# Biogenic Methane Generation from Lignite with Hydrogen Peroxide for Subsurface Cultivation and Gasification

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctorate in Engineering

By

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## Abstract

Increasing energy requirements and decreasing conventional hydrocarbon reserves have led to the development of unconventional hydrocarbon, including biogenic coal bed methane (CBM). Lignite production for power generation is environmentally problematic because the combustion processes contribute to air pollution (e.g., $CO_2$, $SO_X$, $NO_X$, particulate matter, and Hg). However, the lignite has become of global interest for the generation of biogenic CBM via microbial transformation, which is considered to be environmentally friendly energy. Previous studies have indicated that lignite is solubilized by hydrogen peroxide ($H_2O_2$) concomitant with the generation of methanogenic substrates (e.g., acetic acid and formic acid). More recently, the concept of subsurface cultivation and gasification (SCG) was proposed to produce biogenic methane ($CH_4$) by injecting $H_2O_2$ into lignite seams to generate methanogenic substrates. Although this concept has a great promise, its applicability in the field remains uncertain since only batch experiments have been conducted in the laboratory. Therefore, the biogenic methane generations using solution from column reactions of lignite with $H_2O_2$ was studied to demonstrate the potential for the successful *in situ* microbially enhanced CBM generation using $H_2O_2$.

Chapter 1 gives the motivation, importance, and objectives of this study. The recent studies and remaining knowledge gaps regarding biogenic CBM were also discussed.

In Chapter 2, an indigenous microbial consortium associated with coal from the coal-bearing Soya Formation in the Tempoku Coalfield (northern Hokkaido, Japan) was cultivated to reaction solutions of lignite and hydrogen peroxide ($H_2O_2$) (i.e., chemically solubilized lignite) to evaluate *in situ* biogenic methane generation. Column experiments using such reaction solutions achieved maximum concentrations of dissolved organic carbon, acetic acid, and formic acid of 6,330, 612, and 1,810 mg/L, respectively. Cultivation experiments using the above reaction solution as a substrate for methanogens produced nearly 6 $cm^3$ $CH_4$ per g lignite with a maximum rate of 0.14 $cm^3$ per g per day without additional amendments such as nutrients or reducing agents. These findings present a great opportunity to produce biogenic $CH_4$ from the world's lignite seams by injecting $H_2O_2$ into its lignite seams without additional microorganisms (bio-augmentation) or nutrients (bio-stimulation).

After confirming the methane production from the reaction solution of lignite with $H_2O_2$, an indigenous microbial consortium associated with coal from the coal-bearing Soya Formation in the Tempoku Coalfield was identified and discussed in Chapter 3. Pyrosequencing analysis of the microbial consortium after cultivation showed diverse archaeal and bacterial cultures in the vials that would lead to the generation of $CH_4$. The

operational taxonomy units (OTUs) affiliated with the class *Deltaproteobacteria*, *Bacteroidetes*, *Betaproteobacteria*, *Clostridia*, and *Methanomicrobia* were major microorganisms. These results revealed that the biogenic methane was possibly produced through hydrogenotrophic ($CO_2$ reduction), aceticlastic (acetate fermentation), and formate-utilizing methanogenesis pathways, partly following bacterial activities of fermentation, homoacetogenesis, and syntrophic acetate oxidation.

In Chapter 4, the $H_2O_2$-treated lignite, referred to lignite-$H_2O_2$, was also examined in column experiments to confirm the increases in lignite solubilization and organic acids as indicators of enhanced bioavailability. Lignite treated with $H_2O_2$ showed higher concentrations of dissolved organic carbon (up to 84.8 mg/L) and organic acids (up to 18.9 mg/L for acetic acid, and up to 19.9 mg/L for formic acid) than lignite without treatment when reacted with ultrapure water under the column reactions. These results demonstrated the enhanced solubility of lignite after $H_2O_2$ reaction, as well as the generation of reactive structures (e.g., peroxy acids), resulting in the production of organic acids (e.g., acetic acid and formic acid). Thus, the enhanced bioavailability of lignite from reaction with $H_2O_2$ would enhance the biogenic $CH_4$ yield from lignite-$H_2O_2$ reaction solution, which is encouraging for the field application of microbially enhanced CBM generation using $H_2O_2$.

Finally, in Chapter 5, to understand the mechanism of the increased bioavailability of lignite as a result of $H_2O_2$ treatment, the effects of $H_2O_2$ reaction on humic substances of lignite were investigated by characterizing their structures and relative abundance. The results showed that the alkali-soluble carbon content of lignite increased by 4.9 times (from 0.4 to 2.1 g C) after $H_2O_2$ treatment, and the humic acid (HA) content of this fraction increased by 7.7 times (from 0.2 to 1.5 g C). The main cause of the increase in alkali-soluble contents in lignite-$H_2O_2$ could be the breakage of bonds in the lignite macromolecular network, yielding HA, small molecule size fraction (SMSF), or fulvic acid (FA). Specifically, the $H_2O_2$ yields peroxide structures (i.e., ROOH) in lignite, from which alkoxyl radicals (RO•) are formed either by homolytic cleavage or are radical-induced. In HA, which is the dominant regenerated component in the alkali-soluble fraction, O-alky-C content decreased while carbonyl-C content increased in response to the $H_2O_2$ reaction. Therefore, instead of attributing to increased hydrophilicity of the lignite, the enhanced solubility of lignite after $H_2O_2$ treatment might be caused by chemical fragmentation (i.e., $\beta$-fragmentation) of the alkoxyl radicals (RO•), converting O-alkyl-C to carbonyl-C.

In chapter 6, the contents of this research are summarized, and the conclusions are presented.

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# Chapter 1

# GENERAL INTRODUCTION

## 1.1 Biogenic coal bed methane from lignite

Lignite is a low-rank coal with a low calorific value, high ash content, and high water content (Bumpus et al., 1998; Chassapis and Roulia, 2008; Yan et al., 2001). It is formed from the original phytomass by peatification followed by coalification, and is the intermediate between peat and bituminous coal (Doskočil et al., 2014). Currently, 90% of lignite production worldwide is used for power generation (World Energy Council, 2016), but this is environmentally problematic because combustion processes contribute to air pollution (Alpern and Lemos de Sousa, 2002; Breckenridge and Polman, 1994; Huang et al., 2013a; Kaldellis and Kapsali, 2014; Sakulniyomporn et al., 2011). On the other hand, lignite has become of global interest for the generation of biogenic coal bed methane (Fallgren et al., 2013b; Green et al., 2008; Huang et al., 2013b; Jones et al., 2008; Scott, 1999; Strapoć et al., 2008). The combustion processes from a typical natural gas such as methane ($CH_4$) would emit significantly less $CO_2$, $NO_x$, $SO_x$, and mercury than conventional hydrocarbons (U.S. Energy Information Administration, 2017). Moreover, compared with conventional coal mining, biogenic coal bed methane does not require the coal to be mined, processed, and transported, resulting in substantially lower capital and operating cost (Huang et al., 2013a; Yoon et al., 2016).

Coal bed methane (CBM) is mainly contributed by geological (thermogenic) and biological (methanogenic) processes (Formolo et al., 2008; Park and Liang, 2016; Rice, 1993). The thermogenic CBM is attributed by high pressure and temperature through coalification processes, while the biogenic CBM requires metabolic activity of microorganism and is classified into primary and secondary biogenic CBM. The primary

biogenic CBM forms in the peatification, but then vanishes during compaction and coalification process (Butland and Moore, 2008; Formolo et al., 2008; Scott, 1994; 1999). The secondary biogenic CBM forms after the burial, coalification, subsequent uplift and erosion of the basin margin (Huang et al., 2013a). Once the $CH_4$ gas is generated from coal deposits, it is possible to stimulate a new natural gas formation and extend the $CH_4$ production life (Jones et al., 2013, 2008). The occurrences of secondary biogenic CBM were observed in the United States [e.g., San Juan Basin, New Mexico and Colorado (Scott et al., 1994; Strąpoć et al., 2011) and Powder River Basin, Wyoming (Rice et al., 2008)], Canada [e.g., Alberta Basin, Alberta (Bachu and Michael, 2003) and Elk Valley (Aravena et al., 2003)], Australia [e.g., Bowen Basin (Ahmed and Smith, 2001) and Surat Basin (Li et al., 2008; Papendick et al., 2011)], Germany [e.g., Ruhr Basin (Krüger et al., 2008), China [e.g., Xinji area (Tao et al., 2007)], and Japan [e.g., Chiba Prefecture (Mochimaru et al., 2007) and Hokkaido (Shimizu et al., 2007)], suggesting that the active microbes capable of producing $CH_4$ are present in many coal seams.

### 1.2 Bioconversion process of lignite to methane

The generation of biogenic CBM from coal requires multi-step proceses involving a consortium of microorganism such as bacteria and methanogenic archea. Under natural conditions, the bioconversion process can be divided into three steps: (1) soluble organics are released from the coal geopolymer, (2) biodegradation of soluble organics into substrates, and (3) methanogens consume the substrates and produce methane gas (Colosimo et al., 2016; Jones et al., 2010; Ritter et al., 2015; Strapoć et al., 2008). Stimulating the above processes artificially, Scott (1999) proposed the concept of microbially enhanced coalbed methane (MECBM, or MECoM) to enhance coalbed methane recovery. Under natural conditions, the activity of methanogenesis is limited by the availability of the substrates, and the generation of the substrates is limited by coal solubilization (Ritter et al., 2015).

The most common approaches of current MECBM projects are the introduction of nutrients into coal seams (bio-stimulation), the addition of microorganism (bio-augmentation), increasing microbial access to coal, and increasing the bioavailability of coal by chemical treatment (Colosimo et al., 2016; Jones et al., 2013; Ritter et al., 2015). However, since $CH_4$ production in the field-scale pilot test of MECBM still does not commercially meet the expectation, other methods are under development (Ritter et al., 2015). Bio-stimulation and bio-augmentation processes perform the injection of nutrients and microorganism (i.e., bacteria and archaea), respectively, into coal seams to enhance or initiate microbial production. The increase of surface area available for microbial colonialization includes grinding of the *ex situ* coal, fracturing the coal (hydraulic fracturing) and dissolving coal using underground solution to increase porosity (Green et al., 2008; Scott, 1999). Meanwhile, the chemically increasing the coal bioavailability involves the breaking down the coal geopolymers, resulting in the solubilized coal substrates for methanogenesis. The chemical approaches appear to be critical, since it has been suggested that enhancing the rate of coal solubilization is a key factor for successful MECBM (Green et al., 2008; Papendick et al., 2011; Park and Liang, 2016; Wang et al., 2017).

### 1.3 Subsurface cultivation and gasification (SCG)

Previous studies (Mae et al., 2001; Miura et al., 1997) showed that lignite is solubilized by hydrogen peroxide ($H_2O_2$), concomitant with the generation of methanogenic substrates such as acetic acid and formic acids under experimental conditions, which have been identified as immediate precursors to methane production (Chen et al., 2017; Papendick et al., 2011; Strąpoć et al., 2011)). Accordingly, Aramaki et al. ( 2015; 2017) proposed the concept of Subsurface Cultivation and Gasification (SCG; Figure 1-1) using $H_2O_2$ as a solubilizing agent for enhancing the *in situ* biogenic $CH_4$ generations from lignite in the Tempoku coal field, northern Hokkaido, Japan (Figure 1-2). They also confirmed the relatively fast

production (<1 year) of biogenic methane (>2 $cm^3$ per gram of lignite) from the reaction solution of lignite with $H_2O_2$ (by methanogens) in laboratory experiments (Figure 1-3).

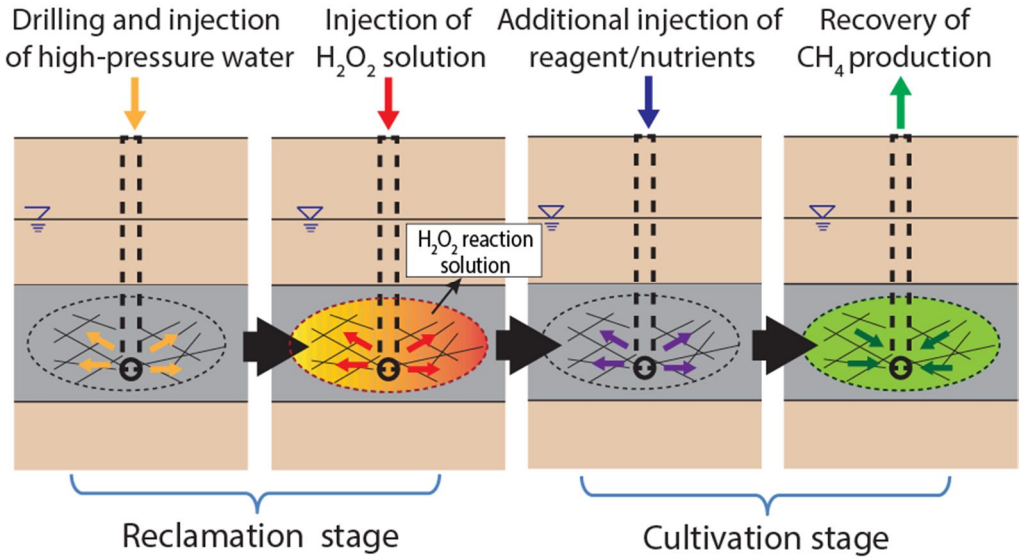


**Figure 1-1** Subsurface cultivation and gasification (SCG) (Aramaki et al., 2015; 2017)

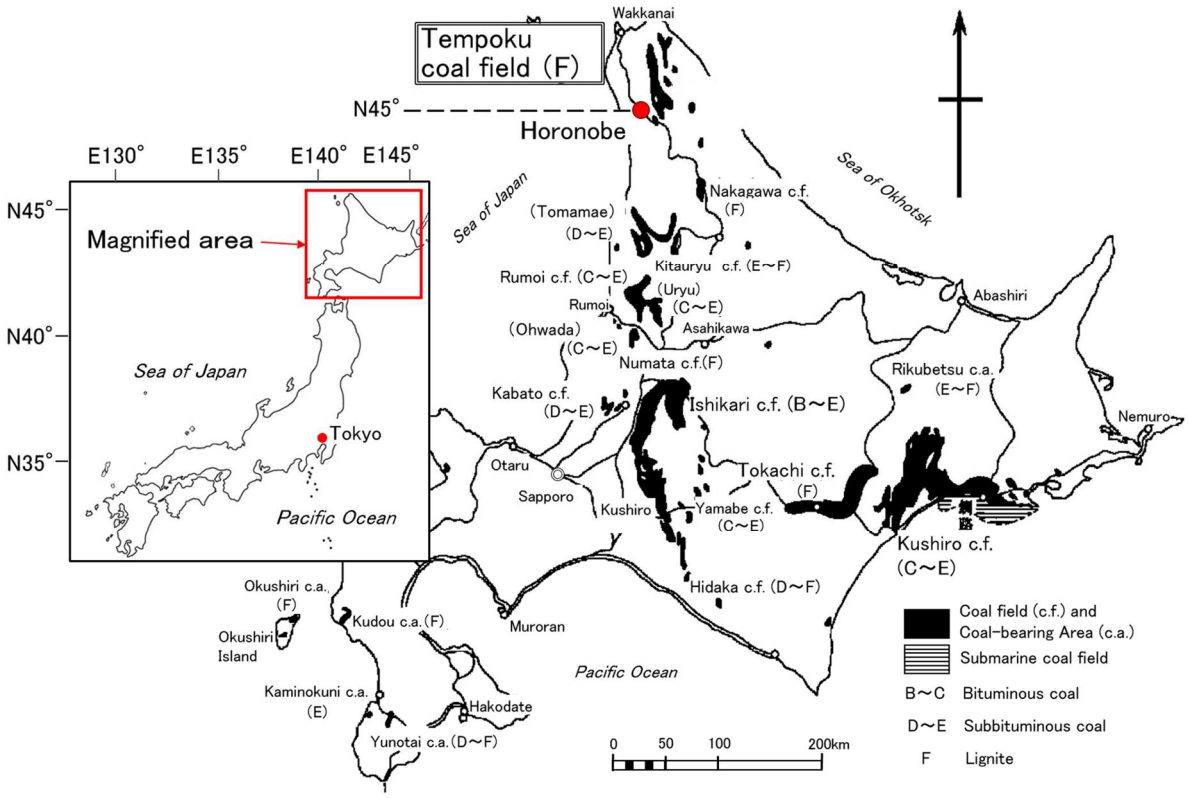


**Figure 1-2** Map location of Tempoku coal field in northern Hokkaido, Japan (Aramaki et al., 2017)

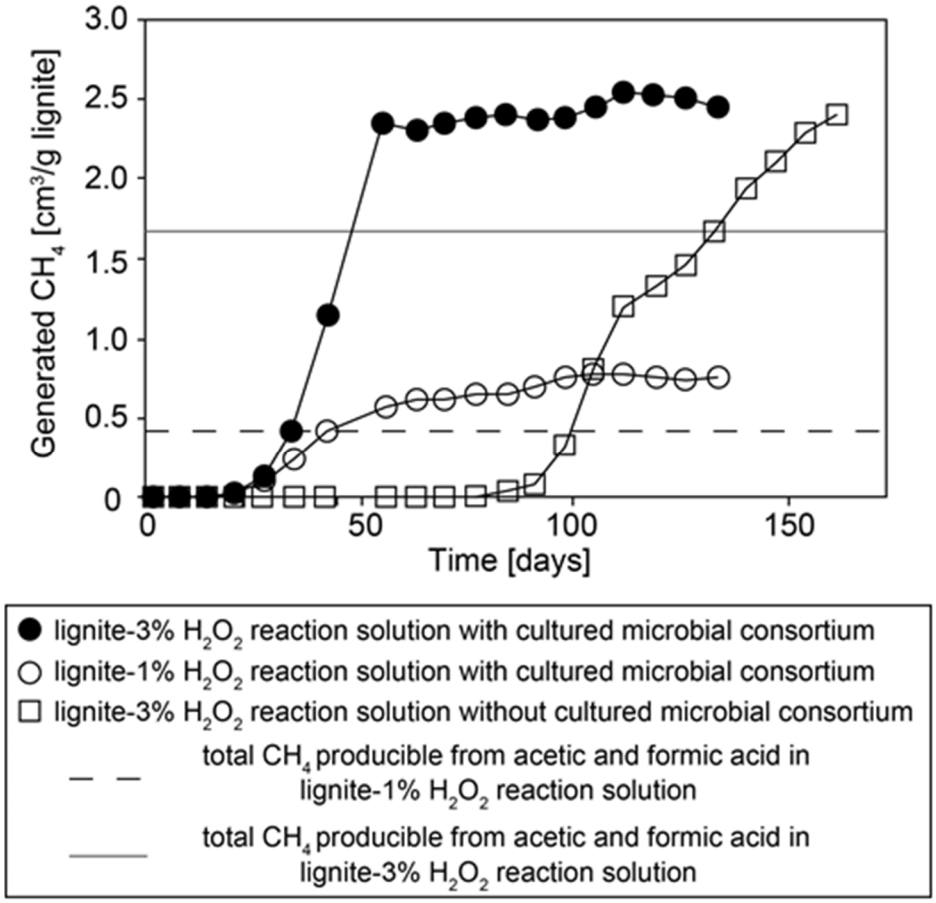


**Figure 1-3**. Produced $CH_4$ ($cm^3$/g lignite) from reaction solutions of lignite with $H_2O_2$ (1% or 3%) under batch experiments in the presence and absence of cultured microbial consortium (Aramaki et al., 2015).

The idea of SCG mainly involves the injection of high-pressure $H_2O_2$ solution into lignite seams to rapidly produce low-molecular-weight organic substances for methanogenesis. During $H_2O_2$ injection, the acidification and oxidation likely occur, which are detrimental to native microorganism (Gallagher et al., 2013; Jones et al., 2013). Therefore, some reagents (e.g., reducing and neutral agents) may also be injected, in case the natural buffering capacities (Heron et al., 1993; Langmuir, 1997) would not achieve the ideal subsurface environment for microorganisms after $H_2O_2$ injection. The injection of additional microorganisms (i.e., bacteria and archaea) may also be considered, depending on the availability and capability of native microorganisms in the formation to produce $CH_4$.

