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Contribution of Yeast Extract, Activated Carbon, and an Electrostatic Field to Interspecies Electron Transfer for the Bioelectrochemical Conversion of Coal to Methane

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Abstract: The bioelectrochemical conversion of coal to methane was investigated in an anaerobic batch reactor containing yeast extract and activated carbon. In anaerobic degradation of coal, yeast extract was a good stimulant for the growth of anaerobic microorganisms, and activated carbon played a positive role. An electrostatic field of 0.67 V/cm significantly improved methane production from coal by promoting direct and mediated interspecies electron transfers between exoelectrogenic bacteria and electrotrophic methanogenic archaea. However, the accumulation of coal degradation intermediates gradually repressed the conversion of coal to methane, and the methane yield of coal was only 31.2 mL/g lignite, indicating that the intermediates were not completely converted to methane. By supplementing yeast extract and seed sludge into the anaerobic reactor, the intermediate residue could be further converted to methane under an electrostatic field of 0.67 V/cm, and the total methane yield of coal increased to 98.0 mL/g lignite. The repression of the intermediates to the conversion of coal to methane was a kind of irreversible substrate inhibition. The irreversible substrate inhibition in the conversion of coal to methane could be attenuated under the electrostatic field of 0.67 V/cm by ensuring sufficient biomass through biostimulation or bioaugmentation.

Keywords: coal; methane; interspecies electron transfer; electrostatic field; irreversible substrate inhibition; biostimulation; bioaugmentation

1. Introduction

Coal is an important fossil fuel that is relatively richly and evenly distributed on earth. However, coal is a pollution-causing fuel, and its use has been decreasing recently [1,2]. Natural gas is an energy source that emits less greenhouse gases than coal or oil, and its main component is methane [3–5]. The conversion of coal to methane could contribute significantly to sustainable development and solution of the energy problem [6,7]. Coal can be gasified into methane thermochemically or biologically. However, the thermochemical gasification that converts coal to methane under high temperature and high pressure requires a large amount of input energy and produces pollutants as by-products [8,9].

Coal is a solid composed of organic matter and fixed carbon, and is classified into peat, lignite, bituminous coal, anthracite, etc., depending on the degree of carbonization. Lignite is a low-grade coal containing a large amount of water and organics; owing to these attributes, biological gasification can

be favorably used to convert it to methane [8,10,11]. In the biological conversion of coal to methane, the organic matter contained in coal is first enzymatically hydrolyzed. The hydrolysis products are degraded by acidogenic bacteria to produce low-molecular compounds, including hydrogen and acetate, which are then finally converted into methane by methanogenic archaea [7,9,12]. The anaerobic microorganisms involved in the biological conversion of coal to methane are usually active in mesophilic (35 °C) or thermophilic (55 °C) conditions [13]. This suggests that the biological conversion of coal to methane is more environmentally friendly than the thermochemical gasification of coal [8,10]. However, presently, the methane conversion rate of coal is too slow, and the methane yield that can be obtained from 1 gram of coal is less than a few milliliters [10,14–16]. The organic components contained in coal are mainly high-molecular hydrophobic substances such as lignin and cellulose [7,12,17]. It has been recognized that the methane conversion of coal is generally controlled by hydrolysis. However, coal hydrolysis products or their degradation intermediates are usually polymeric aromatic and aliphatic compounds, including polycyclic and heterocyclic aromatic hydrocarbons, aromatic nitrogen compounds, and aliphatic compounds [7,14,18]. These intermediate compounds are commonly toxic, and it is difficult for anaerobic microorganisms to directly metabolize them [9,15,17]. The hydrolysis products and their degradation intermediates can easily accumulate in reactors, which can further inhibit their degradation during the conversion of coal to methane [9]. This indicates that the biological conversion of coal to methane relies on the consecutive degradation of the intermediates. Several attempts have been made to improve the conversion of coal to methane, including biostimulation, bioaugmentation, and aerobic pretreatment [17,19,20]. However, the conversion of coal to methane in terms of methane production rate and the yield is still not sufficient for field applications [15,19].

Recently, the conversion of coal to methane was dramatically improved by combining anaerobic digestion with bioelectrochemical technology. When an anaerobic medium containing coal was exposed to an electrostatic field, the methane yield of coal surprisingly increased to 52.5 mL/g lignite [9]. However, the rate of conversion was still quite low, and complete methane production took a long time [9]. In addition, a large amount of soluble organic matter remained in the anaerobic reactor and was not converted to methane [9]. In bioelectrochemical anaerobic reactors, the interspecies electron transfer between electroactive microorganisms, electroactive bacteria (EAB) and electrotrophic methanogenic archaea (EMA), contributes significantly to methane production [21–23]. Based on the presence of a large amount of soluble organic residue, it is likely that the interspecies electron transfer from EAB to EMA was repressed further as the coal degradation intermediates accumulated. This indicates that the conversion of coal to methane is mainly controlled by the inhibition caused by the coal degradation intermediates. However, the mechanisms for inhibiting or improving the interspecies electron transfer for the conversion of coal to methane have not been elucidated in detail yet. The growth of anaerobic microorganisms involved in this interspecies electron transfer could be stimulated by the addition of limited nutrients [19,24]. The interspecies electron transfer could be promoted by an electrostatic field or/and conductive materials such as activated carbon [9,25,26].

In this study, the roles of yeast extract, activated carbon, and an electrostatic field on the interspecies electron transfer for the conversion of coal to methane were studied in an anaerobic batch reactor. The outcomes of the study will contribute to improving the conversion of coal to methane in terms of methane production rate and yield by controlling the inhibition of coal degradation intermediates.

2. Materials and Methods

2.1. Coal, Yeast Extract, Anaerobic Medium, and Seed Sludge

Canadian lignite was obtained from a local agency (Aquajiny Co., South Korea), pulverized with a pestle, and sieved through a 1 mm screen into a powder. The lignite powder was dried at 105 °C for 12 h. The initial properties of the lignite powder were as follows: the ratio of volatile solids (VS) to total solids (TS) 0.28 g/g, chemical oxygen demand (COD) to coal 0.52 g/g, and moisture content 18.4%. Yeast extract (Becton Dickinson and Company; Sparks, MD21152 USA, 1.03 g COD/g) was used as a

biostimulant. Activated carbon in powder form (Power Carbon Technology Co., Ltd., South Korea, specific surface area 1950 m^2/g, particle size 6–8 μm) was purchased from a local distributor and used as a conductive material to mediate the electron transfer. The prepared anaerobic medium contained 2.45 g/L of $NaH_2PO_4 \cdot 2H_2O$, 4.58 g/L of $Na_2HPO_4 \cdot 12H_2O$, 0.31 g/L of NH_4Cl, 0.13 g/L of KCl, 8.4 g/L of $NaHCO_3$, and small amounts of vitamins and trace metals, as described in a previous study [22]. Anaerobic sludge taken from an anaerobic sludge digester (Busan, South Korea) was sieved through a 1 mm screen and then stored at 4 °C in a refrigerator for 24 h to be later used as seed sludge. The initial pH of the seed sludge was 7.27–7.31, and the alkalinity and the volatile suspended solids (VSS) were 3.65–3.68 g/L as $CaCO_3$ and 16.2–16.5 g/L, respectively.

