The Role of Rumen Fluid as A Source of Methanogenic Bacteria on Coalbed Methane Activation

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Abstract. CBM is highly potential as a source of renewable energy that can be activated with methanogenic bacteria. The rumen content is a waste of slaughterhouse can be utilized as a source of methanogenic bacteria. The aims to study the role of rumen fluid as a source of methanogenic bacteria in activating coal bed methane. Descriptive research method using 2 types of media consist of standard media (A) and enriched media (B). The application of rumen fluids for CBM activators are lignite, sub-bituminous, and bituminous coal in biogenic fermentation. The inoculum dose was observed at levels 6\% of rumen media. The technique for counting microbes is through Total Plate Count in anaerobic Hungate tube. VFAs production was measured by titration method and methane production was analysed by gas chromatography (GC). All of parameter observed was measured on days 0, 2, 10, and 15. The highest number of anaerobic bacteria was found on day 10 and methane production was found on day 15. The highest production of VFA and methane occurred in biogenic fermentation of lignite. The results showed that the contents of rumen have the potential for the activation of coal bed methane in biogenic fermentation.

1. Introduction
Indonesia is one of the countries with the largest coal reservoir in the world. Coal mining is scattered in Sumatera, Kalimantan, Sulawesi, and Papua islands. However, 60 percent of total coal reservoirs in Indonesia consist of low quality coal because they have low calorific value (sub-bituminous) [1,2]. Clean coal technology in coal mining will be indispensable in the future and Indonesia is expected to be actively involved in the process as one of the main players in the coal mining sector. One of the activities in this clean technology is the development of coalbed methane (CBM), which has a lot of potential in Indonesia, has begun to get attention lately. CBM is one of the alternative energy sources that can replace fossil fuels that generate during coal formation process [3, 4, 5]. CBM can be formed biogenic through microbial activity such as methanogenic bacteria [6, 7, 8].

Rumen content has potentially used as methanogenic bacteria source [9, 10]. It can be obtain from slaughterhouse waste. The number of bacteria in rumen reach $10^9$ CFU/ml while 2-4\% of total bacteria is methanogens [11, 12]. Methanogens is methane generated bacteria in its metabolism. It live in strictly anaerobic condition such as rumen.

2. Material and methods
2.1. Sample and media preparation
Rumen fluid was taken from the content of rumen from slaughterhouse. Cultured media for anaerobic bacteria was consisted of 2 types of dilution: standard media (A) and enriched media (B). Diluent solvent no. 14 [13] was used to dilute samples. Standard media (A) consisted of nutrient agar, lactose broth,
resazurin, distilled water, and rumen fluid, while enriched media consisted of K$_2$HPO$_4$, NaCl, (NH$_4$)$_2$SO$_4$, KH$_2$PO$_4$, CaCl$_2$, MgSO$_4$.7H$_2$O, glucose, cellobiose, resazurin, bacto agar, rumen fluid, and distilled water.

2.2. Evaluation on total plate count of anaerobic bacteria and methane production

Each sample was cultivated in Hungate tube by using roll tube technique so that the melted media spread evenly in tube wall. After media solidified, all samples were incubated at 37 °C for 15 days. The growth of bacteria was observed on day 2, 5, 10, and 15. Coal used was lignite, subbituminous, and bituminous. Production of methane was measured using gas chromatography (GC). Concentration of VFAs was measured using titration method.

2.3. Statistic Analysis

The research analysis was used descriptive methods. Data was calculated by the average of samples obtained at various sample with triplicates. Data obtained was calculated by using SPSS 17.

3. Result and discussion

Cultured media is a nutrients source that support optimal growth. The quality and quantity of nutrients can determine the growing rate of bacteria. In this study, anaerobic bacteria from rumen content were grown on standard media (A) and enriched media (B). Measurement of the number of anaerobic bacteria was performed on days 2, 5, 10, and 15 (Table 1). The number of anaerobic bacteria on media A and media B increases with time of incubation. However, on day 15 the number of anaerobic bacteria decreases. Based on the bacterial growth curve, it can be explained that the growth of anaerobic bacteria on day 2 is still experiencing an adaptation period in terms of nutrition, pH and temperature of the environment. Furthermore, bacteria experienced a rapid growth process on the 5th day until day 10 so that will produce the largest number of bacteria and reached peak on the day 10 (Fig. 1).

