Pilot Test of a Fermentation Tank for Producing Coal Methane through Anaerobic Fermentation
(Ujian Perintis Penapaian Tangki untuk Menghasilkan Arang Batu Metana melalui Penapaian Anaerob)

Daping Xia, Huaiwen Zhang, Xianbo Su*, Xile Liu & Chaoyong Fu

ABSTRACT
The development and utilization of clean energy has long been a focus of research. In the coal bed methane field, most coal bed biogenic methane experiments are small static sample tests in which the initial conditions are set and the process cannot be batch-fed elements and microbial strains, and the gas cannot be collected in batches. Although significant results have been achieved in the coal-to-biogenic methane conversion in China, findings are restricted to the laboratory scale. No successful commercialization of coal bed biogenic methane production has been achieved yet. This study used a large-capacity fermentation tank (5 L) to conduct biogenic methane experiments. Results were compared to those from the traditional laboratory test. The gas production rate and gas concentration were higher when the 250 mL methane test volume was increased to a 5 L fermentation volume, increasing by 20.9% and 2.3%, respectively. The inhibition effect of the liquid phase products was reduced in the large fermentation tank, and the microbial activity was extended by batch feeding trace elements (iron and nickel) and methane strains and by semi-continuous collection of the gas. However, the gas conversion rate can be increased by retaining the H\textsubscript{2} and CO\textsubscript{2} in the intermediate gas products in the fermentation tank. The gas production rate was increased from 17.9 to 24.6 mL/g, increasing by 37.4%. The simulation pilot test can lay a foundation for the transition from a coal bed biogenic methane laboratory static small sample test to a dynamic pilot test, optimizing the process parameters to improve the reaction efficiency and move forward to commercialization test.

Keywords: Batch-fed trace elements and strains; batch gas collection; coal bed biogenic methane; pilot test

INTRODUCTION
The development and utilization of clean energy has long been a focus of research. In the coal bed methane field, systematic studies have been conducted in China and internationally on the biogenic production of coal bed methane, examining such topics as the gas composition, formation conditions and the accumulation mechanism (Heller et al. 2014; Huang et al. 2013; Kamiński et al. 2003; Lion et al. 2017; Sharma et al. 1988; Park & Liang 2016; Steve et al. 2008; Thararoop et al. 2012). Commercial
development of some coal bed methane reserves has been achieved in the United States and Australia; these projects mainly exploited biogenic methane, which has been considered the most attractive alternative energy source. However, no successful commercialization of coal bed biogenic methane production has been achieved yet. The simulation pilot test can move forward to commercialization test.

**METHOD**

**SAMPLE COLLECTION AND TREATMENT**

The coal sample used in this test was long flame coal from the Yima Mine Area in He’nan Province, China. The sample was pulverized (to 0.25 mm) and baked prior to use. The mine water in the Yima Mine Area also was used to prepare methanogenus-enriched liquid.

**STRAIN ENRICHED LIQUID**

Methanogenus medium: Consisted of beef extract (5 g/L), peptone (3 g/L), NaCl (5 g/L), FeSO$_4$ (0.2 g/L), $K_2$HPO$_4$ (2 g/L) and glucose (maltose, sucrose & lactose). The mine water was enriched with the strain medium to produce methanogenus mine water enriched liquid.

**METHANE PRODUCTION TEST DEVICE**

The small-sample methane device consisted of a 250 mL Erlenmeyer flask that was used as the reactor. In a test a 250 mL Erlenmeyer flask containing 20 g coal and 200 mL enriched mine water culture liquid was sealed using a rubber plug and placed in a thermostat-controlled culture box at 35°C for static cultivation. The gas generated was collected in a collection bottle using the gas drainage port approximately every 3 days and then using drainage for the gas collection.

**ANALYSIS OF THE TEST METHOD**

A Beifen-Ruili Type SP-2100 gas chromatograph (Shanghai) was used for analyzing the gas composition. A Mettler-Toledo acidometer was used to determine pH. The Stove 6-step extraction method was used for determining the occurrence of the trace elements.

**RESULTS**

In the designed test conditions, the test results of the fermentation tank with constant pH and the pilot test with unadjusted pH were compared with the previous 250 mL small-sample test results. The comparison of the gas output, coal mass degradation rate and methane concentration is shown (Figure 1). Figure 1(a) indicates that the gas production rates of the fermentation tank with pH value 7.0 and with unadjusted pH were 17.9 and 12.6 mL/g, respectively. The gas production rate of the fermentation tank with unadjusted pH was 13.4% lower than that of the static small-sample test in the same restrictive conditions. The coal sample mass degradation rate of the fermentation tank main body. The reactor is a 5 L glass structure that is resistant to high temperature. A shaft seal and sealing cushion are used to ensure an anaerobic environment in the reactor. A thermostatically controlled heating jacket is used to heat the reactor to 35°C. The fermentation tank is purged with nitrogen for 3 min prior to the test. A certain head space is reserved at the top of the tank. The temperature, pH and agitation rate during the fermentation are measured and recorded online using a computer.

