Ecological distribution and application research status of methanotroph based on gas control of coal mines

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ABSTRACT

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Gas disaster is considered as a severe problem faced by underground mines. It is seen as a feasible technological means to degrade gases in coal mines by using methanotroph, which is although subjected to a primary research stage in application. Researchers sampled, cultured and identified multiple effective methanotrophs in conventional and extreme natural environments as well as production environment of coal mines. However, the majority of methanotroph is aerobic while minority of them is anaerobic. The designed experimental conditions and scales in the research significantly differed from the actual conditions of coal seams so that it is hard to apply the research on site. This study analyzed the major problems of current researches and put forward the requirement in environmental conditions: the actual conditions of gases of coal mines are fully taken into consideration in the future researches. Moreover, the study pointed out that the extraction environment and cultured conditions of effective methanotroph and is expected to concentrate on improving research efficiency and application value.

1. Introduction

The gas of coal seam is a mixed gas emitted from coal seam and strata during coal mining in which methane (CH_4) take up more than 90%. Therefore, gases of coal mines generally refer to CH_4 (no difference in the study). Gas is flammable, explosive and asphyxiant and various accidents including gas explosion, coal and gas outburst and gas combustion frequently occur during coal mining, resulting in serious casualties and economic loss. Greenhouse effect further aggravates if gases emit to atmosphere, causing the environmental problem, so that governing gas of coal mines is of practical significance and urgency. Gas control of coal mines is recognized as the worldwide difficulty as well as long-term research field of numerous researchers and corporate technicians. Unfortunately, the difficulty has not fundamentally solved yet. Especially in China, the number of casualty caused by gas disasters takes up about one third of the total number of death caused by coal mining accidents [1]. Since 1900s, numerous researchers in the world have carried out experimental researches on degrading methane by using microorganisms with the development of microbial technology. Moreover, they have theoretically verified the feasibility of controlling gases of coal mines using microbial technology, which provides a new channel for controlling gases of coal mines. Finding a kind of methanotroph for degrading methane is the key to controlling gases of coal mines by using microbial technology.

Since methanotroph has been first successfully separated in 1906 [2], 56 species of 21 genera at least are found [3]. The researches in the world reveal that methanotroph widely distributed in natural environment is a kind of microorganism which grows by using methane as single carbon source and energy. It is found that cultured multiple methanotroph exhibits different degrees of oxidative degradation on methane and the process degrading CH_4 is displayed in Fig.1 [4]. The typical characteristic of methanotroph is to catalyze methane by using existing methane monooxygenase (MMO) into various intermediate products (methyl alcohol, methanol, etc.) and eventually produce CO_2 . The methanotroph constantly acquires nutrition and energies for growth in the process [5].

Since the degradation effect of methanotroph on methane has been found, researchers make great efforts to explore the methanotroph with high-efficient degradation efficiency in wide natural environment and preliminarily propose the ecological distribution theory of methanotroph.

Some researchers conducted tests on coal specimens or in situ coal mines in order to further examine the efficacy of acquired methanotroph in degrading gases of coal mines. The study carries out overview based on the ecological distribution of methanotroph and its application in coal mines, aiming to provide references for future researches.

Fig. 1 Degradation process of methane by methanotroph

2. Ecological distribution of methanotroph

2.1 Conventional natural environment

As a greenhouse gas, the production, circulation and regulation of methane in nature are widely investigated. Researches showed that more than 80% of methane is produced by using microbial technology and also part of methane reduces after being degraded by methanotroph. Therefore, methane content in atmosphere equals to the net content of microbe production and amount of methanotroph decomposed [2]. With decade-long efforts, researchers have detected methanotroph from soil, deposition or water sample under various conventional natural environments. The natural environments involve rice field, river, lake, wetland, marsh, grassland, forest and underground water. It is verified through experiments that the cultured methanotroph exerts different degradation effects on methane.

