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Effect of Eutrophic River Water and Trace Element on Oil Gasification into Methane by Indigenous Microbes

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ABSTRACT
Residual oil gasification is a novel approach to extend the life of old oil reservoir using microbes to transform petroleum hydrocarbons into methane which can be exploited or stored *in situ*. The eutrophic river water could be used to promote the oil degradation and producing methane. When the trace element was added, the methane yield and petroleum hydrocarbons degradation rate was better. The best trace element $CoCl_2 \cdot 6H_2O$ concentration was 1.6 mg L^{-1}, $NiCl_2 \cdot 6H_2O$ and Na_2MoO_4 were 0.34 and 2.4 mg L^{-1}, respectively. Meanwhile, the methane yield and oil degradation rate were effected by pressure. Microbes grow better in low pressure condition. The eutrophic river water and trace element have the additive effect on petroleum hydrocarbons degradation. So the eutrophic river water turned useful resource.

Key words: Trace element, eutrophic river water, indigenous microbes, methane

INTRODUCTION

There are various microbes in the oil reservoir and they usually can be divided into several biological communities according to their functions, such as hydrocarbon oxidizing bacteria, fermentative bacteria, nitrate-reducing bacteria, iron-reducing bacteria, sulfate-reducing bacteria and methanogenic archaea (Zengler *et al*., 1999; Anderson and Lovley, 2000; Jones *et al*., 2008; Aitken *et al*., 2004). Most of the microbe can produce some gases in their biological metabolic processes, such as CO_2, H_2, N_2, CH_4, H_2S, etc. The biological metabolic process needs lots of electron acceptor, for example, O_2, nitrate, sulfate, Fe(III), organic acid, etc (Parkes *et al*., 1994; Kniemeyer *et al*., 2007; Chapelle *et al*., 2002; Martini *et al*., 1996). In recent years, it becomes a significance task to make deeply use the old oil field and raise the oil recovery. While the normal technology of enhancing oil recovery has many limitations, for example, the raising extent of oil recovery is relative small and mass of residual oil was stuck underground. The LUCA Company in Colorado of US has been studied biogas for a long time. They tried to convert the coal or petroleum to natural gas. They carried out their experiments in the Monument Butte oil field. There, the microbes were cultivated in the reservoir oil and water mixtures. After 60 days, excess of methane gas was produced. In the following 297 days, the mass of methane reached the maximum value.

MATERIALS AND METHODS

The water was pumped from the oil well area of Dongxin oilfield block to fill several plastic buckets (about 7 L). Reservoir characteristics are continental phase and sandstone. The plastic buckets were tightly closed and kept at 4°C, then sent to the laboratory immediately. The oilfield block characteristics shown in Table 1. The eutrophic river water was taken from a river with many waterweeds and algae (The nutrient content shown in Table 2).

Table 1: Geological information of sampling

Parameters	Values
Porosity (%)	27.0±1.0
Reservoir temperature	50-75
Geothermal gradient	3.5/100 m
Density (g cm^{-3})	0.9354±0.02
Viscosity (mPa•sec)	221.4±5.0
Saturated fraction (%)	33.9±0.7
Aromatic fraction (%)	32.7±0.7
Colloid (%)	28.6±0.6
Asphaltene (%)	4.9±0.1

Data is Mean±SD (n = 3)

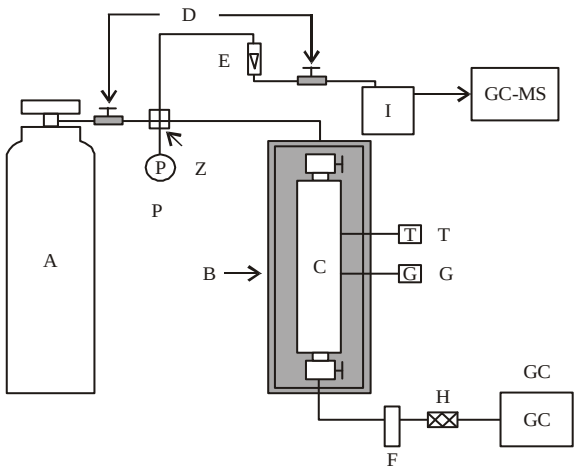

Fig. 1: Oil reservoir conditions simulated system. A: High pressure gas source, B: Temperature control area, C: Main reactor part, D: Pressure control valve, E: Flowmeter, F: Sampler 1, G: Pressure sensor, H: Filtrating equipment, I: Sampler 2, P: Pressure display, T: Temperature sensor, Z: Four-way valve

