



# The Effect of C / N Ratio and Pretreatment in Making Biogas from Tea Waste and Cow Manure in Liquid State Anaerobic Co-Digestion

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**Abstract** - Energy problems in Indonesia are issues that are not easy to solve. If the energy needs dominated by BBM continue to increase without any changes in the pattern of energy use, then Indonesia's sustainability and energy security will be disrupted. Therefore, Indonesia really needs alternative energy. Biogas is an alternative energy produced from the anaerobic degradation of organic compounds and can be a substitute for natural gas and fossil fuels. Solid tea waste can be used as a substrate from anaerobic co-digestion biogas production with cow dung. In general, the purpose of this study is to produce biogas from tea pulp and cow manure by anaerobic co-digestion and have a specific purpose, namely (i) Assessing the effect of pretreatment on the yield of biogas produced, (ii) Assessing the effect of pH on yield of biogas, (iii) Assessing the effect of the value of the C / N ratio on the yield of the biogas produced. This research was conducted by making variations in treatment including physical pretreatment with and without grinding ( $\pm 1$  mm), biological pretreatment with and without addition of 5% v / v microbial consortium, pH controlled (addition of buffer) and uncontrolled, and ratio of C: N waste solid tea (25 and 30). The biogas formation process is carried out for 2 months at room temperature with the quantitative response test results in the form of biogas volume every 2 days. Biogas production in pretreatment tea grounds gives better results than without pretreatment. Optimum biogas production is obtained at a C: N 30 ratio. Comparison of C: N substrate will affect the growth of microorganisms, the microbes that play an anaerobic process need nutrients to grow and develop, in the form of carbon and nitrogen. The highest biogas accumulation produced was 73,167 ml / gr TS on variable C / N 30 ratio, NaOH pretreatment, microbial consortium and smooth size of tea waste that used.

**Keywords** - Biogas, pretreatment, ratio C:N, tea waste

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## 1. Introduction

Energy problems in Indonesia are issues that are not easy to solve. This is of course caused by energy consumption which is still dominant towards oil and gas. The fact is that the national oil reserves as of January 1, 2015 were proven to have decreased by 1.2% compared to the previous year. On the other hand, the rate of consumption of fuel as a processed product continues to increase. While the development of production over the past 10 years showed a downward trend, from 287.30 million barrels or around 800 thousand barrels per day in 2006 to 251.87 million barrels or 690 thousand barrels per day in 2015. The decline in production was due to wells Petroleum production is generally old, while new well production is still relatively limited [1]. If the energy

demand which is dominated by fuel continues to increase without any change in energy usage patterns, then the sustainability and security of Indonesia's energy will be disrupted [2]. To achieve independence and national energy security, as stated in its parent regulation, Government Regulation No. 79 of 2014 concerning the National Energy Policy, the Presidential Regulation RUEN outlines the priorities of Indonesia's energy development which includes several things, namely the maximum use of renewable energy by taking into account the economic level, minimizing the use of petroleum, the optimal use of natural gas and new energy, as well as making

Coal as a mainstay of the national energy supply. Therefore Indonesia needs to develop renewable energy as a solution to these problems.

Biomass energy, which has been a global concern in recent years, is a renewable resource that can be converted into three phases of fuel: gas, liquid and solid [3]. Indonesia is a country that is rich in natural resources both plant and vegetable. Indonesia, which is said to be one of the countries with the richest biomass potential, but still has a relatively low utilization of biomass potential [4]. Most of the biomass energy in Indonesia is also used for household, agriculture, wood and sugar industries, in rural areas for cooking, lighting, rice milling, drying of agricultural products, and heat and electricity generation [5]. One of the biomass-based alternative energy that can be applied in Indonesia is biogas.