Table 1. Growth of Anaerobic Bacteria and Methane Production from Rumen Fluid on Media (A) and Media (B)

| Days | Media A | Media B |
|------|---------|---------|
|      | Anaerobic Bacteria (10$^6$ cfu/ml) | Methane (ppm) | Anaerobic Bacteria (10$^6$ cfu/ml) | Methane (ppm) |
| 2    | 36.67±14.33 | 361.26±101.52 | 33.33±7.18 | 676.55±142.72 |
| 5    | 96.67±18.98 | 1443.42±122.14 | 65.23±22.87 | 1720.94±155.12 |
| 10   | 133.33±50.21 | 11196.67±605.12 | 188.33±39.92 | 42171.69±5431.21 |
| 15   | 98.33±7.18  | 14730.85±930.55 | 136.67±32.53 | 75358.37±4630.99 |

Media A= Standard Media; Media B= Enriched Media

Figure 1. Growth of anaerobic bacteria from rumen fluid on different media
Table 2. Concentration of VFAs and methane production of different coal type

| Type of Coal | Variable       | 2               | 5               | 10              | 15              |
|--------------|----------------|-----------------|-----------------|-----------------|-----------------|
| Lignite      | Methane (ppm)  | 617.54±181.39   | 1514.51±139.11  | 53820.24±6275.52| 90307.66±7895.55|
|              | VFA (mM)       |                 |                 |                 |                 |
|              | Acetate        | 11.73           | 14.67           | 17.67           | 30.62           |
|              | Propionate     | 2.59            | 3.56            | 3.32            | 6.29            |
|              | Iso            | 0.42            | 0.56            | 0.68            | 1.25            |
|              | Butirate       |                 |                 |                 |                 |
|              | N butirate     | 0.54            | 1.15            | 1.15            | 1.77            |
|              | Iso valerate   | 0.22            | 0.35            | 0.37            | 0.62            |
|              | N valerate     | -               | -               | -               | 0.03            |
| Sub-bituminus| Methane (ppm)  | 644.25±158.72   | 1211.35±115.22  | 13610.46±805.44 | 54831.92±6271.71|
|              | VFA (mM)       |                 |                 |                 |                 |
|              | Acetate        | 12.89           | 14.68           | 17.69           | 23.08           |
|              | Propionate     | 1.87            | 2.58            | 4.20            | 5.43            |
|              | Iso butirate   | 0.52            | 0.55            | 0.71            | 1.13            |
|              | N butirate     | 0.50            | 1.35            | 1.26            | 1.75            |
|              | Iso valerate   | 0.19            | 0.26            | 0.34            | 0.74            |
|              | N valerate     | -               | -               | -               | -               |
| Bituminus    | Methane (ppm)  | 505.90±125.11   | 1240.77±120.12  | 17861.22±828.25 | 53182.92±6157.22|
|              | VFA (mM)       |                 |                 |                 |                 |
|              | Acetate        | 11.70           | 13.72           | 15.45           | 19.85           |
|              | Propionate     | 1.21            | 1.89            | 2.74            | 4.18            |
|              | Iso butirate   | 0.40            | 0.48            | 0.72            | 1.81            |
|              | N butirate     | 0.48            | 1.27            | 1.31            | 1.68            |
|              | Iso valerate   | 0.21            | 0.28            | 0.33            | 0.81            |
|              | N valerate     | -               | -               | -               | -               |

On the day 15, nutrient available decrease because of the increasing population of bacteria. This condition is similar to bacteria growth curve [14, 15]. Bacteria needs nutrient to maintain its cell. In this study, the utilization of two different media (standard and enriched media) is to determine the optimal nutrient requirement for anaerobic bacteria.

Enriched media contains more complete nutrient than standard media [13, 16]. The main composition of enriched media is bacto agar and rumen fluid. Bacto agar is purified agar by reducing the content of impurity pigments, salt content (NaCl), and the foreign content (organic and inorganic) as low as possible. Various minerals are added to support the growth of anaerobic bacteria on enriched media [17, 18]. As a result, growth of anaerobic bacteria on enriched media that has more complete nutrients is higher than standard media (Fig. 1).
Measurements of volatile fatty acid (VFA) concentration and methane production in various coal types such as lignite, sub-bituminous, and bituminous were performed on enriched media (Table 2). Methane production was directly proportional to the increase in the number of anaerobic bacteria (Table 2). This illustrates that anaerobic bacteria from rumen fluid grown on enriched media were capable of producing methane while the highest VFAs production after a 15-day incubation period was acetate. Acetate is the main precursor for methane production during anaerobic digestion of organic matter [19]. Volatile fatty acids (VFAs) are important mid-products in the production methane. Their concentrations affect the efficiency of fermentation [20]. High VFAs concentration increases the methane yield [21]. The highest acetate concentration was achieved on 15th day lignite coals (Table 2). Lignite, sometimes called "brown coal", is a low-calorie coal type, with a carbon content of 25-35% and 30-60% water content [22]. The lignite coal response in producing VFAs and methane better than other coal types is unexplainable. However, anaerobic bacteria from rumen fluid can adapt and grow better in lignite than other type.

4. Conclusion
Rumen fluid has a high potential in providing anaerobic bacteria to produce coalbed methane in the biogenic fermentation process. Lignite coal produces the most favorable response in the production of VFAs and methane compared to sub-bituminous and bituminous coal. The 15-day incubation time produces the highest amount of VFAs and methane production, both in lignite, sub-bituminous, and bituminous.

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