**BATCH-FEEDING TRACE ELEMENTS AND STRAINS**

The study was designed such that trace elements and microbial strains were added into the fermentation tank in two modes, i.e. a one-time addition and regular periodic additions (batch-feeding). The total added elements and microbes were the same for both feeding techniques. For trace elements, the batch-feeding mode consisted of a uniform addition of 10 mL of trace element solution (15 mg/L iron and 0.05 mg/L nickel) every day for 20 days (total of 200 mL). For microbes the batch-feeding consisted of daily uniform additions of 50 mL methanogenus enriched liquid for 20 days.

**COMPARISON BETWEEN SMALL-SAMPLE AND FERMENTATION TANK GAS PRODUCTION EFFECTS**

cause analysis showed that small-molecule acids would be formed during the production of biogenic methane. Small-molecule acids would reduce the pH...
value of the reaction liquid, impairing the normal growth of methanogenus. In contrast, constant pH will maintain the metabolic capability of the methanogenus at a vigorous level. The fermentation tank can provide a better anaerobic growth environment than the small-sample static test and also can promote the generation of the nicotinamide adenine dinucleotide reductive pathway, generate more hydrogen and allow the smooth process of carbon dioxide reduction to produce methane.

The strains of the biogenic methane microbes come from the enriched indigenous microbial strains present in the mine water. In the constant pH test, the microbial strains and trace elements were added all at once. Considering the impact of the various side reactions and the special process characteristics of the fermentation tank, trace elements (iron and nickel) and indigenous methanogenus enriched liquid were added in batch-feeding mode during the test. The gas production effects are shown in Figure 2.

**FIGURE 1.** Comparison between (a) small-sample and fermentation tank gas production rates and (b) methane concentration

![Comparison between small-sample and fermentation tank gas production rates and methane concentration](image1.png)

**FIGURE 2.** Gas production effect of batch-feeding trace elements and microbial strains

![Gas production effect of batch-feeding trace elements and microbial strains](image2.png)

Figure 2 indicates that the gas production time during batch-feeding was delayed for 8 days compared with that from the one-time addition because batch-feeding cannot meet the microbial requirements at the beginning of the test. However, the gas production time for batch-feeding was extended to 42 days (from 32 days for the one-time feeding). The gas production rate increased significantly and the gas production rate increased by 4.51 mg/L comparing the fermentation tank with a constant pH. The coal degradation rate increased from 0.88% to 1.34% due to batch-feeding. Periodic batch-feeding was obviously better than one-time feeding.

**ADDITION OF TRACE ELEMENTS AND STRAINS IN BATCH-FEEDING MODE**

A certain amount of denitrifying microbial strains was present in the enriched mine water during the reaction. Denitrifying bacteria will compete for the carbon source during the generation of biogenic methane, reducing the decomposition ability of methanogenus. However, NH$_3$-N and NO$_x$-N substances generated due to the denitrifying bacteria are present in the reaction liquid, and the toxicity of these substances would cause the deactivation of methanogenus. Therefore, improving the prevalence of methanogenus in the fermentation tank is difficult using a one-time addition of these microorganisms. Batch-feeding of high-activity methanogenus in enriched liquid can effectively reduce the competitive effects of denitrifying bacteria and toxic effects of nitrogenous species, and improve the activity of the methanogenus enzyme.

**ANALYSIS OF BATCH-FEEDING MICROBIAL STRAINS**

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**ANALYSIS OF BATCH-FEEDING TRACE ELEMENTS**

Trace elements are important for maintaining the growth of anaerobic microbes, their metabolism and the activity of the anaerobic fermentation enzyme system. Trace elements iron and nickel are the main components of the coenzyme, prosthetic group and cofactor in the bacteria. The occurrence and concentrations of these trace elements in the reactor solution have an impact on the methanogenus growth and biosynthesis.
Not all trace elements are present in a form that can be used by microorganisms after they enter the reaction liquid. Only organic and exchangeable trace elements can be directly used by bacteria. Normally, inorganic trace elements cannot be absorbed by bacteria. The changes of the occurrence of the two trace elements in the coal after reaction can be compared by extracting the trace elements using a stepwise chemical extraction. Figure 3 shows the occurrences of organic states of iron and nickel due to batch-feeding and a one-time addition increased by 17.6% and 10.7%, respectively. Exchangeable states due to batch-feeding also increased by 24.3% and 12.1% compared with the one-time addition, while carbonate state and sulfide state decreased accordingly. Most trace elements were present in the form of sediments or sulfides after entering the system in a one-time addition, and cannot be used by methanogenus in these forms. Batch-feeding can increase the dissolved state and organic state of trace elements and improve the biological absorption of the elements by the methanogenus, therefore increasing the microbial activity and significantly improving the gas production rate.