## 1.4 Statement of the problem and objectives of the study

The above SCG concept for the production of biogenic methane has a great promise; yet, the field applicability of SCG remains uncertain since only batch experiments have been conducted in the laboratory (Tamamura et al., 2016; Aramaki et al., 2017). Also, little is known about the most bioavailable fraction in the coal, and the species of microorganism that is responsible for coal biodegradation and methanogenesis. Therefore, the main objective of this study is to evaluate the conversion of lignite into biogenic methane via $H_2O_2$ under simulated natural conditions. Specifically, this study aims at the following:

a. To confirm the enhance bioavailability of chemically solubilized lignite under simulated groundwater flow (i.e., column experiments)

b. To confirm the methane production by considering the methanogens native to the coal seams of northern Hokkaido, Japan

c. To characterize the composition and structure of each organic fraction of lignite before and after $H_2O_2$ treatment, and

d. To investigate the mechanisms of enhanced bioavailability in lignite after $H_2O_2$ treatment

## 1.5 Outline of dissertation

This dissertation is composed of six chapters. The key contents of each chapter are outlined as follows:

**Chapter 1** introduces the background, statement of the problems, and objectives of this study.

**Chapter 2** presents the investigation of the chemically solubilized lignite from the reaction with $H_2O_2$ solution under simulated groundwater flow conditions, and confirms the methane production from lignite-$H_2O_2$ reaction solution.

**Chapter 3** identifies the microbial communities that are native to lignite seam in northern Hokkaido, and describes the possible methanogenic pathways of biogenic CBM generation from solubilized lignite substrates.

**Chapter 4** discusses the potential optimization of biogenic CBM from lignite after $H_2O_2$ treatment.

**Chapter 5** presents the characterization of lignite and its organic composition before and after $H_2O_2$ reaction to develop reaction mechanisms for the enhanced bioavailability of the lignite after $H_2O_2$ treatment.

**Chapter 6** describes the summary, implications, and conclusion of the dissertation.

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# Chapter 2

# BIOGENIC METHANE GENERATION USING SOLUTION FROM COLUMN REACTION OF LIGNITE WITH HYDROGEN PEROXIDE

## 2.1 Introduction

Considering that coal solubilization is a rate-limiting step for successful MECBM production (Green et al., 2008; Park and Liang, 2016), chemical agents have been used to solubilize coal for subsequent microbial activity (Bumpus et al., 1998; Green et al., 2008; Huang et al., 2013a, 2013b; Papendick et al., 2011). Previous studies (Mae et al., 2001; Miura et al., 1997; Tamamura et al., 2016) have also shown that hydrogen peroxide ($H_2O_2$) can substantially solubilize lignite (low-rank coal) in batch experiments and generate organic acids (e.g., acetic and formic acids), which have been identified as methanogenic substrates (Chen et al., 2017; Strąpoć et al., 2011). In addition, $H_2O_2$ is non-polluting because it easily decomposes without releasing hazardous elements (Doskočil et al., 2014; Klenk et al., 2000; Tamamura et al., 2016). Lignite is also more favorable for bioconversion than other coal ranks (Robbins et al., 2016; Strąpoć et al., 2011) since it is easily biodegraded (Strapoć et al., 2008).

The SCG concept Aramaki et al. (2017, 2015) for the production of biogenic methane has a great promise, yet its applicability in the field remains uncertain since only batch experiments have been conducted in the laboratory (Tamamura et al., 2016; Aramaki et al., 2017). Therefore, this chapter evaluated the reactivity of lignite with $H_2O_2$ under simulated natural conditions using column experimental systems, and confirmed the biogenic methane production from reaction solutions of lignite and $H_2O_2$ by native methanogens in the lignite. The column experiment was adopted to simulate more realistic groundwater flow conditions of an aquifer, which are less likely reproduced by batch experiments.

## 2.2 Materials and methodology

A lignite sample evaluated in this study was collected from the riverbed outcrop of the Miocene coal-bearing Soya Formation in the Tempoku Coalfield in Horonobe, northern Hokkaido, Japan (Figure 1-2). Total organic carbon (TOC) content, volatile matter content, and the calorific value of the lignite are ~69%, 47%–53%, and 6,900–7,200 kcal/kg, respectively, on a dry, ash-free (DAF) basis. Iron (Fe), manganese (Mn) and copper (Cu) contents of the lignite, which can potentially affect reactions with $H_2O_2$ (Gierer et al., 1993), are very low (1.8% Fe, 0.029% Mn, and 0.00047% Cu), as measured by X-ray fluorescence (XRF) analysis. Excluding quartz ($SiO_2$), X-ray diffraction (XRD) analysis could not detect the presence of mineral components (e.g., pyrite) in the lignite.

The sample collection was prepared for column experiments and cultivation experiments. The lignite for column experiments was dried and ground using a mortar and pestle to obtain the size fraction between 0.5–1 mm. For cultivation experiments, clumpy lignite (1000 ~ 10,000 $cm^3$) was dug out from the depths of 5 ~ 40 cm. This lignite was likely situated in an anaerobic condition, based on the existence of active methanogens in the lignite (Aramaki et al., 2017). After retrieval from the riverbed outcrop, the sample was then immediately packed in an anaerobic condition using Anaeropack (Mitubishi Gas Chemical). The lignite was then broken into chunks (~2 cm) in anaerobic chamber, small enough to fit into glass vial opening.

### *2.2.1 Column experiments*

The column used was made of glass with an inner diameter of 1.1 cm and a length of 30 cm**.** The column layer consists of lignite (17 g) and baffles layers composed of spherical glass beads with 2 mm in diameter (Figure 2-1). Hydrogen peroxide solution was injected into the column layer by a peristaltic pump (MP-2000, Tokyo Rikakikai Co., Ltd., Japan).

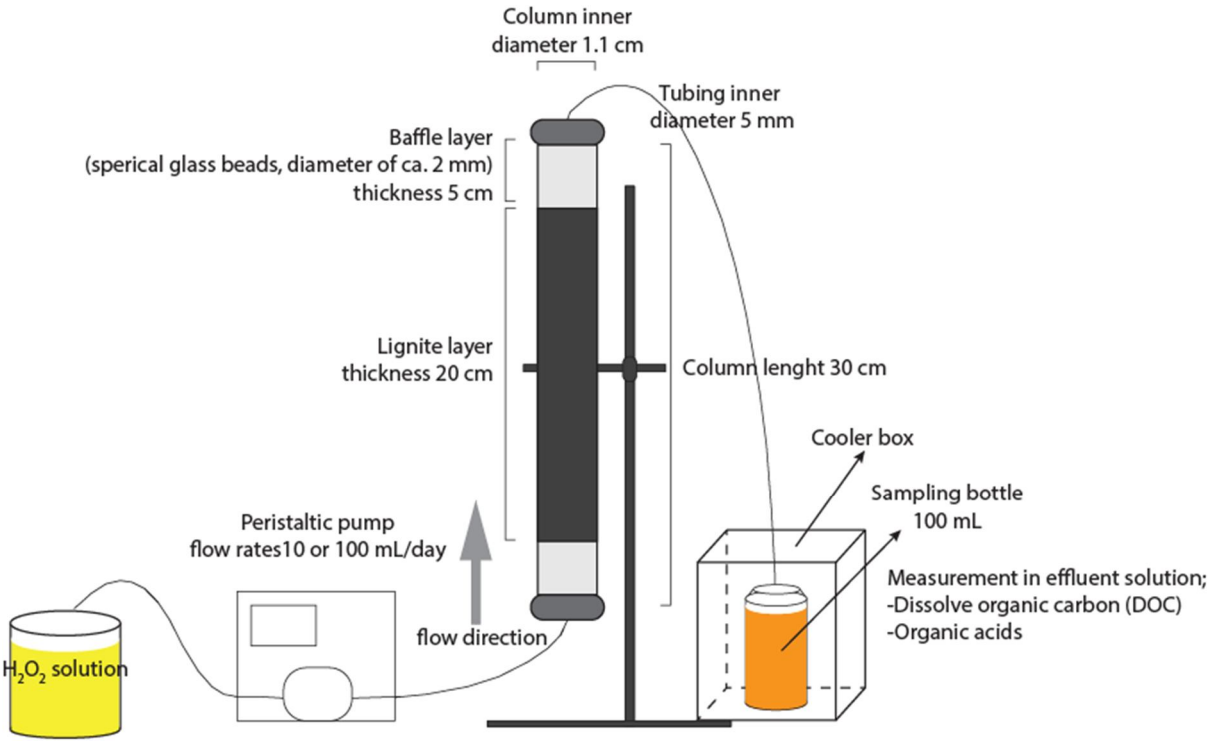


**Figure 2-1.** Setup of the column experiments

Four cases were conducted at room temperature with different $H_2O_2$ concentrations and flow rates (Table 2-1). As a control, a similar column experiment was performed using ultrapure water. These columns were duplicated to evaluate data reproducibility.

**Table 2-1.** List of column experimental conditions

| Case | Influent solution | Flow rate (mL/day) |
|---|---|---|
| 1 | 0.3% $H_2O_2$ | 100 (high flow rate) |
| 2 | 3% $H_2O_2$ | 100 (high flow rate) |
| 3 | 0.3% $H_2O_2$ | 10 (low flow rate) |
| 4 | 3% $H_2O_2$ | 10 (low flow rate) |

The effluent solutions were directly collected in 100 mL bottles kept in a cooler box to retard possible reactions in the effluent until replacement of the bottle for the next sampling. Then, they were provided for the analysis of concentrations of dissolved organic carbon (DOC) and organic acids, as well as $H_2O_2$ concentration, pH and oxidation-reduction potential (ORP). The collection of the effluent was continued until the DOC and organic acid

concentrations were stable. These conditions were achieved before the lignite material was lost by less than 20% weight owing to solubilization.

*2.2.2. Cultivation experiments*

To evaluate methane production from the reaction solution of lignite with $H_2O_2$, methanogen cultivations were set up (Figure 2-2), using the effluent solutions that were collected in the period of the column experiments when the concentrations of organic acids were relatively high (above average). Because the effluent solutions were still harmful for methanogens due to high hydrogen peroxide concentrations (Figure 2-3), as well as high oxidation-reduction potential (ORP) and low pH (Figure 2-4), some treatments were performed.

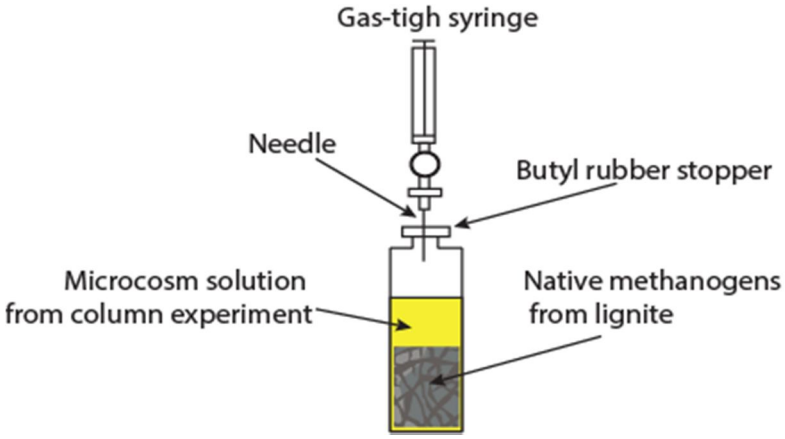


**Figure 2-2.** Setup of the cultivation experiments

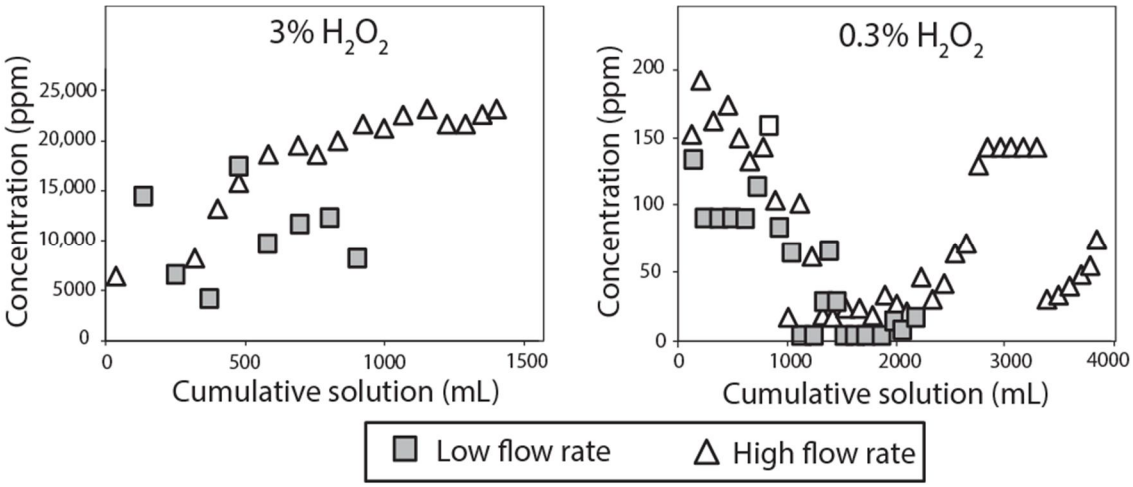


**Figure 2-3.** Hydrogen peroxide concentrations in the effluent solutions

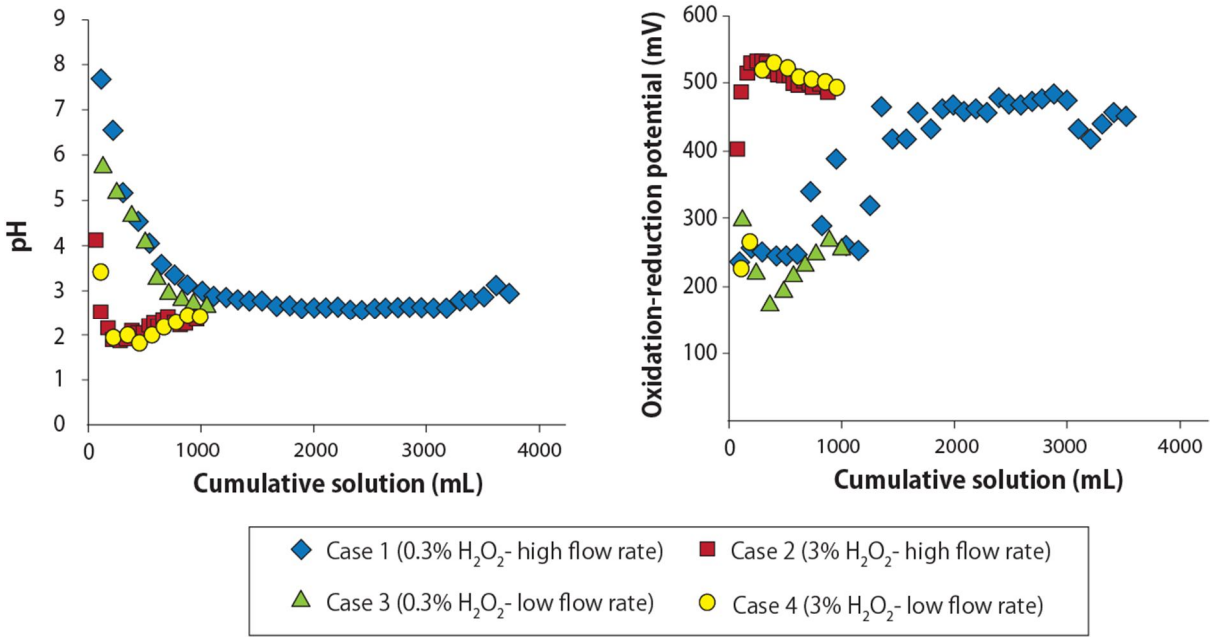


**Figure 2-4.** pH and oxidation-reduction potential (ORP) in the effluent solutions

The effluent solutions in each column case were heated at 70º C for 24 h to reduce $H_2O_2$ concentration by the reaction with DOC components. This heating treatment was not performed for the solution in case 3 (i.e., 0.3% $H_2O_2$ – low flow rate) because the $H_2O_2$ concentration was already low (<0.05 ppm) in the effluent. After heating, the $H_2O_2$ concentration in case 1 became low (<0.05 ppm), whereas the $H_2O_2$ concentration in cases 2 and 4 were still high (>0.5 ppm). Thus, the cultivation experiments were continued only with effluents in case 1 (with heat treatment) and case 3 (without heat treatment).

After vacuum filtration (2 µm filter paper, Kiriyama, Japan) of the collected effluent solutions in cases 1 and 3, phosphoric pH buffer (0.6 M $NaH_2PO_4$, 0.1 mL per 100 mL) was added into them. Eight different cultivation conditions were prepared in these solutions with or without nutrients and a reducing agent (Table 2-2). The nutrients consisted of $NH_4Cl$ (0.5 g/L), $MgCl_2 \cdot 6H_2O$ (0.5 g/L), $CaCl_2$ (0.14 g/L), KCl (0.1 g/L), $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (0.002 g/L), $NaHCO_3$ (2.5 g/L), and trace mineral solution SL-10 (1 mL/L). The trace mineral solution contained $FeCl_2 \cdot 4H_2O$ (1,500 mg/L), $ZnCl_2$ (70 mg/L), $MnCl_2 \cdot 4H_2O$ (100 mg/L), $H_3BO_3$ (6 mg/L), $CoCl_2 \cdot 6H_2O$ (190 mg/L), $CuCl_2 \cdot 2H_2O$ (2 mg/L), $NiCl_2 \cdot 6H_2O$

(24 mg/L), $Na_2MoO_4 \cdot 2H_2O$ (36 mg/L), and 10 ml of 25% HCl. The nutrients were dissolved completely into the effluent. Each effluent, with or without these nutrients, was subsequently neutralized to around pH 7.0 with 2.0 M NaOH.

**Table 2-2.** Treatment conditions used in the cultivation experiments

| Treatment | Solution | Additional amendments |
|---|---|---|
| 1 | 0.3% $H_2O_2$-high flow rate | None |
| 2 | | Nutrients |
| 3 | | Reducing agent |
| 4 | | Nutrients and reducing agent |
| 5 | 0.3% $H_2O_2$-low flow rate | None |
| 6 | | Nutrients |
| 7 | | Reducing agent |
| 8 | | Nutrients and reducing agent |

In an anaerobic chamber, the effluents in the presence or absence of the nutrients (40 mL) were transferred into a 50 mL autoclaved-glass vial in the presence of 35 g fresh lignite as methanogenic inoculum. Then, 0.4 mL of the reducing agent (50 g/L $Na_2S$) was added to some vials in the chamber. After sealing the vials with a butyl rubber stopper and an aluminum crimp, the headspace gas in the vial was substituted with a gas mixture comprising anoxic $N_2/CO_2$ (80:20, by volume) using a gas exchanger (GR-8, Sanshin Industries) before the incubation at 27ºC. Each cultivation treatment was duplicated, and a control with a similar cultivation experiment using ultrapure water was also obtained. During 130 days of the incubation, the $CH_4$ concentration in the headspace was periodically measured by collecting 1 ml of the gas using a gas-tight syringe.

*2.2.3. Analysis*

The DOC concentrations of the sample solutions were analyzed by a total organic carbon analyzer using a TOC-$V_{CHS}$ TOC analyzer (Shimadzu, Japan), with a precision of $< \pm 0.30\%$. Before injecting the sample solution into a combustion tube (720 ºC), the solution

was automatically acidified using HCl and purged with pure air to remove inorganic carbon (IC). Organic acids (formic, acetic, oxalic, malonic, and succinic acid) were measured by an ion chromatograph (761 Compact IC, Metrohm), with a precision of < ± 2.0%. The concentration of $H_2O_2$ was measured by a commercial colorimetric method (Hydrogen Peroxide Assay Kit, CL-204, National Diagnostics) with an accuracy of < ± 3.0%. Gas compositions ($CH_4$, $CO_2$, $O_2$, and $N_2$) were measured by a gas chromatography (GC-14B, Shimadzu) with a precision of < ± 3.5%.

## 2.3 Results

### *2.3.1 Reaction solution of lignite with $H_2O_2$*

Both DOC (Figure 2-5) and organic acids (Figure 2-6) concentrations increased rapidly at the initial stage of the column experiment and stabilized in a similar trend.