Biogas is produced from anaerobic degradation of organic compounds and can be a substitute for natural gas and fossil fuels. Biogas has three main components, namely methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>) and nitrogen (N<sub>2</sub>). But there are also very small byproducts, namely hydrogen sulfide (H<sub>2</sub>S), hydrogen (H<sub>2</sub>), nitrogen (N<sub>2</sub>), ammonia (NH<sub>3</sub>), oxygen (O<sub>2</sub>) and carbon monoxide (CO) [6]. Biogas is renewable energy which means it can be renewed [7]. Turkey produces nearly 2 trillion m<sup>3</sup> of biogas per year and produces 9 million MWh of electricity [8]. By using waste at a low cost even free. One of the processes of making biogas comes from a variety of organic substrates and usually uses an inorganic co-digestion process [9].

Indonesia is the fifth largest tea production country in the world with tea production in the form of dried leaves of 144.02 thousand tons in 2016 [10]. However, to meet domestic needs, Indonesia still imports tea from several countries [11]. Large amounts of tea leaves and tea leaf residues are discharged into the environment through drinking tea every day, instant tea extraction and ready-to-drink tea. The high consumption of tea in Indonesia also causes a lot of waste produced in the form of tea pulp. Tea leaves contain beneficial amino acids, protein, vitamins, tannins and polyphenols. After steeping tea leaves, some nutrients still remain in the tea leaves [8]. Utilization of tea pulp waste so far is still limited so there needs to be development.

[8] says that dried tea leaves are brewed with hot water and the results can be said to be waste. Waste generated in large quantities, disposed of with a mixture of other domestic wastes that have high moisture content can cause environmental pollution and odor. Tea pulp waste continues to increase depending on the amount of consumption. Therefore, it is necessary to develop a new method for the reuse of tea pulp waste. Tea leaves contain amino acids, protein, vitamins, tannins, polyphenols. After brewing the tea leaves, the nutritional content is still left in the tea dregs. Therefore, this waste can be used for biogas production in addition to being used as animal feed and other products.

The population of beef cattle in Indonesia is estimated to be 10.8 million and 350,000 - 400,000 dairy cows and if one cow produces an average of 7 kilograms of dry manure every day, the dry cow dung produced in Indonesia is 78.4

million kilograms per dry day [12]. One cow each day produces manure ranging from 8-10 kg per day or 2.6 - 3.6 tons per year [13]. If this abundant cow dung waste is not managed properly, this dung waste not only affects milk production and quality, but also the surrounding environment [14]. Of all ruminant livestock commodities, cattle are more CH<sub>4</sub> producers compared to other ruminants [15]. It has been reported that the largest contributor to CH<sub>4</sub> gas emissions in Indonesia in the livestock subsector is beef cattle, which is 65.12% of ruminant livestock emissions, or as much as 58.84% of the total CH<sub>4</sub> gas emissions of all livestock commodities. Cow dung has a high nitrogen content and is due to pre-fermentation in ruminant stomach, and has been observed as the most suitable material for high biogas yields through years of research [16]. Cow dung is an excellent substrate for biogas production by co-digestion with other types of waste materials such as organic industrial waste, household waste and sewage sludge even though the yield of methane as a single substrate is low [17]. However, cow manure functions as an excellent "carrier" substrate during mixing of mixed waste and allows concentrated inorganic digestion of industrial waste, which will be difficult to handle separately. a large number of anaerobic bacteria in cow dung function effectively to reduce the organic fraction of cattle dung even though the pH is not regulated [18]. Therefore, in this research, the process of making biogas by anaerobic co-digestion from cow dung and tea dregs will be examined.

## 2. Materials and Methods

The research method used to achieve the objectives of this study was by conducting laboratory-scale experiments conducted at the Chemical Engineering Waste Treatment Laboratory, Faculty of Engineering, Diponegoro University. The process and method of processing are carried out using the anaerobic digestion method in a mesophilic state, ie at ambient temperature (25-35°C) using feed intake with a batch system. The materials used are Flag brand tea grounds collected from Padang Restaurant, cow dung taken from the Faculty of Agriculture and Animal Husbandry, Urea, Microbial Consortium, NaOH, HCl and Water. The tools used are plastic bottles (biodigester), beaker cups, stirrers, plastic hoses, water reservoirs, pH indicators, valves, measuring cups, glue gun, filters, porcelain cups, measuring flask.