Methanotroph in rice field which is an important source of producing methane, can be used to oxidize and degrade a portion of methane. Researchers found different varieties of methanotroph in soil of rice field. India and China with the largest rice areas in the world constantly conduct

new researches about methanotroph. Scholars separately obtained the new varieties of types-I and II methanotroph after they extracted methanotroph from rice fields at the north and west of India [6, 7] while scholars in China also extracted new methanotroph from the rice field with red soil in the south of China [8]. The above researches further enrich the diversity of methanotroph. As the main area of human agricultural activity, rice fields are irrigated and fertilized during human farming which can provide beneficial conditions for ecological environments of methanotroph. Additionally, methanotroph is also detected in soil under similar conditions including vegetable land and orchard while it is different from that of being found from rice field in terms of structure and abundance of microbial community as well as dominant bacteria [8].

Apart from rice field, wetland and marsh are also regarded as the important environments for growth of methanotroph. Scholars found types-I and II methanotroph in plateau wetland with high altitude [9] and peat swamp [10] except for in common wetland and marsh. Researches reveal that there are vegetations in wetlands and marshes with methanotroph which influence the production, consumption and transfer of methane in soil.

Methanotroph is also detected in the bottom mud of some rivers and lakes [11, 12]. Scholars detected methanotroph and methanogens from the depositions in Lake Bourget, France [13], which provide new enlightenments for studying the variety of methanotroph and methane hydrates.

Grassland is also one of the environments for growth of methanotroph: researchers found methanotroph in meadows of Qinghai-Tibet Plateau [14] and Inner Mongolia grassland [15] in China. An analysis reveals that warming and grazing exert an important effect on activity of methanotroph [14].

Researchers also discovered methanotroph from soil and sewage sediments of landfill sites [16, 17] and some researchers found anaerobic archaea and germs [18, 19], which is rarely found in other conventional natural environments.

Researchers also extracted methanotroph in underground water and water-bearing strata. They detected methanotroph from the underground water-bearing strata of a gas field in Japan and proposed that underground water with methanotroph can be used for ecological restoration [20].

The methanotroph grows in conventional natural environments aforementioned, among which, vegetation and water are important environmental conditions. Human production and living make soil fertile which is conducive to the growth of methanotroph. These ecological conditions are beneficial for researchers to favorably culture methanotroph. The methanotroph detected in conventional natural environments is almost aerobic except for anaerobic methanotroph only being detected in waste mud.

2.2 Extreme natural environments

Methanotroph was also detected in multiple extreme natural environments (including acidophilic, alkaliphilic, halophilic and heat-resistant and cold-resistant types) apart from in conventional natural environments aforementioned (as shown in Tab.1). It can be seen from the table that methanotroph under extreme natural environments shows an insignificant difference with that under conventional natural environments in species. However, the adaptation of methanotroph to the extreme natural environments reflects the functions, namely, new functions can satisfy the requirement of biological catalysis under different conditions. Nonetheless, the research remains to be further studied [5].

Tab. 1 methanotrophs in extreme natural environment

extreme environment	Extreme conditions	methanotrophs	References
Acid wetland	pH 3.9-4.7	<i>Methylocystis</i>	[21]
Soda lake	pH 10.3	<i>Methylochromium</i>	[22]
East Sea of Korea	high sodium content	<i>ANME-1b</i>	[23]
Thermal spring	74°C (The maximum)	<i>Methylocystis</i> <i>Methylothermus</i>	[24]
Russian arctic tundra	5-10°C	<i>Methylobacter</i>	[25]

Moscow -11~ -17°C *Methylocystis* [26]

2.2 Production environments of coal mines

Existing ecological researches about methanotroph about mainly concentrate on various natural environments including marsh, lake, forest and grassland in nature while scarcely focus on environments involved human activities. Gases of coal mines are rooted in coal seams and they are constantly emitted from coal seams into atmosphere under coal mining so that coal mines with gases have high methane contents. In recent years, some scholars try have attempted to extract methanotroph in production of coal mines. It aims to analyze the methanotroph community in coal mines in view of ecology, improve the recognition on ecological distributions of methanotroph and acquire the information of methanotroph with special functions. By doing so, it can provide research basis for applying methanotroph to gas control in coal mines.