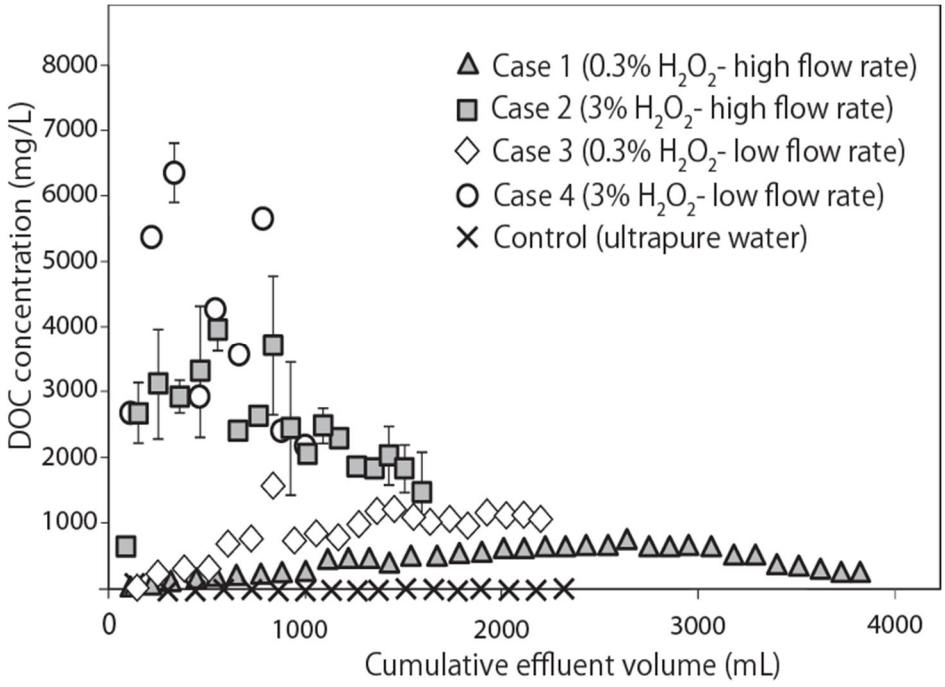


**Figure 2-5.** Dissolved organic carbon (DOC) concentrations in effluent solutions from column experiments in various $H_2O_2$ concentrations (0.3 and 3%) and flow rates (10 and 100 mL/day). Error bars (indicating the difference between the duplicate results) smaller than the symbols are omitted.

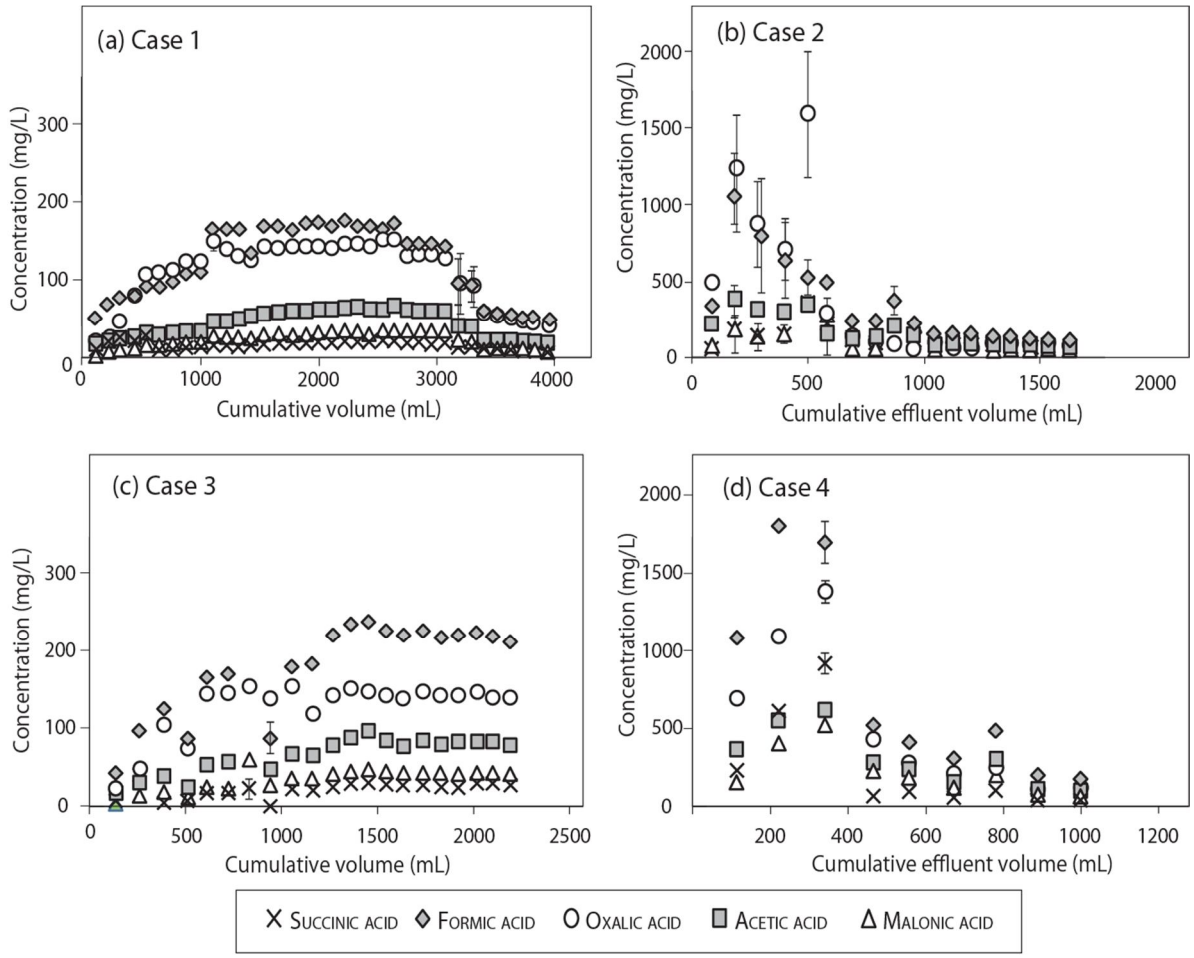


**Figure 2-6.** Organic acid concentrations in effluent solutions from column experiments with (a) Case 1: 0.3 % $H_2O_2$ – high flow rate (100 mL/day), (b) Case 2: 3 % $H_2O_2$ – high flow rate (100 mL/day), (c) Case 3: 0.3 % $H_2O_2$ – low flow rate (10 mL/day), and (d) Case 4: 3 % $H_2O_2$ – low flow rate (10 mL/day). Error bars (indicating the difference between the duplicate results) smaller than the symbols are omitted.

In terms of flow rates, the concentrations of DOC and organic acids were higher at a lower flow rate compared to those at a higher flow rate. At the lower flow rate, the maximum DOC concentration reached 6,330 mg/L, while the maximum concentrations of acetic acid, formic acid, malonic acid, succinic acid, and oxalic acid were 612, 1,810, 528, 919, and 1,380 mg/L, respectively. Meanwhile, at the high flow rate, the maximum DOC concentration of 3,940 mg/L was observed, while the maximum concentrations of of acetic acid, formic acid,

malonic acid, succinic acid, and oxalic acid were 380, 1,030, 393 mg/L, 362 mg/L, and 1,588 mg/L, respectively.

The concentrations of DOC and organic acids were proportional to the initial concentration of $H_2O_2$ in the influent solution, where the reaction of 3% $H_2O_2$ with lignite resulted in higher concentrations of DOC and organic acids in the effluent solution compare to that of 0.3% $H_2O_2$. However, the effluent of 3% $H_2O_2$ was not used as cultivation solution because the remaining $H_2O_2$ concentration in those was still high, as mentioned above. The utilization of 0.3% $H_2O_2$ to generate substrates for methanogens had more advantages in cultivation experiments.

*2.3.2. Biogenic $CH_4$ generation from the reaction solution of lignite with $H_2O_2$*

The $CH_4$ concentrations were shown in Figure 2-7. A high degree of reproducibility was confirmed by relative standard deviations of <1.0% in $CH_4$ concentrations. Carbon dioxide ($CO_2$) concentrations were also presented since $CO_2$ is one of direct $CH_4$ precursors, capable of increasing the methanogenic potential (Opara et al., 2012). Cultivation with the lower flow rate produced $CH_4$ concentrations (11.9%–14.6%) higher than those achieved using the higher flow rate (6.9%–10.2%) in each condition. The production of $CH_4$ was greater with neither minerals nor reducing agents (Figure 2-7a) than when both (Figure 2-7b), minerals only (Figure 2-7c), or reducing agent only (Figure 2-7d) were present. The $CH_4$ production did not result from desorption from the lignite samples, as very low levels of $CH_4$ (<0.02%) were detected from the control.

The maximum $CO_2$ concentration cultivated without minerals or reducing agent (Figure 2-7a) and with a reducing agent only (Figure 2-7d) were both about 10%, similar to their respective $CH_4$ concentrations. In contrast, the $CO_2$ concentrations in the cultivation with minerals (Figure 2-7b) were much greater (1.5–2.0 times) than the respective $CH_4$ concentrations and the $CO_2$ concentrations in the cultivation without minerals or reducing

agent. A similar result was observed in the cultivation with both minerals and a reducing agent (Figure 2-7c), for which the maximum $CO_2$ concentration reached nearly 20%. Nevertheless, high $CO_2$ concentrations (>15%) were also observed in the control in the presence of minerals, implying that most of the $CO_2$ was originally present in the vials and thus not attributable to microbial activity.

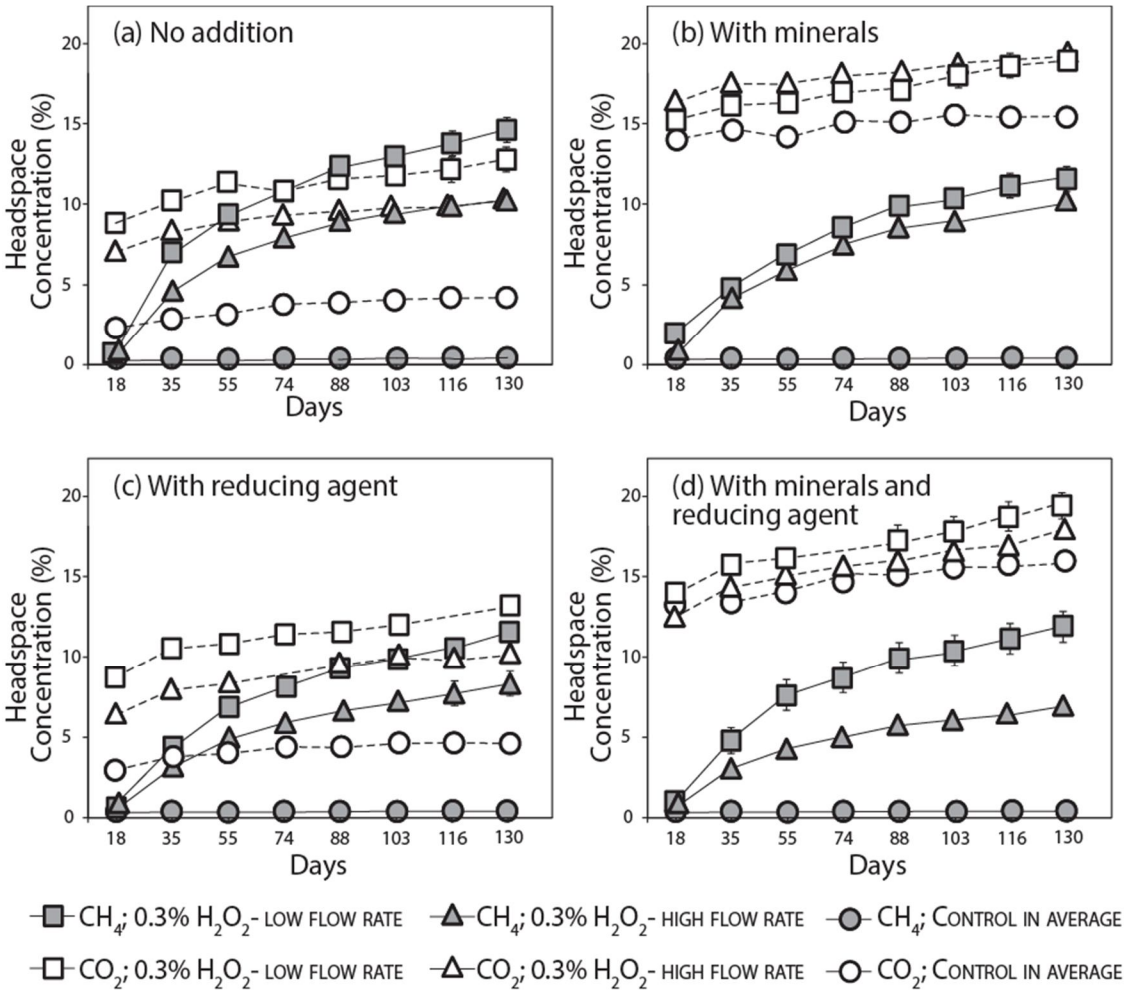


**Figure 2-7.** Generation of biogenic methane ($CH_4$) in various solutions. Error bars (indicating the difference between the duplicate results) smaller than the symbols are omitted.

As compared to the organic acid concentrations just before the cultivation experiments (Table 2-3), these concentrations after the cultivation significantly decreased to <10 mg/L, which was similar to the organic acid concentrations in the control solution. These results

suggest that the dominant carbon sources for microbial growth included these organic acids (e.g., acetic acid and formic acids) in the lignite-$H_2O_2$ reaction solution.

**Table 2-3.** Organic acids before and after cultivation experiments

| | Solution | Organic acids (mg/L) | | | | |
|---|---|---|---|---|---|---|
| | | Acetic | Formic | Malonic | Oxalic | Succinic |
| Before cultivation | Low flow without nutrients | 77.8 | 203 | 41.8 | 141 | 23.7 |
| | Low flow with nutrients | 78.0 | 204 | 41.7 | 133 | 26.3 |
| | High flow without nutrients | 65.5 | 157 | 33.3 | 146 | 18.7 |
| | High flow with nutrients | 65.0 | 157 | 32.7 | 91.5 | 18.7 |
| | Control without nutrients | 0.0 | 5.0 | 1.1 | 5.0 | 0.0 |
| | Control with nutrients | 0.0 | 4.09 | 1.05 | 4.65 | 0.0 |
| After cultivation | Low flow without nutrients | 0.23 | 0.50 | 0.24 | 0.03 | 0.0 |
| | Low flow with nutrients | 4.15 | 9.10 | 4.44 | 0.62 | 0.0 |
| | High flow without nutrients | 2.10 | 4.60 | 2.24 | 0.30 | 0.0 |
| | High flow with nutrients | 0.04 | 0.09 | 0.04 | 0.01 | 0.0 |
| | Control without nutrients | 4.15 | 9.05 | 4.44 | 0.57 | 0.0 |
| | Control with nutrients | 4.15 | 9.05 | 4.44 | 0.57 | 0.0 |

**2.4 Discussion**

The DOC (Figure 2-5) and organic acid (Figure 2-6) concentrations were also consistent with the $CH_4$ generations in the cultivations (Figure 2-7). Specifically, the $CH_4$ concentrations measured from the cultivations with a lower flow rate were higher than those with a higher flow rate, while the DOC and organic acid concentrations in the lower flow rate solution were also higher than those in the higher flow rate.

However, if the concentrations of headspace $CH_4$ (%) from 17 gram of lignite in the column are extrapolated into centimeter cubic of $CH_4$ per gram of lignite as summarized in Figure 2-8, the biogenic $CH_4$ converted from the lignite sample in cultivations with a lower

flow rate was less produced compared to cultivations with a higher flow rate. This is because the column with a higher flow rate provided about twice higher substrate solution (3,960 mL) than the column with a lower flow rate (2220 mL) (Figures 2-5 and 2-6). From the results calculated (Table 2-4), about 6 $cm^3$ of $CH_4$ could be generated per gram of lignite, and the maximum production rate over 130 day was 0.14 $cm^3$/g/day.

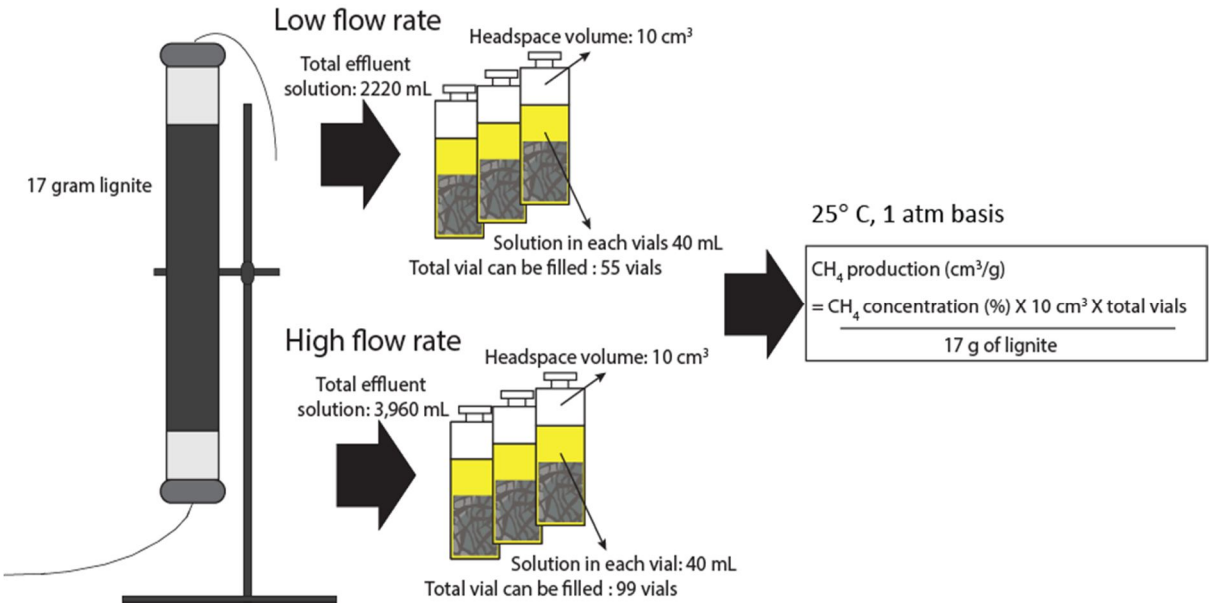


**Figure 2-8.** The calculation of extrapolated $CH_4$ production ($cm^3$/g) from headspace concentration (%)

The $CH_4$ generation potential was mostly higher than produced $CH_4$ from reaction solution of lignite with $H_2O_2$ in batch experiments (Aramaki et al., 2017; Figure 1-3), and previous results using various combinations of microorganisms and coals, which have been reported to vary between 0.006 and 8 $cm^3$/g (Fallgren et al., 2013a, 2013b; Green et al., 2008; Jones et al., 2008; Opara et al., 2012; Papendick et al., 2011; Rathi et al., 2015; Zheng et al., 2017).

Using the following reactions (Ehrlich and Newman, 2009): $CH_3COOH \rightarrow CO_2+CH_2$ and $4HCOOH \rightarrow CH_4+3CO_2+2H_2O$, the maximum theoretical $CH_4$ conversion from acetic and formic acids, respectively, could be 7.8 and 11.3 $cm^3$/g lignite at lower and higher flow

rates, respectively, based on the initial acetic and formic acid concentrations (Table 2-3). They correspond to 1.6-1.9 fold increase, relative to the extrapolated $CH_4$ generation (Table 2-4).

