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Case Report

RESEARCH STATUS AND DEVELOPMENT DIRECTION OF METHANE-OXIDIZING BACTERIA IN ENVIRONMENTAL AND INDUSTRIAL APPLICATION

Tao Zhu, Shiqi Wu, Yanran Shen and Yanxia Wang

School of Chemical & Environmental Engineering, China University of Mining & Technology (Beijing), Beijing 100083, China

ABSTRACT: Methane is the second greenhouse gas which is only lower than carbon dioxide in the atmosphere. Since methanotrophic bacteria contains the unique enzymes of methane monooxygenases (MMO), which could catalyze the oxidation of methane to produce methanol in the nature. And the methanotrophic bacteria are of great value in the industrial biotechnology. Therefore, methanotrophic bacteria have been paid much more attention by the researchers in recent years. This article aims to research the mechanism of the mechanical of methane oxidation by methanotrophic bacteria, and illuminate the problems in the application of catalyzing the oxidation. Then it points out the developing direction of methanotrophic bacteria in the future.

Key words: Methane; Methanotrophic Bacteria, Methane Monooxygenases, Environmental; Oxidation

*Corresponding author: Tao Zhu, [1]Physical-Pharmacy Laboratory, School of Chemical & Environmental Engineering, China University of Mining & Technology (Beijing), Beijing 100083, China, E-mail: bamboozt@cumtb.edu.cn

INTRODUCTION

The studies of methane-oxidizing bacteria's application arouse wide concern between university and the scientific institution of an enterprise because of its multifunctional biological catalytic properties and the special position in industrial biotechnology. There have been a lot of applied basic researches of methane-oxidizing bacteria, including its ecological research and application, genetic engineering, the exogenous expression of genetic engineering of soluble methane monooxygenase and the application of industrial bio-catalysis. With the development of modern molecular biology and systems biology, biotechnology provides an important method to understand the mechanism of the methane-oxidizing bacteria and soluble methane monooxygenase in essence. And it also is the science basis to solve the problem that applies methane-oxidizing bacteria in industry. On the basis of understanding further in the methane-oxidizing bacteria and MMO, the application of them in environment and industry gradually becomes the hot spot of research for scientists.

REVIEW SECTION

The research of methane-oxidizing bacteria in industrial bio-catalysis

The catalytic oxidation of methane to methanol

The main problem of using MMO of the whole cell to produce methanol is that the produced methanol will further oxidation in the role of methanol dehydrogenase. To this end researchers make a great deal of researches in this field to prevent the produced methanol from being oxidized by finding out the reagent or method to inhibit methanol dehydrogenase so that we can realize the extracellular accumulation of methanol.

Dithiothreitol, phenyl hydrazine, metal-chelator, iodoacetic acid $MgCl_2$, high-concentration phosphate and glycidol can be used as methanol dehydrogenase inhibitor (Sheets JP et al, 2015).

Takeguchi etc (Karbin S et al, 2015) have studied that the most effective inhibition in these MDH inhibitors is glycidol.

Because the glycidol has the disadvantage that it is difficult to compound with chemical method and it is unstable under aerobic conditions, Lee etc (Torre A et al, 2015). Add 200 mM NaCl which acted as a methanol dehydrogenase H inhibitor. The bacterium, 0.6 mg dry cell ml^{-1}, in methane/air (1:4, v/v) at 25°C in 12.9 mM phosphate buffer (pH7) containing 20 mM sodium formate and 200 mM NaCl accumulated 7.7 mM methanol over 36 h.

Xin etc (Banerjee R et al, 2015) studied that when transform methane into methanol with M.trichosporium IMV 3011, added CO_2 can be used as a MDH inhibitor to realize the extracellular accumulation of high concentration of methanol. When the concentration of CO_2 in the reaction mixture was 40%, the highest accumulation of methanol concentration optimization of gas phase composition is 20%CH_4, 20%O_2, 20%N_2 and 40%CO_2.However, from the point of product methanol concentration; the largest accumulation concentration is less than 15mM.

