Microbial bioaugmentation from feces of laying hens in biogas formation on anaerobic coal media

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Abstract. The laying hen livestock industry is growing rapidly, along with the increasing demand for eggs for human consumption, produces large amounts of waste. Improper management of laying hens farm waste can interfere with health and environmental pollution, including greenhouse gases (CH₄, CO₂, N₂O), odor disorders, disturbances from rodent animals, disturbances of endoparasites and ectoparasites, pollution of water and soil sources. Waste management that is correct can reduce the risk of pollution of the laying hen's industry to the environment by utilizing the feces of laying hens as a source of the microbial consortium that serves as a starter for biogas formation in anaerobic digester as an environmentally friendly alternative energy source. This study aimed to obtain a bacterial and methanogen consortium from feces of laying hens as a starter for biogas formation with coal media in the anaerobic digester. The study used an experimental method of completely randomized design (CRD) with four inoculum doses with four replicates, further tests of orthogonal polynomials. The study was conducted in two stages: the first stage was pretreatment through in-vitro techniques and adaptation process, and the second stage is the addition of the starter of microbial consortium from feces of the laying feces (bioaugmentation) into liquid media and coal at a dose of 0%, 5%, 10% and 15% incubated at 39°C for 28 days. Observations were conducted every seven days from day 0, day 7, day 14, day 21, and day 28. The parameters measured were the volume of biogas, the number of anaerobic bacteria, and the composition of biogas. This biogas composition was analyzed by Gas Chromatography, the number of anaerobic bacteria planted in Hungate tubes, and calculated using the Ogimoto method. The observations showed that the number of bacteria ranging from 10¹² CFU/ml up to 10¹³ CFU/ml exceeded the starter requirements of 10⁷ CFU/ml, which indicated that the microbial consortium obtained met the requirement.

Keywords: Microbial, Feces, Laying Hens

1. Introduction
Laying hens excreta contains organic matter, can harm the environment and public health because it contains high total organic carbon (16.5%) and nitrogen (4.48%), as well as pathogenic bacteria, especially Staphylococci and Enterobacteriaceae (114,108 and 154,106 CFU/g) [1-4]. Laying hens manure is an organic material rich in nutrients, such as potassium, phosphorus, and nitrogen,
commonly used as organic fertilizer in agriculture. However, agricultural land's direct use can cause various environmental problems, namely eutrophication, groundwater pollution, the spread of pathogens, odors, and greenhouse gas emissions [5]. Anaerobic digestion is a profitable option for stabilizing organic matter in hen manure and biogas production [6, 7]. The feces of laying hens have relatively high energy content than cow and pig manure [8]. Microorganisms in hen excreta can be isolated and used as consortiums of bacteria producing methane and carbon dioxide. Therefore, it is essential to treat excreta waste to prevent an increase in environmental pollution. Organic matter contained in laying poultry excreta waste can be converted into a form of energy. The utilization of excreta into energy can be done by processing it into biogas in an anaerobic digester. One effort to increase methane production from microbial populations sourced from excreta is to add a non-renewable energy source, coal [9]. The presence of methane on coal can be used as a renewable energy source by applying coal in anaerobic fermentation to produce biogas [10]. Anaerobic digestion of organic nitrogen, converted to ammonia, can inhibit and reduce biogas production, which will cause the accumulation of volatile fatty acids (VFA) [11]. Therefore, the formation of ammonia is considered a significant problem in biogas production using laying hens’. Problems that occur from laying hens’ feces in biogas formation using laying hens’. To reduce ammonia resistance in an anaerobic digester, dilute the media with water [12] and reduce the resistance of ammonia formed in anaerobic fermentation with organic raw materials that have low nitrogen content. High carbon content sources need to be added to obtain the optimal C/N ratio in an anaerobic digester originating from laying hens in vitro with a medium of coal containing high organic carbon.

