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ORIGINAL PAPER

Experimental investigation of the use of methanotrophs for the degradation of low-concentration methane

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Abstract

Methanotrophs (methane-oxidizing bacteria) can degrade methane into carbon dioxide and water. In this paper, several types of methanotrophs have been successfully cultivated and sorted out based on microbial technology, and the processes and approaches used are described in detail. By using several types of active methanotrophs, a few groups of experimental investigations have been carried out to determine their degradation effects on methane gas; these studies used different low methane concentrations that were less than 1.5%. The experimental results showed that the methanotrophs studied can still show the characteristics of normal activity under the conditions of a series of low methane concentrations that ranged from 0.25 to 1.5%. In the degradation process, with the increased methane concentrations in the mixed gas, more methane could ultimately be degraded to carbon dioxide. The decrease in methane and increase in carbon dioxide presented approximately linear relationships, and the numerical relationships between carbon dioxide and methane and the microbial degradation times could be described by exponential functions with high correlations. The conclusions in this paper attempt to forge a new path for the prevention of exceeding limited methane concentrations during coal mining by the use of microbial technology.

Keywords Methanotrophs · Flowing gas · Methane · Low concentration · Degradation features

Introduction

Methane, which is a flammable and explosive gas, is largely preserved in underground coal seams (Moore 2012). It is very important to control methane gas during coal mining. First, during the excavation process, large quantities of methane gas continually flow from coal seams and then overflow into underground tunnels and working areas (Moore 2012; Zhou et al. 2019). Conditions occasionally exist that cause high risk of gas combustion and even gas explosions, which, in particular, endanger all workers in the mine. Additionally, methane is a strong greenhouse gas and is very harmful to the global ecological environment if large amounts of methane emissions occur from coal mines (White et al. 2005; Zhou et al. 2020a, b). Mine ventilation is a widely used method for removing methane from coal mines. However, it is costly because of the consistently high power consumption that is needed and often has limited effectiveness for some high methane gas emissions (Gray 1987). Moreover, excessive methane discharges inevitably cause serious pollution problems for the ecological environment (Jessen et al. 2008). Therefore, it is necessary and significant to search for other reasonable approaches to control methane that is carried by airflow in mine shafts.

There are many kinds of microorganisms in nature, which play an important role in the decomposition, absorption, and transformation of garbage (Slezak et al. 2015). Methane emission reduction is an important part of international greenhouse gas emission reduction. There are a large number of methane-oxidizing bacteria in nature, which can oxidize and decompose methane and play an important role in maintaining the balance of atmospheric methane concentration (Aronson et al. 2013). The growth activity and oxidation capacity of

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methane-oxidizing bacteria are affected by environmental factors. The number and activity of methanotroph can be enhanced with the increasing of organic matter content in soil, and the ability of methane oxidation could be improved (Kightley et al. 1995). The ability of gas diffusion would be reduced with the increasing of moisture in growing environment; therefore, the optimum moisture content for the growth of methanotrophs is 25% (Scheutz and Kjeldsen 2004; Scheutz et al. 2009). The oxidation ability of methanotroph is weak in low temperature environment; the optimum temperature for methanotroph growth is 298–308 K (Lefticariu et al. 2006). The application of methanotroph in landfill has been very mature; the application in coal mine is still in the exploration stage. The characteristics of coal are very complex, and the growth features of methanotroph still need to be further studied.

With the rapid development of biotechnology, increasing numbers of scholars around the world have begun to pay close attention to the use of certain selected microbial bacteria for methane degradation. Methane-oxidizing bacteria are microorganisms that can transform methane into water and carbon dioxide by using methane as their sole carbon and energy source for growth (Liang et al. 2008; Reddy et al. 2014; Wang et al. 2011). At present, the degradation principle described in Fig. 1 (Graef et al. 2011) has been accepted in academic circles at home and abroad. The first special bacterium that can oxidize and eliminate methane was successfully cultivated and isolated by Sohngen NL in 1906. Since then, researchers have determined that these bacteria widely exist and live in the natural environment, such as in rice fields, soils near coal mines, sewage sludge, landfills, swamp land, areas of early volcanic eruptions (KANG and FENG 2012; McDonald et al. 1996), and even in the environment under some extreme conditions, such as strong acid or strong alkali conditions (Li and Wang 2007).

Cicerone and Oremland (Cicerone and Oremland 1988) presented the concept of lowering gas concentrations during coal mining by using methane-oxidizing bacteria. Australian researchers sprayed a specified amount of a liquid that contained acclimated microorganisms onto coal walls and found that methane concentrations in the airflow pathway decreased by approximately 66% (Collins et al. 1991).