On the one hand these researches inhibit the activity of the MDH and it realized the biological conversion of methane and extracellular accumulation of methanol. But according to currently known reports about the use of MMO catalyzing methane into methanol, the production of methanol concentration is less than 12mM. The main reason is that MMO catalytic oxidation of methane needs to consume reducing coenzyme NADH. In the normal growth process, NADH can be regenerated by deep oxidation of methanol, at the same time provide reducing power for the first step reaction. But in the oxidation of methane to methanol system, because of the addition of MDH inhibitors that make the methanol oxidation cannot continue to be, and NADH is not renewable. Methane oxidation reaction is to slow or stop. Therefore, when the use of methane oxidative bacteria for whole cell catalysis, it must be by adding sodium format and other external electron donor that can make response to continue; Or when the cell reaction after a period of time, put the cells into the training system, and the oxidation of methane that makes NADH regeneration. Thus, the regeneration of coenzyme NADH is an important factor in the oxidation product of methanol accumulation.

The catalytic oxidation of methane to epoxy propane

Propylene oxide is an important basic chemical raw materials, it is widely used in the production of polyurethane plastics, unsaturated resin and surface active agent. Propylene oxide derivatives are also widely used in food, tobacco, and pharmaceutical and cosmetics industry. There are nearly hundred kinds of production of downstream products, and it is an important raw material of fine chemical products, future development prospects.

In industry acid, peroxide or emulsification was used for the production of epoxy propane currently. These methods have environmental pollution and generate a large number of joint products and other issues. Therefore, looking for a new method of no pollution, low cost, which attracts the attention of the academia and industry. The MMO can be directly used air as oxidant under atmospheric pressure to produce propylene oxide, thus it has the huge market potential and application prospect.

Xin (Hwang IY et al, 2014) in three-phase fluidized bed reactor, uses M. sp. GYJ3, Methylococcus capsulatus IMV 3021 and M. trichosporium IMV 3011 to investigate the change of biofilm formation on Diatomite particles and MMO activity. The production of epoxy propane propylene

catalyzed by MMO has a huge market potential and application prospects, but the product of low concentration, aqueous reaction time was 1.7 mM, water sixteen alkyl two-phase systems, propylene oxide increased slightly, to 2.6 mM.

In order to obtain high methane oxidation system, Xin (Luo MF et al, 2007) uses sampling from the agricultural soil, with methane as the sole carbon source for selective subculture, Get methane oxidation mixed bacteria which has a stable growth performance and MMO activity, and it is superior to the M. richosporium OB3b pure culture. Based on the mixed bacteria catalyst can realize production epoxy propane of oxidation of propylene. The concentration of propylene oxide can be effectively improved by reducing the concentration of phosphate. The maximum can reach 5mM.

The above two processes need the participation of NADH, and they were limited by reducing power levels within the system. Therefore, the preparation of propylene oxide by catalytic oxidation needs two-stage method (Catalytic oxidation and cultivate compound). Cells which have been catalyzed for a period of time can produce NADH in the environment of methane.

Methane oxidative bacteria of application environment

Distribution and role of methane oxidizing bacteria

Methane is second to the carbon dioxide in the earth's atmosphere, greenhouse gases. Years of atmospheric methane production, transportation, circulation and regulation of the study showed that more than 80% of the methane is produced by microbial activity, part before entering the atmosphere to be absorbed by the oxidation of methane bacteria use, reduced the methane into big volume. So far, the most part the net content of methane in the atmosphere is methane-producing microbes and methane oxidative bacteria the result of the interaction.

Therefore methane oxidative bacteria to cause the attention of ecologists, its distribution in all kinds of ecological environment and its impact on large oxygen environment are studied, involving the natural environment including ocean (De Angelis MA, Baross JA 1991), tourism (Rahalkar M, Schink B 2007), lake (Horz HP et al, 2005), grassland, marshes (Hirayama H et al, 2005), paddy field (Horz HP et al, 2001), forest (Pester M et al, 2004), the landfill (Nikiema J et al, 2005), etc.

According to the people understanding of methane oxidative bacteria, containing methane environment generally there are a lot of methane oxidative bacteria. Therefore, the coal mine methane oxidative bacteria community parsing, can improve understanding of methane oxidative bacteria distribution in the environment, has the special function of methane oxidation bacteria information; At the same time for prevention and control of methane oxidative bacteria was applied to coal mine gas provides research foundation.

Compared with other natural environment, coal mine gas tends to have higher ethane concentrations. When people mining coal mine, a large amount of methane gas from the coal mine release into the atmosphere. According to the understanding of methane oxidative bacteria, there are a lot of methane oxidative bacteria in methane environment. We can further understand the distribution of methane oxidizing bacteria in the environment by the study of coal mine methane oxidative bacteria, and obtain some information of methane oxidizing bacteria which has special functions. At the same time, it can provide the research basis for the application of methane oxidizing bacteria in the prevention and control of coal mine gas.