GAS COLLECTION IN BATCHES

Figure 4 shows that in the initial period of gas production, the gas production rate in the continuous collection system with constant pH was higher than that in the system of gas collection. However, the gas production rate in the batch gas collection system increased quickly after Day 16. Gas production in the batch collection system did not exceed that in the system with constant pH until approximately Day 30.

Gas contents and coal mass decomposition rates in the continuous gas collection system and in the batch collection system were calculated after the end of gas production, as shown in Table 1:

Compared to methane production rate during continuous gas collection (17.29 mL/g), the methane production rate from the batch collection system (19 mL/g) by 9.9%. The H₂ concentration and CO₂ concentration during batch gas collection were 9.3% and 12.5% lower, respectively, than in the continuous gas collection system. Mass degradation was 0.76% higher in the batch gas collection system than in the continuous collection system. Therefore, gas collection in batches is obviously better than continuous gas collection.

### TABLE 1. Comparison of gas production during continuous gas collection and gas collection in batches

<table>
<thead>
<tr>
<th>Gas collection mode</th>
<th>CH₄ Production rate (mL/g)</th>
<th>H₂ Concentration (%)</th>
<th>CO₂ Concentration (%)</th>
<th>Coal mass degradation rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous collection</td>
<td>17.92</td>
<td>16.70%</td>
<td>23.40%</td>
<td>0.88%</td>
</tr>
<tr>
<td>Collection in batches</td>
<td>19</td>
<td>7.40%</td>
<td>10.90%</td>
<td>1.61%</td>
</tr>
</tbody>
</table>
The data available from Pan et al. (2009) has proven that there are two approaches to the production of coal bed biogenic methane. One is acetic acid decomposition by acidophilus methanogenus. The other is CO\textsubscript{2} reduction by hydrogenophilus methanogenus. The output of coal bed methane mainly depends on the reduction of CO\textsubscript{2}. In CO\textsubscript{2} reduction, the H\textsubscript{2} and CO\textsubscript{2} outputs and concentrations are critical factors affecting the gas production rate. The intermediate gas products in the fermentation tank include H\textsubscript{2} and CO\textsubscript{2}; these gases cannot combine and generate more methane if they are released from the system on a timely basis. However, high H\textsubscript{2} partial pressure can also impact a smooth reaction. Therefore, in this study a semi-continuous gas collection method was used involving a gas collection device such that the system was opened approximately every 3 days. The gas produced is remained in the system during the intervening times, which allowed the H\textsubscript{2} and CO\textsubscript{2} in the fermentation tank to develop in the direction of CO\textsubscript{2} reduction. Therefore, the H\textsubscript{2} and CO\textsubscript{2} concentrations obtained were lower and the methane production rate and concentration were higher at the end of the reaction than when continuous gas collection was used.

GAS COLLECTION IN BATCHES AND BATCH-FEEDING TRACE ELEMENTS AND STRAINS SIMULTANEously

Considering the commercialization requirements for gas production rates and methane concentrations, batch-feeding of trace elements, microbial strains and gas collection in batches were used simultaneously based on the chemical action and reaction mechanism inside the fermentation tank (Figure 5). Batch-fed trace elements and strains can significantly increase methane yield, extend the coal degradation gas production time and increasing the substrate coal degradation rate. Gas collection in batches can increase the H\textsubscript{2} and CO\textsubscript{2} reaction time, significantly increase methane production rate. Therefore, production performance of biogenic methane can be improved significantly by combining batch-feeding and batch gas collection.

