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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.

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.

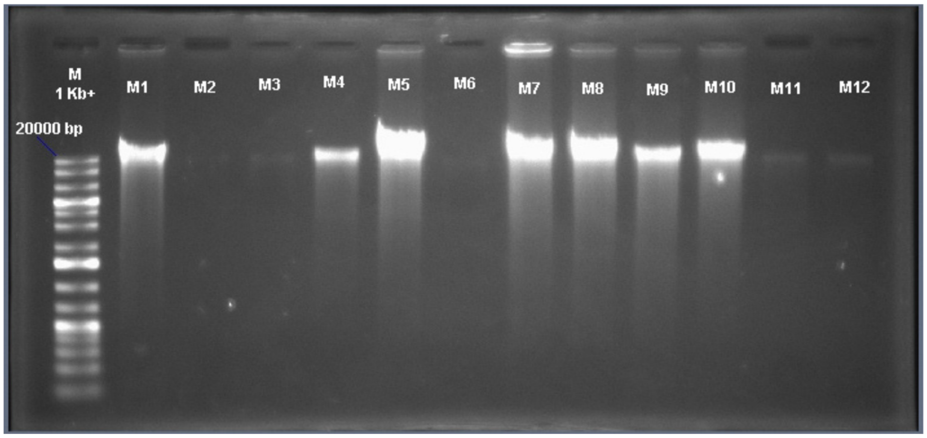

Fig.1. Bacterial genome in five liquid samples of Biogas Digester (DNA isolation gave an electrophoresis band at 20.000 bp)

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, dan Pseudomonadaceae. Sample from DH-JcL and SC-JcL inlet digester was dominated by Azotobacteraceae (coccobassil shaped), then came Azotobacteraceae (bassil shaped) and the lowest was Pseudomonadaceae. Bacterial diversity in outlet which DH-JcL as biogas feedstock, it was relatively more, namely Pseudomonadaceae, Halobacteriaceae, Azotobacteraceae (coccobassil shaped), and azotobacteraceae (bassil) 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% (Coccobassil) and 8.92% (Bassil) on the outlet side, while the inlet SC-JcL digester has a similar condition, specifically 87.87% (Coccobassil) and 9.09% (Bassil) 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].

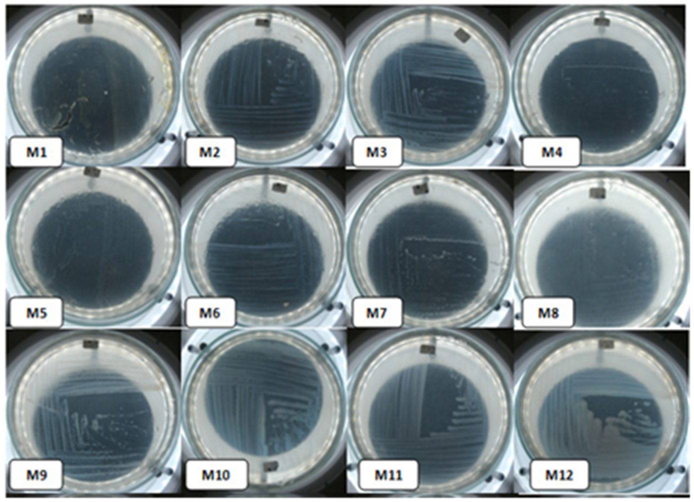

Fig. 2. Twelve isolates of bacteria in five liquid samples from Biogas Digester

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

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

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

[1] Sumiarso, L. "Global energy crisis and solution via biogas development." In *Green Productivity–Biogas Seminar. KADIN. Jakarta*. 2011.

[2] Warnika, Kardaya. "Potensi peluang & peranan energi nabati dalam mewujudkan kedaulatan energi nasional." In *Simposium & Seminar Bersama PERAGI-PERHOTI-PERIPI-HIGI mewujudkan kedaulatan pangan & energi berkelanjutan*, pp. 1-2.

[3] Kusdiana, D. Pendampingan, Monitoring dan Evaluasi Pelaksanaan Desa Mandiri Energi BBN. Coordination Meeting of Desa Mandiri Energi BBN, Malang, 19th November 2012.

[4] Kismurtono, Muhammad, and B. Paul Naiola. "The challenges of biofuel implementation in Indonesia: environmental prospect." *Natural gas* 212, no. 832.0 (2008): 619-2.

[5] Salafudin, Adinurani PG, T. Liwang, L. O. Nelwan, Y. Sakri, and R. Hendroko. "Study biorefinery capsule husk from Jatropha curcas L. waste crude jatropha oil as source for biogas." In *World Renewable Energy Congress Indonesia, International Conference and Exhibition on Renewable Energy and Energy Efficiency in Nusa Dua Bali*. 2011.

[6] World Bank. *One Goal, Two Paths: Achieving Universal Access to Modern Energy in East Asia and Pacific*. World Bank Publications, 2011.

[7] Soerawidjaja, Tatang H. "Prospek dan potensi teknologi pencernaan anaerobik di dalam perekonomian berbasis nabati." In *Seminar Nasional Green Productivity II "desa produktif berwawasan lingkungan*. 2011.

[8] Sivasamy, A., S. Zinoviev, P. Foransiero, S. Miertus, F. Müller-Langer, M. Kaltschmitt, A. Vogel, and D. Thraen. "Bio-fuels technology status and future trends." *Technology Assessment and Decision Support Tools* (2007).

[9] Deublin, Dieter, and Angelika Steinhauser. "Biogas from waste and renewable resources." (2008).

[10] Hendroko, Roy, Tony Liwang, Praptiningsih G. Adinurani, Leopold O. Nelwan, Yosephianus Sakri, and Satriyo K. Wahono. "The modification for increasing productivity at hydrolysis reactor with Jatropha curcas Linn capsule husk as bio-methane feedstocks at two stage digestion." *Energy Procedia* 32 (2013): 47-54.

[11] Li, Cai-Yan, Rakshit K. Devappa, Jian-Xin Liu, Jian-Min Lv, H. P. S. Makkar, and K. Becker. "Toxicity of Jatropha curcas phorbol esters in mice." *Food and Chemical Toxicology* 48, no. 2 (2010): 620-625.

[12] Cappuccino and Sherman. Bergey's Manual of Determinative Bacteriology". Eighth Edition. 2005.

[13] Saraswati, Rasti, and Sumarno Sumarno. "Pemanfaatan mikroba penyubur tanah sebagai komponen teknologi pertanian." *Iptek Tanaman Pangan* 3, no. 1 (2015).

[14] Nihorimbere, Venant, Marc Ongena, Maïté Smargiassi, and Philippe Thonart. "Beneficial effect of the rhizosphere microbial community for plant growth and health." *Biotechnologie, Agronomie, Société et Environnement= Biotechnology, Agronomy, Society and Environment [= BASE]*15, no. 2 (2011): 327-337.