2.2. Experimental Setup for Bioelectrochemical Methane Conversion of Coal

The bioelectrochemical anaerobic batch reactor (cylindrical type, effective volume 0.5 L, diameter 8.5 cm, height 10 cm) was prepared with acrylic resin (Figure 1). The top of the reactor was covered with an acrylic plate which was sealed by a flange. A blade was installed inside the reactor, and the blade was connected to a DC motor on the cover plate using a steel shaft. Sampling ports that collected small amounts of biogas and liquid were installed on the cover plate. The sealing tubes were attached to the bottom of the cover plate holes for liquid sampling and to the steel shaft, respectively. The sealing tubes were extended into the liquid phase to block the gas flux between the outside and the inside of the reactor. The off-gas valve was connected with a rubber tube to a floating type gas collector. The gas collector was filled with acidified saline water to prevent the dissolution of biogas. Two titanium foil pieces (0.1 T, Grade2; Baoji HongYaDa Nonferrous Metal Materials Co., Ltd; Baoji, China) with a large size (26 cm × 9 cm) and a small size (6.5 cm × 7 cm), were prepared and their surfaces were coated with a dielectric polymer (G2140, Printec Co., Ltd; Daegu, Korea) that insulated the surface electrodes. The titanium electrodes were rolled into an annular and installed on the inner wall of the reactor and the outer wall of the sealing tube of the steel shaft. The distance between the electrodes was 3 cm.

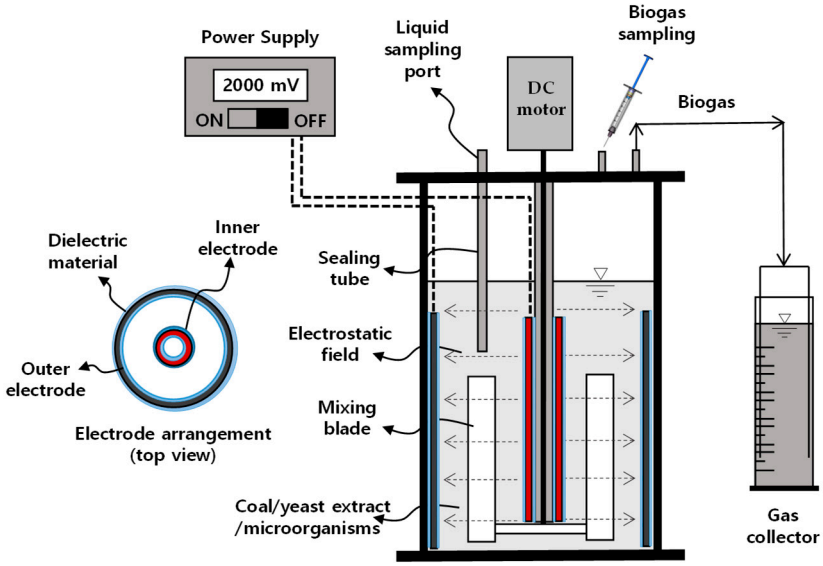


Figure 1. Schematic diagram of the bioelectrochemical anaerobic batch reactor for the conversion of coal to methane.

For the experiment, five anaerobic batch reactors were prepared and investigated (Table 1). A coal-free anaerobic medium (250 mL) containing yeast extract as a biostimulant was added to the anaerobic batch reactor used as the blank for coal. The anaerobic medium containing yeast extract and coal was added to the anaerobic batch reactor and used as the control for the activated carbon. The activated carbon was further mixed with the medium of the control and then added to the

anaerobic batch reactor named AC. The other two anaerobic batch reactors containing the AC medium, named AE33 and AE67, were prepared, and the electrodes were polarized with a DC voltage source to expose the bulk solutions to electrostatic fields of 0.33 V/cm and 0.67 V/cm, respectively. The initial concentrations of yeast extract, coal, and activated carbon were 1.0 g/L, 5 g/L, and 3.0 g/L, respectively, in the anaerobic batch reactor. The anaerobic batch reactors were inoculated with seed sludge (250 mL), and placed in a temperature controlled room (35 ± 1 °C), and the blade was rotated at 120 rpm to mix the anaerobic medium. When the production of methane from the coal in the anaerobic batch reactors was completed, the conversion of the coal degradation intermediates to methane was examined by adding the seed sludge and yeast extract to the residual solution.

Table 1. Experimental conditions for the bioelectrochemical coal conversion to methane.

Content	Blank	Control	AC	AE33	AE67
Medium (mL)	250	250	250	250	250
Sludge (mL)	250	250	250	250	250
Activated Carbon (g)	-	-	1.50	1.50	1.50
Electrostatic field (V/cm)	-	-	-	0.33	0.67

2.3. Analysis and Calculations

The liquid sample was taken from the anaerobic batch reactor at the start and the end of the experiment, and physical properties including TS, VS, VSS, total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), and alkalinity were measured according to standard methods [27]. The pH of the liquid sample was also measured using a pH meter (YSI pH1200 laboratory pH meter 115–230 V (T1), USA). The biogas production from the coal degradation in the anaerobic batch reactor was intermittently monitored during the operation. The composition of the biogas was analyzed using a GC (Gas Chromatograph Clarus 580, PerkinElmer Co., Ltd.) with Porapak-Q column (6ft × 1/8", SS) and thermal conductivity detector. The methane production in the anaerobic batch reactor was estimated using Equation (1) from the biogas amount monitored in the gas collector and its composition.

$$V_{CH4} = C_{CH4}(V_{RH} + V_{GT} + V_{GC}) \quad (1)$$

where V_{CH4} is the cumulative methane production (mL), C_{CH4} is the methane percentage (%) in the biogas, V_{RH} is the volume of the reactor head space (mL), and V_{GT} is the volume of the rubber tube between the reactor and the gas collector (mL). The cumulative methane production was converted into the value at standard temperature and pressure using Equation (2).

