Bacterial Biodiversity in Anaerobic Digestions of Capsule Husk and Seed Cake of *Jatropha curcas* Linn and Cow Dung

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**ABSTRACT**

Biogas has big contributions to the environment since it produces renewable energy, prevents methane’s release as greenhouse gas, and at the same time, the slurry or sludge from outlet digester can be used as organic fertilizer or animal feed ingredients. This research was carried out at PT Bumimas Ekapersada, Bekasi, and West Java of Indonesia. Liquid samples were derived from inlet digester of hydrolysis of crude Jatropha oil (CJO) waste and also taken from outlet digester of methanogenesis of husk and seed cake. The liquid samples from digester of cow dung were also taken from its outlet. The observations of inlet and outlet liquid samples from digesters showed the presence of 12 bacteria isolates that comprise of three families, which are all negative gram bacteria such as Halobacteriaceae, Azotobacteraceae, dan Pseudomonadaceae. The samples from outlet methanogenesis digester, that generated by waste of CJO showed the highest population of Pseudomonadaceae family. The percentage of Pseudomonadaceae in husk based methanogenesis digester was 53.57%; while in digester from methanogenesis of seed cake was 78.57%. The highest population obtained from all digester was digestion of cow dung based; it is Azotobacteraceae (75.86%) and Pseudomonadaceae (24.13%). There was a shift of bacterial diversity in the inlet of digester that dominated by Azotobacteraceae compared to the outlet of digester which was dominated by Pseudomonadaceae that using CJO waste as a feedstock. The liquid from outlet digester of CJO and digester of cow dung could be used as an organic fertilizer because it contains Plant Growth Promoting Rhizobacteria (PGPR) bacteria such as Azotobacteraceae and Pseudomonadaceae.

**Keywords:** Biogas, bacteria biodiversity, cow dung, capsule husk, seed cake, *Jatropha curcas* Linn
1. Introduction

Currently, the usage of Indonesia's energy relies only on fossil oil. Considering the availability of fossil oil and the increasing of price, addition of energy subsidies, and ecologically factor, Indonesia government plans to switch into renewable energy gradually [1]. In the 25/25 program, bioenergy/biofuels will occupy the largest share, which is 8.9% [2]. That is a relatively hard target, because some evaluated data stated that biodiesel and bioethanol program as a fuel substitute is constrained until 2012, so it does not reach the target. Inability to reach this target, it was also reported in the Desa Mandiri Energi (Self-Sufficient Energy Village) program, especially for the usage of cassava in order to provide bioethanol and *Jatropha curcas* Linn. (JcL) as the material of biodiesel production. The evaluation states that only biogas program is running well [3].

Biogas is categorized as gaseous fuel, while World Bank states that biogas is a modern energy in Asia [4-6]. Soerawijaya [7] stated that biogas is a fuel, which is energetically most efficient. In the biogas production process, it is not generating a gaseous fuel only, but also produce slurry and sludge, which can be used as organic fertilizer and an animal feed additive. In that consideration of the biogas product (stock) it can produce more impact, so that the development of biogas industry it should be supported [8].

In Indonesia, generally biogas use cow dung (manure) as feedstock. However, it is hard to supply cow dung in some regions because the area is not the dairy farms, cattle breeding habits is not ground, and any other reason. Deublein and Steinhauser [9] and Hendroko et al., [10] suggested that utilizing waste of JcL, especially dried husk capsules (DH-JcL) for some consideration such as biorefinery to increase revenue for JcL farmers and spur on the development of JcL cultivation for biodiesel, in addition to obtain biogas and slurry as fertilizer.

Actually, biogas is derived from series processes of microorganisms consortium. In order to support the utilization of DH-JcL waste, it is necessary to conduct the preliminary studies of microbes that occupy a role in the biogas digester. Similarly, studies are needed to determine that digester slurry or sludge as organic fertilizer, especially about presentation of useful microbes for agricultural land. It is also known that JcL contains a number of anti-nutrients such as phorbol esters which are toxic for livestock and humans [11].