Table 2: Eutrophic river water nutrient content

Item	Content (mg L^{-1})	Item	Content (mg L^{-1})
Nitrogen	494.3±14.6	Iron	29.2±0.4
Phosphorus	408.8±12.4	Cobalt	-
Potassium	509.8±11.2	Nickel	-
Sulfur	278.8±8.9	Molybdenum	-
Magnesium	45.6±2.6	Calcium	81.6±1.1
Copper	10.3±0.7	Manganese	10.5±0.6
Zinc	14.7±0.6		

-: None or quantity was small, The data is Mean±SD (n = 3)

First, primary culture in which 50 mL water samples and 30 mL inorganic salt culture medium were both added into 120 mL sterile anaerobic bottle. At the same time, the oxygen was removed by Hungate (Balch and Wolfe, 1976; Gu *et al*., 2013), to maintain strictly anaerobic condition. Hundred days later, methane was detected by gas chromatography. Inorganic salts culture medium contained: KH_2PO_4, 5.0 g; K_2HPO_4, 5.0 g; NH_4Cl, 5.0 g; NaCl, 1.0 g; $MgCl_2$,2.0 g; $CaCl_2$, 0.1 g; Yeast, 1.0 g; deionized water volume to 1 L and pH: 7.0-7.2.

Second, enrichment culture in which 5 mL inoculum (the upper clear liquid was filtered) above mentioned was added into the oil reservoir simulation system reactors (The volume: 120 mL), shown in Fig. 1. Five gram crude oil, 50 mL eutrophic river water which was sterilized were added into reactors. The inoculum which was sterilized was as control group. Meanwhile the pressure was set 6, 7, 8, 9 and 10 MPa and the temperature was set 30, 45 and 60°C (The culture time was 185 days).

According to the Table 2, the trace element was added into the reactors under 45°C and standard atmospheric pressure. The $CoCl_2 \cdot 6H_2O$ (C) factor with six levels, C1: 0 mg L^{-1}, C2: 0.8 mg L^{-1}, C3: 1.2 mg L^{-1}, C4: 1.6 mg L^{-1}, C5: 2 mg L^{-1}, C6: 2.4 mg L^{-1}; the $NiCl_2 \cdot 6H_2O$(N) factor with six levels, N1: 0 mg L^{-1}, N2: 0.16 mg L^{-1}, N3: 0.22 mg L^{-1}, N4: 0.28 mg L^{-1}, N5: 0.34 mg L^{-1}, N6: 0.4 mg L^{-1}. The Na_2MoO_4(M) factor with six levels, M1: 0 mg L^{-1}, M2: 0.6 mg L^{-1}, M3: 1.2 mg L^{-1}, M4: 1.8 mg L^{-1}, M5: 2.4 mg L^{-1}, M6: 3 mg L^{-1}. The inoculum which was sterilized was as control group. The culture time was 80 days.

Gas measurement: Gas composition detection was done by SHIMADZU gas chromatograph. Carrier gas was 99.99% helium, 50 kPa, combustion gas was hydrogen 50 kPa, supporting gas was air 40 kPa, detector was FID 300°C, gasifier injector 300°C; column was PONA elastic quartz capillary column (50 m×0.2 mm×0.5 μm), column initial temperature was of 35°C, 15 min, 2°C/min heating to 220°C, 5 min injection volume: 0.5 mL, the standard gas is diluted with pure nitrogen to different concentrations, the analysis was under the above conditions, the gas content was quantified by modified area normalization method, data acquisition and handling was computer assisted (Gu *et al*., 2013).