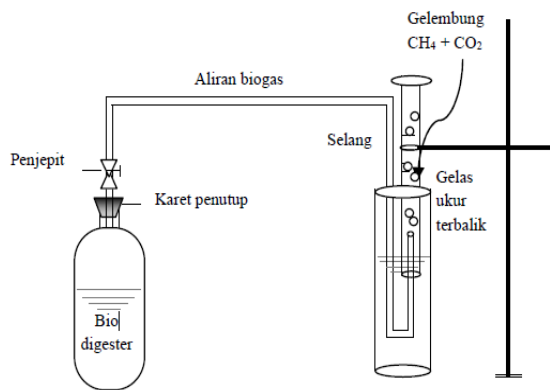


Figure 1. Series of Research Tools

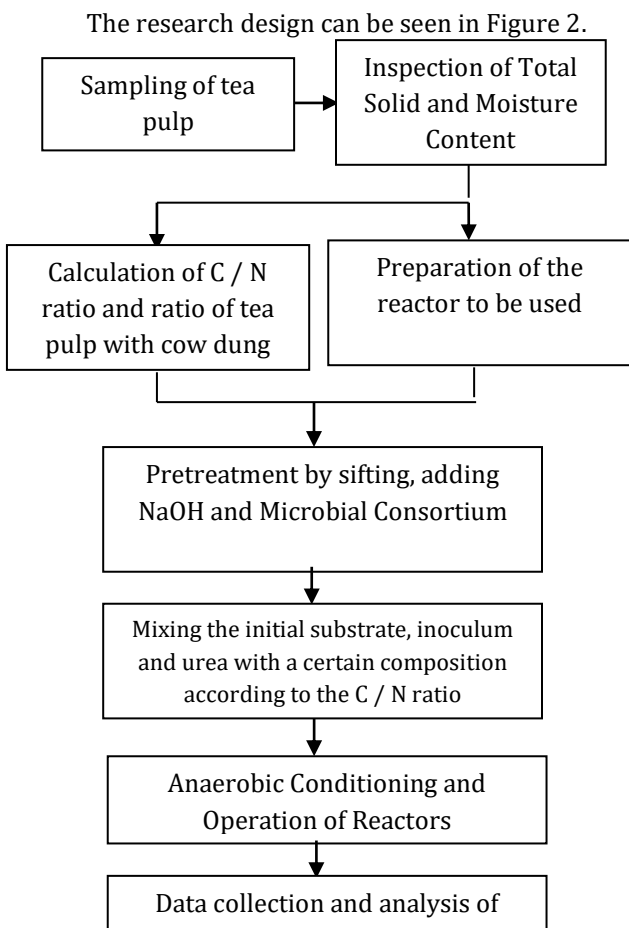


Figure 2. Research design

**2.1 Control Variable**

1. Operating conditions (batch)
2. Initial pH neutral (7)
3. Time of observation (for 40 days).
4. Ambient temperature (25-35oC) and atmospheric pressure (1 atm)
5. Chemical pretreatment with 4% NaOH g / g TS
6. Reactor volume 600 ml
7. The ratio of the amount of tea pulp and cow dung 1: 1

8. Liquid state 10% gr TS / V

**2.2 Independent variable**

1. Physical pretreatment by sifting ± 1 mm in order to obtain a smooth size (pass the sieve) and rough (do not pass the sieve).
2. Biological pretreatment with the addition of microbial consortium 5% v / v and without the addition of microbial consortium.
3. Chemical pretreatment with the addition of NaOH and without the addition of NaOH
4. C / N ratio (25 and 30)

**2.3 Dependent variable**

The response observed from this study was the total biogas volume once every 2 days for 40 days.

**2.4 Preparation phase**

The tool is prepared and designed according to the research variables. Take tea pulp and cow dung in a ratio of 1: 1 weighed 60 grams each total solid and mix with water to a base volume of 600 ml and then put in a container as many as the total variable number (16 variables).