After consulting literatures, it can be seen that there are only a finite number of researches about acquiring methanotroph with strong activity from coal environments and scholars in China make efforts due to the seriousness of gas disasters in coal mines, China. Hao Jiang et al. [27] detected aerobic methanotroph in soil near the exit of air shaft of a coal mine, China and Han Bing et al. [5, 28] found amount of types-I and II methanotroph in alkaline soil of coal mines, showing great activity absorbing methane. Huang Xia et al. [29] found multiple strains of methanotroph from acid soil of coal mines and successfully extracted the strains H4 with strong ability to degrade methane. Mao Fei [30], Zhang Ruilin et al. [31] also detected methanotroph in soil of coal mines.

Numerous researchers extracted methanotroph in production environments of coal mines in recent years, however do not get much progress. Methanotroph is mainly detected from the ground soil of coal mines in these researches and little research about the detected methanotroph from the underground coal mines is reported.

2.3 Distributions of anaerobic methanotroph

In the long term, researchers suggest that the oxidation of methane in depositions can be conducted only under aerobic conditions. However, they find that methane can be oxidized under anaerobic or anoxic conditions with research advancement, which can be called anaerobic oxidation of methane (AOM). Researchers demonstrate that the anaerobic oxidation of methane occurs based on two approaches [32, 33]: on the one hand, it is conducted under the drive of methanotroph and the symbiont of sulfate reducing bacteria; on the other hand, it is carried out by using methanotroph and nitrate denitrification. Their chemical equations are shown as follows:

Most anaerobic methanotroph is detected from ocean sediments and researchers have also detected anaerobic methanotroph from soil and sewage depositions of landfill sites [18, 19] in recent years. Min Hang et al. in Zhejiang University in China found the bacteria strain AMOB3 in rice fields which can cause the anaerobic oxidation of methane and suggest that the bacteria strain is a new species which differs from existing methanotroph. Part of methanotroph enables to oxidize methane both under aerobic and anoxic conditions. Roslev et al. [35] illustrated that the type-II methanotroph can be stored for 6 days under lacking of methane and oxygen while the oxidation on methane can be recovered within several hours after adding methane and oxygen. Liu Guosheng, Zhang Ruilin et al. [11] cultured methanotroph AEN1235 in canal mud in Wei River of Xinxiang in Henan province of China and it was found through methane degradation experiment that the strain enables to oxidize the gases adsorbed in coal specimens both under aerobic and anoxic conditions.

Therefore, there is also anaerobic methanotroph distributed in nature apart from common aerobic methanotroph. The above researches further enrich the diversity of methanotroph and ecological distribution. It is worth noting that discovering anaerobic methanotroph exerts an important significance on controlling gases of coal seam. This is because gases are derived from coal seam which hardly contains oxygen so that the coal seam is suitable for growth of anaerobic methanotroph. However, it is difficult to extract and identify the anaerobic methanotroph and therefore the biochemical character, approaches of oxidation and mechanisms of methanotroph remain to be further investigated.

3. Application of methanotroph in gas control of coal mines

3.1 Existence of gases of coal mines

As the secondary products of coal-production, gases are stored in coal seam and constantly emitted into mining space influenced by coal mining to discharge into atmosphere with the mine air. Gases of coal mines are expressed as three states: firstly, gases inside coal seam (source of gases of coal mines); secondly, gases of mine air; thirdly, gases in goaf. Most of gases inside coal seam are adsorbed onto the internal surface of pores and fractures of coal masses and there are a few free gases, hardly containing oxygen. The gases of mine air are emitted to mining space from coal seam and mixed with mine air with a low concentration (normal concentration is not larger than 0.75%). The gases are sealed in goaf, showing high gas concentration and low oxygen concentration. The gases of coal mine under three states are shown in Fig.2. It can be seen that the gases of coal mines are degraded by using methanotroph need to consider the specific environments of gases under different states. Especially, it is necessary to purposefully study the specific requirements of different oxygen-bearing conditions under three states on activity and degradation efficacy of methanotroph.