![Figure 5](image.png)

**FIGURE 5.** Comparison of gas production effects between combination of two modes and batch-feeding alone

<table>
<thead>
<tr>
<th>Mode</th>
<th>Methane production rate(%)</th>
<th>Methane concentration</th>
<th>Coal mass degradation rate</th>
<th>Average methane production rate (mL/g·d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant pH</td>
<td>17.9</td>
<td>49.80%</td>
<td>0.88%</td>
<td>0.56</td>
</tr>
<tr>
<td>Two kinds of fed-batch Collection</td>
<td>21.6</td>
<td>50.20%</td>
<td>1.34%</td>
<td>0.51</td>
</tr>
<tr>
<td>in batches</td>
<td>19</td>
<td>51.30%</td>
<td>1.61%</td>
<td>0.59</td>
</tr>
<tr>
<td>Two kinds of fed-batch + collection in batches</td>
<td>24.6</td>
<td>52.40%</td>
<td>1.82%</td>
<td>0.61</td>
</tr>
</tbody>
</table>

**TABLE 2.** Methane production performance in different pilot fermentation process conditions

Figure 5 and Table 2 indicate that batch-feeding of trace elements and microbial strains can significantly increase the methane gas production time, and the microbial trace elements can be fully absorbed. The strains can be fully used. Gas collection in batches can mainly increase the methane production rate. The test results indicate that gas collection in batches and batch-feeding of trace elements and microbial strains simultaneously can optimize the gas production, increasing it by 37.43%. Two kinds of batch-feeding can increase the production rate by 20.6% compared with that without optimization, while gas collection in batches alone (without batch feeding) can only increase the production rate by 6.1%.

**DISCUSSION**

The study of coal-to-biogenic methane conversion in China and elsewhere has mainly focused on the methane bacteria separation, purification and cultivation, as well as on simulation tests of gas production the coal bed biogenic methane production process has been simulated in the laboratory and the coal degradation ability of methanogenus has been studied. The methane production maximization method and an efficient methanogenus colony were examined by testing the microbial colony characteristics and factors influencing gas production. The laboratory test focused on a small 500 mL sample. The defect of the small-sample static test is that the designed
conditions are the initial conditions, which cannot be varied based on the reaction effects. The production scale is small and cannot meet the requirements of a commercialization test (Faison et al. 1992; He et al. 2007; Hu et al. 2009; Ijaz & Yasin 2017; Li et al. 2001; Pan et al. 2009; Opara et al. 2012; Roslee et al. 2017; Sharma et al. 1988; Su et al. 2013b).

In the study reported in this paper, the small static-sample test procedure was improved using a 5 L fermentation tank. The improved pilot test was studied in terms of coal-to-biogenic methane conversion. The test was closely monitored and adjustments were made to the intermediate products and process to further accelerate the commercialization of coal-to-methane production through fermentation. The aim was to master and control the operational parameters in the commercialization test and fully utilize of the optimal gas production effects and substrates. The gas production difference between the improved pilot test and traditional small-sample test was compared and the coal-to-biogenic methane process was further optimized to achieve higher methane production efficiency and improved coal degradation rates. The gas collection mode and batch-feeding mode were improved during the pilot test and compared with the static small-sample test to enable the reaction to take place in the way that was conducive to methane generation and improvement of methane yield.

**CONCLUSION**

The fermentation tank test process was optimized based on the static small-sample test. The study results justify the following conclusions.

The accumulative methane production rate and concentration of methane from the 5 L fermentation tank with constant pH increased by 17.9 mL/g (49.8%) compared to that from the traditionally used 250 mL small-sample methane device. The big sample test effect without any treatment is not satisfactory. Amounts of exchangeable and organic forms of trace elements can be increased through batch-feeding of the trace elements, thereby improving the availability of the trace elements. The gas production time due to batch-feeding of microbial strains can be extended and the total gas output can be increased. The methane production rate increased from 17.9 mL/g to 21.6 mL/g and the methane concentration increased by 3.5% when both microbes and trace elements were batch fed, compared to when they were added all at once. Methane production through CO₂ reduction can be fully achieved by collecting gas in batches and the side reactions due to high H₂ partial pressure can be avoided so that the methane production rate and methane concentration can be increased. The batch-feeding of trace elements and microbial strains can significantly extend the gas production time while gas collection in batches can significantly increase the methane yield. Coal-to-biogenic methane conversion can be optimized when the three methods are used together and the gas production rate can reach 24.8 mL/g.

The coal-to-biogenic methane fermentation tank test is a pilot-scale test device and is an important step in the transition of the biogenic methane test from a laboratory process to full-scale industrialization in China. The reaction process was controlled based on the test results to allow it develop in the direction that was conducive to the substrate utilization and gas production. The level of control significantly improved the limitation of the small-sample test and increased the reaction efficiency. In addition, the fermentation tank creates an environment that is close to the actual on-site test conditions and can provide an important process reference for the industrialization test in the future.

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**REFERENCES**