2. Materials and methods
This study consisted of two stages. The first stage was the in-vitro procedures and the adaptations process. In this stage, the bacterial starter was obtained that would be used for activities on the second stage. The second stage was an experimental method, namely Randomized Experiment Design, with four treatments of bacterial consortium doses form Laying hens excreta, namely P1 (0%), P2 (5%), P3 (10%), and P4 (15%) with five replications, wherein each tube coal as much as 10% was added of the media volume. The parameters observed were the volume of gas, the proportion of biogas consisted of methane (CH₄), and carbon dioxide (CO₂), the number of anaerobic bacteria, and the content of Volatile Fatty acid (VFA). The number of bacteria and the volume of biogas were taken from weeks 7, 14, 21, and 28. Observation of the proportion of biogas was carried out from week 14, 21, and 28. This research had been carried out at the Laboratory of Microbiology and Livestock Waste Management, Faculty of Animal Husbandry, Universitas Padjadjaran. Analysis of the proportion of biogas utilized GC TDC in the Laboratory of Chemical Engineering, University of Indonesia. The calculation of anaerobic bacteria was through the roll-tube method. The gas obtained for calculating the volume of gas was drawn using a Syringe volume of 50 ml. The results were analyzed using the analysis of variations to see the difference between treatments.

3. Result and discussion

3.1. Number of anaerobic bacteria
Bacterial growth generally refers to the total increase in bacterial cell mass [14]. Anaerobic bacteria do not use oxygen as the last electron acceptor in respiration [15]. Figure 1 displaying the average number of anaerobic bacteria. The results of statistical calculations show that the starter dose treatment has the same effect on the number of bacteria.
This study used coal as microbial growth media that serve as high carbon elements substrate. Lignite was low-grade coal with a carbon content of <69%, had Rv between 0.5-2.0 and porosity 9.20% [16, 17]. The coal samples used in this study consisted of 88.53% dry matter, 58.05% ash, and a total N of 3.54%. The organic content of coal in the form of long-chain alkanes was converted to fatty acids with long chains and acetic acids through bacterial respiration, in contrast, the single ring aromatics component of coal was fermented into phenol and benzoic compounds by fermentative bacteria [18]. Excreta and intestinal fluids of laying hens contained many cellulolytic bacteria that converted crude fiber in feed to methane to increase Coal Bed Methane (CBM) production. Laying hens, including poultry, which could not digest crude fiber, only about 20% of raw fiber could be digested in the cecum so that crude fiber that could not be digested would make nutrients come out with feces [19]. Cellulolytic bacteria would convert the crude fiber into CH₄ as a material for biogas formation in the intestine (cecum). CH₄ could increase and decrease. It is influenced by the presence of anaerobic bacteria and methanogens and the availability of substrates that is very important for the growth of anaerobic bacteria in producing methane.

3.2. Biogas volume

Biogas is a natural gas-rich in methane and is formed from organic material through the anaerobic fermentation process [20]. Biogas derived from organic matter contains 30% -40% CO₂; 60% -70% CH₄, H₂ and <1% N₂ and N₂O [21]. Figure 2 displayed the average total gas volume. The statistical analysis of the data showed that the treatment of starter doses has the same effect on the gas volume.

The highest biogas production was obtained at P3 (10% dose). The non-significant statistical analysis was an indication that the addition of microbial inoculums at various doses could accelerate the biogenic process of biogas formation in coal substrates. In line with opinion [22], the acceleration
of gas production per unit time was in line with microorganisms' activities. The faster the gas production was, the more active the microbes degraded the available nutrient source.

3.3. Production of methane and carbon dioxide

Enteric fermentation does not occur in the digestion of poultry. Methane and CO$_2$ are formed when manure is stored in an anaerobic condition [23]. When stored under anaerobic conditions, the addition of starter microorganisms from poultry can produce biogas consisting of methane (CH$_4$) and carbon dioxide (CO$_2$), N$_2$O, along with nutrients, salt, and organic matter. This process consists of four stages, in which a specialized bacterial consortium is responsible for determining the first phase of hydrolysis, acidogenesis, and acetogenesis, while the fourth step, methanogenesis, is carried out by the methanogenic groups of archaea.