Methane oxidative bacteria in extreme environments

Methane oxidizing bacteria in an environment of low methane can be concentrated (meadows, lakes, etc.), and even in many extreme environment (such as hot springs, volcanoes, etc.) are also widely exist. The oxidation of methane bacteria can not only absorb the methane in environment greatly, reduce the greenhouse effect. At the same time, they are also great methane oxidative bacteria repository. In these environments people found a wide variety of methane oxidative bacteria (acidophilic (Dedysh SN et al, 2000, 2002), basophilic (Eshinimaev BT et al, 2002), halophilic (Yakimov MM et al, 2002), thermophilic (Tsyrenzhapova IS et al, 2007), psychrophilic (Pacheco-Oliver M et al, 2002). The methane oxidizing bacteria in these extreme environments can meet the needs of bio-catalysis under different conditions as shown in Table 1. Therefore, the discovery of the new methane oxidizing bacteria species is highly paid concern and attention.

The potential industrial application of the methanotrophic bacteria is to oxidize alkanes of C_1-C_{20} partly, produce chiral aliphatic alcohol and epoxy alkanes by using olefin of C_2-C_{10} and it can be used in the contaminated groundwater and soil bio-remediation which are polluted by chloroform, trichloroethylene, methylene chloride and other toxic halogenated hydrocarbons (Luo MF et al, 2007). Which must be solved in the bio-catalysis are the separation of the product and the microorganisms' tolerance of catalytic reaction conditions and product and so on. For example, in the process of producing epoxy propane by using MMO to catalysis propylene, epoxy propane will hinder the catalyzed reaction due to the obstruction of the product. Whether can we separate epoxy propane simply in the reaction system are needed to be resolved. Therefore, it is necessary to separate a new kind of methanotrophic bacteria from different natural environment by using analytical methods of environment, which can make the methanotrophic bacteria and MMO meet the needs of the different reaction conditions in the industrial bio-catalysis.

The research method of microbial molecular ecology

With the development of molecular biology and biological informatics, especially in recent years the fledgling macro genomics technology, it accelerates the understanding of methanotrophic bacteria. A large number of molecular ecology methods which are based on ucleic acid and functional genes, fatty acid were applied to resolve methanotrophic bacteria, such as diversity of DNA restriction fragment analysis, fluorescence in hybridization (Eller G et al, 2001), denaturing gradient gel electrophoresis (Henckel T et al, 1999), biochip (Bodrossy L et al, 2003), phospholipid fatty acid analysis (Sundh I et al, 2000), stable isotopic probe techniques (Radajewski S et al, 2000), etc. Molecular ecology can get the information of the bacteria in the environment and help separate special features of methanotrophic bacteria, without the need to cultivate microorganisms.

Table 1: Extreme methanotrophic bacteria and the optimal growth conditions.

Methane oxidative bacteria	Optimal growth conditions	Environment	References
Thermophilic			
Methylococcus capsulatus Bath	42°C	----	Whittenbury R, Phillips KC (1970)
Methylococcus thermophilus	55°C	----	(Malashenko YR et al, 1975)
Methylocaldum gracile	42°C	----	(Bodrossy L et al, 1997)
Methylocaldum tepidum	42°C	Farmland	(Bodrossy L et al, 1997)
Methylocaldum szegediense	55°C	Hot spring through the natural gas field	(Bodrossy L et al, 1997) (Bodrossy L et al, 1995)
Methylothermus sp. HB	55-62°C	Hot spring	(Bodrossy L et al, 1999)
Psychrophilic			
Methylobacter psychrophilus	5-10°C	Northern Russia	(Omelchenko MV et al, 1996)
Methylomonas scandinavica	15°C	Underground water	(Kalyuzhnaya MG et al, 1999)
Methylocystis rosea	5-27°C	Arctic wetland	(Wartiainen I et al, 2006)
Acidophilic			
Methylocella palustris	pH 5.0-5.5	Acidic swamps	(Dedysh SN et al, 2000)
Methylocapsa acidophila	pH 5.0-5.5	Acidic swamps	(Dedysh SN et al, 2002)
Halophilic			
Methylomicrobium pelagicum	0.5-2%NaCl	Deep sea	(Sieburth JM et al, 1987)
Methylomicrobium mdestohalophilum	2%NaCl	Alkali lake	(Kalyuzhnaya MG et al, 1999)
Basophilic			
Methylomicrobium alcalkphilum	pH 9	Alkali lake	(Khmelenina VN et al, 1997)
Methylomicrobium buryatense	pH 7.5-9.5	Alkali lake	(Kaluzhnaya M et al, 2001)
Methylomicrobium sp. AMO1	pH 9-10	Alkali lake	(Sorokin DY et al, 2000)

The problems in the application of methanotrophic bacteria

As an important functional microorganism catalyst, methanotrophic bacteria has a tremendous potential application, it has drawn many research institutions' attention. But in the process of application, it also exist some problems which need to be addressed.