Measurement of petroleum hydrocarbons degradation rate: The crude oil culture after degradation was transferred to a 250 mL separating funnel and was then acidified with hydrochloric acid to a pH value of ≤2, followed by washing with 20 mL of CCl_4, with the extract transferred to an Erlenmeyer flask and the remainder left in the separating funnel. The extract after being diluted to a definite factor was analyzed by infrared spectroscopy to measure the hydrocarbon content (HJ 637-2012, China) and the petroleum hydrocarbons degradation rate was calculated (Zhao *et al*., 2011; McFarlin *et al*., 2014).

Statistical analyses: The data was mapped using Origin 8.0 and all of the statistical analyses were performed using SPSS statistical software (SPSS Inc., Chicago, IL).

RESULTS

Effect of temperature and pressure on methane yield: With the increase of temperature, the methane yield decreased. With 120 days adjustment time, the methane yield at 60°C treatment reached 16 μmol in 185 days old culture (Fig. 2). Whereas, for 100 days adjustment time, the methane yield at 30°C could reach 28 μmol and 90 days adjustment time, the methane yield at 45°C was 35 μmol in 185 days old culture. After the adjustment time, the methane yield rate increased significantly and at 45°C treatment the methane yield was the highest.

It is shown in Fig. 2 that with the increasing of cultivation days, in different pressure conditions, the methane yield has obviously changed. Specifically, when the pressure was 6 MPa, methane yield was the largest, through the cultivation 185 days and was 25 μmol. While the pressure is 7, 8 and 9 MPa through the cultivation 185 days, the methane yield were 20, 18 and 14 μmol, respectively and especially, when the pressure is 10 MPa, methane yield was 10 μmol and was far lower than others. It can be known through the slope