Fig. 2 State sketch of methane in coal mine

3.2 Research advancement

Scholars from different countries have made some achievements on degrading gases of coal mines using methanotroph with three different states.

In the early 1970s, microbiologists in Russian Academy of Sciences (RAS) and researchers from Mexico Mining Research Institute tackled key problems together and proposed a gas control technique using microorganisms which can effectively control the methane contents in coal seams. The mechanism of the technique is described as follows: a biological filter layer of oxidized bacteria with the effect of eliminating gases is formed by spraying active biological fungi for eliminating gases in goaf. On the other hand, it is attempted to inject active biological fluids for eliminating gases into coal seam through special drilling system [36]. The industrial test data of Donetsk (Ukraine) and Kuznetsk (Russian) coal fields display that the methane content in coal seam can decrease by 30%~60% by using the technique [37].

In 4th international symposium on mine ventilation in 1988, Chakravorty et al., scholars in Canada, announced that they extracted the bacteria for degrading and oxidizing methane from the water sample of coal mines in the west of Canada. They carried out tests after the methanotroph was added in the simulated ventilation tunnel separately on the conditions of opening the tunnel, closing the tunnel and constant air supply. The best test result showed that the methane content of wind flow without being treated by bacteria is 5% while that treated by bacteria declines to 0.05% at least [38].

Researchers in Australia obtain some achievements in this aspect at the same period. Researchers sprayed the bacterial fluids for degrading methane onto coal wall. Bacteria can be constantly reproduced by taking methane as only carbon source and energy. The methane content of underground wind flow decreased by 66% after 20 days [39]. Chris A et al., scholars in Australia, sprayed methanotroph fluids into goaf so that the oxidation and biological filter layer was formed to degrade gases in goaf. By doing so, the methane content in gases emitted from goaf was decreased and further certain achievements were obtained [40]. Researches revealed that the removal rate of a kind of methanotroph on methane under having gases is up to 99% and that still remains 97% after 7 days [41].

Scholars in China have carried out multiple researches on gas control of coal mines using methanotroph. Wang Lu, Chen Dongke et al. [42] from University of Science and Technology Beijing separately carried out gas degradation tests on 12 coal specimens bearing gases taken from different coal mines by using the mixed bacteria of M3011 and GYJ3 which were screened, cultured and naturalized. The test result showed that the gas content desorbed by coal specimens can be degraded from 100% to 4.8% by employing nethanotroph, showing the degradation rate and average degradation rate of 52% and 44%, respectively. Zhang Ruilin et al. [31] in Henan University of Engineering sprayed different contents of methanotroph fluids on multi-group of dried coal specimens which were put into steel can in advance by using self-made hybrid plant with high-pressure gases. On this basis, they concentrated on studying the change characteristics of adsorption (desorption) quantity of coal specimens on methane and CO₂ productions under the supply condition of pressure-bearing and hybrid gases with low oxygen. Mao Fei [30] in Chongqing University extracted methanotroph from waterflooding rice field and injected bacteria fluids into coal walls at underground coal mines. By detecting relevant indexes, it can be seen that gas content of returned wind separately reduced by 22.54% and 77.23% while those of tons of coal decreased by 39.67% and 13.45%, respectively. Yu Haixia [43] from Zhejiang University extracted a hybrid system of methanotroph in soil of rice field and designed a bio-trickling filter for simulating the flow environment of mine air. The test result showed that it is safe, steady and reliable to treat gases of coal mines by using methanotroph system and various harmful gases (including CO, H₂S and SO₂) of mine air have an insignificant influence on activity of methanotroph.

