Coal Utilization as a Growth Medium of Microbial Consortium from Dairy Cow Feces

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Abstract. Many uneconomical coal mining areas which have low calorific value can be found in Kalimantan, Indonesia. In those areas, coalbed methane (CBM) mainly methane (CH\textsubscript{4}) is formed, trapped and accumulated in the pores or cleats during the lifetime coal formation. This condition can potentially be utilized as an alternative energy source. Adding a source of methanogenic bacteria and organic matter is an option to maximize methane in CBM. High carbon content causes young coal to become a source of nutrients for methane-forming microorganisms. Methanogen type microorganisms play an important role in the manufacture of CBM and they can only work on anaerobic conditions. The purpose of this research is to know the relationship between coal characteristic and coal function as microbial growth media to gas formation which can be utilized as environmentally friendly alternative energy. The research used explorative method with descriptive analysis. The research parameters were three coal characteristics, methane production, and Volatile Fatty Acids. The results indicated that coal characteristics support symbiosis and microbial growth with total bacterial counted > 10\textsuperscript{10} CFU/ml, while the amount of acetic acid larger than butyric acid and propionic acid which support sustainable methane gas formation.

1. Introduction
Coal is a complex heterogeneous stone with various physics and chemical characteristics. It composes of organic and inorganic materials. The organic content of coal consists of carbon, oxygen, hydrogen, and sulfur. The carbon content is around 65%-95% of coal and increases as the coal-forming process. During coal formation process, the level of oxygen and hydrogen decrease approximately about 2%-30% and 2%-7% respectively while nitrogen and sulfur decrease around 1%-4% [1]. Microorganisms can grow on coal utilizing organic material contained in coal as a substrate. Complex microbes degrade C\textsubscript{3}, n-C\textsubscript{4}, hydrocarbon liquids, and solid organic mixtures contained in coal, to the stage of biogas formation [2]. Coalbed Methane (CBM) can be offer as a new and renewable energy source. Based on potential study conducted on 10 coal basins, Indonesia has a potential CBM of 337 tcf [3]. This is a potential to be developed, considering Indonesia is also one of the countries with the largest coal reserves in the world. CBM is methane contained in coal and trapped in micro pores of coal through biogenic or thermogenic process during coal formation at 600-1000 m depth. CBM is a clean and renewable energy source, as it will continue to be produced as long as coal seams are available. The yield of coal excavation will produce the remains of coal that is left piled up. This coal can be utilized as a medium of microbial growth in biogas digester through bio augmentation and bio stimulation process.
Bioaugmentation is carried out by using microbial feces from livestock waste with addition of coal (lignite, bituminous, and subbituminous) media. Characteristics of coal include cleats, pores and permeability. The content of organic matter in coal can be used as nutrient source to support microbial growth so that methane production increases with a longer time. Similar process on anaerobic fermentation such as hydrolysis, acidogenesis, acetogenesis and metanogenesis occurs in coal. The similarity of process with anaerobic digestion becomes the basis of bioaugmentation using microbial from livestock waste. The purpose of this study is to determine the characteristics of lignite, bituminous and subbituminous coal, and to what extent coal can be utilized as a medium for microbial growth from dairy cattle waste.

2. Materials and methods

2.1. Materials
The research materials were three types of coal namely lignite, bituminous and subbituminous. The data of coal characteristic obtained through scan process on each coal. Other research materials used were dairy cow feces, medium 985 for anaerobic microbes, and diluent solution no. 14 [4].

2.2. Research procedure
The research procedures were divided into several steps. First, preparation of dairy cow feces sample. Sample of feces was taken from dairy cow farm in Faculty of Animal Husbandry, Universitas Padjadjaran. Fresh feces was taken from the middle part of feces pile which contained relatively low oxygen contamination. After that, sample of feces was squeezed using gauze so that the liquid part separated and put into closed flask. Second, activation of microbes from dairy cow feces. Third, adaptation of microbes on enriched medium. Fourth, preparing of dairy cow feces microbial inoculum. Fifth, addition of microbial consortium from dairy cow feces into coal medium. As much as 70 ml of medium 985 were put into a 100 ml bottle, followed with coal about 10% of media volume. Microbial consortium was added using syringe. The study was conducted with six treatments of microbial consortium addition, namely 0%, 2%, 4%, 6%, 8%, and 10% of medium volume with triplicates. Each sample was observed on day 2, 5, 10, and 15. CO₂ was passed during the process to create anaerobic condition.