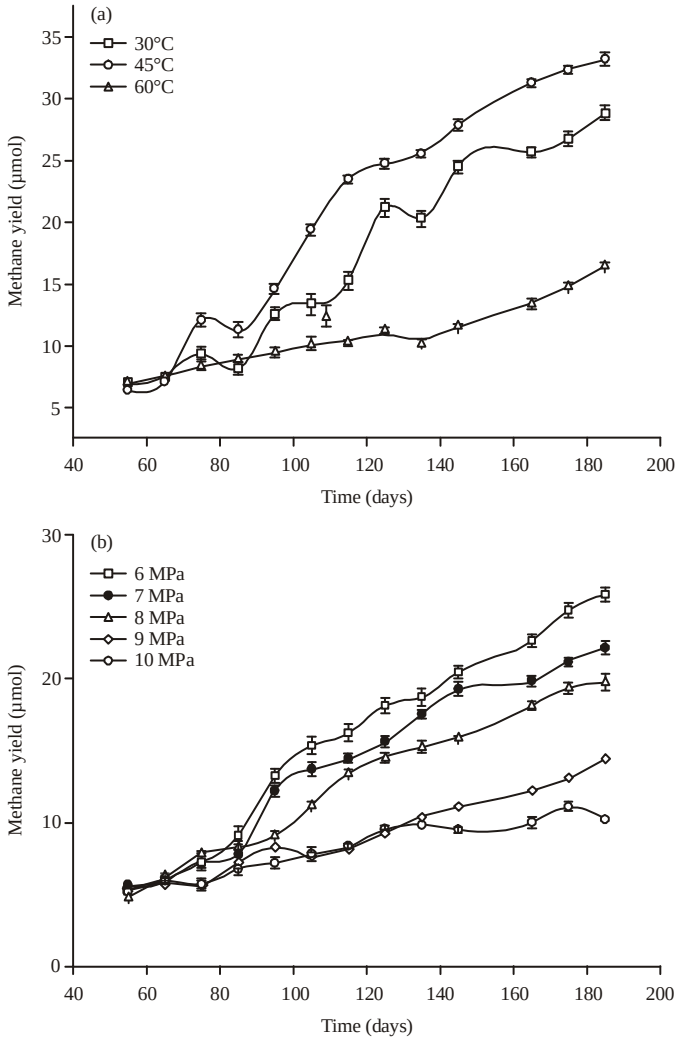

Fig. 2(a-b): Methane yield of different (a) Temperature and (b) Pressure with time as horizontal ordinate. Five milliliter inoculum, 5 g crude oil and 50 mL eutrophic river water which was sterilized were added into reactors after 185 days culture under 5 MPa and 45°C, respectively

that after 80-90 adjustment days, the methane yield rate rose obviously and the methane yield rate was largest in 6, 7 MPa treatment. So, the pressure could effect the methane yield by indigenous microbes degradation. The microbes were willing to grow in low pressure.

Effect of trace element on methane yield and degradation rate: Under the 45°C and standard atmospheric pressure condition, after 80 days culture, with the trace element concentration increasing, the methane yield and petroleum hydrocarbons degradation rate were both increasing too. The methane yield and petroleum hydrocarbons degradation rate of adding trace element treatment were higher than the treatment which was no adding trace element (Fig. 3a-c). Specifically, when the trace element $CoCl_2 \cdot 6H_2O$ concentration was 1.6 mg L^{-1}, the methane yield was highest 245.6 μmol and the petroleum hydrocarbons degradation rate was the highest, so the methane yield was related to the petroleum hydrocarbons degradation rate. After 80 days culture the petroleum hydrocarbons degradation rate was highest 30.2%.

When the trace element $NiCl_2 \cdot 6H_2O$ concentration was 0.34 mg L^{-1} the methane yield and petroleum hydrocarbons degradation rate were highest, 241 μmol, 30.6%, respectively. Meanwhile, when the Na_2MoO_4 concentration was 2.4 mg L^{-1} the methane yield and petroleum hydrocarbons degradation rate were highest, 269.1 μmol, 29.6%, respectively. So, the best concentration of $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$ and Na_2MoO_4 were 1.6, 0.34 and 2.4 mg L^{-1}, respectively. At the same time, the control group was no methane yield and petroleum hydrocarbons degradation rate (Fig. 4).

A and B group experiment: In the A group the eutrophic river water was used to promote the oil degradation and producing methane. The eutrophic river water had many nutrient (Table 2), so this water was useful for microbes to degrade oil. The Cobalt, Nickel and Molybdenum were none,