**Table 2-4.** Extrapolated $CH_4$ generations in $cm^3$/gram of lignite over 130 days

| Treatment | Solution | Additional amendments | $CH_4$ production ($cm^3$/g) | Maximum $CH_4$ rate ($cm^3$/g/day) |
|---|---|---|---|---|
| 1 | 0.3%$H_2O_2$ - high flow rate | None | 5.92 | 0.14 |
| 2 | | Nutrients | 5.85 | 0.12 |
| 3 | | Reducing agent | 4.90 | 0.10 |
| 4 | | Nutrients and reducing agent | 4.02 | 0.10 |
| 5 | 0.3%$H_2O_2$ - low flow rate | None | 4.79 | 0.12 |
| 6 | | Nutrients | 3.85 | 0.06 |
| 7 | | Reducing agent | 3.81 | 0.07 |
| 8 | | Nutrients and reducing agent | 3.88 | 0.08 |

These inconsistencies between the theoretical and extrapolated $CH_4$ production values might be caused by the microbial assimilation (Alber et al., 2006; Clifton, 1951) and the unsaturation of $CH_4$ concentration in the headspace. For the former, it is possibility that a part of acetate (and formate) may be used for cell growth (assimilation), not for methanogenesis. For the latter, since the measurement was conducted 2 weeks after the last measurements of $CH_4$ concentration (day 130), it is possible that acetate and formate were not be completely utilized for methanogenesis during the time of measurement. Although this study did not exactly represent the *in-situ* conditions due to, for example, substantially greater pressures in subsurface coal beds ($>2\times10^3$ atm at a burial depth >455 m; Davis and Gerlach, 2018; Zhao et al., 2016), the $CH_4$ productions in the cultivation experiments confirmed that the enrichment substrates in the reaction solution of lignite-$H_2O_2$ could support the indigenous microorganisms for methanogenesis.

One of the interesting parts in this study was that the highest $CH_4$ productions were observed in the cultivation without additional nutrients and reducing agent (Figure 2-7). In many cases, the addition of amendments (e.g., nutrients and a reducing agent) with appropriate concentrations would stimulate the microbial growth (Colosimo et al., 2016; Fallgren et al., 2013b; Jones et al., 2013; Ritter et al., 2015); however in this study, they did not result in higher $CH_4$ production.

The presence of some nutrients such as $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in the cultivations likely resulted in the lower pH (Drever, 1997; Wurts and Durborow, 1992). Such a low pH may favor homoacetogenesis relative to methanogenesis, leading to the decrease of $CH_4$ production when aceticlastic methanogenesis is not the main pathways (Harris et al., 2008; Phelps and Zeikus, 1984). Nevertheless, since the pH differences in the cultivation without nutrients (pH 6.3) and with nutrients (pH 6.0) were not significant (Table 2-5), it is not clear that the lower pH be the driving force of homoacetogenesis in this study.

**Table 2-5.** pH measurements in the cultivation experiments. The average pH in the absence of nutrients was 6.32, while in the presence of nutrients was 6.07.

| Treatment | Mirocosm solution | Additional amendments | pH |
|---|---|---|---|
| 1 | 0.3% $H_2O_2$-high flow rate | None | 6.28 |
| 2 | | Nutrients | 6.05 |
| 3 | | Reducing agent | 6.27 |
| 4 | | Nutrients and reducing agent | 6.17 |
| 5 | 0.3%$H_2O_2$-low flow rate | None | 6.17 |
| 6 | | Nutrients | 6.01 |
| 7 | | Reducing agent | 6.26 |
| 8 | | Nutrients and reducing agent | 6.1 |
| 9 | Control (ultrapure water) | None | 6.46 |
| 10 | | Nutrients | 5.94 |
| 11 | | Reducing agent | 6.52 |
| 12 | | Nutrients and reducing agent | 6.18 |

## 2.5 Conclusions

The findings of this research through column experiments and cultivation experiments are the following:

(1) The reaction of lignite with $H_2O_2$ under simulated groundwater flow conditions substantially solubilized lignite and generated organic acids, comparable with those of batch tests.

(2) Microbial consortia associated with lignite from the Soya formation utilized solubilized organic substances from the lignite with $H_2O_2$ to produce nearly 6 $cm^3$ $CH_4$ per gram of lignite over 130 days, with the maximum rate of 0.14 $cm^3$ per gram per day.

These findings present a great opportunity to produce biogenic $CH_4$ from lignite seams in the world. In case of the Tempoku coal field, at least 7 billion $m^3$ of $CH_4$ would potentially be recovered by injecting $H_2O_2$ into lignite seams without additional microorganism (bio-stimulation) and amendments (bio-stimulation), judging from cultivation experimental results.

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# Chapter 3

## POSSIBLE METHANOGENIC PATHWAYS FROM THE REACTION SOLUTION OF LIGNITE WITH HYDROGEN PEROXIDE

### 3.1. Introduction

The generation of biogenic $CH_4$ from coal requires multi-steps proceses involving the interaction of bacteria and archaea (Jones et al., 2010; Park and Liang, 2016; Ritter et al., 2015; Strąpoć et al., 2011; Thielemann et al., 2004). Under anaerobic conditions, methanogenic archaea generates biogenic $CH_4$ by only consuming simple carbon compounds as substrates (e.g., acetate, formate, $CO_2/H_2$). These methanogenic substrates are provided by complex microbial activities, which mainly consist of fermentations and acetogenesis. Fermentative bacteria first initiates the release of soluble organics from coal, and then biodegrades them into intermediates (e.g., fatty acids, alcohol) and simple by-products (e.g., acetates, $CO_2/H_2$) (Davis and Gerlach, 2018; Schink, 2006). More acetate can be produced by acetogenic bacteria from (i) $H_2$-using acetogenesis by consuming $H_2/CO_2$ and (ii) $H_2$-producing acetogenesis by converting fatty acids (Green et al., 2008; Park and Liang, 2016). The above natural process may be different from the generation of biogenic methane from the reaction solution of lignite with $H_2O_2$. Therefore, in this chapter, we identified the microbial communities that are native to lignite seam in Northern Hokkaido to evaluate the possible methanogenic pathways producing biogenic methane from the reaction solution of lignite with $H_2O_2$.

### 3.2. Materials and methodology

Bulk DNA was extracted from lignite slurry samples (2 mL) of cultivation experiments, which were described in Chapter 2, using a MoBio Powersoil® DNA isolation kit (MoBio Laboratories, USA) according to the manufacturer's instructions. The samples were collected

from vials by a syringe after disinfecting the butyl rubber and shaking each vial to mix the sample. To increase the DNA recovery, the method was modified by repeating (2–3 times) the addition of "Solution 3 (200 µL)" from the isolation kit to precipitate humic substances as impurities. The extracted DNA was amplified and sequenced at FASMAC Laboratory, Kanagawa, Japan. V4 regions of 16S rRNA genes were amplified using a universal primer set: 515f (5′- GTGBCAGCMGCCGCGGTAA-3′) and 805r (5′- GACTACHVGGGTATCTAATCC-3′). Polymerase chain reaction (PCR) was performed according to the following protocol: 94 °C for 2 min; 23 cycles at 94 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s; and a final step at 72 °C for 2 min. This process produced until about million copy of DNA. After purification of the PCR products, they were sequenced using an Illumina Miseq Next Generation Sequencer, which determine the precise order of DNA nucleotides (genetic codes). The sequences were clustered into operational taxonomic units (OTUs) using a cut-off of 97% identity to the 16S rRNA gene sequences. After taxonomy assignments, diversity indices (Chao1) and coverage values were calculated. The latter were calculated using equation [1-($n$/$N$)], where $n$ is the number of OTUs in a single read and $N$ is the total number of reads (Narihiro et al., 2014).

### 3.3 Results

The 16S rRNA gene pyrosequencing analysis using a universal primer set showed diverse archaeal and bacterial cultures in the vials that would lead to the generation of $CH_4$. In total, 31,900 archaeal and 948,000 bacterial sequence reads were generated (Table 3-1) with the average number of reads of 3,990 and 118,000, respectively. The Chao1 estimates of the total microbial diversity (i.e., archaea and bacteria) were considerably higher (63 – 84%) than the observed number of OTU, suggesting that further sequencing archaea and bacteria would lead to additional OTUs, and reveal more genera/species (Barnhart et al., 2016; Guo et al., 2012). The coverage values were >99%, implying that the OTUs retrieved in this study

were sufficient for estimating the microbial diversity (Narihiro et al., 2014). The OTUs affiliated with the class *Deltaproteobacteria*, *Bacteroidetes*, *Betaproteobacteria*, *Clostridia*, and *Methanomicrobia* were major microbial constituents in the methanogenic culture with lignite-$H_2O_2$ reaction solution.

**Table 3-1.** Summary of reads, operational taxonomic units (OTUs), and diversity estimates

| Treatment | Solution | Additional amendments | *Archaea* | | *Bacteria* | | Chao1 | Coverage (%) |
|---|---|---|---|---|---|---|---|---|
| | | | Reads | OTUs | Reads | OTUs | | |
| 1 | 0.3% $H_2O_2$-low flow rate | None | 6,150 | 10 | 93,800 | 331 | 541 | 99.7 |
| 2 | | Nutrients | 846 | 11 | 99,500 | 417 | 532 | 99.6 |
| 3 | | Reducing agent | 2,920 | 11 | 104,000 | 488 | 633 | 99.5 |
| 4 | | Nutrients and reducing agent | 3,980 | 11 | 96,400 | 412 | 502 | 99.6 |
| 5 | 0.3%$H_2O_2$-high flow rate | None | 8,160 | 12 | 200,000 | 492 | 623 | 99.8 |
| 6 | | Nutrients | 2,750 | 12 | 125,000 | 496 | 657 | 99.6 |
| 7 | | Reducing agent | 4,740 | 17 | 136,000 | 563 | 714 | 99.6 |
| 8 | | Nutrients and reducing agent | 2,420 | 14 | 92,500 | 445 | 575 | 99.5 |

### *3.3.1 Phylogenetic classification of archaeal sequences*

The phylogenetic classification of archaeal sequences is given in Figure 3-1. The family *Methanoregulaceae* (20–68% OTUs) and *Methanosarcinaceae* (16–52% OTUs) were the most prevalent methanogens in all cultivation treatments, followed by *Methanocellaceae* (1.7–16% OTUs). *Methanobacteriaceae*, *Methanosaetaceae*, and *Methanomassilicocaceae* were also present as minor archaea (< 5% OTUs). In contrast, few methanogens (< 8% of total archaeal) were detected in the control culture.

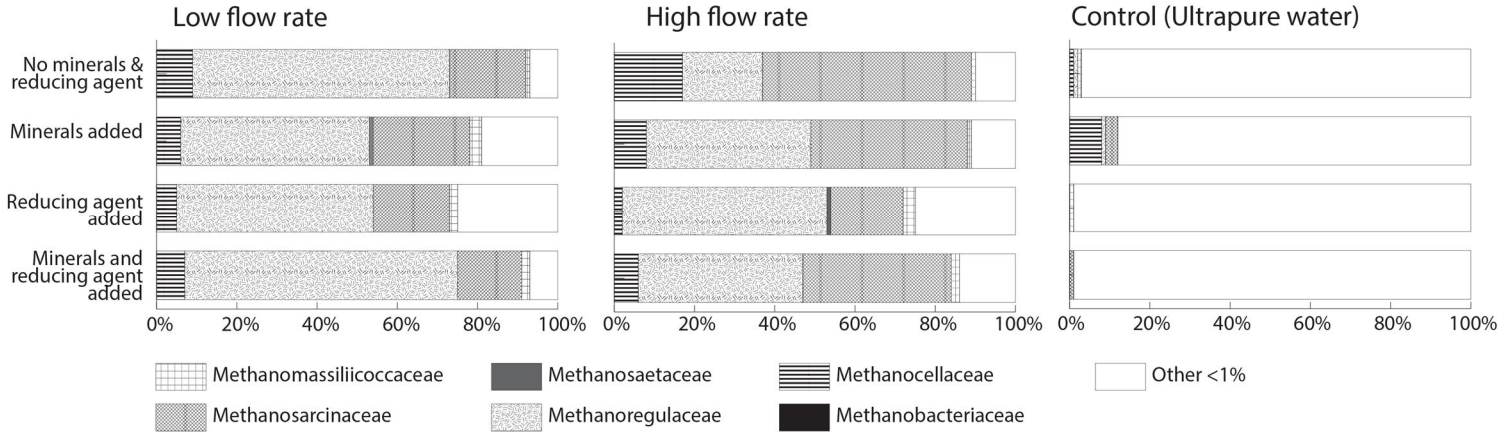


**Figure 3-1.** Phylogenetic classification of archaeal sequences in the cultivation experiments with solution of (a) lignite-$H_2O_2$ at low flow rate, (b) lignite-$H_2O_2$ at high flow rate, and (c) ultrapure water

*3.3.2 Phylogenetic classification of bacterial sequences*

The phylogenetic classification of bacterial sequences is given in Figure 3-2. Bacterial sequences from all cultivation treatments were diverse since about 40% OTUs at a genus level were <1% of relative abundance, mentioned as “other”. They mostly consisted of anaerobic fermentative, acetogenic, and syntrophic bacteria.

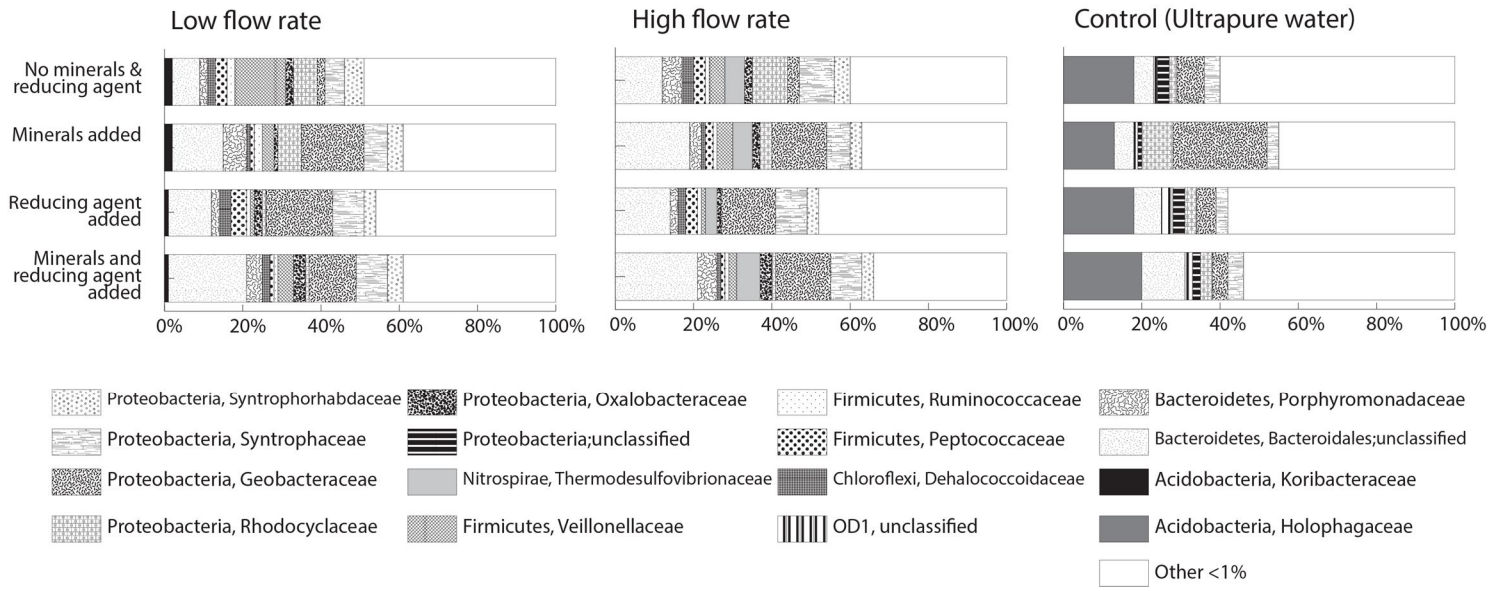


**Figure 3-2.** Phylogenetic classification of bacterial sequences in the cultivation experiments with solution of (a) lignite-$H_2O_2$ at low flow rate, (b) lignite-$H_2O_2$ at high flow rate, and (c) ultrapure water

At a phylum level *Proteobacteria* (29-46% OTUs) followed by *Bacteroidetes* (13-30%) and *Firmicutes* (7.5-15% OTUs) were dominant. At a family level, *Geobacteraceae* and *Syntrophaceae* that belong to phylum *Proteobacteria* dominated the total community with an abundance of 2-17% OTUs and 5-8% OTUs, respectively, followed by *Veillonellaceae* (1-12% OTUs) and *Clostridiaceae* (1 – 2% OTUs) of phylum *Firmicutes*. In the cultivations without nutrients and reducing agent, the family *Rhocyclaceae* (*Dechloromonas*) was identified with relatively high abundance of 6 – 8% OTUs. Meanwhile, the family *Porphyromonadaceae* (*Paludibacter*) and *Spirochaetaceae* (*Treponema*) were also observed with relative abundance of 3 – 6% OTUs and 2% OTUs respectively, in the presence of nutrients-only and a reducing agent-only.

### 3.4. Discussion

The identification of diverse microbial communities (Table 3-1) provided information about the likely pathways involved in the $CH_4$ generation in this study. The microbial consortium native to lignite from the coal-bearing Soya Formation contained a substantial proportion of methanogens (Figure 3-1) that are different from the methanogens found in Yubari coal mine, central Hokkaido (Shimizu et al., 2007). Methanogens of the family *Methanoregulaceae*, associated with a hydrogenotrophic pathway (Oren, 2014a), were found to be the most abundant in this study. Some can utilize $H_2$ and formate (*Methanoregula formicica*; Yashiro et al., 2011); others are strictly $H_2$-utilizing methanogens (*Methanoregula boonei*; Bräuer et al., 2011). The second most abundant methanogen family was *Methanosarcinaceae*, which is a versatile genus of acetate-utilizing methanogens. They are not only capable of cleaving acetate to $CH_4$ and $CO_2$, but also of converting $H_2/CO_2$ to $CH_4$ and growing on methylotrophic substrates (Oren, 2014b; Zinder, 1993).

While the reaction solution of lignite with $H_2O_2$ provided acetates and formates as readily usable substrates for direct methanogenesis, the methanogenic pathways might also be

affected by the activities of bacterial population (Figure 3-2), which was dominated by *Proteobacteria*, *Bacteroidetes*, and *Firmicutes*. These bacteria were commonly found in coal bed consortium (Green et al., 2008; Jones et al., 2008; Robbins et al., 2016; Strapoć et al., 2008). Among these bacterial groups, the family of *Geobacteraceae* was the most abundant enrichment in the cultivations, especially in the presence of nutrients and a reducing agent. They can syntrophically oxidize organic acids and aromatic hydrocarbon to produce $CO_2$ (Coates et al., 1995; Lovley and Lonergan, 1990; Röling, 2014). The second most abundant bacterial group was the family of *Syntrophaceae*, which grows only in the presence of $H_2$/formate utilizing partners in syntrophic association and is capable of oxidizing acetate to $H_2/CO_2$ completely (Kuever, 2014). In contrast, *Clostridiaceae* may act as homoacetogens that are able to produce acetate by consuming $H_2/CO_2$ (Green et al., 2008; Park and Liang, 2016; Zhang et al., 2015). In addition, *Paludibacter*, *Dechloromonas*, and *Treponema*, which were detected from all methanogenic cultures in this study, are fermentative bacteria capable of producing $CO_2/H_2$, formates and acetates from a wide range of organic substrates (Jones et al., 2013; Robbins et al., 2016; Strąpoć et al., 2011; Wang et al., 2017; Zheng et al., 2017).

From the above discussion, the transformation pathways of lignite in the Tempoku coal field via $H_2O_2$ into biogenic $CH_4$ can be summarized in Figure 3-3. During the reaction of lignite with $H_2O_2$, organic substrates such as acetate, formate, and other substrates (e.g., succinic and oxalic acids) are produced from lignite. After $H_2O_2$ decomposition and following pH neutralization, fermentative bacteria converts part of the organic substrates into $H_2/CO_2$ and acetates. Owing to the presence of syntrophic acetate-oxidizing bacteria, some acetates should also be converted into $H_2/CO_2$ (Hattori, 2008). Finally, biogenic $CH_4$ is possibly generated from these acetates, $H_2/CO_2$, and formates by the hydrogenotrophic, aceticlastic, and formate-utilizing methanogenesis pathways (Ehrlich and Newman, 2009; Zinder, 1993).