$$V_{CH4}(mL, STP) = V_{CH4}(at\ T) \times \frac{273}{(273 + T)} \times \frac{(760 - W)}{760} \quad (2)$$

where T is the temperature (35 °C), and W is the water vapor pressure at T °C. At the end of the experiment, electrochemical tests including cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were performed on the bulk solution. Small pieces of stainless steel mesh were used as the working and the counter electrodes, and an Ag/AgCl electrode was used as the reference electrode. In the CV test, the voltage was scanned at 10 mV/sec from −1.0 V to 1.0 V using a potentiostat (ZIVE SP1, WonA Tech, Korea). The redox peak was obtained from the voltammogram using Smart manager software (ZIVE BP2 Series, WonATech, Korea) [22]. For the EIS experiment, the potential wave signal was 100 mV, and the frequency range was from 100 Hz to 0.3 MHz. The equivalent circuit fitted to the EIS data was explored using ZMAN 2.4 software (ZIVE LAB, WonA-Tech, Korea). The suitable equivalent circuit (L-R_s-Q|(R_{ct}-W)) was obtained by the connection of three components in series: (i) an inductance element (L), (ii) a solution resistance (R_s), and (iii) a constant phase element (Q) connected with an impedance of the faradic reaction in parallel. The impedance of the faradic reaction was the charge transfer resistance (R_{ct}) connected with the Warburg element (W) in series.

When the production of methane from coal was completed, a liquid sample was taken from the bulk solution and filtered with 0.45 μm GF/C. The filtrate was diluted based on the 1 mg/L of DOC (dissolved organic carbon), and the excitation emission matrix (EEM) spectrum was obtained using a spectrofluorphotometer (RF-6000 SHIMADZU; wavelength: excitation (Ex) 220–400 nm/emission (Em) 250–600 nm) to investigate the intermediates of coal degradation [28].

2.4. Microbial Community

At the end of the experiment on the conversion of coal degradation intermediates to methane, Microbiome Taxonomix profiling for the suspended microorganisms in the bulk solution was performed to investigate microbial communities using 16S rRNA. DNA was extracted from the sample using a Power soil DNA isolation kit (MO BIO Laboratories, Inc., CA, USA) according to the kit protocol. Next, 16s rRNA was amplified from Methagenomic DNA, pooled, and sequenced on a MiSeq Personal Sequencer (Illumina, San Diego, CA, USA). A fusion primer was used to amplify the variable region (V3V4 for bacteria, V1V9 for archaea) of the 16s rRNA in genomic DNA. Amplification, construction of the sequencing library, and bioinformatic analysis were performed as described previously [29]. The chimera was checked, and taxonomic assignments of these readings were done using the EzBioCloud database (http://ezbiocloud.net/). Microbial community and statistical taxonomical assignments were obtained via operational taxonomic units (OTUs). Comprehensive bioinformatic analyses such as species-level classification of microbes, cluster analysis, microbial origin tracking, hierarchical clustering, and various indicators of species diversity were conducted using EZ Biocloud (Chunlab, Inc., Seoul, Korea). The similarity between microbial communities was obtained from the principal component analysis using the factoextra package in R.

3. Results and Discussion

3.1. Production of Methane from Coal

The production of methane from coal in the anaerobic batch reactors was greatly influenced by the presence of yeast extract and activated carbon in the medium, and the strength of the electrostatic field (Figure 2). The methane production in the blank increased quickly with no lag phase to 140.0 mL. The electron sources in the blank for methane production were mainly organics contained in the yeast extract. The seed sludge may also have contained some organic matter, but in generally small enough quantities to be negligible. It seems that yeast extract was a good biostimulant that promoted the growth of anaerobic microorganisms. In the control, the initial methane production from coal and yeast extract increased exponentially to a maximum value of 172.2 mL. This indicates that the initial methane production was stimulated by yeast extract as in the blank. The maximum methane production in the control was more than that in the blank, which indicates that not only the organics in the yeast extract, but also in the coal were converted to methane. However, the cumulative methane production in the control gradually decreased from its maximum value over time. Some methanogenic and anaerobic methane-oxidizing archaeal species, called methanotrophs, can oxidize methane by enzymatic back flux when methanogenesis is kinetically and thermodynamically limited [30–32]. The methanotrophic species can be grown by the co-oxidation of methane and a wide range of substrates, including aromatics and aliphatics [32,33]. The methane conversion of coal degradation intermediates can be repressed by the accumulation of toxic intermediates [9,15]. In the control, it seems that the reverse methanogenesis that oxidizes methane overwhelmed the methanogenesis as coal degradation intermediates accumulated [9,33].

It is well known that the interspecies electron transfer between EAB and EMA for methane production can be mediated by conductive materials including activated carbon, biochar, and magnetite particles [34–36]. AC is the anaerobic batch reactor added with activated carbon in the control. In AC, the pattern of cumulative methane production was similar to the control, but the maximum value was slightly lower. This indicates that activated carbon alone did not have a positive effect on the coal

conversion to methane. It seems that the intermediates of coal degradation accumulated more in AC than in the control, further inhibiting methane production. In AE33, cumulative methane production initially increased to 177.9 mL, which was higher than the control. The decrease rate in methane over time in AE33 was also slower than in the control. This indicates that methane production from the coal degradation intermediates was improved by an electrostatic field of 0.33 V/cm. The positive influence of the electrostatic field on methane production from the coal degradation intermediates was more evident in AE67. The cumulative methane production in AE67 exponentially increased to 219.1 mL. In AE67, reverse methanogenesis was not observed externally. The methane yield of coal on the basis of the saturated cumulative methane production was 31.2 mL/g lignite. In a previous study, the methane yield of coal could be improved by the electrostatic field alone without yeast extract and activated carbon, but it took a long time to produce methane from coal [9]. This suggests that yeast extract and activated carbon contributed significantly to the conversion of coal into methane under an electrostatic field of 0.67 V/cm.

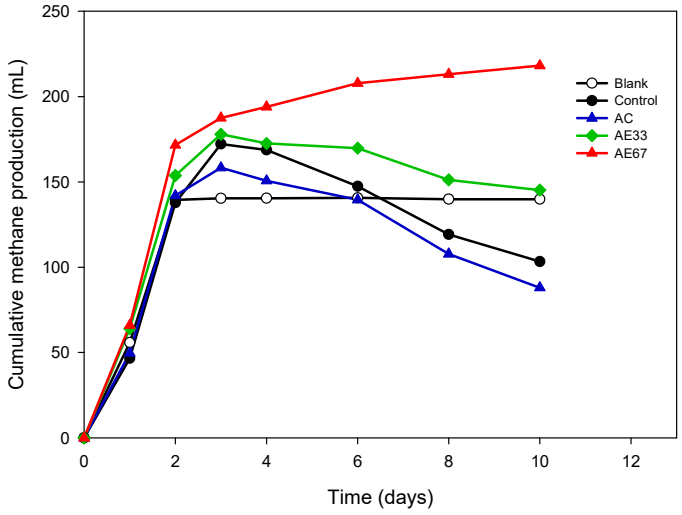


Figure 2. Cumulative methane production from coal in the anaerobic batch reactor.