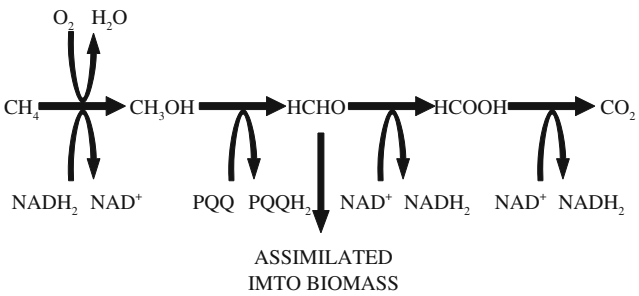

Fig. 1 The principle of microbial degradation of methane

Researchers from the USA domesticated some bacteria that were collected from acidic swamps, named them Bayesian Lin Keshi bacteria in their experiments and found that these bacteria could clear approximately 90% of methane in the atmosphere (Lipscomb 1994). In recent years, some domestic scholars (HOU et al. 2008; Jiang et al. 2010; Jiang et al. 2014; Liu et al. 2016; Tang et al. 2012; Ward 1987; Wu et al. 2014) have carried out a large number of experiments using different approaches, and all verified that methane-oxidizing bacteria can degrade and eliminate methane from coal seams. Considering their feasibility and lower costs for controlling coal mine gas, microbial techniques should be widely applied for this problem in the future. For this reason, related theoretical research is especially needed and urgent.

Methane is a serious disaster in coal mining. It is easy to form gas explosion and coal and gas outburst, which seriously endangers the safety of workers. In order to improve the effect of gas control, a large number of technologies were proposed, such as CO_2 injection and water injection in coal seams. However, these technologies will be limited in the application. The permeability of coal seams in China is very low, the drainage range of boreholes is small, and the drainage period is short. Therefore, the gas drainage cannot be carried out for a long time. Water injection has been widely used in coal mining, and gas is expelled by injecting water into the coal seam. It is very difficult for water molecules to enter the micropores of coal, and it is likely to block the gas in the micropores. The methanotroph is a kind of microorganism that consumes methane as energy. By adding the methanotroph into the water and injecting into the coal seams, this technology can achieve the purpose of water injection in coal; the methanotroph can enter into the pores of coal and continuously consume the methane.

In previous research on methane-oxidizing bacteria, most theoretical discussions have mainly focused on their degradation features for pure methane (i.e., concentrations exceeding 99%) (Bykova et al. 2007; Ghorai et al. 2014; Li and Shao 2010; Ma et al. 2012; Siavash and Hossein 2013). However, little academic literature has been published that aims to eliminate low-concentration methane present in airways in coal mines by exploring the degradation features for low methane concentrations (i.e., methane concentrations less than 1.5%) (Li et al. 2007; Ting-xiang 2009; Xianfeng et al. 2010; Zhang et al. 2013). In this paper, comprehensive experimental analyses have been carried out regarding the microbial degradation of low-concentration methane, and the research has been discussed in detail, which mainly includes sample collection, bacterial cultivation, and experimental investigations of methane degradation. The conclusions are expected to contribute to the development of new methods and technologies to prevent methane gas disasters during coal mining.

The cultivation and separation of the methane-oxidizing bacteria

Sample and medium selection

Based on the suitable conditions for the growth and survival of methane-oxidizing bacteria, sampling sites should have rich occurrences of methane and a presence of oxygen. Some previous, related studies have stated that large numbers of methane-oxidizing bacteria are always present in topsoil below 15 cm depths (Pol et al. 2007). Based on abovementioned factors, the experimental samples were collected from muds or soils in the bed of the Shuanglei River in Xinyang City, Henan Province. During the sampling process, various types of gravel debris were removed, and the sampling times along with the locations and surrounding environmental conditions were recorded. All collected samples were placed into special bags or boxes and were immediately sent to the laboratory.

To prepare and culture bacterial liquids, 500 g of mud or soil samples were placed in a conical flask, which was disinfected and sterilized in advance. Ultra-pure sterilized water was poured into the flask, and the flask was shaken to fully soak and evenly mix the sample. After allowing the suspension to stand for 24 h, 4 ml of the liquid supernatant was removed, and this was injected into a bottle with 50 ml of culture medium in a special super-clean experimental setup. This mixed liquid is referred to as IBS (initial bacterial solution). It is extremely important to provide an appropriate culture medium to obtain sufficient quantities of methane-oxidizing bacteria. Although the proportions of culture media used in previous research were not strictly identical, nitrogen sources and some microelements are necessary for the growth and survival of methane-oxidizing bacteria. On the other hand, considering a key point in of the following experiment that the obtained bacteria must use methane as the sole carbon source for their survival and propagation, the inorganic salt culture medium that was used in the experiments described in this paper was common NMS (New Murashige & Skoog) medium, which does not contain any carbon sources. The proportions of the culture medium used in this paper are shown in Table 1 and Table 2 below, and the reagents used in the components were all of high purity.