2.3. Research methods
This research was used explorative methods with descriptive explanation. Parameter observe were coal characteristics (porosity and permeability), total number of microbes, volatile fatty acids (VFA), and methane content.

3. Result and discussions

3.1. Coal characteristics
Coals were obtained from different location. Bituminous coals (Tanjung formation) were obtained from Muser Village, Paser District, East Kalimantan. Subbituminous coals (Pamaluan formation) were obtained from Liburinding Village, Paser District, East Kalimantan. Lignite coals (Sajau formation) were obtained from Kasai Village, Berau District, East Kalimantan. The analysis results of coal characteristics can be seen in Table 1.

Table 1 described the higher rank of coal, the lower the coal's ability to store and passed fluids rich in volatile substances, including methane. Methane in coal was stored through absorption. If porosity and permeability were low, the liquid would not be stored properly. Lack of absorbed fluid would also reduce methane production by coal bed [5].

Lignite had an average distance between clit (b) 0.55 mm with standard deviation 0.0079 mm; opening clit 0.12 mm. While subbituminous had average distance between clit 0.32 mm with standard deviation 0.0336 mm; clit openings ranged from 0.05 to 0.12 mm with an average of 0.0247 mm. Bituminous coal (Tanjung formation) had a distance between clit range of 0.29 to 0.39 mm with an
average of 0.34 mm; with the average value of clit opening was 0.07 mm with standard deviation 0.0317 mm. The presence of clit would support the escape of liquid from the coal bed. The higher the maturity level of coal, the less clit visible in coal was. Lignite had the largest average distance between clit compared to bituminous and subbituminous coal.

The gas content in coal increased with the additional level of coal hardness associated with the maturity level of coal. The permeability value would decrease along with the addition of coal hardness level. The pores were part of the total volume of coal that could be filled by the fluid. Coal pores were classified by macropores (> 500Å), mesopores (20-500Å), and micropores (8-20Å). The volume and size of the pores would shrink as the maturity level of coal increases. Most macropores were filled with water, free gas, and water-soluble gases. In the micropores structure the water rate and permeability capacities were low, whereas in the clusters of water rate and permeability capacity were larger therefore coal was a reservoir having a double porosity system [6]. The observation of coal characteristics such as porosity, permeability and clit appearance could be a basis for utilizing coal as a growth medium for microbial consortium from dairy cow feces.

### Table 1. Sampling site and coal characteristics

| Type of coals | Bituminous | Subbituminous | Lignit |
|---------------|------------|---------------|--------|
| Coal characteristics | Black in color, black scratched, glossy glass, containing many fractures, hard and solid, conchoidal shards | Black colored, black scratch, glossy glass, contains many fractures, compact, conchoidal shards | Black brownish colored, black scratch, glossy earth glass, containing fractures, soft, conchoidal shards |
| Permeability | 30.2 md<sup>a</sup> | 7.05 md<sup>a</sup> | 37.21 md<sup>a</sup> |
| Porosity | 9.2 % | 5.20 % | 9.20 % |

<sup>a</sup> md: millidarcy

3.2. The number of anaerobic bacteria

Microorganisms can grow on coal using the organic material contained as a substrate. Complex microbes degrade C₃, n-C₄, hydrocarbon liquids, and solid organic mixtures contained in coal until the stage of biogas formation [7]. The proximate analysis by Applied Microbiology Laboratory-Biotechnology LIPI, Cibinong, showed that sample of coal contained 88.53% dry matter component, 11.47% water content, 58.05% ash and 3.54% total nitrogen. These components support the growth of microbes. Data on the number of anaerobic bacteria is shown in Figure 1.

The highest amount of anaerobic bacteria on the lignite medium was achieved at a concentration of 4%, while the highest anaerobic bacteria on subbituminous and bituminous coal media were achieved at a concentration of 6%. Increase in the number of bacteria and the presence of gas formation showed that the microbes from dairy cattle feces can adapt in coal media and the existence of microbial symbiosis between dairy cow feces microbes and coal microbe. Basically methanogens are microorganisms that can only utilize simple carbon components as substrates. During the process of biogas formation of coal, methanogens need to collaborate with other bacterial species that can degrade complex organic materials in coal to a simpler carbon component [8].
Figure 1. The number of anaerobic bacteria

3.3. VFA production

The formation of VFA is started by a consecutive processes namely hydrolysis, acidogenesis and acetogenesis. VFA, apart from acetate, specifically propionate and butyrate is converted into acetate and hydrogen by acetogenic bacteria such as *syntrophomonas* or *syntrophobacter*. Acetate and hydrogen are used by methanogens to form methane [9]. Generally, the accumulation of VFA in the biogas formation process can decrease the pH value, but this depends on the buffer content formed, and the type of biomass used. Methane formation can be inhibited by high VFA concentration [10]. VFA production can be seen in Figure 2 to Figure 4.