However, it is necessary to better understand the mechanism of the production of methane from coal and coal degradation intermediates to further improve yield and rate. The aromatic and aliphatic compounds that are the main components of coal degradation intermediates could be degraded by oxidative ring-opening or chain-breaking reactions in the presence of electron acceptors [37–39]. However, the decomposition of the coal degradation intermediates is thermodynamically unfavorable in the anaerobic condition. In a bioelectrochemical anaerobic reactor, electroactive microorganisms, including EAB and EMA, could be enriched under an electrostatic field [9,40]. Methane production could be bioelectrochemically promoted by the interspecies electron transfer between EAB and EMA through direct contact or via the mediation of abiotic redox substances [21,22]. Therefore, the following hypotheses on the mechanism of the conversion of coal to methane in AE67 were proposed: (i) electroactive microorganisms, such as EAB and EMA, are enriched in the anaerobic medium containing yeast extract and activated carbon by an electrostatic field of 0.67 V/cm, (ii) coal in particulate form is first enzymatically hydrolyzed, (iii) the hydrolysis products are consecutively broken down by EAB to release electrons, and (iv) the electrons are transferred to EMA under an electrostatic field of 0.67 V/cm, which then produce methane.

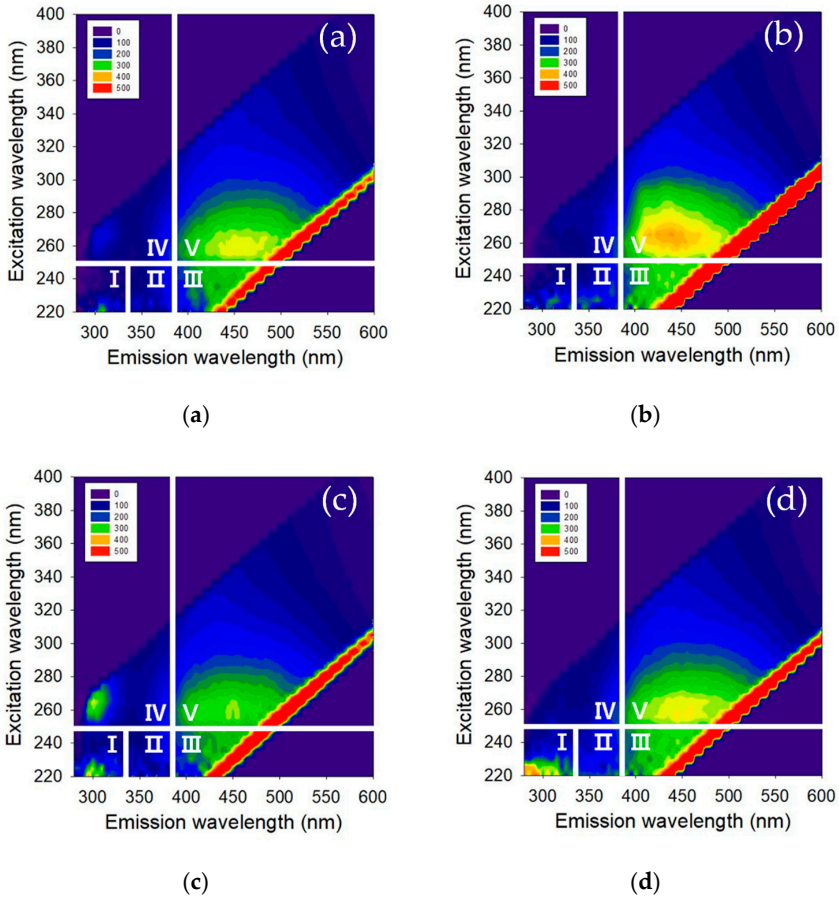
3.2. Intermediates of Coal Degradation

After the production of methane from coal, the concentrations of soluble organic residues in the anaerobic batch reactors were as high as 2600–3200 mg COD/L, depending on the presence of activated carbon and the strength of the electric field (Table 2). The soluble organic residues were the intermediates

that were produced from the consecutive decomposition of the hydrolysis products of coal. Based on the fluorescence peaks in the EEM spectra, the common components observed in the intermediates of coal degradation were fulvic-acid-like compounds (region III) and humic-acid-like compounds (region V) (Figure 3). This indicates that the fulvic-acid-like and humic-acid-like compounds are the main components in the hydrolysis products of coal, as previous studies have reported [15,18].

In the control, the concentration of the soluble organic residue was 2686.3 mg COD/L, and in addition to the main components of the hydrolysis products of coal, small fluorescence peaks were also observed in the EEM spectrum for aromatic protein (region I). However, in AC, the concentration of the soluble organic residue was 2856 mg COD/L, slightly higher than the control, and the fluorescence peaks for the intermediates were more varied (Figure 3). This indicates that the coal degradation intermediates in AC were slightly more diverse than those in the control, and their concentrations were also higher, which further repressed methane production from the degradation of the intermediates (Figure 2).

In AE33, the soluble organic residue had a concentration of 3043.5 mg COD/L, and fluorescence peaks were observed in the EEM spectrum for tyrosine- and protein-like compounds (region IV), which were higher than those in the AE67. It is likely that the biodegradability of tyrosine- and protein-like compounds was poor under a weak electrostatic field of 0.33 V/cm. In AE67, the soluble organic residue had a concentration of 3127.8 mg COD/L, which was higher than that in AE33 (Table 2). The fluorescence peak for aromatic protein was higher in AE67 than in the other anaerobic batch reactors. Interestingly, there was a clear correlation between methane production (Figure 2) and the peaks of the aromatic protein in the anaerobic batch reactors (Figure 3). It seems that the hydrolysis products of coal, including the fulvic-acid- and the humic-acid-like compounds, were first decomposed into tyrosine- and protein-like compounds, and then into aromatic protein during methane production.



(**a**) (**b**)

(**c**) (**d**)

Figure 3. Excitation emission matrix for coal degradation intermediates: (**a**) control, (**b**) AC, (**c**) AE33, and (**d**) AE67.