Table 1 Components of the NMS culture medium

Number	Component	Mixture ratio
1	KNO_3	1.000 g
2	NH_4Cl	0.250g
3	KH_2PO_4	0.272g
4	$CaCl_2{\cdot}6H_2O$	0.200g
5	$MgSO_4{\cdot}7H_2O$	1.000g
6	$Na_2HPO_4{\cdot}12H_2O$	0.717g
7	Microelement solution	1mL
8	Distilled water	1000mL

Experimental methods

The culture and separation of methane-oxidizing bacteria

For the culture containing methane-oxidizing bacteria, first, the pre-made IBS (initial bacterial solution) was divided into three portions, which were poured into three bottles, each with a capacity of 250 ml. The bottles were then filled with the mixed gas containing air and methane, and the methane concentrations were maintained at approximately 50%. Next, these three bottles were placed on a rotary platform; a temperature of 30°C was maintained, and a shaking speed of 120 r/min was used for 5 to 7 days. The process was observed until the solutions became slightly turbid, which indicated that large quantities of methane-oxidizing bacteria were growing and propagating in the solutions. Next, three pipettes were used to extract 5 ml of the liquid supernatant from each of the culture solutions, and these samples were then transferred to new culture bottles. To completely remove impurities from the solutions and to obtain more stable methane-oxidizing bacteria, the above transfer step should be repeated at least three times.

To ensure that the final methane-oxidizing bacteria were as pure as possible in the following experiments, treatments to purify and separate the bacteria were needed. For this purpose, the flat plate coating method for solid culture media was used as described below. The first step placed a 2% concentration of agar into a conical flask along with the liquid culture medium, and this mixture was continuously shaken until the agar was evenly mixed. The second step consisted of using six flat plates that were placed in an incubator while maintaining the temperature at 30°C. For the third step, mixed air and methane with a methane concentration of 50% were filled as the sole

Table 2 Trace element components

Number	Component	Mixture ratio
1	H_3BO_3	0.030g
2	Na_2EDTA	0.500g
3	$CoCl{\cdot}6H_2O$	0.020g
4	$NiCl_2{\cdot}6H_2O$	0.002g
5	$FeSO4{\cdot}7H_2O$	0.200g
6	$CuSO_4{\cdot}5H_2O$	0.030g
7	$ZnSO_4{\cdot}7H_2O$	0.003g
8	$MnSO_4{\cdot}4H_2O$	0.003g
9	$NaMnO_4{\cdot}2H_2O$	0.003g
10	Distilled water	1000mL

carbon source for the cultures containing the methane-oxidizing bacteria. Because of their slow growth speeds at the beginning of the culture process, the methane-oxidizing bacteria could generally be seen on the coatings of the culture dishes after 7 to 10 days. The fourth step, which took place in a sterile environment, attempted to locate single colonies with better growth by using a vaccination ring. The fifth step consisted of gathering the selected single colonies and placing them on a new culture dish, and the plates were streaked immediately. Finally, the last two steps were repeated at least three times until satisfying the requirement for single bacterial colonies was successful.

Microscopic observations of methane-oxidizing bacteria growth

The bacteria were selected from the liquid medium, and the methanotroph was screened out by streak plate method. After repeated purification, the selected methanotroph was transferred into the liquid medium. After a 5–7-day culture period, with naked-eye observations, white turbid matter gradually began to appear in the culture liquid, and large numbers of bacteria attached to the inner wall of the bottle, while some flocculated micro-precipitates fell to the bottom of the culture flask. Moreover, those that were in a liquid medium that was not transformed into methane-oxidizing bacteria remained clear. These results illustrate that these bacteria can survive and grow very well by depending on methane as the sole carbon source.

A small amount of bacterial liquid was taken and dropped onto a glass slide, and it was then made into a sample smear by drying and fixing. This sample smear was stained by using a red liquid compound with carbolic acid and was then placed under a high magnification microscope for observation. It was observed that the stained bacteria exhibited a short rod shape, which is shown in Fig. 2 below.

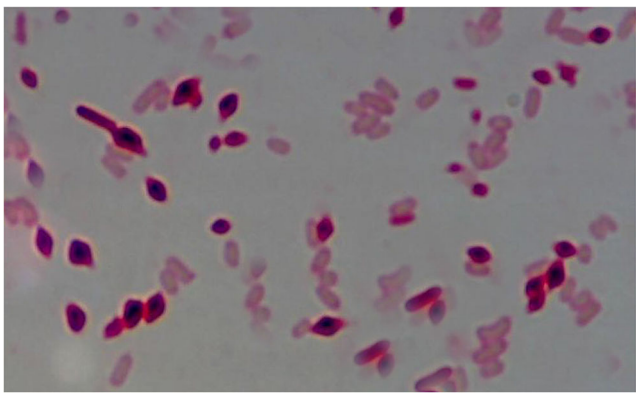

Fig. 2 Microscopic observation of the stained bacteria (magnified 200 times)

Experimental on growth characteristics of selected bacteria

The selected bacteria were used to observe their growth characteristics. (1) The pH of the culture medium was set to 7, and the culture temperature was set to 30°C. The relationship between bacterial growth and time was obtained. (2) Different culture temperatures and pH levels were used to observe the effects of temperature and pH on bacterial growth. Optical density (OD) was used to indicate bacterial concentrations. When light passes through a substance to be tested, there are energy differences before and after passing through a substance. The OD values represent these differences. The more energy that is absorbed, the greater the concentration of the substance. Therefore, the OD values of the bacterial solutions reflect the numbers of living bacteria in the culture medium. A spectrophotometer (model uv-3600 with a voltage and frequency of AC 220 V ± 22 V and 50 Hz ± 1 Hz, respectively) was used to measure the OD values in the bacterial solutions.