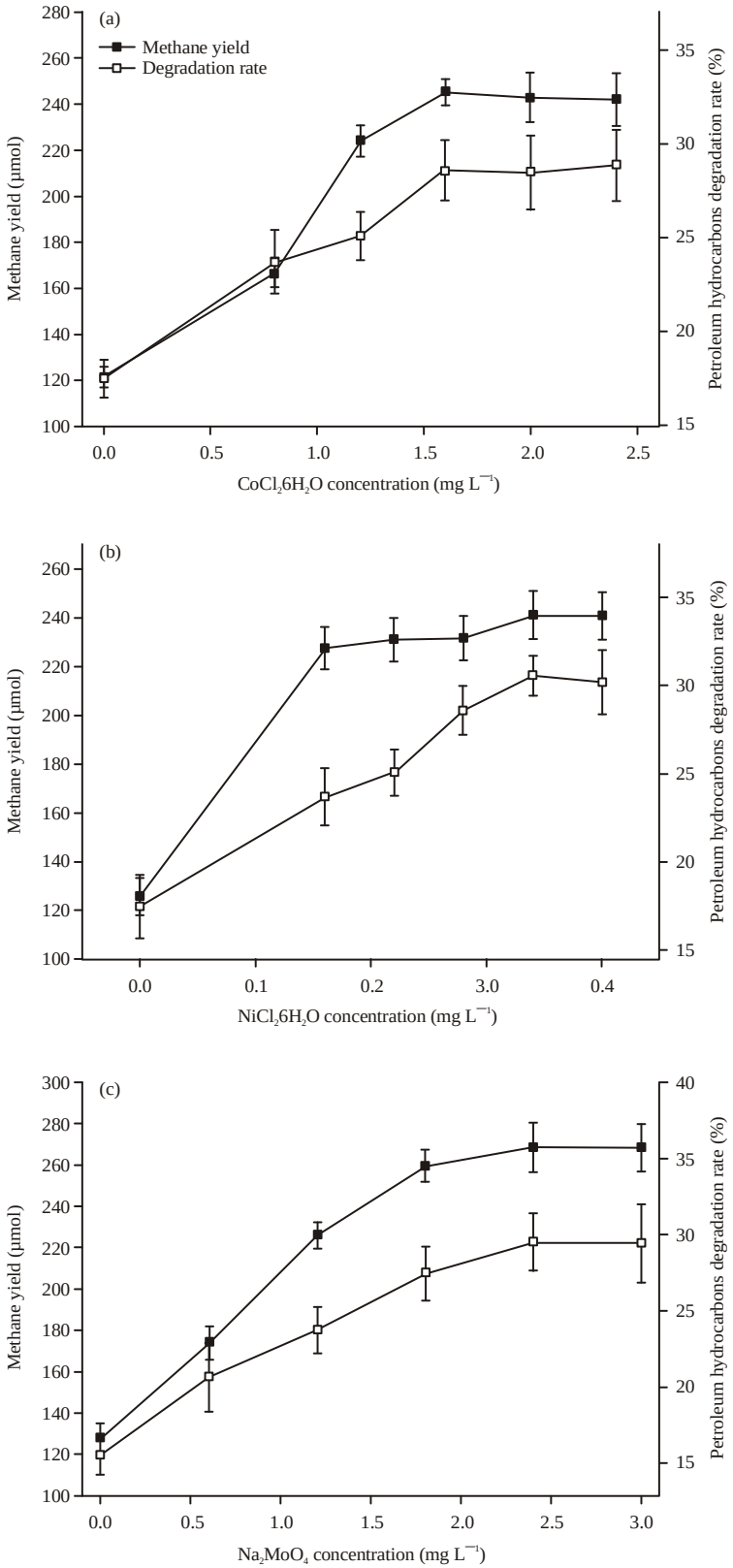

Fig. 3(a-c): Methane yield and petroleum hydrocarbons degradation rate of different (a) $CoCl_2 \cdot 6H_2O$, (b) $NiCl_2 \cdot 6H_2O$ and (c) Na_2MoO_4 concentration. Five milliliter inoculum, 5 g crude oil and 50 mL eutrophic river water which was sterilized were added into reactors after 80 days culture under 45°C, standard atmospheric pressure

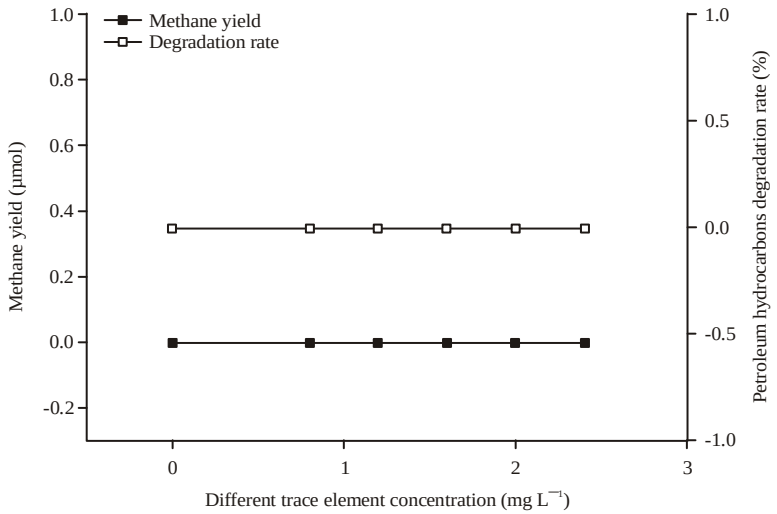

Fig. 4: Methane yield and petroleum hydrocarbons degradation rate of different trace element concentration. Five milliliter inoculum, 5 g crude oil and 50 ml eutrophic river water which was sterilized were added into reactors after 80 days culture(sterilization control group) under 45°C, standard atmospheric pressure