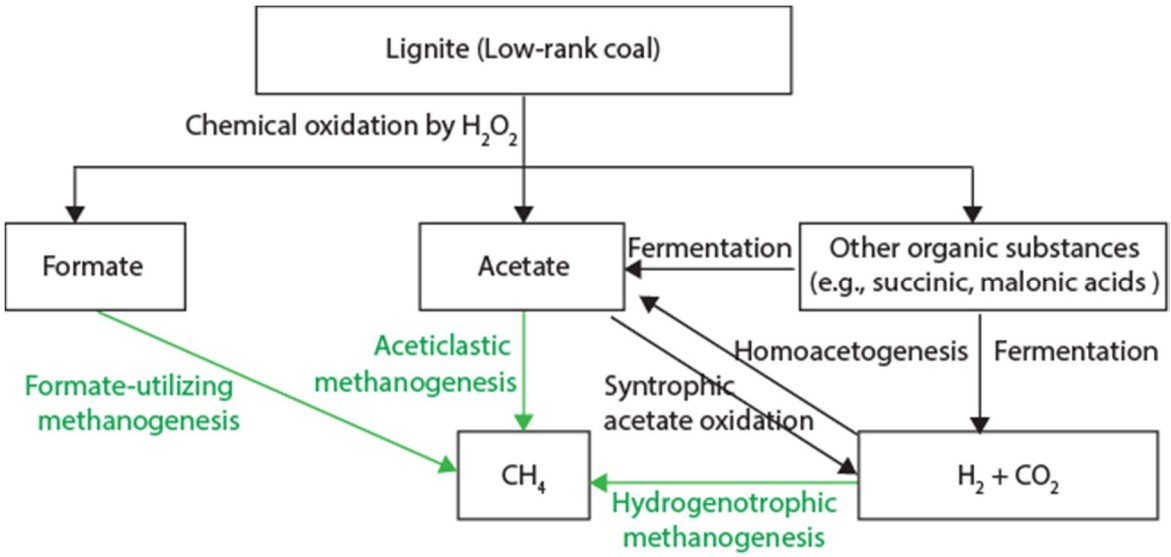


**Figure 3-3.** Possible methanogenic pathways in the cultivation experiments based on archaeal and bacterial cultures with lignite-$H_2O_2$ reaction solution

### 3.5 Conclusion

Microbial consortia associated with lignite from the Soya Formation could utilize solubilized organic substances resulting from the lignite and $H_2O_2$ to produce biogenic $CH_4$. This methanogenic process possibly involves multiple pathways; hydrogenotrophic, aceticlastic, and formates-utilizing methanogenesis, partly following bacterial activities of fermentations, homoacetogenesis, and syntrophic acetate oxidation.

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# Chapter 4

# BIOAVAILABILITY OF LIGNITE AFTER HYDROGEN PEROXIDE TREATMENT

## 4.1 Introduction

The bioavailability of lignite after hydrogen peroxide ($H_2O_2$) treatment is of interest for successful Subsurface Cultivation and Gasification (SCG) since it would optimize the biogenic $CH_4$ production from the reaction solution of lignite with $H_2O_2$. The bioavailability enhancement involves breaking down organic matter (e.g., coal and lignite) into methanogenic intermediates, and is considered a rate limiting step in methanogenesis (Colosimo et al., 2016; Scott, 1999; Strąpoć et al., 2011). Various methods including physical, chemical, or biological pre-treatments can be employed to enhance the bioavailability, otherwise effective microbially enhanced coal bed methane (MECBM) generation will remain difficult (Papendick et al., 2011). Laboratory experiments have suggested the addition of surfactant (Green et al., 2008; Papendick et al., 2011), oxidants (Huang et al., 2013a, 2013b), acids (Huang et al., 2013a), bases (Huang et al., 2013b), and chelating agents (Bumpus et al., 1998) to increase the solubilization of coal. In case of subbituminous coal from Powder River Basin, it has also been suggested that potassium permanganate ($KMnO_4$) is more effective agent than $H_2O_2$ and nitric acid ($HNO_3$), or sodium hydroxide (NaOH) to solubilize and produce bioavailable organics (Huang et al., 2013a). On the other hand, $H_2O_2$ can substantially solubilizes lignite (Allard and Derenne, 2007; Doskočil et al., 2014; Mae et al., 2001; Miura et al., 1997; Tamamura et al., 2016) to produce organic acids (Mae et al., 2001; Miura et al., 1997; Tamamura et al., 2016).

Arctech Inc. (1990) and Jones et al., (2013) showed that the rate of microbial conversion of coal into biogenic methane was higher for $H_2O_2$-treated coal than for untreated coal. Furthermore, Kaneko (2016) observed an increase in organic acid concentration in a

water solution in which $H_2O_2$-treated lignite (referred to as lignite-$H_2O_2$) was immersed. These observations implied the presence of reactive intermediates in the lignite-$H_2O_2$, which subsequently yielded organic acids in pure water. Therefore, this chapter confirmed the enhanced bioavailability in the effluent from lignite-$H_2O_2$ under column experiments compared to untreated lignite (referred to as lignite-original), and discussed the implications for *in situ* biogenic methane production.

## 4.2 Materials and methodology

### *4.2.1 Lignite*

A lignite sample was collected from the middle Miocene coal-bearing Soya Formation in the Tempoku Coalfield in Horonobe, northern Hokkaido, Japan, as mentioned in chapter 2. The sample was prepared in two parts: lignite-original and lignite-$H_2O_2$. Both samples were dried and ground to obtain a 0.5–1 mm diameter size fraction. To make lignite-$H_2O_2$, lignite-original (50 g) was immersed in 750 mL of 3% $H_2O_2$ at room temperature (22°C) until the organic acid concentrations in the reaction solution were stabilized (Figure 4.1).

The concentration of $H_2O_2$ used in this study was fixed at 3%. At the ratio of lignite to $H_2O_2$ used in this study, a higher concentration of $H_2O_2$ would result in over-oxidation of the lignite, while a lower concentration would result in negligible oxidation. Following $H_2O_2$ treatment, the lignite solids were separated from the solution, rinsed with ultrapure water to remove residual dissolved organics and $H_2O_2$, and then dried.

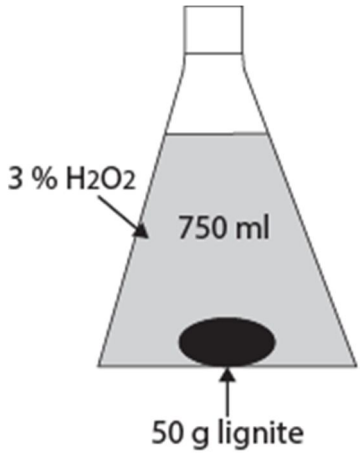


**Figure 4-1**. Experimental setup for the lignite-$H_2O_2$ preparation

To ensure that the lignite solids were rinsed completely, the 3% $H_2O_2$ solution (750 mL) was spiked with 1 mL of concentrated NaCl solution immediately after the reaction with lignite. After discarding the supernatant, the lignite was mounted on a filter paper in a funnel and rinsed with pure water repeatedly. Rinsing continued until the leached amount (mg) of chloride ion ($Cl^-$) from the lignite-$H_2O_2$ was two orders of magnitude less than the amount of initial $Cl^-$ (Table 4-1). The lignite-$H_2O_2$ was then dried in an oven at 50°C for subsequent experiments.

Table 4-1. Details of the lignite-$H_2O_2$ rinse solution

| Compound | Original leachate | | Leachate in the $1_{st}$ rinse solution | | Leachate in the $2_{nd}$ rinse solution | | Leachate in the $3_{rd}$ solution | |
|---|---|---|---|---|---|---|---|---|
| | No. 1 | No. 2 | No. 1 | No. 2 | No. 1 | No. 2 | No. 1 | No. 2 |
| Acetic acid [mg] | 232 | 235 | 13.4 | 16.9 | 2.03 | 1.53 | 0.765 | 0.72 |
| Formic acid [mg] | 594 | 606 | 25.5 | 33.8 | 3.17 | 2.35 | 1.31 | 1.23 |
| $Cl^-$ [mg] | 6.63 | 6.64 | 1.17 | 1.18 | 0.32 | 0.31 | 0.06 | 0.05 |
| $SO_4^{2-}$ [mg] | 57.7 | 57.7 | 2.70 | 3.04 | 0.271 | 0.26 | 0.16 | 0.16 |
| Malonic acid [mg] | 227 | 224 | 11.9 | 14.1 | 2.37 | 2.01 | 0.93 | 0.92 |
| Succinic acid [mg] | 163 | 152 | 4.64 | 5.90 | 2.03 | 1.64 | 0.61 | 0.59 |
| Oxalic acid [mg] | 703 | 709 | 38.8 | 46.3 | 9.50 | 8.39 | 4.89 | 4.93 |
| TOC [mg] | 1460 | 1480 | 85.7 | 94.5 | 25.2 | 22.9 | 15.4 | 15.6 |
| TN [mg] | 47.1 | 48.5 | 3.23 | 3.78 | 0.71 | 1.07 | 0.49 | 0.46 |

*4.2.2. Column experiments*

To confirm the enhanced solubility and the possible generation of organic acids after $H_2O_2$ treatment, the lignite-$H_2O_2$ was placed in a column (inner diameter of 1.1 cm and length of 30 cm) through which ultrapure water was flowed at a constant rate of 100 mL/day for 18 days (Figure 4-2).

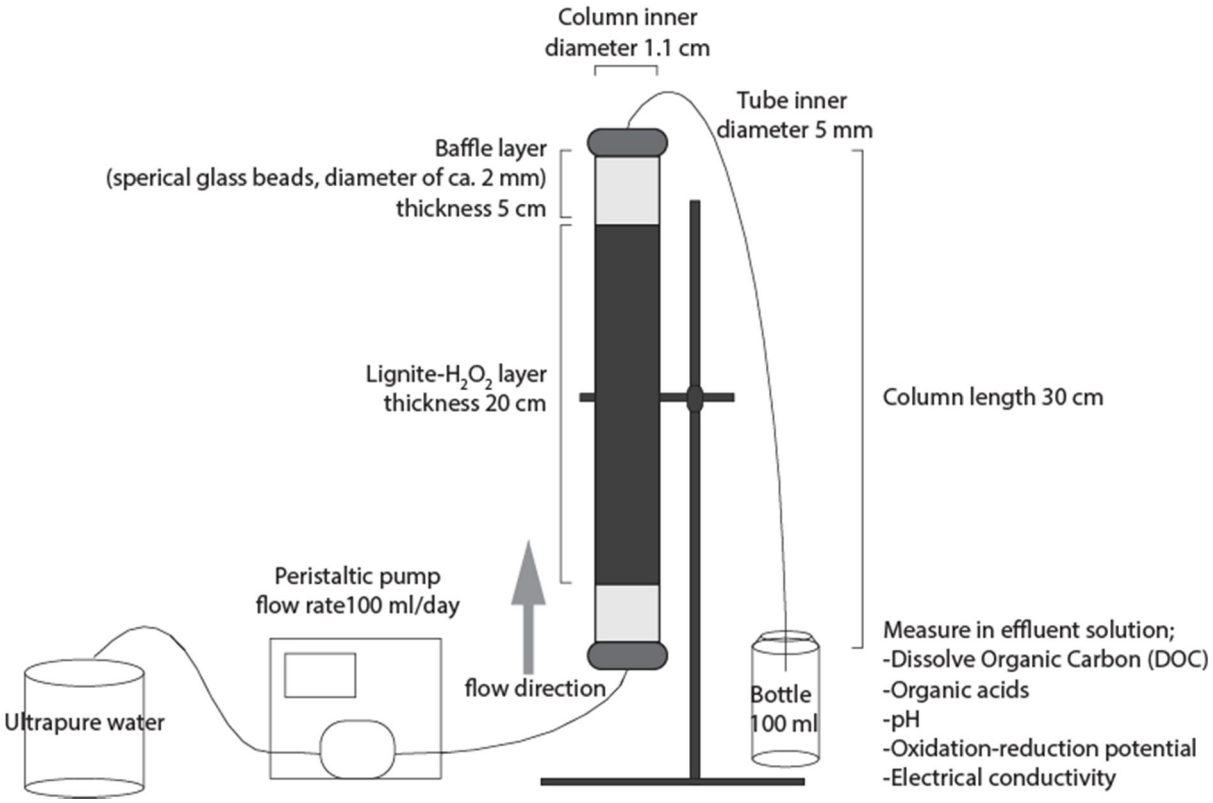


**Figure 4-2.** Setup of the column experiment for lignite-$H_2O_2$

The column system was selected because it simulates the *in situ* conditions of natural coal more closely than batch experiments. Here, up-flow mode (i.e., pumping water from the bottom to the top) was applied to achieve saturated conditions. An advantage of the up-flow mode in column experiments is that as the solution moves from the bottom to the top, it displaces the air in the lignite pores. Hence, saturated conditions are better achieved than downflow mode (Gilbert et al., 2014). The lignite-$H_2O_2$ (17-18 g) was packed between baffle layers composed of spherical glass beads with a diameter of 2 mm. The dissolved organic

carbon (DOC) and organic acid concentrations in the effluent were measured periodically. Collection of the effluent continued until the DOC and organic acid concentrations were stable. As a control, a similar column experiment was performed using the lignite-original sample. These columns were duplicated to evaluate data reproducibility.

*4.2.3 Analysis*

The DOC concentrations of the sample solutions were determined using a Shimadzu TOC-$V_{CHS}$ TOC analyzer. The total carbon (TC) and inorganic carbon (IC) contents of the solid samples were determined using the same device equipped with a SSM-5000A solid sample combustion unit. The TOC content of the solid samples was calculated from the difference between the TC and IC contents (TOC = TC – IC). The relative standard deviation for DOC was 0.23% ($n$ = 5) as determined by a standard solution (potassium hydrogen phthalate at 10 mgC/L). The standard deviation for solid TC was 0.66% ($n$ = 7) relative to a standard sedimentary sample (LKSD-2, Canadian Certified Reference Materials Project), and that of solid IC analysis was less than 1% ($n$ = 4) relative to calcium carbonate (Wako Chemicals, Japan).

**4.3 Results**

*4.3.1 Dissolved Organic Carbon (DOC) concentrations*

The lignite-$H_2O_2$ sample reached a higher dissolved organic carbon (DOC) concentration than the lignite-original sample (Figure 4-3). The DOC increased rapidly until the maximum of 84.8 mg/L was reached after two days of flow. Then, it decreased gradually and eventually stabilized at 30 mg/L after 11 days. The fact that the stabilized DOC concentration of lignite-$H_2O_2$ was higher than that of lignite-original (<0.59 mg/L) indicates that the solubility of lignite-$H_2O_2$ is greater than that of lignite-original.

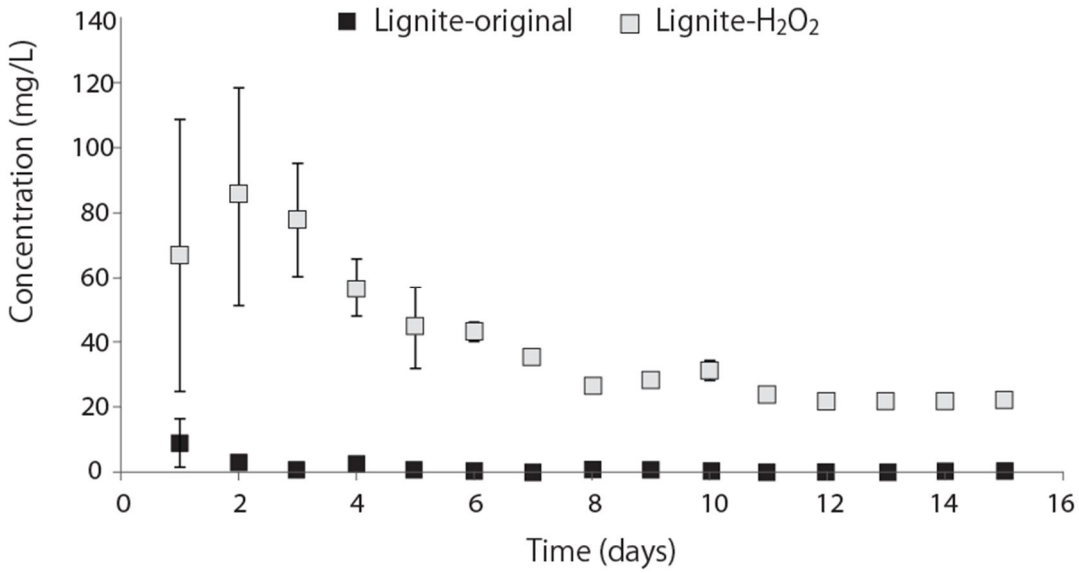


**Figure 4-3.** DOC concentration of lignite before and after $H_2O_2$ treatment in the column experiment

*4.3.2 Organic acid concentrations*

The enhanced bioavailability is also indicated by higher organic acid (i.e., acetic acid and formic acid) concentrations in the effluent solution of lignite-$H_2O_2$ than in that of lignite-original (Figure 4-4). At the start of the experiment, the acetic and formic acids concentrations of the lignite-$H_2O_2$ treatment were 18.9 and 19.9 mg/L, respectively. A dramatic decrease in concentration was observed after six days (<2.45 mg/L), and after 12 days the organic acid concentrations recorded from lignite-$H_2O_2$ and lignite-original were identical (<0.05 mg/L).

After 6 days of the column experiment, the concentrations of organic acids from lignite-$H_2O_2$ were low (<2.45 mg/L; Figure 4-4) and similar to those of the control solution, indicating exhaustion of the peroxy acid structures. However, the concentration of DOC remained relatively high (20 mg/L; Figure 4-3). At this stage, lignite-$H_2O_2$ may release the remaining alkali-soluble carbon.

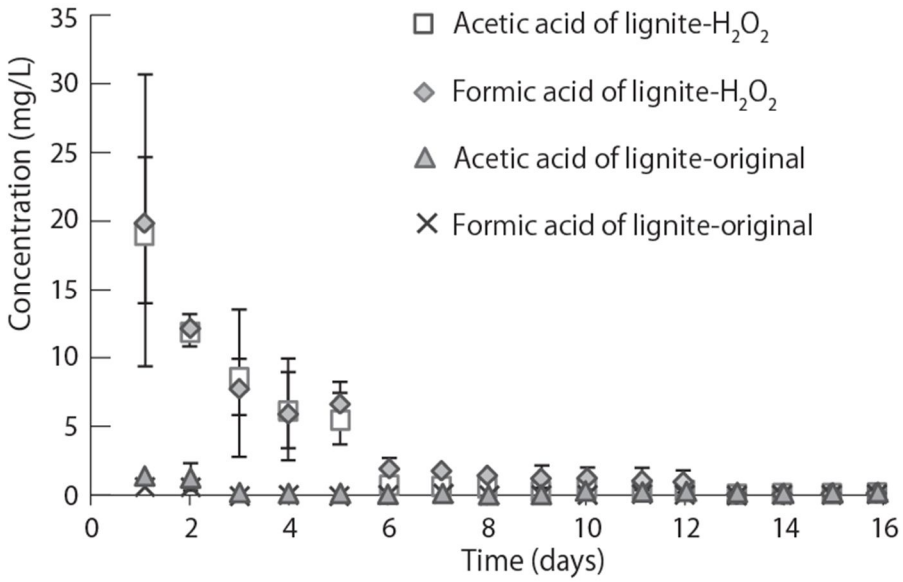


**Figure 4-4.** Organic acid concentrations before and after $H_2O_2$ treatment in the column experiment

### 4.4 Discussion

The column experiments indeed showed higher DOC concentrations (Figure 4-3), suggesting enhanced solubility in the effluent from lignite-$H_2O_2$ compared with lignite-original. The increased bioavailability of lignite-$H_2O_2$ was also confirmed by the increased concentrations of organic acids in the effluent solution (Figure 4-4). The DOC and organic acids are unlikely to be derived from leaching of the primary solution in the lignite pores. This is indicated by the higher ratio of DOC/$Cl^-$ (397 mg/mg; Table 4-2) in the effluent solution of the column experiment than that of the $H_2O_2$ rinse solution before the column experiment (275 – 290 mg/mg; Table 4-3). In fact, the $Cl^-$ concentration of the lignite-$H_2O_2$ effluent was low (<0.20 mg/L) and similar to that of the control solution.

