHB production performance. Afterwards, the gas-phase condition in PHB production phase was also optimized. Finally, high-throughput sequencing technique was employed to investigate the composition of methanotrophic–heterotrophic communities, while most of previous analyses on methane-driven microbial consortia were merely focused on the characteristics of methanotrophs (Chidambarampadmavathy et al., 2015; Karthikeyan et al., 2015b; López et al., 2014; Pieja et al., 2011).

1. Materials and methods

1.1. Bacterial inoculum

To confirm the feasibility of the enrichment method, activated sludge was obtained from the secondary sedimentation tank of two different municipal wastewater treatment plants, namely Fujiazhuang (seed sludge I) and Lingshui (seed sludge II) Wastewater Treatment Plant, Dalian, China. The experiments were conducted in two steps: (1) selecting enrichment of methanotrophic–heterotrophic cultures under different conditions; (2) assessing PHB synthesis and accumulation capacity of enriched cultures under growth-limiting conditions.

1.2. Enrichment experiments

All methanotrophic–heterotrophic cultures were enriched with nitrate free mineral salt (NFMS), containing (per liter) KH_2PO_4 0.272 g, $Na_2HPO_4{\cdot}12H_2O$ 2.868 g, $MgSO_4{\cdot}7H_2O$ 0.10 g, $CaCl_2{\cdot}6H_2O$ 0.20 g and 2 mL of trace element solutions. The trace element solution was composed of (per 100 mL): Na-EDTA 25 mg; $FeSO_4{\cdot}7H_2O$ 50 mg; Fe-EDTA 38 mg; $ZnSO_4{\cdot}7H_2O$ 40 mg; H_3BO_3 1.5 mg; $MnCl_2{\cdot}4H_2O$ 2 mg; $Na_2MoO_4{\cdot}2H_2O$ 26 mg; $CuCl_2{\cdot}2H_2O$ 30 mg; $NiCl_2{\cdot}6H_2O$ 1 mg; $CoCl_2{\cdot}6H_2O$ 5 mg. 5 μmol/L or no Cu were implemented to control the expression of pMMO or sMMO. The initial pH of the medium was adjusted to 6.8 with 1 mol/L sodium hydroxide. The experiments were conducted in a series of identical batch bottles with a total volume of 300 mL each. Firstly, seed sludge I and II were washed and diluted with NFMS medium. Fifty-milliliter diluted seed sludge was transferred to one bottle. Methanotrophic–heterotrophic communities were enriched under four conditions: 5 μmol/L Cu with 0.1 atm O_2, 5 μmol/L Cu with 0.2 atm O_2, 0 μmol/L Cu with 0.1 atm O_2 and 0 μmol/L Cu with 0.2 atm O_2. All bottles were capped, sealed and placed on orbital shakers (150 r/min, 30°C) to improve the gas transfer rate. To ensure the bottles were not substrate-limited, methane and oxygen were supplied on semi-continuous basis where the headspace gas of each bottle was replaced every 24 hr. The bottles were subjected to vacuum and introduced with 0.25 atm CH_4 (160.6 g/m^3), 0.1 or 0.2 atm O_2 (128.5 or 257.0 g/m^3) and adequate N_2 (618.5 to 730.9 g/m^3) to restore ambient atmospheric

Please cite this article as: Zhang, T., et al., Enrichments of methanotrophic–heterotrophic cultures with high poly-β-hydroxybutyrate (PHB) accumulation capacities, J. Environ. Sci. (2017), http://dx.doi.org/10.1016/j.jes.2017.03.016