Figure 2. VFA content in lignite coal media

Figure 3. VFA content in bituminous coal media
The highest VFA produced on the lignite medium was acetic acid. In acetogenetic phases about 25% acetate and 11% hydrogen was formed [11]. Acetate, H₂, CO₂, formate, methanol, methylamine, and dimethyl sulfide are substrate in methane formation. The process of converting polymers such as polysaccharides, proteins, amino acids, and fats to CO₂ and CH₄ was called methanogenesis that involves interaction of various species of microorganisms. Methane was formed through conversion of acetate, formate, and hydrogen into methane and CO₂ with the help of methanogenic microbes [11]. The process of acetate formation represented the effectiveness level of biogas production, because about 70% of methane was formed from the conversion of acetate to methane. Only a few types of bacteria were capable of producing methane from acetate, but the majority of CH₄ was formed from the process of digestion of acetate by heterotrophic methane bacteria [12]. Only about 30% of methane was formed from CO₂ and H₂ reduction by methane autothrophic bacteria [13,14].

The concentration of VFA was aligned with the activity of microorganisms in substrate degradation [15]. The addition of 4% microbial inoculum in lignite and bituminous coal medium and addition of 6% microbial inoculum on subbituminous coal media showed optimal results in producing VFA. This related to the population and the ability of microbes to degrade organic matter contained in coal. Too dense microbial population caused competition in obtaining substrate, otherwise a small microbial population could cause non optimal substrate degradation. Competition between microorganisms because of population density might cause dominance in one species [16]. According to [17], if two species of microorganisms competed for a limited source of nutrients they could not co-exist with a constant population and a superior species would dominate.

Such dominance induced the production of VFA during incubation to be not maximal. VFA was formed by the activity and symbiosis between microbial species through 2 stages of formation. Stage one was the degradation of organic matter into simple sugars by microbial enzymes. The second stage was the conversion of pyruvic acid into VFA through microbial intracellular metabolism process. VFA results were influenced by the types of microorganisms that played role in degrading pyruvic acid into acetate, butyrate, or propionate [15]. There were at least 4 genera of bacteria consisting of Bacteroides, Geobacter, Clostridium, and Sporochaeta which were known to have a role in the hydrolysis geopolymer process in coal [8].

Coal organic content was converted into fatty acids with long chains and acetic acid through the process of bacterial respiration, while the single ring aromatics component of coal was fermented into phenol and benzoate compounds by fermentative bacteria. Long-chain fatty acids as well as phenol and benzoate compounds were then converted to propionate and butyrate through the activity of syntrophic bacteria [18].

3.4. Methane production
CH₄ is formed in two ways: acetic acid fermentation and CO₂ reduction using formic acid or hydrogen [19]. Acetate is formed during the acidification process of VFA formation and VFA conversion of long
carbon chains through the process of acetogenesis [10]. In addition, [10] states that if VFA concentration too high, it may inhibit gas formation. High VFA concentrations should be accompanied by high methanogen populations so that VFA conversion process becomes balanced with VFA concentration levels. VFA that accumulates in the medium can decrease the pH value. pH is one of the environmental indicators that affect the growth of methanogens. Most methanogens grow optimally at neutral pH, but can still survive at a pH between 3-10 [10,20].

![Figure 5. Methane content on various inoculum concentration](image)

The addition of 4% inoculum treatment might increase methane gas production in lignite, bituminous and sub bituminous coal media. This was an indication that bioaugmentation could accelerate the biogenic process of biogas formation on the coal substrate. The addition of selected bacterial and methanogen consortia could increase methane production by 0.1 µmol methane/day/g coal in the first 50 days of incubation period, and 3 µmol methane/day/g coal in the 50-70 day of incubation period [18]. Research conducted by [21], states that the addition of Na-Acetic nutrients can increase methane production to 5,034 mmol/g of coal on day 24.

4. Conclusion
Coal can be utilized as a growth medium for microbial consortium from dairy cow feces because it has porosity, permeability and clit that can accommodate liquid and moisture. Bioaugmentation with microbes from dairy cattle feces on the three types of coal media can produce VFA with the highest acetic acid to support the production of methane gas and the growth of sustainable anaerobic bacteria.

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