2 Methodology

Preliminary studies on biogas production was conducted in PT Bumimas Ekapersada, Bekasi, and West Java of Indonesia. Slurry samples were taken from the three digesters outlet that are digester of dried husk capsule (DH-JcL), digester of seed cake (SC-JcL) and digester of cow dung. Similarly, the samples were also taken from the inlet of two digesters namely digester of DH-JcL and digester of SC-JcL. The five of slurry samples were observed in the laboratory of PT Smart Tbk in Bogor. The observations conducted based on standard procedure given by Li et al., [11], which was isolated by pour plate method, where the smallest dilution of $10^{-1}$ to $10^{-8}$ using Nutrient Agar (NA) media and Lauria Agar(LA) media with two duplicates. Pour plates were incubated at the temperature of 37°C in overnight and then it counted for the population colonies at each dish. Then, the biochemical test, catalase test, indole test, and phenilalanine test were conducted as in standard procedure given [12].

3 Results

The samples from digester of DH-JcL and SC-JcL were obtained from inlet hydrolysis digester and were taken from outlet methanogenesis digester. The samples from digester of cow dung were also...
extracted from the outlet. The observations of sample were based on standard method and it are presented as in Figure 1, Figure 2 and Table 1.

![Image of DNA gel electrophoresis](image)

**Fig.1.** Bacterial genome in five liquid samples of Biogas Digester (DNA isolation gave an electrophoresis band at 20,000 bp)

Based on observation of the result, the inlet and outlet liquid digesters show the presence of 12 bacteria isolates comprising of three families, which are all negative gram bacteria such as Halobacteriaceae, Azotobacteraceae, and Pseudomonadaceae. Sample from DH-JcL and SC-JcL inlet digester was dominated by Azotobacteraceae (coccobasill shaped), then came Azotobacteraceae (basill shaped) and the lowest was Pseudomonadaceae. Bacterial diversity in outlet which DH-JcL as biogas feedstock, it was relatively more, namely Pseudomonadaceae, Halobacteriaceae, Azotobacteraceae (coccobasill shaped), and azotobacteraceae (basill) compared to two families of bacteria isolated from SC-JcL and cow dung based digester.

Halobacteriaceae was only found in the outlet side of the digester, which used CJO waste as feedstock, both are DH-JcL and SC-JcL. In addition, Pseudomonadaceae also experienced rapid growth in the outlets. Sample from methanogenesis outlet which used waste of CJO, it showed the highest population of Pseudomonadaceae. The percentage of Pseudomonadaceae in husk methanogenesis digester was 53.57%, while seed cake methanogenesis digester was 78.57%. On the other hand, Azotobacteraceae growth in contrast with Pseudomonadaceae in digester of CJO waste. Azotobacteraceae population located in the inlet DH-JcL digester was 100% changed into 14.28% (Coccobasill) and 8.92% (Basill) on the outlet side, while the inlet SC-JcL digester has a similar condition, specifically 87.87% (Coccobasill) and 9.09% (Basill) turned out to be the almost non-existent on the outlet side. It stated that methanogenesis of CJO waste is the right medium and appropriate conditions for Halobacteriaceae and Pseudomonadaceae growth, whereas the opposite condition happened to Azotobacteraceae.

Bacteria biodiversity, which is grown in digester of cow dung, it was different from digester of CJO waste, which Azotobacteraceae was more dominant than Pseudomonadaceae. The population of the digester with cow dung as a feedstock was 75.86% of Azotobacteraceae and 24.13% of Pseudomonadaceae. In spite of there were different types of bacteria composition in digester of cow dung but in digester of CJO waste had similarities populations of Azotobacteraceae and Pseudomonadaceae. The slurry/sludge which taken from the three digesters have a potential as organic fertilizer since both types of bacteria were found in the digester, then it have the ability to be a Plant Growth Promoting Rhizobacteria (PGPR) which can enhance the plant productivity and can be used as a biological control agents [13,14].
Fig. 2. Twelve isolates of bacteria in five liquid samples from Biogas Digester

Table 1
Bacteria Colonies Characteristic from Five Samples of Biogas Digester Liquid