Table 2. Summary of the methane conversion of coal in an anaerobic batch reactor.

Content		Control	AC	AE33	AE67
Coal	Cumulative CH_4 (mL)	172.2	158.3	177.9	218.1
	CH_4 yield (mL/g lignite)	-	-	12.2	31.2
	Organic residue (mg SCOD/L)	2686.3	2855.9	3043.5	3127.8
Organic residue	Cumulative CH_4 (mL)	82.4	49.3	52.4	171.0
	CH_4 yield (mL/g lignite)	-	-	-	66.8
Total CH_4 yield (mL/g lignite)		-	-	12.2	98.0

3.3. Methane Production from the Intermediates of Coal Degradation

The methane production from the coal degradation intermediates was examined by adding yeast extract and seed sludge to the anaerobic batch reactors. The main features of methane production from the intermediates were quite similar to those of methane production from coal. This suggests that the conversion of coal to methane was governed by the consecutive degradation of coal intermediates [9]. In the control, AC, and AE33, the cumulative methane produced from the coal degradation intermediates slowly increased to a maximum and then gradually decreased to less than the blank over time (Figure 4). In AE67, however, methane production from the intermediates exponentially increased without a lag phase and gradually saturated to 171.0 mL. This indicates that the production of methane from coal degradation intermediates was significantly promoted by the addition of yeast extract and seed sludge under an electrostatic field of 0.67 V/cm. It seems that EAB participated in the ring-opening of aromatic compounds and the chain-breaking of aliphatic compounds that occurred during the degradation of the intermediates by transferring electrons to EMA. However, it is worth noting that even after the conversion of coal to methane, large amounts of coal degradation intermediates remained, and they were not broken down to produce methane (Figure 2). This suggests that the conversion of coal to methane was severely inhibited by the intermediates of coal degradation. The production of methane from the intermediates only resumed after the yeast extract and the seed sludge were replenished. However, the inhibition of methane production from the coal degradation intermediates could be alleviated by dilution of the intermediates [9]. The inhibition of methane production from coal degradation intermediates is likely to be an irreversible substrate inhibition. However, it is believed that the irreversible substrate inhibition could be partially overcome by operating the anaerobic reactor at a low loading rate of coal or by a periodic biostimulation and bioaugmentation to maintain active anaerobic microorganisms.

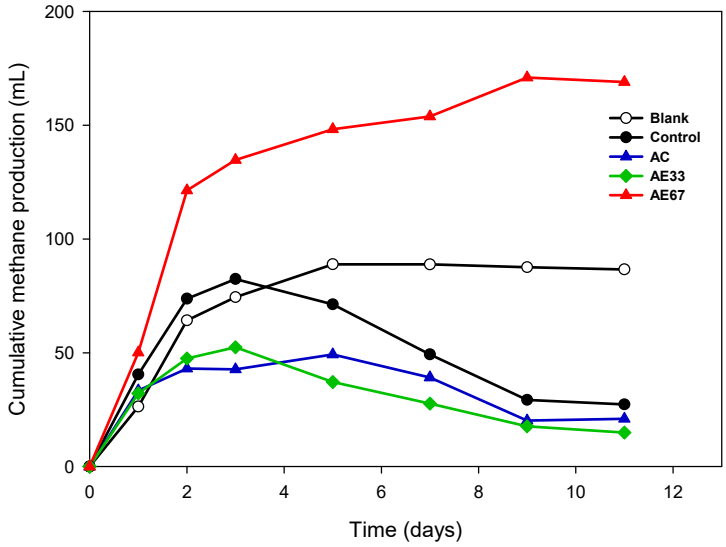


Figure 4. Cumulative methane production from the intermediates of coal degradation in the anaerobic batch reactor.

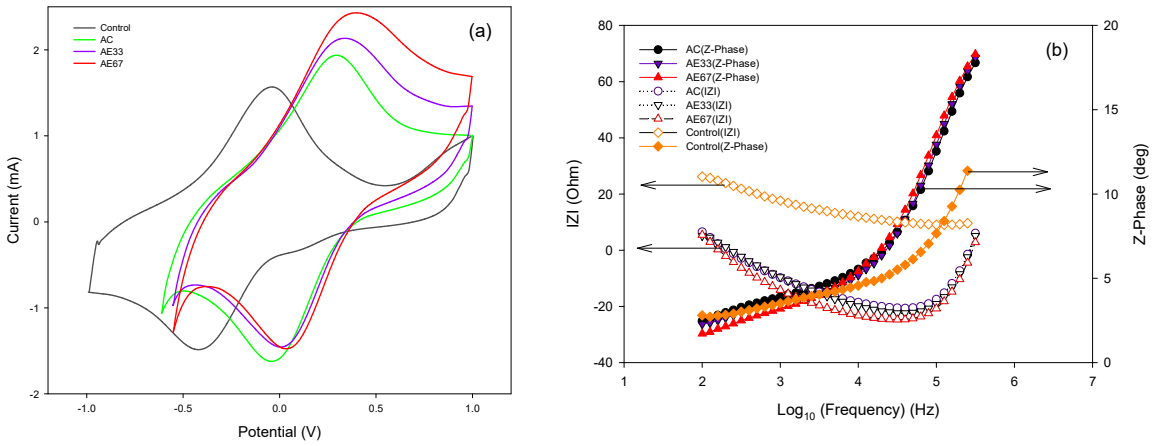
3.4. Cyclic Voltammetry and EIS

The CV curve for the anaerobic medium of the anaerobic batch reactor provided useful information on the redox substances involved in the electron transfer between EAB and EMA [22,41]. The redox substances indicated the presence of electroactive microorganisms and abiotic redox substances in the medium. The electroactive microorganisms were EAB and EMA, and the abiotic redox substances included flavin- and sulfur-based compounds, phenazines, quinones, and humic substances, which are exogenous or secreted endogenously by microorganisms [21,22,41]. In the voltammograms for AC, AE33, and AE67, the peak heights were 0.71–1.12 for oxidation and 1.14–1.39 for reduction (Table 3). However, the oxidation and reduction peaks in the control were also comparatively high, at 0.58 and 0.64, respectively. It is possible that the redox peaks in the control were caused by the abiotic redox substances. In the bioelectrochemical anaerobic reactor, the formal potentials for the redox peaks were generally observed in the range of –0.43 V vs. Ag/AgCl and 0.5 V vs. Ag/AgCl in the voltammogram [9,22,42]. In AE67, the formal potentials was 0.22 V vs. Ag/AgCl, which was in the range of the formal potentials mentioned above. However, compared to those of AE67, the formal potentials for AE33 shifted to more negative values, and those for AC and the control became considerably negative. It is likely that the abiotic redox substances and the electroactive microorganisms contributed considerably to the redox peaks for AC, AE33, and AE67. This suggests that electrons were transferred directly by the physical contact between EAB and EMA, or indirectly by the mediation of activated carbon and abiotic redox substances during the degradation of coal to produce methane.

