Journal of Industrial and Engineering Chemistry 35 (2016) 8–13

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

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

Review

Metabolic versatility of microbial methane oxidation for biocatalytic methane conversion

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ARTICLE INFO

Article history:
Received 11 November 2015
Received in revised form 5 January 2016
Accepted 20 January 2016
Available online 28 January 2016

Keywords:
Aerobic oxidation
Methane
Methanotroph
Metabolic versatility
Reverse methanogenesis

ABSTRACT

Methane is utilized aerobically and anaerobically as a carbon and energy source by methanotrophs. Microbial methane oxidation can play a key role in mitigating methane as a greenhouse gas and converting methane to value-added chemicals and biofuels. Recently, genomic and metabolomic analyses of aerobic oxidation in model bacteria have revealed their metabolic versatility in assimilating methane to generate value-added metabolites. Anaerobic reverse methanogenesis metabolism can also be employed to produce liquid biofuels. In this review, the metabolic versatility of aerobic and anaerobic methane oxidation is compared and discussed.

Contents

Introduction

Methane is a greenhouse gas with a global warming potential 20 –30 times greater than carbon dioxide, and thus needs to be mitigated [1]. At the same time, methane is considered to be a next-generation chemical feedstock because it is abundant in natural/shale gas and biogas from anaerobic digestion. As there has been a rapid increase in worldwide gas production, the amount of methane available has continuously increased.

Currently, methane is converted to methanol by a costly two-step process: methane to syngas by means of steam reforming and then syngas to methanol. Steam reforming of methane to syngas is conducted at high temperatures (800–1000 °C), making it an energy-intensive process. The conversion of syngas to methanol also requires a high pressure of 50 bar and a temperature of 250 °C [2]. Other chemical processes for the conversion of methane to value-added chemicals have also been developed such as the Fischer–Tropsch process to produce long-chain hydrocarbons. However, these processes require multiple-unit operations, with high capital expenses upwards of $20 billion [2]. Although chemical conversion has been successfully commercialized at large scale, it is not applicable to marginal oil fields that produce

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http://dx.doi.org/10.1016/j.jiec.2016.01.017
1226-086X/

methane. If no pipeline is available to such remote gas sources, small-scale on-site conversion of methane to chemicals or methane liquefaction methods need to be developed [3]. Direct biological conversion of methane to chemicals may be a solution.

Biological methane conversion can be exploited at small scales with low capital cost. Especially, biological conversion can be applicable to methane that is frequently vented or flared at marginal oil fields [4]. Methane is a very unreactive molecule due to its high C–H bond-dissociation energy of 435 kJ/mol [5]. Oxygen-dependent activation of methane is carried out by methane monooxygenases (MMOs) in mild conditions. In addition, methane is activated via methyl transfer in the anaerobic oxidation of methane (AOM). Methanotrophs can be used as the biocatalyst for methane bioconversion. Regarding biotechnological applications of methanotrophs, they have attracted much attention over the last 30 years as biocatalysts for the bioremediation of halogenated hydrocarbons such as trichloroethylene [6,7]. In this paper, the metabolic versatility of microbial conversion of methane via aerobic and anaerobic oxidation is reviewed. Advantages and limitations of exploiting metabolic versatility for the conversion of methane are also discussed.

Aerobic methanotrophs

Methanotrophs, which can use CH_4 as their sole carbon and energy source, are widespread in aquatic and terrestrial habitats where methane and oxygen are present [8]. Methane is oxidized to carbon dioxide by methanotrophs that utilize methane for energy generation (as an electron donor) and carbon assimilation. Aerobic Gram-negative methanotrophs are classified into three types, on the basis of their intracytoplasmic membranes, formaldehyde assimilation pathways, and 16S rRNA sequences. Type I methanotrophs belong to the Gammaproteobacteria class and employ the ribulose monophosphate (RuMP) cycle (http://www.methanotroph.org/). They convert methane to methanol, catalyzed by particulate methane monooxygenase (pMMO), and then convert the methanol to formaldehyde. The formaldehyde is then assimilated into cell biomass by means of the RuMP cycle. Type II methanotrophs belong to the Alphaproteobacteria class and use the serine cycle for converting formaldehyde into biomass [9]. There are notable differences in the internal membrane ultrastructure of Gammaproteobacteria and Alphaproteobacteria. The intracytoplasmic membranes (ICM) of Gammaproteobacteria arrange in bundles (lamellar stacks) perpendicular to the cell periphery, whereas those of Alphaproteobacteria form stacks packed parallel to the cell periphery. Type II methanotrophs have higher levels of GC content (60–65 mol%) than Type I methanotrophs. Type X methanotrophs belong to the *Verrucomicrobia* [10]. The phylum *Verrucomicrobia* can survive at pH of 1–2 and temperatures up to 65 °C [11]. They express the RuMP cycle and low levels of the serine cycle and Calvin cycle together [12].