Experiments to examine methane consumption by bacteria

The methane-oxidizing bacteria were transferred to five flasks containing a medium culture, and these flasks were shaken on a rotary platform with a maintained temperature of 30°C and shaking speed of 120 r/min; culturing the bacteria continued for 5 to 7 days. During this interval, the methane-oxidizing bacteria gradually begin to enter a logarithmic growth phase. In China, there are strict rules for the maximum methane concentration in every place of the coal mine. The allowable methane concentration is usually less than 1.5%, and it is even lower than 0.5% in some places. Therefore, the values of methane concentration in tests were set to less than 1.5%. The five bacterial culture flasks were filled with gas at 0.25%, 0.5%, 0.75%, 1%, and 1.5% methane concentrations, respectively, and the changes in gas compositions of methane and carbon dioxide in each bacterial culture bottle were measured by a gas chromatograph every 2 h.

Experimental results

Results of bacterial growth characteristics

There are many factors that affect bacterial growth characteristics, and temperature and pH are the most important factors. Due to the influence of bacterial metabolism, the growth activity of the bacteria decreased with time. The OD values for the different experiments are shown in Fig. 3. With the extension of time, the OD values first increased and then decreased, as shown in Fig. 3a. At 5–7 days, the bacterial concentrations in the solutions were the highest, and bacterial growth was the

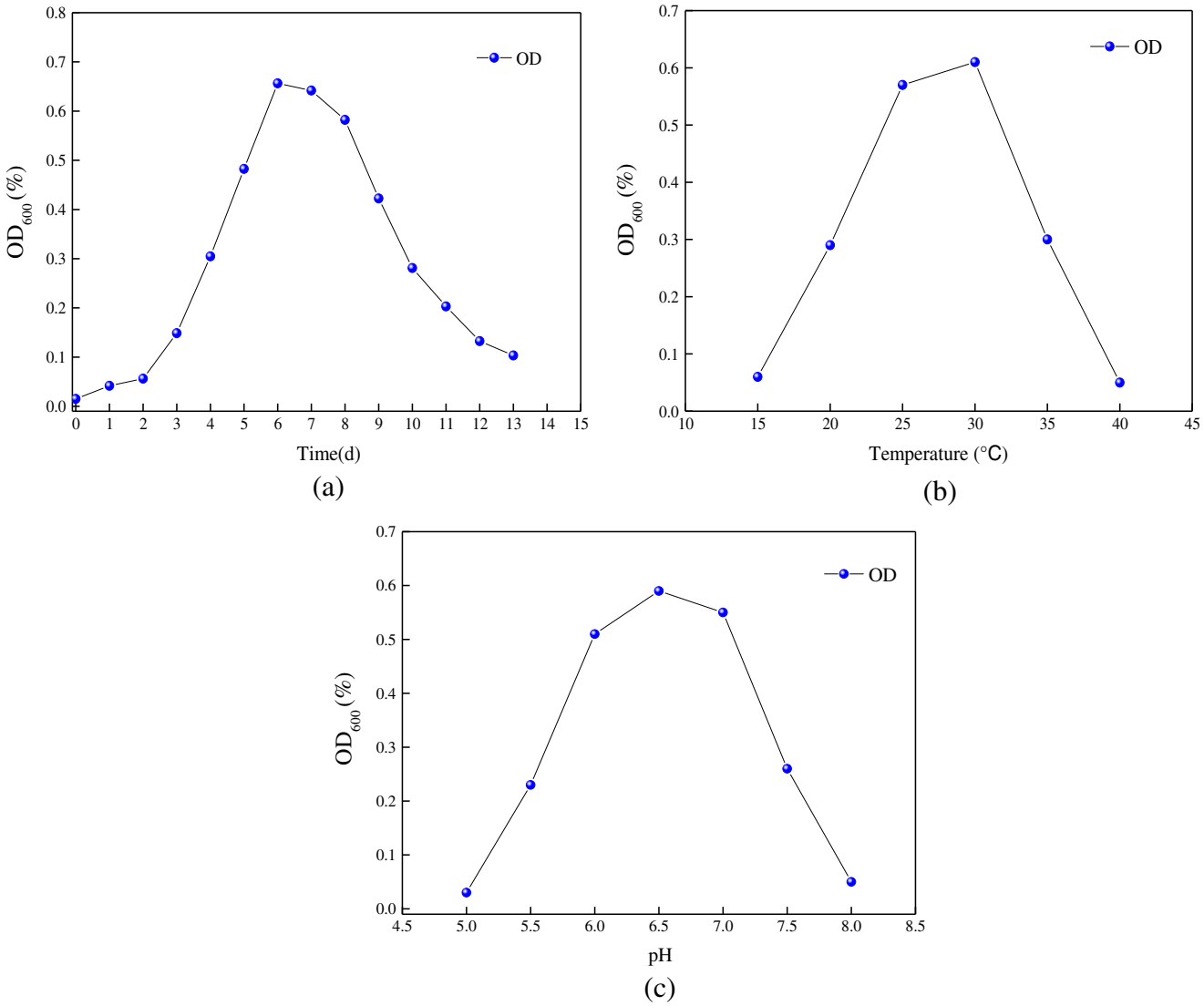

Fig. 3 The growth characteristics of bacteria. **a** Growth time for methanotroph. **b** Growth temperature for methanotroph. **c** Value of pH for methanotroph growth