**2.5 Data Collection and Analysis Phase**

Biogas volume data is analyzed in the form of graphs of the relationship of biogas volume to time. Graphical data analysis and theoretical phenomena that occur on the effect of pretreatment and comparison of the ratio of tea pulp with cow dung respectively to the results obtained on the graph of research results.

**3. Results and discussion**

**3.1 Effect of NaOH Addition on Tea Pulp on Biogas Production**

In the biogas research that is produced in terms of chemical pretreatment with the addition of NaOH. NaOH is mixed with the substrate to speed up the process of delignification or lignin removal process. In Figure 3.1 and Figure 3.2 we can see the comparison of biogas produced between coarse tea pulp with the addition of NaOH C / N ratio of 25 and 30.

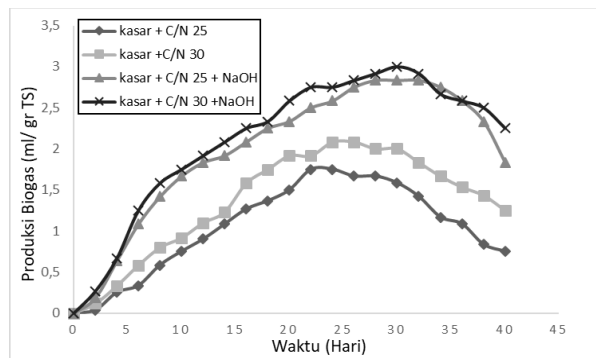


Figure 3. Daily biogas production of coarse tea pulp with and without the addition of NaOH at a C / N ratio of 25 and 30

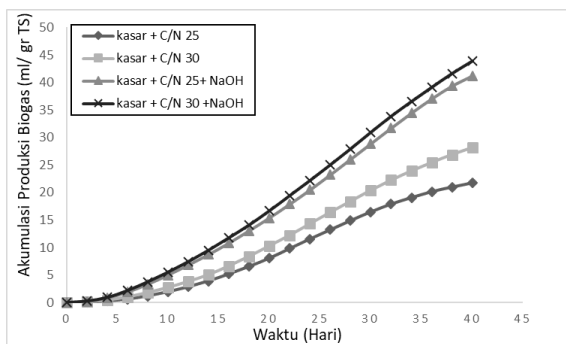


Figure 4. The accumulation of biogas production of coarse tea pulp with and without the addition of NaOH at a C / N ratio of 25 and 30

In Figure 3 and Figure 4 can be seen the comparison of the amount of biogas production from coarse tea pulp with the addition of NaOH with different C / N ratios of 25 and 30. The total production of coarse tea extract biogas without addition and with the addition of NaOH on the C / N ratio 25 respectively is 21.73 ml / gr TS and 41.23 ml / gr TS, while the total production of crude tea pulp biogas without addition and with the addition of NaOH at a C / N ratio of 30 respectively is 28.13 ml / gr TS and 43, 85 ml / gr TS.

Giving NaOH which is useful to help the process of destroying the structure of lignin is commonly called the delignification process [19]. Alkaline pretreatment using a base, with NaOH is one of the most popular, to make the lignocellulose matrix easily degraded by microbes, through removal of parts of lignin and hemicellulose. It also can reduce the degree of polymerization, crystallinity, and can damage the chain between lignin and other polymers [20]. The main mechanism of this method is saponification and the cleavage of the relationship between lignin and carbohydrates [21]. The efficiency of alkaline hydrolysis depends on the substrate and treatment conditions as it is more effective for biomass with low lignin content such as agricultural residues [5]. NaOH particles will enter the material and break down the structure of lignin so that lignin is more soluble which results in decreased levels of lignin [22], [23].

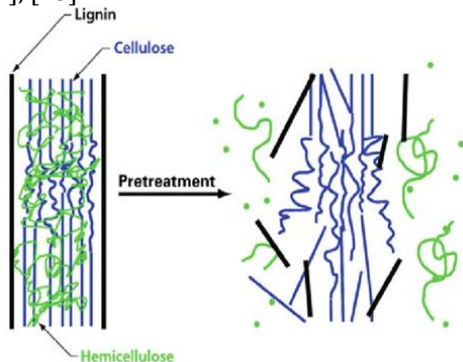


Figure 5. Schematic of the process of damaging the structure of lignin [24].