In the bioelectrochemical anaerobic reactor, the contribution of the redox substances to the interspecies electron transfer could be further described by the electrochemical impedance spectrum. The impedance spectrum for the bulk solution was generally affected by ohmic resistance, charge transfer resistance, and a double layer capacitor or constant phase element. However, in the Bode plots for AC, AE33, and AE67, the impedance amplitudes and the phase angle shifts were significantly increased in the high frequency range (Figure 5). This indicates that there was an inductive element in the anaerobic batch reactor with the activated carbon. However, further studies are needed to describe the inductive element observed in the anaerobic reactor for the conversion of coal to methane. The solution resistance (R_s), obtained by fitting the EIS data to a suitable equivalent circuit, L-R_s-Q|(R_{ct}-W), was 7.86 Ω in the control. However, the solution resistance decreased to 3.02 Ω in AC, and decreased slightly further in AE33 and AE67 (Table 3). Electroactive microorganism have conductive proteins such as pili or C type cytochromes that extend to the outer membrane [43,44]. The solution resistance (R_s) can be decreased by an abundance of electroactive microorganisms in the bulk solution [21,44,45]. The charge transfer resistance (R_{ct}) is the electrical element associated with the activation overpotential for the electron transfer. The charge transfer resistances for AC, AE33, and AE67 were in the range of 1.20 and 1.45 Ω, while the control had a charge transfer resistance of 10.62 Ω. However, the production of methane from coal was significantly affected by the strength of the electrostatic field (Figure 2). This suggests that the interspecies electron transfer required for methane production was mainly promoted by the electrostatic field.

Table 3. Electrochemical properties in electrochemical impedance spectroscopy (EIS) of the bulk solution.

Electrochemical Properties			Control	AC	AE33	AE67
CV	E_f (vs. Ag/AgCl)		−0.25	0.13	0.17	0.22
	$I_{p,ox}/I_{p,red}$ (mA)		0.58/0.64	0.71/1.39	0.87/1.19	1.12/1.14
Equivalent circuit for EIS data, (L-R_s-Q|(R_{ct}-W))	L (Ω)		3.33 u	3.62 u	3.44 u	3.58 u
	R_s (Ω)		7.86	3.02	2.42	2.74
	Q	Q_y	0.27 m	94.47 u	45.50 u	25.29 u
		Q_a	0.57	0.83	0.92	0.94
	R_{ct} (Ω)		10.62	1.55	1.19	1.45
	W (Ω/√s)		2.02 m	8.40 m	7.35 m	8.64 m
	r^2		0.998	0.999	0.999	0.998



(**a**) (**b**)

Figure 5. (**a**) Cyclic voltammogram and (**b**) Bode plots of the electrochemical impedance spectra of the bulk solutions of the anaerobic batch reactors.

3.5. Microbial Communities

The microbial communities in the bulk solution were analyzed at the end of the experiment with regards to methane production from coal degradation intermediates. The bacterial OTUs were in the range of 1485 and 1642, which was significantly higher than those of the archaea (90–228). Based on Shannon and Jackknife indices, the diversity of bacteria was also higher than that of the archaea (Table 4). Bacterial evenness and richness were the highest in AC, and were similar in AE33 and AE67. These values were all higher than those of the control. It seems that the bacterial species diversity was increased by activated carbon, but slightly selected by the electrostatic field. In the archaeal community, the diversity in AC based on the OTUs and the Jackknife and Shannon indices was higher than that in the control. This indicates that the archaeal diversity, like that of the bacteria, was increased by activated carbon. The selection of archaeal species under the electrostatic field, however, was clearer in AE67 than in AE33.

In the bacterial community, the dominant phyla were Bacteroidetes, Proteobacteria, Firmicutes, and Cloacamonas_p (Table 5). At the phylum level, however, the bacterial groups were not clearly different owing to the effects of the activated carbon or electrostatic field. At the genus level, *GQ396981_g*, *Cloacamonas*, *BBZD_g*, *Porphyromonadaceae_uc* were abundant in the control, AC, AE33, and AE67. However, the genus *DQ415754_g* was abundant in the control and AE67, while the genus *Acrobacter* was abundant in AE33 and AC.

Table 4. Operational taxonomic units (OTUs) and diversity indices for microbial species in the anaerobic reactors.

Contents	Bacteria				Archaea			
	Control	**AC**	**AE33**	**AE67**	**Control**	**AC**	**AE33**	**AE67**
Valid reads	42,387	49,041	46,822	42,770	22,536	18,365	19,376	18,970
OTUs	1485	1642	1568	1535	90	207	228	153
Jackknife	1697	1873	1741	1757	96	219	236	168
Shannon	5.001	5.138	5.015	5.024	2.189	2.796	2.932	2.562

In the principal component analysis (PCA) for the bacterial species, the variance was mostly explained by the two principal components PC1 and PC2, which captured 81.9% and 13.5% of the variance, respectively (Figure 6a). In the biplot, PC1 was mainly affected by *GQ396981_g CU921187_s* (Accession CU921187), *BBZD_g_uc*, *Porphyromonaceae_uc*, and *Cloacamonas acidaminovorans*, and the features of the bacterial community were all positively correlated to PC1. PC2 was significantly affected in the positive direction by *DQ415754_g_uc* and in the negative direction by *Acrobacter EU234123_s*

(Accession EU234123). The community features in the control and AE67 were positively correlated to PC2 by *DQ415754_g_uc*. The influence of the activated carbon or electrostatic field on PC2 was not clear. The community features of AC and AE33 were significantly correlated in a negative direction to the abundance of *A. EU234123_s*. The bacterial species *G. CU921187_s* is an uncultured species isolated from a mesophilic anaerobic digester for municipal wastewater sludge [46]. *BBZD_g_uc*, belonging to the Bacteroidetes phylum, are uncultured species that can be isolated from various sources including anaerobic co-digestion of cassava pulp and pig manure, microbial fuel cell, lake sediment, PAH contaminated soil, and paddy field soil [46,47]. *Porphyromonaceae_uc* belong to the Porphyromonaceae family and are uncultured species.

Table 5. Microbial phyla, genera, and species in the anaerobic reactors used for methane conversion of coal.