most active. Bacterial growth is also limited by temperature and pH, and the OD results for different temperatures and pH levels are shown as Fig. 3 b and c, respectively. According to the relationship between bacterial growth and time, the OD values of the bacterial solutions were obtained on the seventh day. The OD values for different temperatures and pH were similar to a para-curve. Too high or too low temperatures and pH levels reduce the growth activity of bacteria. The temperature was approximately 30°C, the pH was approximately 6.5, the concentration of bacteria in the medium was the highest, and growth was the most active.

The consumption of methane by methanotroph is persistent and closely related to the characteristics of its growth. The best growth conditions of selected methanotroph were determined by tests. Due to the coalification, the differences between different coal seams are significant, such as temperature and pH. The survival of methanotroph in coal seams is inevitably affected by environment. According to the properties of coal seam, the bacteria which can grow vigorously under this condition can be selected.

Results of methane consumption

By following the process for the experiment described in section 1.4, the survey data are recorded and summarized in Table 3, as shown below.

The change law of methane content during the degradation process

Based on the data determined from gas chromatography shown in Table 3, the numerical relationships between the initial methane concentrations and microbial degradation times are shown in Fig. 4. As the degradation time increased,

Table 3 Changes in gas components during the degradation process

Group	Time (h)	Concentration of CH_4 (%)	Contents of CH_4 (ml)	Concentration of CO_2 (%)	Contents of CO_2 (ml)
1	0	0.2500	0.2100	0.0000	0.0000
	2	0.1464	0.1230	0.0000	0.0000
	4	0.1087	0.0913	0.0023	0.0019
	6	0.0797	0.06670	0.0026	0.0021
	8	0.0507	0.0426	0.0034	0.0028
	10	0.0492	0.0413	0.0042	0.0034
	20	0.0000	0.0000	0.0079	0.0066
2	0	0.5000	0.4200	0.0000	0.0000
	2.2	0.2928	0.2459	0.0031	0.0026
	4.1	0.2160	0.1815	0.0038	0.0032
	6.15	0.1556	0.1307	0.0051	0.0043
	8.32	0.1457	0.1224	0.0062	0.0052
	10.15	0.0821	0.0689	0.0083	0.0070
	22	0.0000	0.0000	0.0171	0.0144
3	0	0.7500	0.6300	0.0000	0.0000
	2.23	0.4371	0.3672	0.0042	0.0035
	4.25	0.2807	0.2358	0.0047	0.0039
	6.13	0.2342	0.1967	0.0090	0.0075
	8	0.1855	0.1559	0.0100	0.0084
	10.25	0.1211	0.1018	0.0126	0.0105
	20.35	0.0000	0.0000	0.0238	0.0200
4	0	1.0000	0.8400	0.0000	0.0000
	2.35	0.4984	0.4187	0.0039	0.0033
	4.23	0.3724	0.3128	0.0051	0.0043
	5.85	0.3223	0.2708	0.0081	0.0068
	8.03	0.2255	0.1894	0.0161	0.0135
	9.7	0.1967	0.1653	0.0195	0.0163
	22.65	0.0000	0.0000	0.0291	0.0244
5	0	1.5000	1.2600	0.0000	0.0000
	2.35	0.9767	0.8204	0.0063	0.0053
	4.45	0.6962	0.5848	0.0151	0.0127
	6.65	0.3953	0.3321	0.0168	0.0141
	8.5	0.2968	0.2493	0.0223	0.0187
	10.35	0.1194	0.1003	0.0301	0.0253
	21.35	0.0410	0.0345	0.0498	0.0419

the contents and concentrations of methane in every bottle showed a decreasing trend. The values of methane and carbon dioxide concentration are tested using the gas chromatograph (Agilent Technologies 7890B GC System). All these data are filled into the Origin software. These data were analysed by using the least square method, and it was revealed that the methane contents in every group of culture bottles numerically changed by following an exponential function with the passage of time and that the correlations were very high between the above two variables. When the initial concentration of methane (ICM) in a culture bottle was set to 0.25%, the numerical relationship between the instantaneous content of methane (W) and the duration time (t) fits the formula $W = 0.2021e^{-0.1930t}$. The same method was also used to analyse the other four groups of data. When the initial methane concentrations were set to 0.5%, 0.75%, 1%, and 1.5%, in different culture bottles, the numerical relationships between the instantaneous methane contents (W) and duration time (t) fit the formulas $W = 0.4040e^{-0.1801t}$, $W = 0.6109e^{-0.1954t}$, $W = 0.8000e^{-0.2018t}$, and $W = 1.2765e^{-0.1951t}$, respectively. All correlations for the fitting results were greater than 95%. These correlation characteristics are more clearly shown in Fig. 3,