Aerobic methane oxidation and assimilation via metabolic pathway

MMO activates molecular oxygen and inserts one oxygen atom into a C–H bond of methane to form methanol [13,14]. Membrane-bound pMMO containing copper is ubiquitous in methanotrophs and uses ubiquinol as the most likely physiological electron donor [15–17]. pMMO consists of three integral membrane polypeptides encoded by *pmoC*, *pmoA*, and *pmoB*. The 27 kDa subunit (encoded by *pmoA*) harbors the active site [18]. This three-gene operon has a σ^{70}-type promoter and is present in duplicate copies. pMMO is expressed only when the copper-to-biomass ratio is high. Cytoplasmic soluble methane monooxygenase (sMMO) is a non-heme, diiron catalytic center-containing enzyme complex and requires electrons supplied by NADH for methane oxidation. sMMO consists of three components: a hydroxylase, a regulatory protein (protein B) and a reductase (protein C). The hydroxylase has three subunits: α (60 kDa), β (45 kDa) and γ (20 kDa), which are arranged in an $\alpha2\beta2\gamma2$ configuration. sMMO is encoded by the six-gene cluster of *mmoX* (α subunit), *mmoY* (β subunit), *mmoB* (a regulatory protein), *mmoZ* (γ subunit), *mmoD* (*orfY*, function unknown), and *mmoC* (a reductase). sMMO has higher broad substrate specificity than pMMO, and thus is used as the enzyme for biotransformation and bioremediation applications. Conversion of methanol to formaldehyde by periplasmic pyrroloquinoline quinone (PQQ)-dependent methanol dehydrogenase (MDH) generates one reduced cytochrome *c*L by returning electrons back through a membrane-bound electron transport chain via a pyrroloquinoline quinone cofactor [19]. Formaldehyde is further oxidized to formate and formate to carbon dioxide by formaldehyde dehydrogenase and formate dehydrogenase, respectively, thereby yielding two NADH molecules. Cytochrome *c*L and NADH are oxidized to generate a proton-motive force for ATP generation.

In addition to the aforementioned pathway, methanotrophs have multiple other options in oxidizing formaldehyde (Fig. 1). One of the most widespread pathways utilizes the cofactor

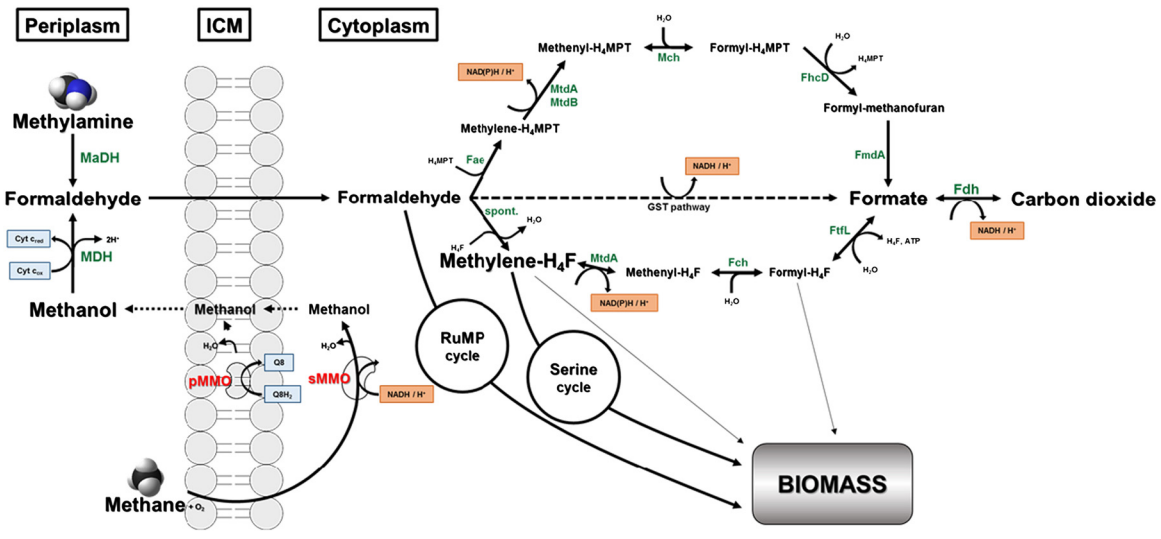

Fig. 1. A simplified diagram of aerobic methane oxidation to CO_2 in methanotrophs. (modified from Refs. [20–22]).