- As methanotrophic bacteria and MMO are applied in chemical industry, the production of methanol, propylene oxide or other chemical substances, the MMO must be produced in a large number. But there are several problems about the methanotrophic bacteria, such as the low growth rate, the low cell density, the long fermentation cycle and the cells are 6gL-1 after fermentation. Due to methane and oxygen which are gases are necessary substrates for the growth of methanotrophic bacteria, the solubility of methane and oxygen in water are about 24mgL-1 and 9mgL-1 under the normal conditions, so the using rate of methane and oxygen is low. It limits the growth of cells as well causes that MMO can't be produced in a large scale in industrial.
- In the process of using the MMO for biocatalysis, the methanotrophic bacteria's expression of sMMO or pMMO protein will be low due to the coding sMMO and pMMO gene copy number is limited in the methanotrophic bacteria. So it can't satisfy the catalytic need in industry when the concentrations of methanotrophic bacteria cells are low. And sMMO protein's expressions are affected by the concentration of Cu^{2+}, it can't express when the concentration of Cu^{2+} is high. According to the analysis of pMMO crystal structure, Cu^{2+} is the essential metal ion for pMMO protein's folding. It is likely to be pMMO metal active center, but it's unclear that Cu^{2+} is how to influence the pMMO expression.

- Reducing co-enzyme NADH is required in the catalytic oxidation processes, but it's expensive and NADH regeneration in vitro technology is not mature. In the process of using the whole cell MMO oxide methane to produce methanol, the main problem is that it needs further oxidation to provide energy for the growth of cells. Therefore, in the process of the whole cell catalysis, it must regenerate the co-enzyme NADH by cell refolding when the catalytic oxidation comes to a certain stage. So the problems of co-enzyme regeneration can also restrict its application.
- The number of identified methanotrophic bacteria is limited, while the industrial bio-catalysis often require microorganisms can tolerant certain acid/alkali, cold/hot, organic solvents, etc. In order to meet the needs of industrial bio-catalysis, we need to find richer resources of methanotrophic bacteria flora.

CONCLUSION

According to the characteristics of methanotrophic bacteria and the aim to control greenhouse gases and the application of industrial biological catalysis, it will be significant to conduct a deep research about the distribution, training of the methanotrophic bacteria and the MMO gene expression with the demand of people for environmental protection and economy coordinated development. As a potential industrial biological catalyst, on the one hand we can produce the bulk chemicals by converting the methane. On the other hand we can use methane to produce high value-added fine chemicals, such as chiral aliphatic alcohol, epoxy alkanes, etc.

In order to meet the deep application of make the methanotrophic bacteria, we need to study from the following aspects:

- By using the microbial molecular ecology and biological informatics methods, we can analyze all kinds of environment which maybe in rich of methanotrophic bacteria, to obtain methanotrophic bacteria and the biological information of MMO, then separate the methanotrophic bacteria which adapted to different industrial catalytic conditions.
- In order to produce commodity chemicals, we can improve the ethanotrophic bacteria growth rate, the concentration of the cell and the expression of MMO. By promoting the expression of MMO gene and with the application of the genetic engineering and metabolic engineering By promoting the expression of MMO gene, methanotrophic bacteria can grow quickly and strength the oxygen and methane transfer rate. In common genetically engineering bacteria, excessive heterologous can express MMO protein. Taking advantage of the growth of the host and the enzyme regeneration system can obtain high MMO catalytic cells. And it can be used in biological catalysis process.
- With the modification of the method and technology platform of the methanotrophic bacteria or MMO, it can produce high value-added chemicals. By using metabolic engineering technology, reconstructing methanotrophic bacteria intracellular or strengthening the producing way of high-value-added chemicals, then it can produce fine chemicals with cheap methane. According to the point mutations or molecular modification of MMO, it can improve the enzyme activity or change MMO substrate scope so as to produce high value-added chemicals, to meet the special catalytic reaction conditions such as organic phase catalysis and high temperature catalysis.

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