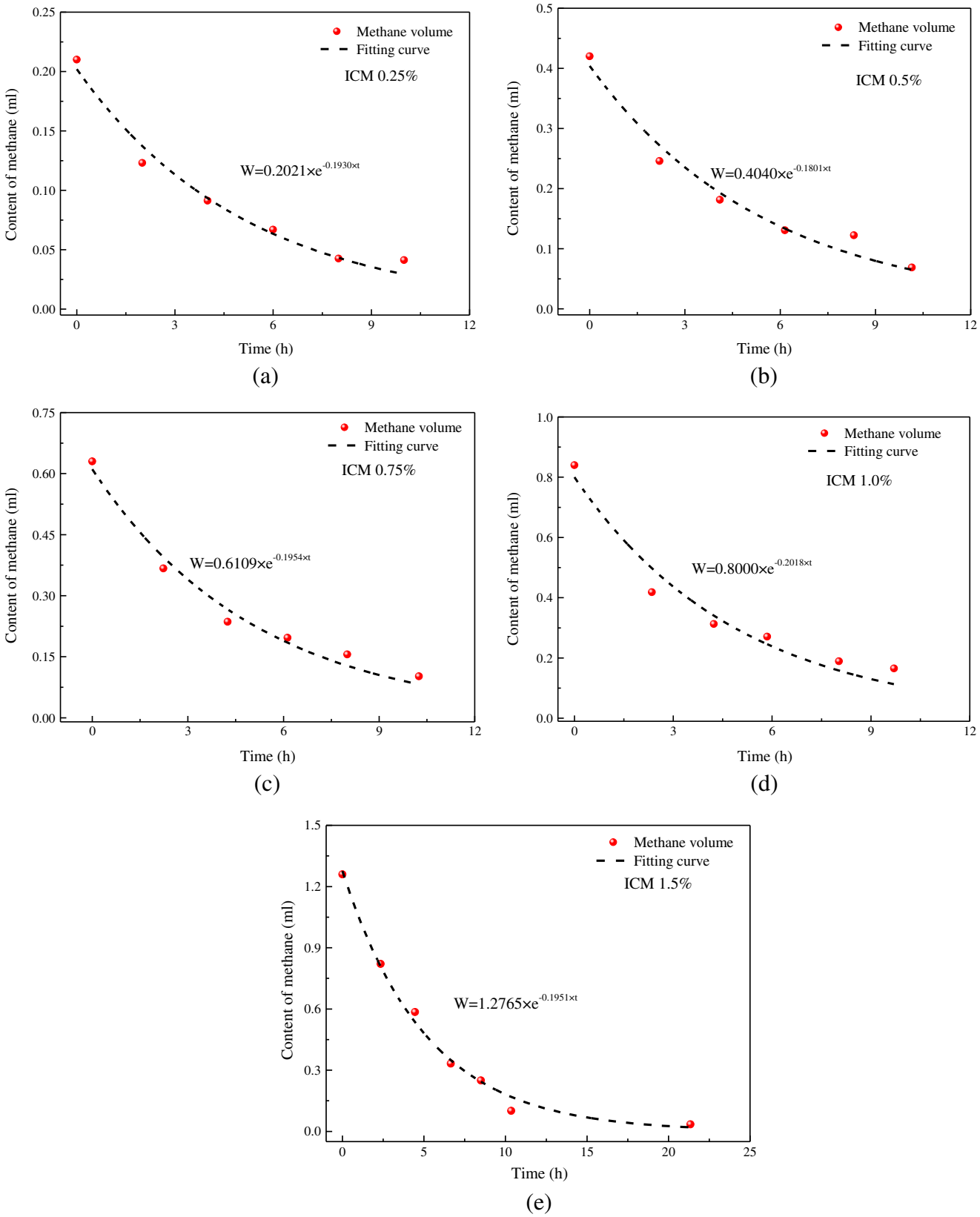

Fig. 4 Relationship between methane volume and time

from which useful patterns were also determined. Methane consumption by methanotroph is a continuous process. The volume of methane in the environment will continue to decrease until it is completely oxidized by methanotroph. With the decrease of methane concentration in the culture environment, the energy required by methanotroph is gradually insufficient. However, the oxidation ability of methanotroph will decrease at the end of experiment. This is because the quantity of degraded methane reached approximately 50% of the total quantity within the first 3 h, but only approximately 50% was reached within the next 18 h. Therefore, for the promotion effect of degradation in the actual application process, increasing the supply of fresh methane is needed as opposed to simply extending the degradation time. That is, this technique of microbial degradation of methane is more suitable for flowing methane gas, for example, for airflow in coal mines.