Table 4-2. Normalized concentrations with respect to $Cl^-$ after lignite-$H_2O_2$ rinse

| Compound | Original leachate [mg/mg] | | Leachate in the $1_{st}$ rinse solution [mg/mg] | | Leachate in the $2_{nd}$ solution [mg/mg] | | Leachate in the $3_{rd}$ solution [mg/mg] | |
|---|---|---|---|---|---|---|---|---|
| | No. 1 | No. 2 | No. 1 | No. 2 | No. 1 | No. 2 | No. 1 | No. 2 |
| Acetic acid/Cl | 35.1 | 35.4 | 11.5 | 14.4 | 6.46 | 4.98 | 13.76 | 13.4 |
| Formic acid/Cl | 89.7 | 91.4 | 21.8 | 28.8 | 10.1 | 7.65 | 23.6 | 22.8 |
| $SO_4^{2-}$/Cl | 8.70 | 8.69 | 2.32 | 2.59 | 0.86 | 0.84 | 2.79 | 2.91 |
| Malonic acid/Cl | 34.2 | 33.7 | 10.3 | 12 | 7.53 | 6.53 | 16.7 | 17.1 |
| Succinic acid/Cl | 24.6 | 22.9 | 3.97 | 5.02 | 6.46 | 5.35 | 10.8 | 11.1 |
| Oxalic acid/Cl | 106 | 106 | 33.2 | 39.3 | 30.2 | 27.4 | 87.8 | 91.8 |
| DOC/Cl | 219 | 222 | 73.3 | 80.3 | 80.1 | 74.7 | 275 | 290 |
| TN/Cl | 7.09 | 7.30 | 2.77 | 3.21 | 2.26 | 3.48 | 8.81 | 8.62 |

Table 4-3. Normalized concentration with respect to $Cl^-$ in the effluent solution of lignite-$H_2O_2$ under column experiment

| Day | Acetic acid [mg/mg] | Formic acid [mg/mg] | DOC [mg/mg] |
|---|---|---|---|
| 1 | 135 | 168 | 397 |
| 2 | 60.2 | 72.5 | 652 |
| 3 | 16.2 | 24.3 | 332 |
| 4 | 12.3 | 19.1 | 264 |
| 5 | 17.5 | 28.6 | 175 |
| 6 | 11.7 | 19.4 | 222 |
| 7 | 7.65 | 11.9 | 183 |
| 8 | 5.97 | 1.36 | 159 |
| 9 | 0.15 | 0.64 | 154 |
| 10 | 0.312 | 0.759 | 155 |

The column experiments in this study demonstrated enhanced solubility of lignite after $H_2O_2$ reaction, as well as the production of organic acids (e.g., acetic acid and formic acid). Theoretically, methanogens consuming the acetic and formic acids produced in the column experiments could create 0.13 $m^3$ of $CH_4$ (25°C, 1 atm basis) per ton of lignite-$H_2O_2$ (4.87 μmol $CH_4$/g) via Equations 1 and 2 (Ehrlich and Newman, 2009; Zinder, 1993).

$$CH_3COOH \rightarrow CO_2 + CH_4 \quad (1)$$

$$4HCOOH \rightarrow CH_4 + 3CO_2 + 2H_2O \quad (2)$$

This production is lower compared with $CH_4$ yields from coal reported from laboratory coal-to-methane cultures (0.24–320 μmol $CH_4$/g coal; Zheng et al., 2017). However, the enhance bioavailability of lignite-$H_2O_2$ would contribute to the biogenic $CH_4$ yield from the reaction solution of lignite with $H_2O_2$ (Chapter 2), thereby encouraging the field application of SCG.

### 4.5 Conclusion

In the column experiments, lignite treated with $H_2O_2$ showed higher DOC (up to 84.8 mg/L) and organic acid (up to 18.9 mg/L for acetic acid and up to 19.9 mg/L for formic acid) concentrations than lignite without treatment. These findings indicate the great potential to optimize $CH_4$ production from the MECBM operation using $H_2O_2$.

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# Chapter 5

# REACTION MECHANISMS OF ENHANCED LIGNITE BIOAVAILABILITY AFTER HYDROGEN PEROXIDE TREATMENT

## 5.1 Introduction

As confirmed in Chapter 4, the lignite after hydrogen peroxide ($H_2O_2$) treatment (lignite-$H_2O_2$) was still bioavailable. The reaction with $H_2O_2$ may generate hydrophilic functional groups in the lignite structure, especially O-bearing groups (e.g., hydroxyl, ketone, and carboxyl groups), which are more readily utilized by microbes for methanogenesis (Strapoć et al., 2008). Therefore, the enhanced bioavailability of the lignite-$H_2O_2$ could reflect increased O-bearing functional groups in the coal structure. However, the effect of reaction with $H_2O_2$ on the content of O-bearing functional groups in lignite has not been elucidated, and the mechanisms that underlie the increase in bioavailability remains unknown.

The reaction between $H_2O_2$ and coal has been widely investigated; yet the reaction mechanisms remain uncertain. The general agreement is that the reaction generates radicals in the coal matrix either through the Fenton reaction (Doskočil et al., 2014; Huang et al., 2013; Miura et al., 1997) or quinone reaction (Nohl and Jordan, 1987; Tamamura et al., 2016). The radicals then gradually degrade the coal substances by breaking them down into progressively smaller molecule species (Berkowitz, 1979; Martínez and Escobar, 1995). Simultaneously, the radicals may propagate a reaction to generate organic acids (e.g., acetic and formic acids) (Clemens et al., 1991; Fossey et al., 1995; Parsons, 2000; Wang et al., 2003).

Humic substances are an essential part of natural organic matter (Jackson et al., 1996). They can be divided into fulvic acid carbon (FA) that is soluble at any pH, humic acid carbon (HA) that is insoluble at pH < 1, and humin carbon that is insoluble under all pH conditions

(Kononova, 1966; Stevenson, 1994). HA and FA are regenerated in response to the natural oxidative weathering of sedimentary organic matter (Estévez et al., 1990; Klika and Kraussová, 1993; Kurková et al., 2004; Tamamura et al., 2015). However, it is not certain whether $H_2O_2$ treatment regenerates HA and FA with or without structural modifications.

The objectives of this chapter are to characterize the composition and structure of each humic fraction of lignite before and after $H_2O_2$ treatment. Each humic fraction is investigated because the humic composition of lignite is probably sensitive to $H_2O_2$ treatment, and more information is obtained in this way than by simply analyzing the bulk lignite. The properties of the humic substances are used to develop reaction mechanisms for the enhanced bioavailability of the lignite-$H_2O_2$ that was obtained from column experiments in Chapter 4.

## 5.2 Materials and methodology

### *5.2.1 Lignite*

A lignite sample was collected from the middle Miocene coal-bearing Soya Formation in the Tempoku Coalfield in Horonobe, northern Hokkaido, Japan, and was prepared in two parts: original lignite (referred to as lignite-original) and lignite-$H_2O_2$ as explained in Chapter 4. The different part was that the both lignite samples were prepared to particle size <106 µm.

### *5.2.2 Extraction experiments*

The humic substances were extracted from both the lignite-original and lignite-$H_2O_2$ samples following the method of Tamamura et al., (2015) (Figure 5.1). The steps of this fractionation are outlined below.

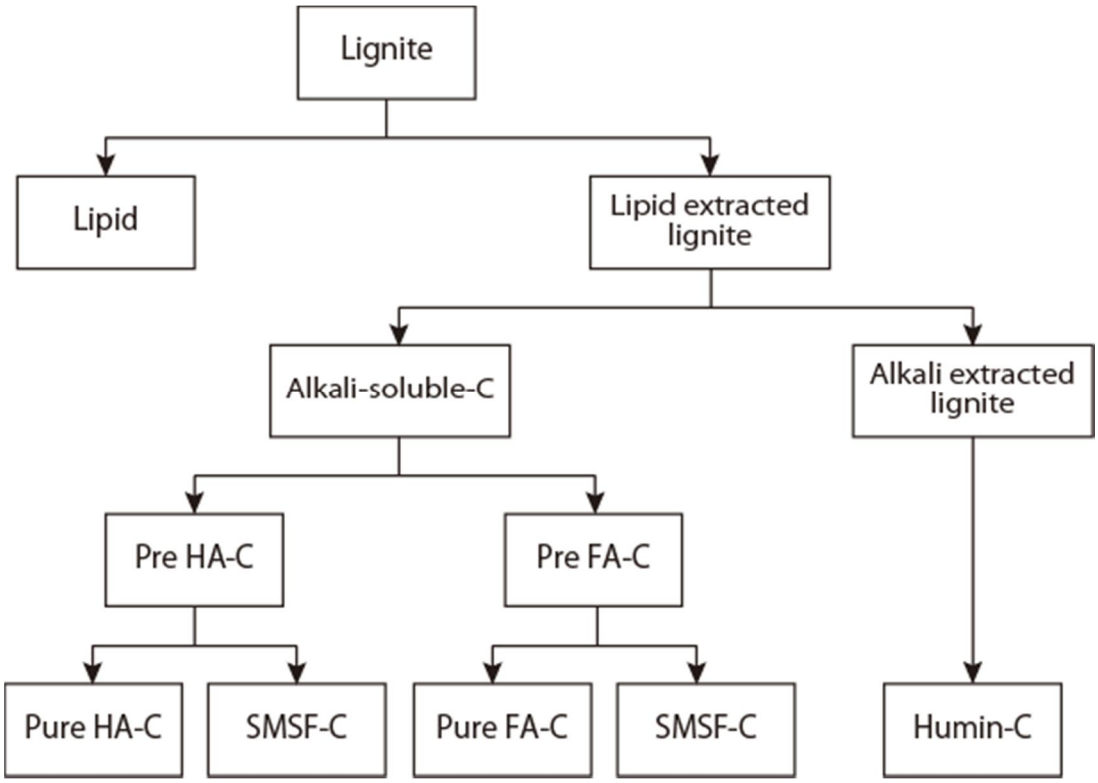


**Figure 5-1.** Humic fractionation of lignite

*Lipid isolation*

The lignite sample (25 g) was mixed with benzene and methanol (3:2 solution ratio) and ultrasonically treated for 20 min at 25°C in combination with manual stirring with a glass rod. The lipid was in solution and was separated from the residue (i.e., lipid-extracted lignite) by vacuum filtration (1 µm filter paper). The residue was re-extracted under the same conditions. The mixtures (first and second extractions) were filtered again (0.50 µm PTFE filter), and the solvents were evaporated in a beaker in a vacuum. The remaining residue in the beakers was weighed as the lipid. The lipid-extracted lignite was dried at 70°C for the subsequent alkali extraction.

*Humic acid isolation*

The lipid-extracted lignite (20 g) was mixed with 250 mL of 0.5 M NaOH and shaken for 24 hours at 25°C using a reciprocal shaker. The alkali-soluble carbon (solvents) and alkali-extracted lignite (residue) were separated via centrifugation (9,500 rpm for 10 minutes).

This process was repeated 5 times until the supernatant became pale in color. The final residue was stored as pre-humin.

The alkali-soluble carbon was acidified to pH 1.0 with 6 M HCl. The precipitated pre-humic acid (pre-HA) was centrifuged (3,000 rpm, 15 min) to separate the pre-HA from the supernatant, and the pre-HA was dialyzed (molecular weight cutoff (MWCO) 1,000 Da membrane) with ultrapure water until $Cl^-$ was undetected by the AgCl test in the outer solution of the dialysis membrane. The humic acid in the membrane was freeze-dried as purified humic acid (HA).

*Fulvic acid isolation*

The supernatant of the acidified alkali-soluble carbon was filtered (0.50 μm PTFE filter) and collected as the pre-fulvic acid (pre-FA) solution. After neutralization of the pre-FA solution by 1–2 M NaOH, the pre-FA solution was freeze-dried to reduce the volume (2–3 L) of the pre-FA solution. The pre-FA was re-extracted from the freeze-dried powder with a much smaller volume (200 mL) of 1 M NaOH, and this pre-FA solution was dialyzed (MWCO 1,000 Da) with 0.01 M NaOH to maintain Si in a dissolved state. After the Si concentration in the outer solution of the membrane was reduced to < 1.0 mg/L, the outer solution was changed to ultrapure water. This second round of dialysis continued until the Si concentration was < 0.01 mg/L. The remaining fulvic acid was freeze-dried as purified fulvic acid (FA).

*Humin isolation*

The pre-humin (10 g), obtained from the alkali extraction process, was treated with a solution of 92 mL of 46% hydrofluoric (HF) and 8.3 mL of 12 M hydrochloric (HCl) acid at 80°C for 1 hour to decompose contaminated silicates. After centrifugation (2,500 rpm, 10 min), the pre-humin residue was reacted with 100 mL of 1 M HCl at 60°C for 2 hours to remove fluoride contaminants. The solution was then centrifuged, and the residue was reacted

again with the same volume of 1 M HCl at 60°C for 2 hours. After further centrifugation, the pre-humin was dialyzed (MWCO 1,000 Da) with ultrapure water until the pH of the outer solution rose above 4. The remaining humin in the membrane was freeze-dried as purified humin.

*SMSF quantification*

The small molecule size fraction (SMSF) is defined as the organic molecules to which the dialysis membrane was permeable (i.e., <1,000 Da) in the pre-HA and pre-FA dialyses (Figure 5-1). This fraction corresponds to the small molecule size fraction (<1,000 Da) in the alkali-soluble carbon from the lignite (Figure 5-1), and is quantified by measuring the TOC concentration of the outer membrane solution in the pre-HA and pre-FA dialyses.

*5.2.3. Analysis*

The dissolved organic carbon (DOC) concentrations of the sample solutions were determined using a Shimadzu TOC-$V_{CHS}$ TOC analyzer. The total carbon (TC) and inorganic carbon (IC) contents of the solid samples were determined using the same device equipped with a SSM-5000A solid sample combustion unit. The TOC content of the solid samples was calculated from the difference between the TC and IC contents (TOC = TC – IC). The relative standard deviation for DOC was 0.23% ($n$ = 5) as determined by a standard solution (potassium hydrogen phthalate at 10 mgC/L). The standard deviation for solid TC was 0.66% ($n$ = 7) relative to a standard sedimentary sample (LKSD-2, Canadian Certified Reference Materials Project), and that of solid IC analysis was less than 1% ($n$ = 4) relative to calcium carbonate (Wako Chemicals, Japan).

The elemental compositions of the lignite, humin, HA, and FA before and after $H_2O_2$ treatment were determined with a Vario EL III (Elementar, Germany) elemental analyzer. The relative standard deviations for C, H, and O analyses were typically less than 1.0%, 2.7%, and 1.5%, respectively. The $^{13}C$ cross-polarization–magnetic angle spinning (CP–MAS)

nuclear magnetic resonance (NMR) analysis of lignite, purified humin, HA, and FA was performed using a JNM-GX270 (JEOL, Japan) spectrometer at 67.8 MHz. A contact time of 1 ms cross-polarization (CP) was utilized for all samples. The magnetic angle spinning frequency was set at 11 kHz. The number of scans was 10,000–25,000 with a repetition delay of 2 s. The $^{13}C$ chemical shift was calibrated externally with hexamethylbenzene (17.4 ppm). The relative standard deviation of the peak area of the hexamethylbenzene was less than 4.8% ($n$ = 12). The ratio of alkyl-C (0–45 δ), O-alkyl-C (45–110 δ), aromatic-C (110–160 δ), and carbonyl-C (160–190 δ) in the samples was evaluated from the ratio of the corresponding peak area of the $^{13}C$ NMR spectrum.

Fourier transform infrared (FTIR) spectra of the lignite, purified humin, HA, and FA were measured using a Nicolet iS5 spectrometer (Thermo Scientific) equipped with an iD5 attenuated total reflection (ATR) probe with a Ge crystal (refraction index = 4.0). The sample absorbance was recorded between 4,000 and 650 $cm^{-1}$ using 20 scans at a resolution of 4 $cm^{-1}$. The spectra were corrected by the "advanced ATR correction" function of the spectral analysis software (OMNIC, Thermo Scientific), under the assumption that the samples had a refractive index of 1.5.

## 5.3. Results

### *5.3.1. Humic substance composition*

Humin, lipid, alkali-soluble, HA, FA, and SMSF carbon were extracted in duplicate from both lignite-original and lignite-$H_2O_2$. A high degree of reproducibility was confirmed by relative standard deviations of less than 5.0% in the amounts of each component extracted from both lignite samples. The only exception was the relative standard deviation of 11.5% for HA extracted from the lignite-original sample.

The contents and fractions of humin, lipid, and alkali-soluble carbon are presented in Figures 5-2 and 5-3, respectively. After $H_2O_2$ treatment, the alkali-soluble carbon content

increased from 433 to 2,130 mg, its fraction increasing from 3.37% to 16.2%. The lipid content in lignite-$H_2O_2$ increased by ~1.5 times relative to lignite-original, from 382 to 563 mg C. Conversely, the humin content decreased from 12,000 to 10,400 mg C, with its fraction dropping from 93.7% to 79.5%.

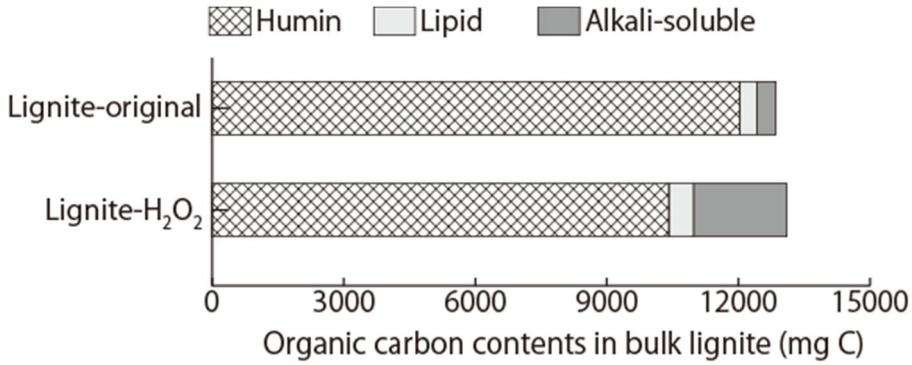


**Figure 5-2.** Humin, lipid, and alkali-soluble carbon contents (mg C) in 25 g of the bulk lignite samples. Lignite-original had 12.9 g of C, and lignite-$H_2O_2$ had 13.1 g of C.

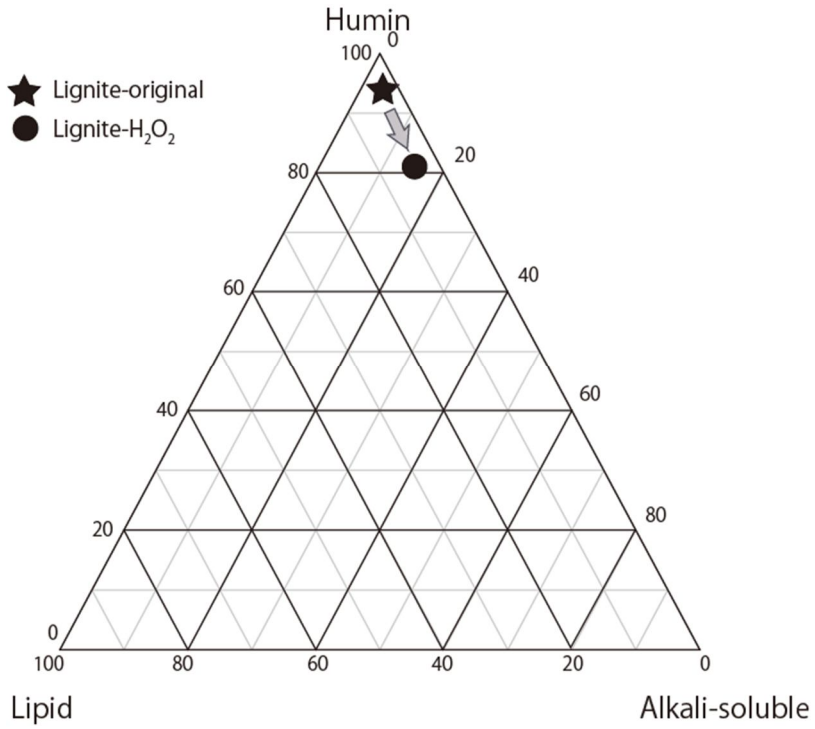


**Figure 5-3.** Fractions of humin, lipid, and alkali-soluble carbon in the bulk lignite samples

The contents and fractions of HA, FA, and SMSF carbon are presented in Figures 5-4 and 5-5, respectively. After $H_2O_2$ treatment, the HA content of the alkali-soluble carbon fraction increased from 198 to 1,530 mg C, with its fraction rising from 48.1% to 71.7%. The

FA and SMSF carbon contents also increased, from 66.2 to 129 mg C and from 169 to 469 mg C, respectively. However, in this case, both fractions decreased, with a drop from 14.4% to 6.0% for FA and 37.5% to 22.33% for SMSF. The ratio of HA to FA also increased after $H_2O_2$ treatment, from 3.0 in lignite-original to 11.9 in lignite-$H_2O_2$.