Classification	Taxonomic Composition	Control (%)	AC (%)	AE33 (%)	AE67 (%)
Bacteria					
Phylum	Bacteroidetes	44.3	45.6	44.9	45.1
	Proteobacteria	18.0	24.6	23.3	23.0
	Firmicutes	10.7	9.8	9.7	10.4
	Cloacamonas_p	9.1	6.7	8.1	6.5
	Others	17.9	13.3	14.0	15.0
Genus	*GQ396981_g*	10.1	10.0	9.2	9.9
	BBZD_g	7.3	7.5	7.2	7.0
	Cloacamonas	7.4	6.2	7.6	6.0
	DQ415754_g	6.1	1.8	0.5	6.0
	Porphyromonadaceae_uc	5.3	5.2	4.7	5.6
	Thermomonas	2.4	2.0	1.9	2.9
	Others	61.5	67.3	68.9	62.5
Species	*GQ396981_g CU921187_s*	10.0	9.9	9.1	9.8
	BBZD_g_uc	6.5	7.0	6.6	6.5
	DQ415754_g_uc	6.1	1.8	0.5	6.0
	Porphyromonadaceae_uc	5.3	5.2	4.7	5.6
	Cloacamonas acidaminovorans	4.5	3.3	4.3	3.2
	Thermomonas carbonis	2.1	1.7	1.6	2.5
	Others	65.5	71.1	73.1	66.4
Archaea					
Phylum	Euryarchaeota	96.1	93.3	95.0	94.7
	Bathyarchaeota	3.8	6.7	5.0	5.2
	Others	0.1	0.1	0.0	0.1
Genus	*Methanosaeta*	44.4	35.7	30.1	39.9
	LNJC_g	20.7	13.5	18.7	16.9
	Methanomassiliicoccus	15.9	11.4	10.3	13.9
	DHVE4b_c_uc	2.3	22.6	22.1	13.2
	Others	16.7	16.9	18.8	16.2
Species	*Methanosaeta concilii*	33.1	27.2	22.0	30.0
	LNJC_g LNJC_s	20.6	13.5	18.7	16.8
	Methanomassiliicoccus_uc	15.9	11.3	10.3	13.8
	DHVE4b_c_uc	2.3	22.6	22.1	13.2
	Methanosaeta JN397687_s	8.7	6.6	6.2	7.5
	AF424768_g CU917078_s	3.4	5.8	4.2	4.5
	Others	15.9	13.1	16.6	14.2

C. acidaminovorans is a syntroph that captures energy and carbon from amino acid fermentation. *C. acidaminovorans* is an exoelectrogen frequently observed in bioelectrochemical reactors [48,49]. *DQ415754_g_uc* belongs to the genus *DQ415754_g* and is an uncultured species. The species in the genus *DQ415754_g* have been isolated in Frasassi sulfidic cave stream biofilm and upper sediment [50].

A. EU234123_s is the species isolated in anaerobic processes in the wastewater treatment plant (WWTP) for treating penicillin G production wastewater [51]. It seems that the species significantly affecting the features of PC1 and PC2 contributed to the degradation of the coal degradation intermediates, but the features of bacterial communities were not characterized by the activated carbon or the electrostatic field. However, in the EEM fluorescence spectrum for the intermediates of the control, as in that for AE67, high peaks were observed in regions III and IV. It seems that the abundant bacterial species were dependent on the intermediates of coal degradation.

In the archaeal communities, the most dominant phylum was Euryarchaeota (>93%), followed by Bathyarchaeota (>3.8%) (Table 5). At the genus level, the abundant bacteria in AC, AE33, and AE67 were *Methanosaeta*, *LNJC_g*, and *Methanomassiliicoccus*. However, compared to AC, AE33, and AE67, the control exhibited an increased abundance of genus *Methanocorpusculum* and a reduced abundance of genus *Methanosaeta*. In the PCA, the variance of the archaeal community was explained by two principal components (PC1 95.1% and PC2 3.6%) (Figure 6b). PC1 was characterized by the archaeal species *Methanosaeta concilii*, *LNJC_g LNJC_s* (Accession LNJC01000028), *Methanomassiliicoccus_uc*, *DHVE4b_c_uc*, and *Methanosaeta JN397687_s*. However, PC2 was significantly affected by *DHVE4b_c_uc*. Interestingly, the archaeal community feature of AE67 was very close in the positive direction to PC1. The features of AC and AE33 were also positively correlated with PC1, but for PC2, they were negatively correlated, owing to *DHVE4b_c_uc*. The features of AC and AE33 were very similar, but the variance of AE33 was slightly higher than that of AC. The archaeal community feature of the control was correlated with PC1 and PC2 in the positive direction owing to the relative low abundance of *DHVE4b_c_uc*, which was different from the AC and AE33. The species *M. concilii*, known as an acetoclastic methanogen, is an EMA observed in bioelectrochemical anaerobic digesters [23,52,53]. According to GenBank, *M. JN397687_s* is an uncultured archaeon isolated from river banks. *Methanomassiliicococus spp.* including *Methanomassiliicoccus luminyensis* and *Methanomassiliicoccus intestinalis* are methanogenic archaea species that utilize hydrogen and methanol [54,55]. Recently, the genus *Methanomassiliicoccus* was shown to be an EMA able to degrade antibiotic compounds [56]. The species *L. LNJC_s* were isolated from a methanogenic purified terephthalic acid process wastewater treatment bioreactor, but are possibly electrotrophic methanogens observed in bioelectrochemical anaerobic reactors [22,57]. *DHVE4b_c_uc*, which significantly affected the community feature of PC2, are uncultured species belonging to the class DHVE4b_c. It seems that the archaeal species, including *M. concilii*, *L. LNJC_s*, *Methanomassiliicoccus_uc*, *DHVE4b_c_uc*, and *M. JN397687_s*, that affected the community feature of PC1 were the EMA involved in the direct interspecies electron transfer (DIET) or mediated interspecies electron transfer (MET) during the degradation of coal intermediates.