The process of converting polymers such as polysaccharides, proteins, amino acids, and fats to CO$_2$ and CH$_4$ is called methanogenesis and involves the interaction of various species of microorganisms. Methane is formed by converting acetate, formate, and hydrogen to methane and CO$_2$ with the methanogenic microbes/methanogens [24]. Figure 3 showed data on the average production of methane and carbon dioxide. The statistical analysis results showed that the starter dose treatment significantly affected methane production and the same effect on carbon dioxide formation.

![Figure 3. The average production of methane and carbon dioxide](image)

Microbes played a role in the process of anaerobic digestion under anaerobic conditions, digestion of various organic substrates operated by a consortium of microorganisms converting organic material into biogas, a mixture consisting of methane (CH$_4$) and carbon dioxide (CO$_2$) along with nutrients, additional cell substances, salts, and salts organic matter. This process consisted of four stages, in which a particular bacterial consortium was responsible for determining the first phase of hydrolysis, acidogenesis, and acetogenesis, at the same time the fourth step, methanogenesis, was carried out by methanogenic groups of archaea. The process of converting polymers such as polysaccharides, proteins, amino acids, and fats to CO$_2$ and CH$_4$ was called methanogenesis and involved the interaction of various species of microorganisms. Methane was formed by converting acetate, formate, and hydrogen into methane and CO$_2$ with the help of methanogen microbes [21].

3.4. Volatile fatty acid (VFA)

VFA other than acetate, i.e., propionate or butyrate, was converted to acetate and hydrogen by acetogenic bacteria such as syntrophomonas or syntrophobacter. The process of acetate formation illustrated the level of efficiency of biogas production because about 70% of methane was formed from the conversion of acetate into methane. In the acetogenic process, about 25% acetate and 11% hydrogen were formed [25].
Figure 4. The average of volatile fatty acid (VFA)

The increase of VFA was a result of the conversion of the organic content contained in coal in the form of long-chain alkanes into long-chain fatty acids and acetic acid through the process of bacterial respiration. In contrast, the single ring aromatics component of coal was fermented into phenol and benzoic compounds by fermentative bacteria. Long-chain fatty acids and phenols and benzoate compounds were then converted to propionate and butyrate through the activity of syntrophic bacteria [18]. In methane formation, VFA was one of the critical indicators. VFA concentrations were in line with the activity of microorganisms in degrading the substrate [26]. Overpopulation of microbes led to competition in obtaining substrate.

Meanwhile, under the population of microbes caused degradation of the substrate not optimal. The competition between microorganisms caused by population density resulted in dominance in one species. That is, if two species of microorganisms compete for a limited source of nutrition, they cannot coexist with a stable population, and one superior species will dominate [27, 28]. This dominance causes VFA production during incubation to be suboptimal. VFAs were formed by the activities and relationships between microbial species through two stages of formation. Stage one was the degradation of organic matter into simple sugars by enzymes produced by microbes. The second stage was the conversion of pyruvic acid to VFA through the process of intracellular microbial metabolism. VFA results were influenced by the types of microorganisms that play a role in degrading pyruvic acid to acetate, butyrate, or propionate [26]. At least four bacterial genera were composed of Bacteroides, Geobacter, Clostridium, and Sporochaeta, which were known to play a role in the process.

4. Conclusion
The consortium of bacteria from the fecal laying hens can be used as a biogas starter, which is $>10^7$ CFU/ml, by anticipating problems that can inhibit the formation of biogas by using coal as a carbon source, the highest methane, and VFA gas content is obtained at a dose of 10%. The number of bacteria increases with increasing starter dose.

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