The relationship between degradation time and the final products

The microbial degradation mechanism for methane shows that the methane-oxidizing bacteria continuously require methane as the sole energy source for their growth and reproduction. In this process, methane is successively converted to formaldehyde, then to formate, and finally to carbon dioxide. For the relatively confined working spaces in coal mines, because of the potential adverse effects, the existence and production of carbon dioxide must be of great concern. Therefore, the productive characteristics of carbon dioxide, in particular, have been determined and analysed during this experimental process.

The experimental data show that carbon dioxide contents gradually increase with extension of the time needed for bacteria to degrade methane. Under different ICM conditions, the numerical relationships between carbon dioxide production and microbial degradation time accurately fit exponential functions with very high correlations. When the initial methane concentration in a culture bottle was set to 0.25%, the fitting formula was $C = 0.0004t^{0.9553}$. When the initial methane concentrations were set to 0.5%, 0.75%, 1%, and 1.5%, the fitting formulas were $C = 0.0008t^{0.9201}$, $C = 0.0013t^{0.8975}$, $C = 0.0017t^{0.8699}$, and $C = 0.0032t^{0.8435}$, respectively. The above numerical characteristics are more clearly shown in Fig. 5.

The findings from the above experiments also show that carbon dioxide production is relatively high in the early degradation stage. As the degradation time increases, there is a gradual downward trend for carbon dioxide production, and the changes in methane quantities obey a similar law. Therefore, it can be inferred that the methane decrement and carbon dioxide increment in the experimental container are in agreement with a positive proportional relationship during the bacterial degradation process.

On the other hand, by analysing the above experimental data, it is also evident that the molecular weight of methane is very different from that of carbon dioxide. The methane decrement and carbon dioxide increment during the microbial degradation process are not always in accord with a one-to-one numerical relationship. In the initial 3 h, the volume ratio between the decrement of methane and increment of carbon dioxide reached approximately 120:1 and then gradually presented a rapid decreasing trend, while the volume ratios were 40:1 at the sixth hour, 12:1 at the eighth hour, and 8:1 at the tenth hour.

The relationship between CH_4 consumption and CO_2 output

Methane-oxidizing bacteria consume CH_4 and produce CO_2 at the same time. Considering the actual concentration range of air flows for mining faces and for return airways in coal mines, the initial methane concentrations for the experimental investigation in this paper were set to be within the lower part of the values that ranged from 0.25 to 1.5%. The ratios of CH_4 consumption to CO_2 output were all obtained, as shown in Fig. 6. The results show that the CH_4 content in group 5 was not completely consumed after 48 h. This methane was not completely converted into CO_2, and there were many intermediate products, such as methanol and ethanol. It can be concluded that this is a common pattern, although this numerical ratio could exhibit some small variations at different degradation times. By applying this principle, some new technologies for the microbial degradation of methane can be developed and successfully applied to prevent exceeding the limit of methane concentrations in air return pathways or at mining faces.

Gas control is an important work in coal mining. The water injection and gas injection have been widely used; however, the technologies cannot achieve the expected purpose in some special cases. In recent years, biotechnology has been greatly developed in environmental governance. Methanotrophs have been applied in treatment of methane emission from landfill sites; the application of this technology in coal mining is still in the exploration stage. The energy needed by methanotroph can only be obtained by consuming methane. With the decreasing of methane content, the growth rate of methanotroph was inhibited. After the coal body has been excavated, the amount of coal in goaf is very little. The concentration of methane in the air of goaf is very low. Even so, it will still cause danger to the working face. However, it is impossible to drill holes and extract methane. In order to effectively reduce methane concentration in goaf, the methanotroph could be added in water. Then the liquid containing methanotroph is injected into the goaf and coal seam. Methane is consumed continuously by