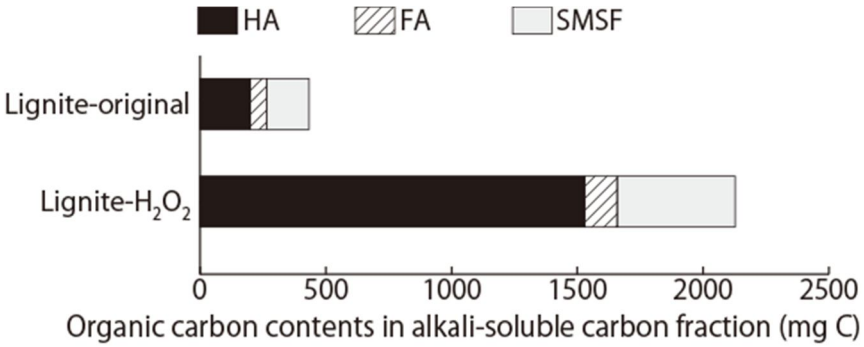


**Figure 5-4.** Contents of humic acid (HA), fulvic acid (FA), and the small molecule size fraction (SMSF) in the alkali-soluble carbon fraction of both lignite samples. The lignite-original (25 g) contained 0.4 g of alkali-soluble carbon, and the same amount of lignite-$H_2O_2$ contained 2.1 g.

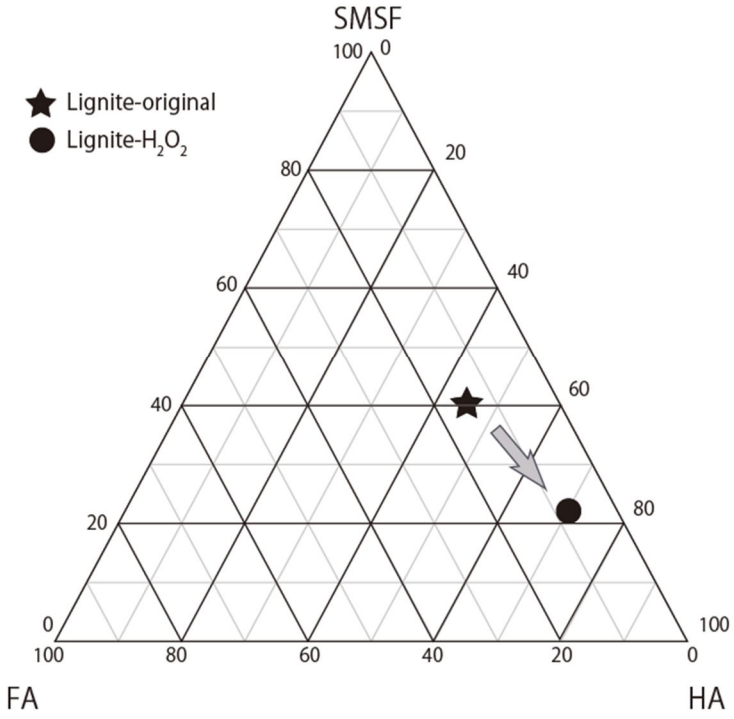


**Figure 5-5.** Fractions of humic acid (HA), fulvic acid (FA), and the small molecule size fraction (SMSF) in the alkali-soluble carbon fraction in both lignite samples

*5.3.2. Elemental analysis*

Table 5-1 lists the elemental compositions of the bulk lignite and its humic fractions (HA, FA, and humin) before and after $H_2O_2$ treatment. After $H_2O_2$ treatment, the H/C and O/C ratios of the lignite samples increased from 0.96 to 0.98 and 0.45 to 0.48, respectively. In contrast, a decrease in the H/C ratio from 1.01 to 0.94, and in the O/C ratio from 0.66 to 0.54, was observed for HA. In FA, the H/C ratio decreased from 1.08 to 1.05, while the O/C ratio increased from 0.82 to 0.93. In humin, the H/C ratio decreased from 0.91 to 0.88 while the O/C ratio remained constant at 0.39.

**Table 5-1.** Elemental compositions of lignite and its humic fractions before and after $H_2O_2$ treatment

| | **Sample** | **C** | **H** | **O** | **N** | **H/C** | **O/C** |
|---|---|---|---|---|---|---|---|
| | | **wt %** | | | | | |
| Before $H_2O_2$ (lignite-original) | Lignite | 52.9 | 4.25 | 31.7 | 1.08 | 0.96 | 0.45 |
| | HA | 44.3 | 3.73 | 38.7 | 1.10 | 1.01 | 0.66 |
| | FA | 37.9 | 3.42 | 41.5 | 0.90 | 1.08 | 0.82 |
| | Humin | 60.3 | 4.57 | 31.0 | 1.25 | 0.91 | 0.39 |
| After $H_2O_2$ (lignite-$H_2O_2$) | Lignite | 54.3 | 4.44 | 34.5 | 1.13 | 0.98 | 0.48 |
| | HA | 50.3 | 3.93 | 37.8 | 1.27 | 0.94 | 0.56 |
| | FA | 34.2 | 2.99 | 42.6 | 0.75 | 1.05 | 0.93 |
| | Humin | 60.2 | 4.43 | 31.7 | 1.25 | 0.88 | 0.39 |

*5.3.3. Carbon structural characteristics*

The structural composition of lignite and its humic fractions can be described in terms of alkyl-C, aromatic-C, carbonyl-C, and O-alkyl-C contents, as determined by $^{13}C$ NMR analysis. The changes in the structural composition of lignite and its fractions before and after $H_2O_2$ treatment in terms of absolute amounts of each functional group are shown in Figure 5-6. The carbon content of the “lipid + SMSF” fraction was determined by subtracting the sum of carbon from humin, HA, and FA from the bulk lignite carbon.

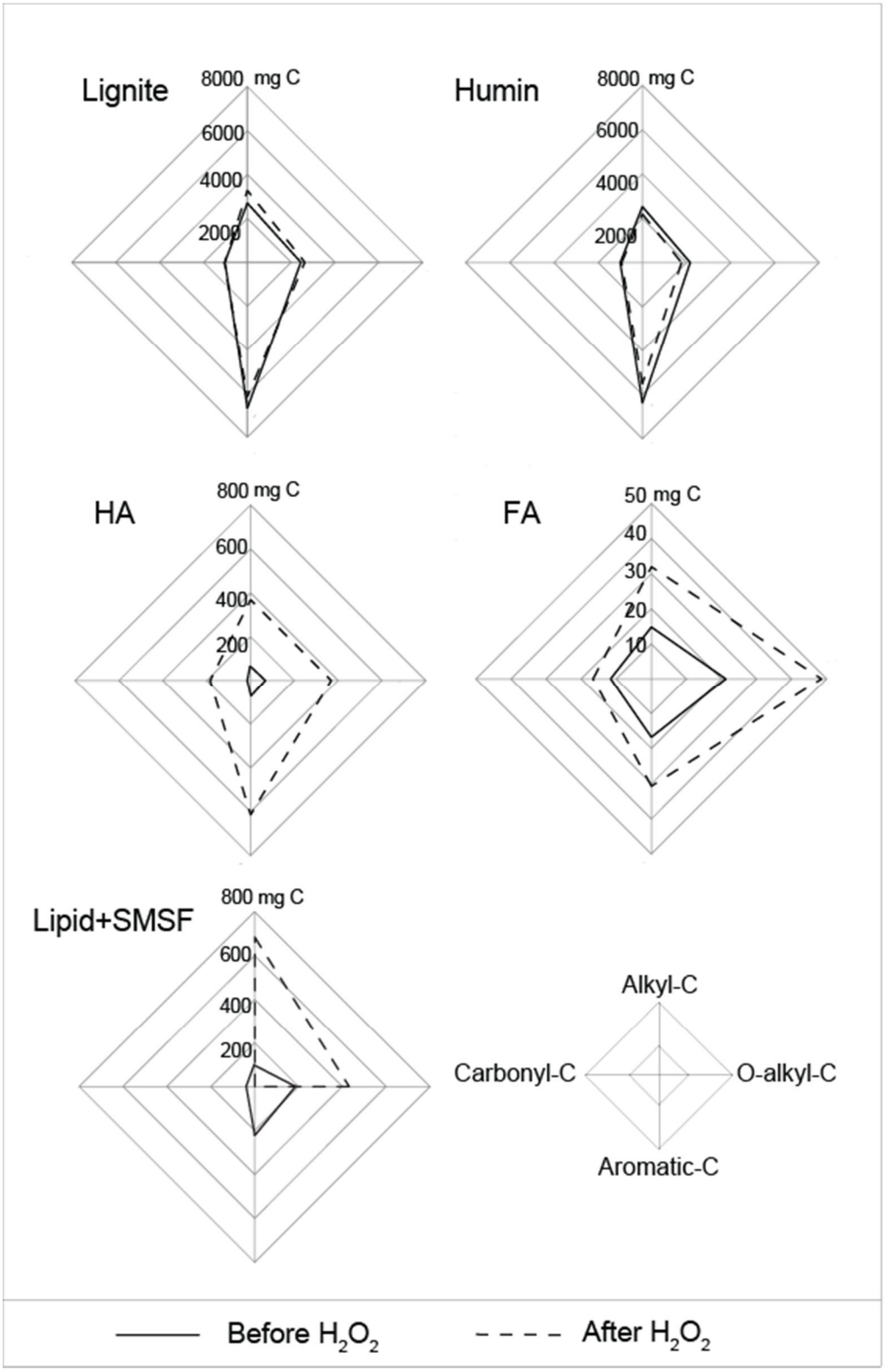


**Figure 5-6.** Structural composition of lignite and its humic fractions before and after $H_2O_2$ treatment, in terms of the absolute amounts (mg) of four carbon functional groups (alkyl-C, carbonyl-C, O-alkyl-C, and aromatic-C). The lignite sample (25 g) contained 12.9 g carbon before $H_2O_2$ treatment, and 13.1 g carbon after $H_2O_2$ treatment.

For the bulk lignite, the amount of alkyl-C and O-alkyl-C increased after $H_2O_2$ treatment from 2,700 to 3,280 mg, and from 2,440 to 2,620 mg C, respectively. The content of aromatic-C decreased from 6,680 to 6,160 mg, in agreement with the results of Tamamura et al. (2015). In humin, the overall content of carbon functional groups decreased by less than 20% after $H_2O_2$ treatment. For example, O-alkyl-C and carbonyl-C decreased from 2,170 to 1,770 mg and 962 to 937 mg, respectively. On the other hand, the overall content of carbon functional groups in HA increased by more than five times in response to $H_2O_2$, and by less than two times in FA. For example, in HA, O-alkyl-C and carbonyl-C increased from 68.6 to 368 mg and from 15 to 184 mg, respectively, while in FA, they increased from 21.3 to 45.6 mg and 11.6 to 16.6 mg, respectively. An increase in alkyl-C (from 96.65 to 689 mg) and O-alkyl-C (from 187 to 433 mg) was found in the lipid + SMSF fraction after $H_2O_2$ treatment, accompanied by a decrease in the aromatic-C and carbonyl-C contents.

The $^{13}C$ NMR analytical results for HA, FA, humin, and the lipid + SMSF fraction before and after $H_2O_2$ treatment in terms of relative changes in functional group contents were presented in Figure. 5-7. Carbonyl-C content increased from 7% to 12% and O-alkyl-C content decreased from 32% to 24% in HA after $H_2O_2$ reaction. In contrast, the carbonyl-C content of FA decreased from 18% to 13% and the O-alkyl-C content increased from 33% to 38%. The structural composition of humin before and after $H_2O_2$ treatment was almost identical. The lipid + SMSF fraction was unique in its increase of alkyl-C and decrease of aromatic-C contents after $H_2O_2$ treatment.

The percent distributions of alkyl-C, aromatic-C, O-alkyl-C, and carbonyl-C, amongst humin, HA, FA and the lipid + SMSF fraction are shown in Figure 5-8. Before $H_2O_2$ treatment, the functional groups were almost exclusively contained in humin, because humin is the dominant component (~85%) of lignite (Figure 5-3). However, after $H_2O_2$ treatment, the carbon functional groups were changed to the other fractions. The alkyl-C distributed in

HA increased from 2.3% to 11%, and that of the lipid + SMSF fraction increased from 3.6% to 21%. The O-alkyl-C distributed in HA and lipid + SMSF also increased from 2.8% to 14%, and from 7.7% to 17%, respectively. The aromatic-C and carbonyl-C distributed in HA increased from 1.0% to 10% and from 1.5% to 18%, respectively. However, $H_2O_2$ treatment appeared to have no effect on the distribution of functional groups of the FA fraction, which were negligible (<1%) amounts both before and after reaction.

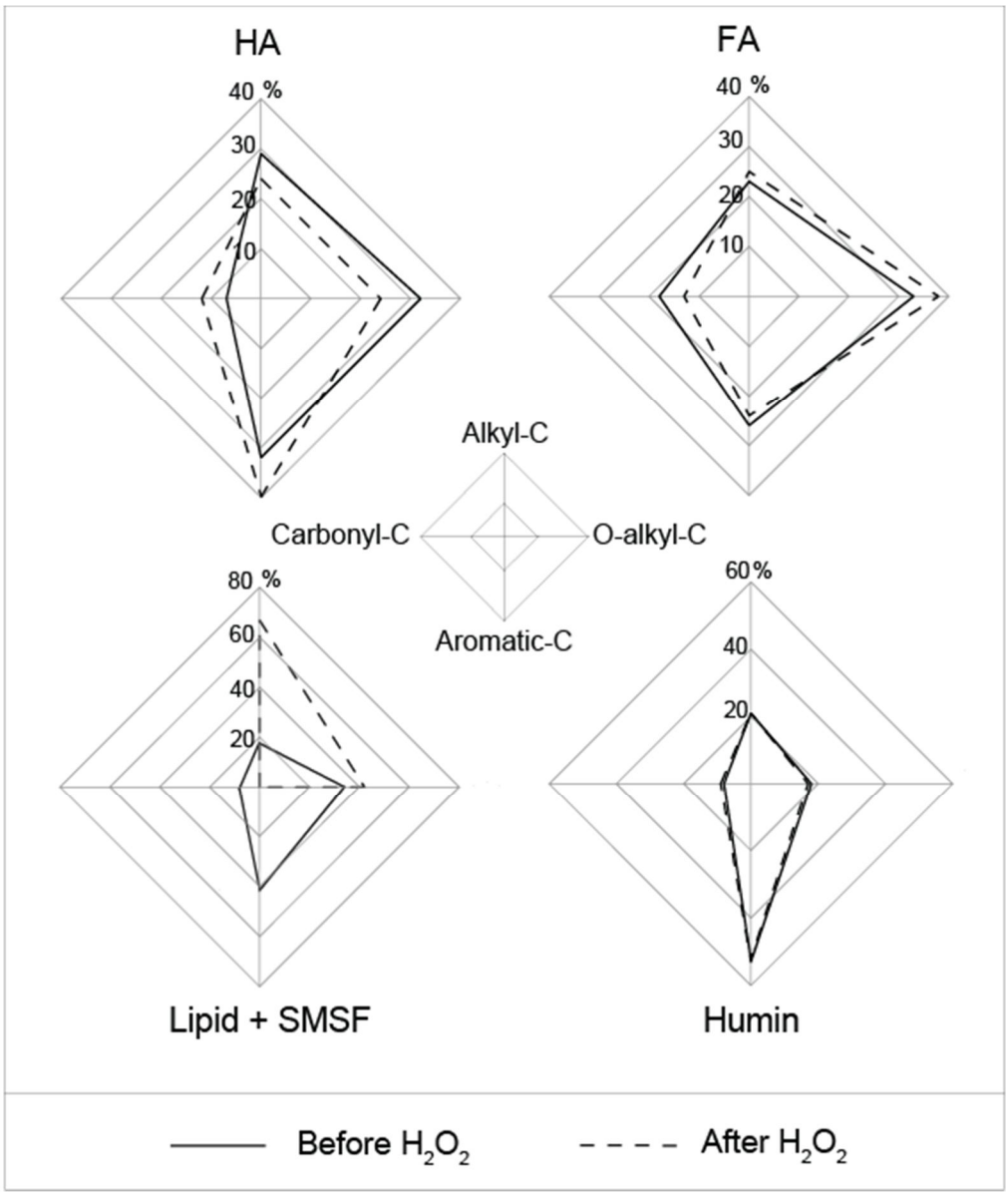


**Figure 5-7.** Structural composition of lignite and its humic fractions before and after $H_2O_2$ treatment in terms of the relative abundances (%) of four carbon functional groups (alkyl-C, carbonyl-C, O-alkyl-C, and aromatic-C)

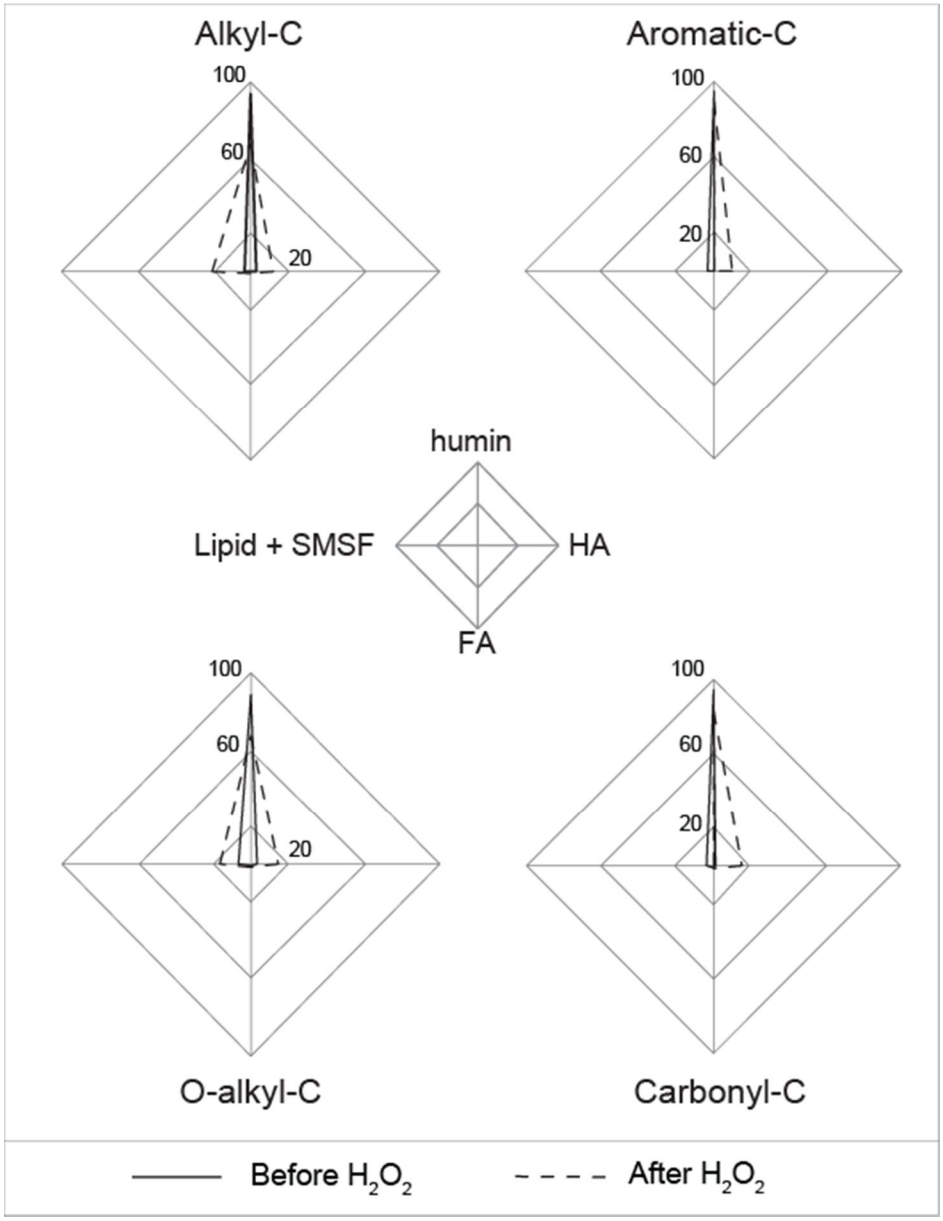


**Figure 5-8.** Percent distribution of the four carbon functional groups amongst the humic fractions of lignite

*5.3.4 FTIR spectroscopy*

The FTIR spectra of the lignite and its humic fractions before and after $H_2O_2$ treatment are shown in Figure 5-9. After $H_2O_2$ treatment, absorption bands around 1,700 $cm^{-1}$ appeared in lignite and all fractions except FA. These bands may be due to the increase of carbonyl groups (C=O) relative to the aromatic group, as reported in FTIR spectra of coal after oxidation (Calemma et al., 1994; Hayashi et al., 1997; Lynch et al., 1987).