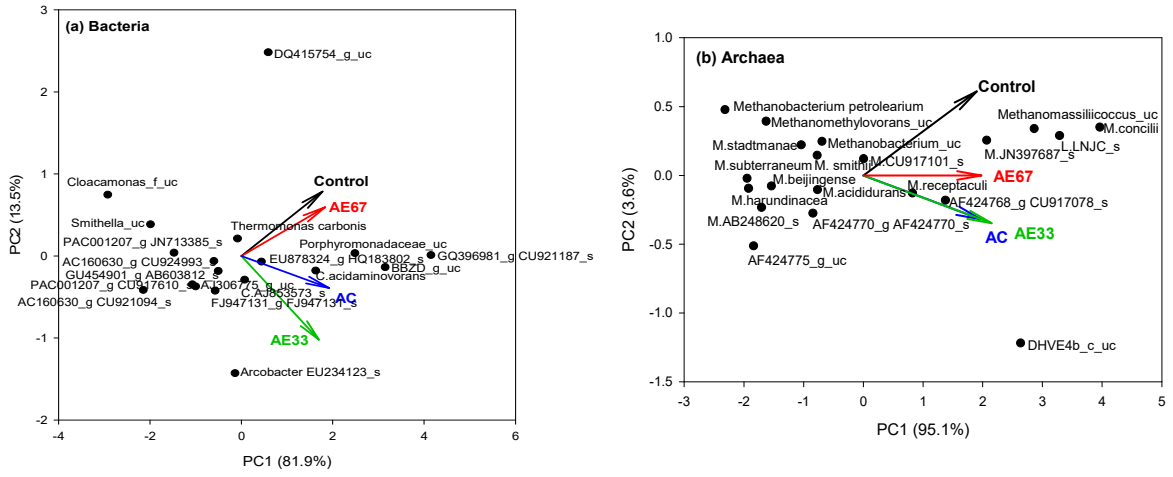


Figure 6. Biplot of the microbial species communities in the bulk solution: (**a**) bacteria and (**b**) archaea.

However, the features of the archaeal community in AE67 were similar to those in the other reactors, including the control, AC, and AE33, except for the abundance of *DHVE4b_c_uc*. The archaeal species *DHVE4b_c_uc* were more abundant in the reactors with the activated carbon, i.e., AC, AE33, and AE67, than in the control. This indicates that *DHVE4b_c_uc* are the archaeal species involved in the electron transfer mediated by the activated carbon. It seems that the methane production from coal and coal degradation intermediates was affected more by the electrostatic field than by the specific microbial community, although the feature of archaeal species producing methane from coal was slightly affected by the activated carbon.

3.6. Implications

The intermediates of coal anaerobic degradation are usually polymeric compounds that are commonly toxic and difficult for anaerobic microorganisms to metabolize directly [7,9,14,17,18]. Therefore, to date, the biological conversion of coal to methane has been slow, and the methane yield has also been too low to justify its use in industrial applications [19,58–60]. Recently, the methane yield of coal was successfully improved by about 52.5 mL/g lignite in a bioelectrochemical anaerobic reactor [9]. However, the conversion rate of coal to methane was still very low. This was due to the inhibitory effect of coal degradation intermediates on methane production from the intermediates. In this study, anaerobic microorganisms were sufficiently activated by yeast extract and activated carbon to play a positive role in the degradation of the intermediates. In the anaerobic medium containing yeast extract and activated carbon, the methane conversion rate of coal was greatly improved by an electrostatic field of 0.67 V/cm, and the final methane yield was also increased to 98 mL/g lignite. Based on the mass balance, the percentage of COD converted to methane was 67.3%, and the residuals in the form of soluble COD and particulate COD were 17.3% and 15.4%, respectively. The aromatic and aliphatic compounds that make up the coal degradation intermediates can be generally oxidized by the ring-opening and chain-breaking reactions that occur in the presence of an electron acceptor [37–39]. It seems that coal degradation intermediates are generally difficult to degrade under anaerobic conditions that do not have readily available electron acceptors. The bioelectrochemical conversion of coal to methane can be explained as follows: (i) coal is first enzymatically hydrolyzed into intermediates, (ii) the intermediates are consecutively broken down under an electrostatic field of 0.67 V/cm by EAB through ring-opening and chain-breaking reactions, and (iii) the electrons released are transferred to EMA to produce methane. Based on electrochemical analyses, including CV and EIS, the direct physical contact between EAB and EMA, together with the mediation of activated carbon and abiotic redox mediators, contributed to the interspecies electron transfer. However, it is worth noting that, although the conversion of coal to methane in a medium containing yeast extract and activated carbon was improved by an electrostatic field of 0.67 V/cm, large amounts of coal degradation intermediates remained in the medium. Interestingly, the intermediates could be quickly converted to methane by replenishing yeast extract and the anaerobic seed sludge in the presence of an electrostatic field of 0.67 V/cm. The diversity of the microbial species was increased by the activated carbon, and the microbial species were slightly more selected by the high strength of the electrostatic field. However, the correlation between the conversion of coal to methane and the structure of the microbial community was unclear. It seems that the microbial metabolism of coal degradation intermediates for methane production was repressed by a form of irreversible substrate inhibition. This implies that the conversion of coal to methane could be controlled by preventing the accumulation of the intermediates and by maintaining an active biomass able to degrade the intermediates. The accumulation of the intermediates could be prevented by using a low loading rate of coal. The active biomass can be maintained by supplying anaerobic microorganisms from outside or by promoting their growth. Yeast extract was a good stimulant for the growth of anaerobic microorganisms, but a variety of readily available and inexpensive materials could be used as biostimulants. Generally, the microbial activity for biodegradation of organic matter increased with increasing the temperature in the medium. The inhibition of the coal degradation intermediates to the thermophilic microorganisms was different

from that of the mesophilic microorganisms. Methane production in subsurface coal beds is generally attempted under thermophilic conditions. This suggests that the aromatic and aliphatic compounds might be broken down more efficiently by thermophilic microorganisms [61]. Coal conversion to methane under thermophilic conditions is a further research topic that must be explored in order to significantly improve the production of methane from coal.

4. Conclusions

The conversion of coal to methane was significantly improved in an anaerobic medium containing yeast extract and activated carbon by an electrostatic field of 0.67 V/cm. Yeast extract is a good biostimulant for anaerobic microorganisms, and activated carbon contributes to microbial diversity and plays a positive role in the degradation of coal. Electroactive microorganisms including EAB and EMA were enriched by the electrostatic field, and coal degradation intermediates were broken down by EAB with the help of the electrostatic field. The electrons released from the degradation of the intermediates were transferred to EMA, which produced methane. However, the microbial metabolism of the coal degradation intermediates that were converted to methane was repressed by a kind of irreversible substrate inhibition. Further conversion of coal to methane depended on control of the irreversible substrate inhibition of the coal degradation intermediates.

Author Contributions: Y.-C.S., D.-H.K., and B.-U.B. conceived the original idea. Y.-C.S. and D.-M.P. designed the study. D.-M.P. and G.-G.O. carried out the experiment and collected the data. Y.-C.S. and D.-H.K. interpreted the data and developed the theory. All authors discussed the data and contributed to the preparation of final manuscript.

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