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Code</th>
<th>Family</th>
<th>Gram</th>
<th>Form Cell</th>
<th>Margin</th>
<th>Elevation</th>
<th>Form</th>
<th>Colour</th>
<th>CFU/mL (x 10^5)</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outlet digester with JcL Husk as feedstock</td>
<td>M1</td>
<td>Halobacteriaceae</td>
<td>Negative</td>
<td>Coccus</td>
<td>Entire</td>
<td>Convex</td>
<td>Circular</td>
<td>Yellow</td>
<td>1.299</td>
<td>23.21</td>
</tr>
<tr>
<td>M2 Azotobacteraceae</td>
<td></td>
<td></td>
<td>Negative</td>
<td>Coccobassil</td>
<td>Entire</td>
<td>Umbonate</td>
<td>Circular</td>
<td>Orange</td>
<td>0.795</td>
<td>14.28</td>
</tr>
<tr>
<td>M3 Azotobacteraceae</td>
<td></td>
<td></td>
<td>Negative</td>
<td>Bassil</td>
<td>Entire</td>
<td>Convex</td>
<td>Circular</td>
<td>White</td>
<td>0.498</td>
<td>8.92</td>
</tr>
<tr>
<td>M4 Pseudomonadaceae</td>
<td></td>
<td></td>
<td>Negative</td>
<td>Bassil</td>
<td>Undulate</td>
<td>Flat</td>
<td>Circular</td>
<td>Transparent</td>
<td>2.996</td>
<td>53.57</td>
</tr>
<tr>
<td>Outlet digester with JcL Seed Cake as feedstock</td>
<td>M5</td>
<td>Pseudomonadaceae</td>
<td>Negative</td>
<td>Bassil</td>
<td>Lobate</td>
<td>Raised</td>
<td>Circular</td>
<td>White</td>
<td>1.099</td>
<td>78.57</td>
</tr>
<tr>
<td>M6 Halobacteriaceae</td>
<td></td>
<td></td>
<td>Negative</td>
<td>Coccus</td>
<td>Entire</td>
<td>Convex</td>
<td>Circular</td>
<td>White</td>
<td>0.299</td>
<td>21.42</td>
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<tr>
<td>Inlet digester with JcL Husk as feedstock</td>
<td>M7</td>
<td>Azotobacteraceae</td>
<td>Negative</td>
<td>Bassil</td>
<td>Undulate</td>
<td>Convex</td>
<td>Circular</td>
<td>White</td>
<td>0.600</td>
<td>100</td>
</tr>
<tr>
<td>Inlet digester with JcL Seed Cake as feedstock</td>
<td>M8</td>
<td>Azotobacteraceae</td>
<td>Negative</td>
<td>Coccobassil</td>
<td>Entire</td>
<td>Convex</td>
<td>Circular</td>
<td>White</td>
<td>5.794</td>
<td>87.87</td>
</tr>
<tr>
<td>M9 Azotobacteraceae</td>
<td></td>
<td></td>
<td>Negative</td>
<td>Bassil</td>
<td>Filamentous</td>
<td>Convex</td>
<td>Irregular</td>
<td>White</td>
<td>0.594</td>
<td>9.09</td>
</tr>
<tr>
<td>M10 Pseudomonadaceae</td>
<td></td>
<td></td>
<td>Negative</td>
<td>Bassil</td>
<td>Lobate</td>
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<td>Circular</td>
<td>White</td>
<td>0.198</td>
<td>3.03</td>
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<td>Outlet digester with Cowdung as feedstock</td>
<td>M11</td>
<td>Pseudomonadaceae</td>
<td>Negative</td>
<td>Bassil</td>
<td>Undulate</td>
<td>Flat</td>
<td>Circular</td>
<td>White</td>
<td>3.494</td>
<td>24.13</td>
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<tr>
<td>M12 Azotobacteraceae</td>
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<td></td>
<td>Negative</td>
<td>Bassil</td>
<td>Filamentous</td>
<td>Convex</td>
<td>Irregular</td>
<td>White</td>
<td>10.991</td>
<td>75.86</td>
</tr>
</tbody>
</table>
4. Conclusions

There was a shift of bacterial diversity in the inlet of digester where it was dominated by Azotobacteraceae compared to the outlet of digester that was dominated by Pseudomonadaceae, where it used CJO waste as a feedstock for biogas production. Outlet liquid from digester of CJO waste and digester of cow dung can be used as organic fertilizer because it contains Plant Growth Promoting Rhizobacteria (PGPR), which can enhance the plant productivity and be as a biological control agent such as Azotobacteraceae and Pseudomonadaceae. This observation are only conducted for the species identification, and isolation of anaerobic bacteria involved in biogas (methane) production.

References