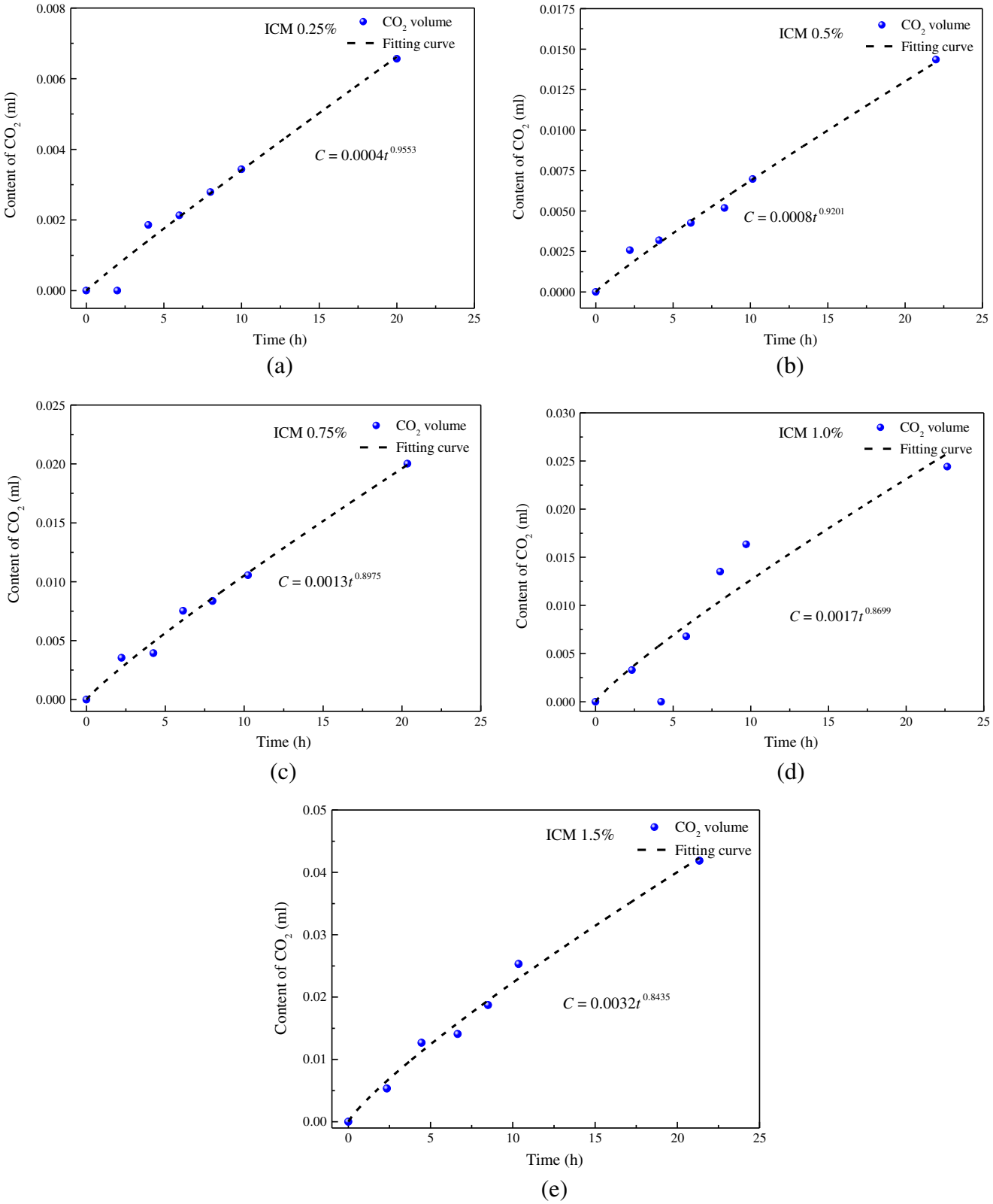

Fig. 5 Relationship between CO_2 volume and time

microorganisms. This method can be used in complex environment and to achieve good results. The use of microorganisms in the underground can also reduce the investment in gas control.

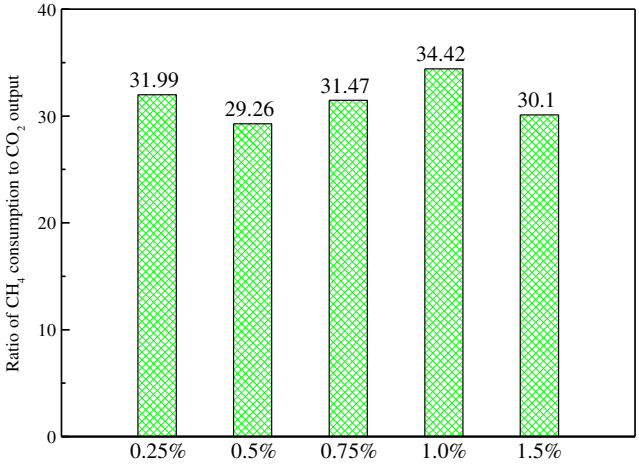

Fig. 6 Ratio of CH_4 consumption to CO_2 output

Conclusions

It is difficult to reduce the gas content of coal; the technology for gas control needs to be improved as soon as possible. Microbial plays an important role in environmental pollution control, especially in landfill sites. In order to reduce methane emission from coal mines to the atmosphere, the methanotroph has been selected to consume the methane.

1) The methanotroph was selected from the mud, and the growth conditions were also tested. The values of optimum temperature and pH for methanotroph growth are 30°C and 6.5. Under this condition, the growth ability of methanotroph is the strongest.
2) The methanotroph is a microorganism that consumes methane as energy. Methane concentration is set to less than 1.5%, and the methane consumption capacity of methanotroph is tested. The results show that the methane concentration decreased continuously under the action of methanotroph. In the whole microbial degradation process, the numerical relationship between the methane decrement and degradation time fits certain exponential functions.
3) Methane is consumed by methanotroph to produce carbon dioxide and other substances. The methanotroph is added in water and injected into the coal seams. New practical technologies for the microbial degradation of methane can be developed and successfully applied to prevent exceeding the methane concentration limits in air return pathways or at mining faces.

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Declarations

Conflict of interest The author(s) declare that they have no competing interests.

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