Table 3: Methane yield and petroleum hydrocarbons degradation rate of A group and B group

Groups	Methane yield (μmol)	Petroleum hydrocarbons degradation rate (%)
A	124.3±6.6	17.4±0.9
B	488.9±15.2	38.2±1.4
Control	0.0	0.0

Data is Mean±SD (n = 3). A group: 5 mL inoculum, 5 g crude oil and 50 mL eutrophic river water which was sterilized were added into reactors after 80 days culture under 45°C, standard atmospheric pressure. B group: According to Fig. 3a-c, the c concentration was set 1.6 mg L^{-1}, N concentration was set 0.34 mg L^{-1} and the M concentration was set 2.4 mg L^{-1} in the reactors. And 5 mL inoculum, 5 g crude oil and 50 mL eutrophic river water which was sterilized were added into reactors after 80 days culture under 45°C, standard atmospheric pressure

but the trace element was useful for microbes to live. From Table 3 the methane yield and petroleum hydrocarbons degradation rate were 124.3 μmol, 17.4%, respectively after 80 days culture under 45°C, standard atmospheric pressure.

But when adding the trace element (Table 3) the C was set 1.6 mg L^{-1}, N was set 0.34 mg L^{-1} and the M was set 2.4 mg L^{-1} in the reactors. The methane yield and petroleum hydrocarbons degradation rate were 488.9 μmol and 38.2%, respectively. It showed that the eutrophic river water could be used for microbes to degrade oil. When the trace element was added the methane yield and petroleum hydrocarbons degradation rate was better.

DISCUSSION

In this study, a oil reservoir simulation system was innovatively designed to study the process by using indigenous microbes that could transform residual oil into methane under different pressure and temperature (Fig. 1). Microbes commonly exist in a petroleum reservoir, with lower than 80°C. The methanogenic microbes were hard to survive when the temperature is above 80°C (Orphan *et al*., 2000; Larter *et al*., 2006) while under 40°C, their growth rate is maximum. Hydrocarbon degradation was often effected by phosphorus, potassium and nitrogen (Rogers and Bennett, 2004). The oil degradation was related to the phosphorus dissolving from mineral in the reservoir by microbes (Ehrenberg and Jakobsen, 2001). The concentration of ammonium ion in the reservoir was very low and it would accelerate methane generation if improving the concentration of ammonium ion (Manning and Hutcheon, 2004). In order to promote the methane generation rate of residual oil and reduce the degradation of methane, it was necessary to improve the microbe's activity. By study the oil reservoir environment, it could be concluded that chemical additives can activate underground microbes. During this approach, some chemical additives and nutrients or electron acceptor were feed to the microbes to activate their function on producing methane (Gray *et al*., 2009). Additives may include nitrogen and phosphorus nutrient which could be quickly dispersed into the oil reservoir. The microbes can also be separate from the oil sands or oil shale and the nutrients can be other carbon or nitrogen source (Lambo *et al*., 2009; Gray *et al*., 2009; Gieg *et al*., 2008; Jones *et al*., 2008).

CONCLUSION

The eutrophic river water could be used to promote the oil degradation and producing methane. And When the trace element was added the methane yield and petroleum hydrocarbons degradation rate was better. The best trace element $CoCl_2 \cdot 6H_2O$ concentration was 1.6 mg L^{-1}, $NiCl_2 \cdot 6H_2O$ and Na_2MoO_4 were 0.34 and 2.4 mg L^{-1}, respectively. The pressure could effect the methane yield and petroleum hydrocarbons degradation. Microbes grow better in low pressure condition.

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