Researchers investigated gas control of coal mines under three states and verified the feasibility through tests. However, scholars in China have carried out productive researches in recent years and obtained progression especially on the degradation of gases inside coal masses (gas source). Generally speaking, the research on degradation of gases of coal mines using methanotroph is still subjected to the primary phase and some achievements are obtained based on ideal conditions. The research do not consider multiple practical conditions involving storage stress, temperatures, humidity and oxygen bearing about coal masses and therefore the achievements fail to have application value.

3. The limits of previous research

Researchers made sampling in wide natural environments (including extreme environments) and obtained multiple species of methanotroph having different degrees of capacities for degrading methane through laboratory culture. Therefore, the varieties of methanotroph are further enriched. However, the laboratory culture exhibits high risk or uncertainty and the acquired methanotroph lack of application value in degrading gases of coal mines. The reasons are drawn as follows:

(1) Researchers found that certain environmental conditions (including plants, water, temperatures, fertility and oxygen bearing) in different natural environments shows similarities, which can provide guidance for laboratory to favorably culture methanotroph. However, researchers have not found the action mechanism between the environmental conditions and methanotroph and have an unclear recognition on the scale disparity of environmental conditions on laboratories and fields [44]. Moreover, to obtain the maximum efficacy of methanotroph on degrading methane, researchers optimized experimental conditions as far as possible. However, the optimized experimental conditions significantly differ from the storage environmental conditions of gases of coal mines and the correlation between natural environments of methanotroph and storage environment of gases of coal mines was not deeply analyzed. Therefore, it fails to provide the guidance for exploring the methanotroph with high degradation efficacy.

(2) There are three different storage states of gases of underground mines and the three states show different environmental conditions. Where, oxygen-bearing condition exerts an important influence on selection of methanotroph, which is not deeply studied in current researches. Most methanotroph for degrading methane is aerobic and there are only a few researches about anaerobic methanotroph. Coal seam is the source of gases of coal mines and it is generally considered that there is not oxygen in it. Thus, it is promising to control gases of coal mines by using anaerobic bacteria while the ecological distribution of anaerobic methanotroph has not been clear. It is still difficult to culture, extract and identify the anaerobic methanotroph which is inferior to aerobic methanotroph in variety. The influences of multiple environmental conditions (ground stress, water-bearing condition, environmental temperature, humidity and pH value) of coal seam on activity and degradation efficacy of methanotroph, except for the influence of oxygen-bearing condition, lack of systemic. The influences of multiple common conditions (such as

temperature, humidity and pH value) are studied in laboratory at most. However, the comprehensive influence of ground stress and multiple factors has not been conducted. It is an important reason why current achievements do not have application value.

5. Future research direction

By deeply analyzing the problems during researches, it is necessary to consider the practical environments of storing gases of coal mines when gases of coal mines are degraded by using methanotroph. Research is further conducted from the following two aspects.

(1) The application requirement is clarified to explore the methanotroph (including aerobic and anaerobic types) which can purposely adapt to specific environments. It is necessary to explore the correlation between distributed natural environments of methanotroph and storage environments of gases of coal mines. It aims to effectively guide and further culture methanotroph with favorable activity and high degradation efficacy, avoid mass-election and accidental success and improve the research efficiency.

(2) It is necessary to further investigate the influence of environmental conditions of gases of coal mines under different storage states on the activity and degradation efficacy of methanotroph. To achieve such goal, the efficacy and mechanism of methanotroph on degrading gases of coal seam under comprehensive effect of multiple conditions need to be explored. Especially, it is necessary to study dynamic changes of ground stresses and mine ground pressures of coal seam as well as the influence of the change of physical structures of coal on methanotroph under the effect of environmental conditions.

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