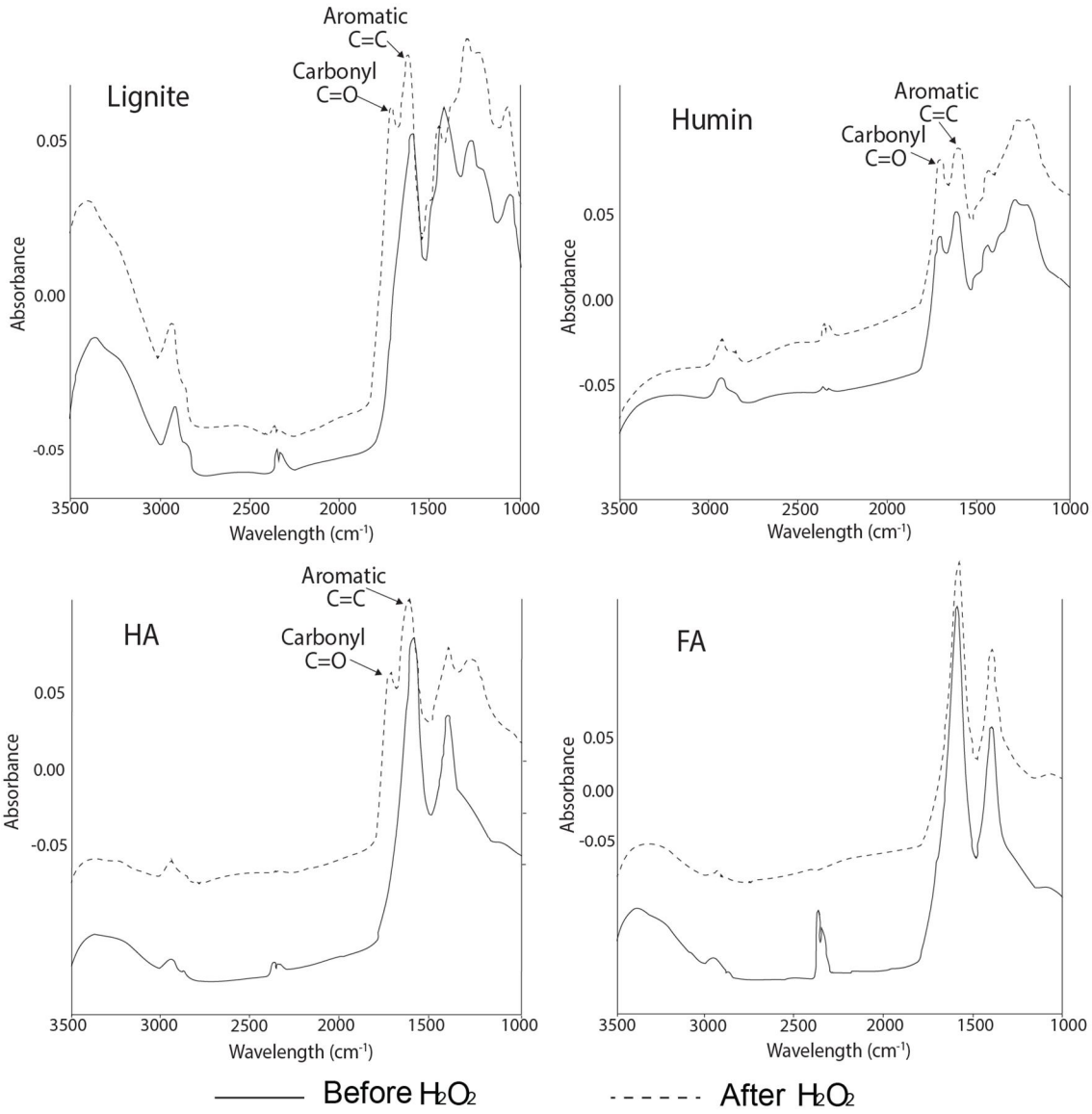


**Figure 5-9**. FTIR spectra in the range 1,000–3,500 $cm^{-1}$ for lignite, humin, HA, and FA, before and after $H_2O_2$ treatment

### 5.4 Discussion

The most notable change in the humic composition of lignite after $H_2O_2$ treatment was the significant increase in alkali-soluble carbon content (Figure 5-2). This fraction was comprised predominantly of HA (70%; Figure 5-4). Previous studies have demonstrated that oxidation of $H_2O_2$ depolymerizes coal by breaking C-C and C-O linkages, and simultaneously introducing oxygen functional groups (Mae et al., 2000; Miura et al., 1997). However, the increase in alkali-soluble carbon observed in this study would not be related to an increase in

oxidized hydrophilic functional groups in lignite-$H_2O_2$, because increases in both the O/C ratio (from 0.45 to 0.48, Table 5-1) and the content of O-bearing functional groups of lignite (Figure 5-6) were insignificant. In fact, the O/C ratio (from 0.66 to 0.56, Table 5-1) and O-alkyl-C content of HA (Figure 5-7) decreased after $H_2O_2$ treatment.

Generally speaking, the smaller the size of the organic molecules is, the greater their solubility is (Israelachvili, 1991). Following this concept, the main cause of the increase in alkali-soluble contents in lignite-$H_2O_2$ could be the breakage of bonds in the lignite macromolecular network, yielding HA, SMSF, or FA (Berkowitz, 1979; Martínez and Escobar, 1995). Because $H_2O_2$ is a radical initiator in organic reactions (Fossey et al., 1995; Parsons, 2000), the reaction of lignite with $H_2O_2$ would involve a radical intermediate (Doskočil et al., 2014; Huang et al., 2013; Miura et al., 1997; Tamamura et al., 2016). Specifically, the $H_2O_2$ yields peroxide structures (i.e., ROOH) in lignite, from which alkoxyl radicals (RO•) are formed either by homolytic cleavage (Equation 1; Clemens et al., 1991) or are radical-induced (Equations 2a–c; Wang et al., 2003).

$$ROOH \rightarrow RO\bullet + HO\bullet \quad (1)$$

$$ROOH + R\bullet \rightarrow ROR + HO\bullet \quad (2a)$$

$$R\bullet + HO\bullet \rightarrow ROH \quad (2b)$$

$$ROH + R\bullet \rightarrow RO\bullet + R\text{-}H \quad (2c)$$

After the production of RO•, the nature of subsequent reactions depends on the radical's structure, acidic–basic conditions, and temperature (Clemens et al., 1991; Klenk et al., 2000). In HA, which was the dominant regenerated component in the alkali-soluble fraction (Figure 5-4), O-alky-C content decreased while carbonyl-C content increased in response to the $H_2O_2$ reaction (Figure 5-6). Therefore, the dominant mode of fragmentation of the lignite would be $\beta$-fragmentation of the alkoxyl radicals (RO•), converting O-alkyl-C to carbonyl-C (Figure 5-10).

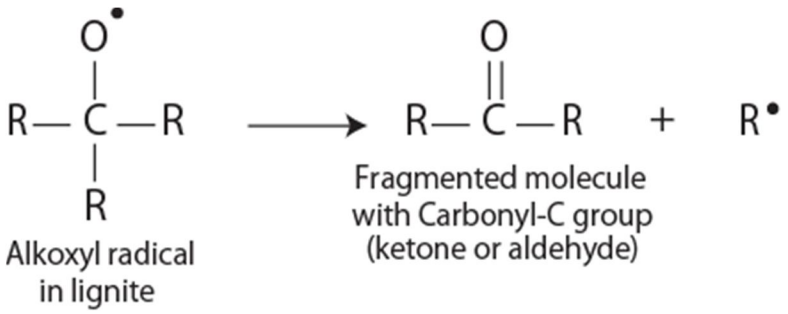


**Figure 5-10.** *β*-fragmentation of the alkoxyl radical in lignite, where the substituent (R) denotes segments of the aromatic or aliphatic structure or hydrogen

The increased bioavailability of coal after $H_2O_2$ treatment (Arctech Inc., 1990; Jones et al., 2013) may be the result of enhanced solubility of the coal from chemical fragmentation (*β*-fragmentation), rather than from increased O-bearing functional groups in the lignite. The column experiments in Chapter 4 indeed showed higher DOC concentrations, suggesting enhanced solubility in the effluent from lignite-$H_2O_2$ compared with lignite-original, where the presence of less-soluble HA (compared with FA and SMSF) is indicated by the yellow-brownish color of the solution. Furthermore, the dissolved components (HA, FA, and SMSF) were enriched in oxidized moieties (O-alkyl-C and carbonyl-C; Figure 5-7) relative to humin, and are therefore more bioavailable than the humin.

The production of organic acids may be related to the peroxy acid structure (R(CO)OOH) in lignite-$H_2O_2$. This structure can be formed from the reaction of carbonyl groups with $H_2O_2$ (Fossey et al., 1995; Parsons, 2000) and through radical propagation. Regarding the latter, the hydroperoxide (ROOH) would induce the radical reactions (Equations 1 and 2), resulting in *β*-fragmentation of the alkoxyl radical (Figure 5-10) to produce ketone and aldehyde. Acyl radical (R(CO)•) would form from the aldehyde, and this radical would subsequently react with oxygen ($O_2$), resulting in the peroxy acid structure (Clemens et al., 1991; Fossey et al., 1995; Parsons, 2000; Wang et al., 2003). These peroxy acids (i.e., derived from the reaction of carbonyl groups with $H_2O_2$ and radical propagation)

could then oxidize the carbonyl group (ketone or aldehyde) in lignite-$H_2O_2$ and its solubilized components (e.g., HA) to produce organic acids (Figure 5-11).

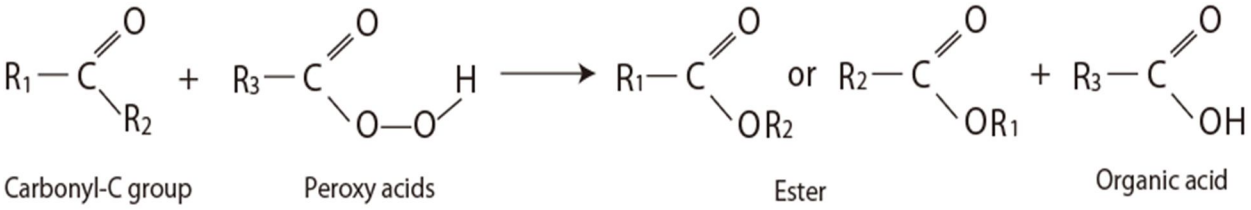


**Fig. 5-11** Organic acid production from the peroxy acid reaction (Krow, 1991; Plesnicar, 1978), where the substituent (R) denotes segments of the aromatic or aliphatic structure of lignite

## 5.5 Conclusions

In this study, the composition and structure of humic substances were characterized before and after $H_2O_2$ treatment of coal. The results obtained are the following.

(1) After $H_2O_2$ treatment, the alkali-soluble carbon content of lignite increased by 4.9 times. The alkali-soluble fraction consisted mainly (70%) of regenerated HA.

(2) Radical fragmentation (β-fragmentation) was the main mechanism of lignite solubilization via $H_2O_2$, and was the process by which fragmented molecules with a carbonyl-C group (e.g., ketones and aldehydes) was formed.

(3) The peroxide structure in lignite-$H_2O_2$ could become the source of organic acids.

(4) The increases in dissolved components (e. g., HA, FA, and SMSF) after $H_2O_2$ treatment would promote their bio-utilization with enriched O-functional groups (O-alkyl-C and carbonyl-C) as compared with the insoluble fraction (humin). This may lead to the enhanced production of substrates for methanogens.

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# Chapter 6

## GENERAL CONCLUSIONS

The present study was undertaken with the main objective of enhancing biogenic methane generation using hydrogen peroxide ($H_2O_2$) to support the field application of the subsurface cultivation and gasification (SCG) concept. Chapter 1 presents the introduction of biogenic methane from coal and current coal bed methane (CBM) enhancement technology through the injection of $H_2O_2$ solution into lignite seams in the subsurface environment. Not only the technology is expected to provide a new stable source of energy, but also contribute to the mitigation of greenhouse gases. It builds on previous investigations showing that $H_2O_2$ substantially solubilized lignite and generated organic acids in batch experiments, resulting in a significant increase of lignite bioavailability. Several laboratory experiments were conducted to simulate natural conditions.

In chapter 2, the reactivity of lignite with $H_2O_2$ solution was investigated by column experiments. Biogenic methane generation was also evaluated by applying an indigenous microbial consortium associated with coal from the Soya coal-bearing formations in the Tempoku Coalfield, northern Hokkaido, Japan, to the reaction solution of lignite and $H_2O_2$ that was produced by the column experiments. The concentrations of dissolved organic carbon (DOC) and organic acids were proportional to the initial concentration of $H_2O_2$ in the influent solution: the reaction of 3% $H_2O_2$ with lignite resulted in higher DOC and acid concentrations in the effluent solution compared with those of 0.3% $H_2O_2$. However, since $H_2O_2$ is detrimental to microorganism, the effluent of 3% $H_2O_2$ was not used as cultivation solution because the remaining $H_2O_2$ concentration was still high. Interestingly, in the

cultivation experiments, the highest methane ($CH_4$) productions were observed without additional minerals or a reducing agent.

Chapter 3 summarized the possible the transformation pathways of lignite via $H_2O_2$ into biogenic $CH_4$. Diverse archaeal and bacterial cultures in the vials were identified by the 16S rRNA gene pyrosequencing analysis using a universal primer set. The abundance of methanogens in the cultivation with lignite-$H_2O_2$ solution suggests that the dominant carbon sources for methanogenesis were organic acids. However, while the reaction solution of lignite with $H_2O_2$ provided acetates and formates as readily solubilized substrates for direct methanogenesis, the methanogenic pathways might also be affected by the activities of bacterial population. During the reaction of lignite with $H_2O_2$, the organic substrates were produced from lignite. The organic substrates were then converted into $H_2/CO_2$ and acetates fermentative bacteria. Some acetates could also be converted into $H_2/CO_2$. Therefore, biogenic $CH_4$ was possibly generated by the hydrogenotrophic, aceticlastic, and formate-utilizing methanogenesis pathways.

After confirming the biogenic $CH_4$ generation from the reaction solution of lignite-$H_2O_2$ and the methanogenic processes involved, the bioavailability of lignite after $H_2O_2$ treatment (lignite-$H_2O_2$) was investigated in Chapter 4. Lignite-$H_2O_2$ would optimize the biogenic $CH_4$ production (from the reaction solution of lignite with $H_2O_2$) and may serve as a by-product of SCG. High DOC and organic acid concentrations in the effluent solution of lignite-$H_2O_2$ were observed when it was reacted with ultrapure water under column experiment conditions. These results suggest that the bioavailability of lignite after $H_2O_2$ reaction was still enhanced. Although the potential $CH_4$ production was lower compared with $CH_4$ yields from coal reported from laboratory coal-to-methane cultures, the enhance

bioavailability of lignite-$H_2O_2$ would contribute to the biogenic $CH_4$ yield in SCG, thereby encouraging the field application of SCG.

Finally, in Chapter 5, the reaction mechanisms for the enhanced bioavailability of the lignite-$H_2O_2$ treatment, which was implied in Chapter 4, were developed. The composition and structure of each humic fraction of lignite before and after $H_2O_2$ treatment were characterized. The most notable change in the humic composition of lignite after $H_2O_2$ treatment was the significant increase in alkali-soluble carbon content, in which humic acid (HA) was comprised dominantly of 70%. The main cause of the increase in alkali-soluble contents in lignite-$H_2O_2$ could be the breakage of bonds in the lignite macromolecular network, yielding HA, small molecule size fraction (SMSF), and fulvic acid (FA). O-alky-C content in HA decreased while carbonyl-C content increased in response to the $H_2O_2$ reaction. This means that the dominant mechanism would be $\beta$-fragmentation of the alkoxyl radicals (RO•), converting O-alkyl-C to carbonyl-C. More importantly, the increase in dissolved components (e.g., HA, FA, and SMSF) after $H_2O_2$ treatment would promote their bio-utilization with enriched O-functional groups (O-alkyl-C and carbonyl-C) as compared with the insoluble fraction (humin). This may lead to the enhanced production of substrates for methanogens.

These findings present a great opportunity to produce biogenic $CH_4$ from the world's lignite seams. In the case of the Tempoku coal field, at least 7 billion $m^3$ of $CH_4$ is potentially recoverable by injecting $H_2O_2$ into its lignite seams without additional microorganisms (bioaugmentation) or minerals (biostimulation), estimated by the results of $CH_4$ production in this study. Furthermore, the enriched O-functional groups in the solvable components of lignite after $H_2O_2$ reaction would promote the additional production of substrates, thereby enhancing the biogenic $CH_4$ yield of SCG as illustrated in Figure 6-1.

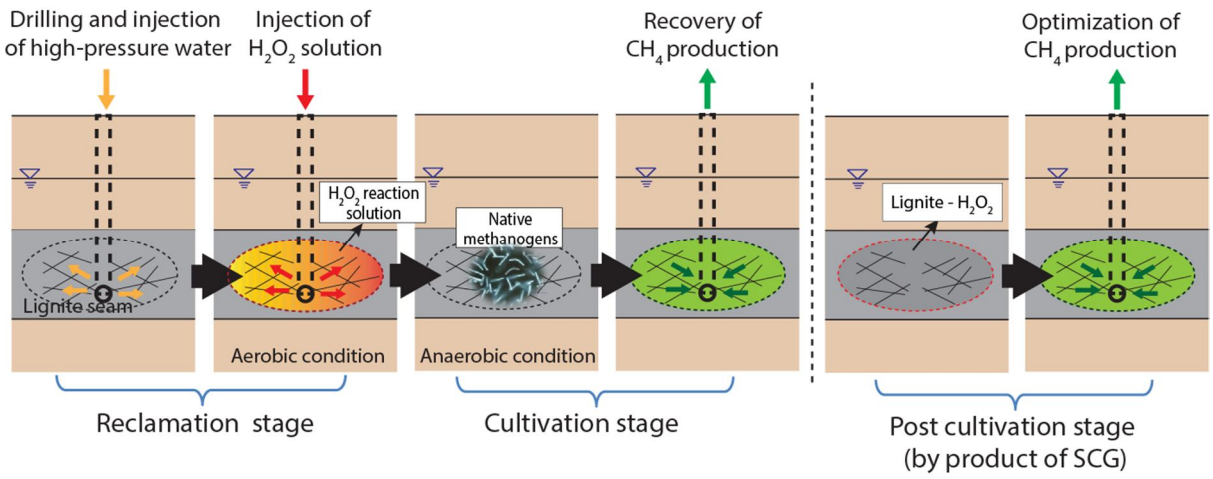


**Figure 6-1.** Proposed SCG technique with the optimization of biogenic $CH_4$ production from lignite-$H_2O_2$ as a by-product

The practical application of SCG indeed faces environmental issues. One of them, toxic substances may leach from the lignite and could contaminate the groundwater. Besides, acidified groundwater could also release toxic metals from the sediments surrounding the lignite due to the increase of metal solubility. Depending on the subsurface conditions, if these pollutants are likely to appear, some countermeasures (e.g., groundwater monitoring, immobilization of contaminated groundwater, and barriers) should be taken.

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