Lignocellulose consists of 3 main components including cellulose, hemicellulose, and lignin. Among these three main elements, cellulose and hemicellulose are polymeric forms of sugar and can be hydrolyzed. Whereas lignin forms a protective layer which limits the biodegradability of cellulose and hemicellulose. In the delignification process, a number of lignin will be dissolved. This process is the process of saponification of the intermolecular ester bonds surrounding xylan, hemicellulose and other components, such as lignin and other hemicelluloses. The delignification process causes damage to the structure of lignin and releases carbohydrate compounds [20]. The process of damaging the structure of matter with lignocellulose content is one step to convert lignocellulose into sugar compounds. The delignification process is believed to be a potential process as a preliminary process in the preparation of raw materials [25]. Therefore, the raw material of tea pulp that has been given NaOH pretreatment produces more biogas compared to the others.

NaOH pretreatment can increase cellulose breakdown and sugar degradation more significantly than acid pretreatment, but in its application it is constrained at high costs [26]. Pretreatment of NaOH solution against lignocellulosic material causes swelling or swelling, increased surface area, reduction in the degree of polymerization, reduction of crystallinity, separation of structural bonds between lignin and carbohydrates and interference with lignin structure [27].

### 3.2 Effects of Addition of Microbial Consortium on Tea Leaves on Biogas Production

The study aims to determine the amount of biogas produced by conducting biological pretreatment by adding microbial consortium. Pretreatment carried out aims to accelerate the process of cellulose degradation. Figure 6 and Figure 7 are the results of biogas between coarse tea pulp with the addition of a microbial consortium at a C / N ratio of 25 and 30.

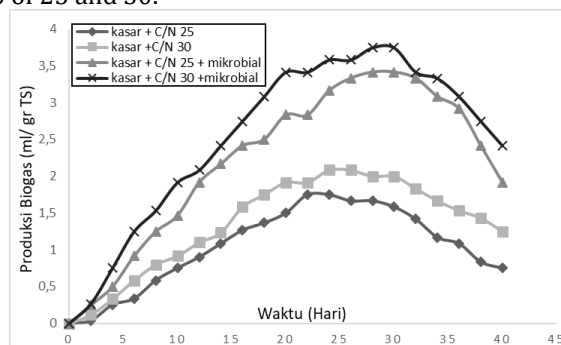


Figure 6. Daily production of coarse tea pulp with and without the addition of a microbial consortium at a C / N ratio of 25 and 30

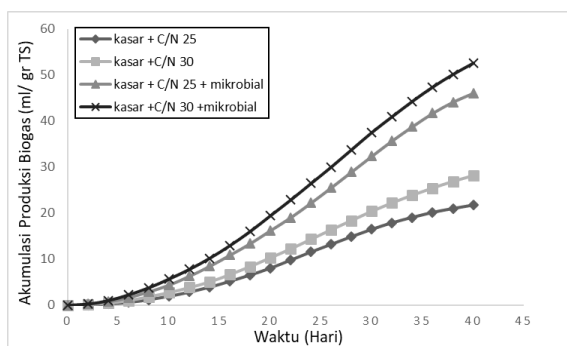


Figure 7. The accumulation of biogas production of coarse tea pulp with and without the addition of a microbial consortium at a C / N ratio of 25 and 30

In Figure 6 and Figure 7 we can see a comparison of the amount of biogas production from coarse tea pulp at C / N ratio of 25 and 30 with the ratio of the addition of microbial consortium and without microbial consortium. Total biogas production at C / N ratio 25 with the addition of microbial consortium and without microbial consortium respectively for coarse tea pulp reached 46.05 ml / gr TS and 21.73 ml / gr TS. While the total biogas production in the C / N 30 ratio with the addition of microbial consortium and without microbial consortium respectively for coarse tea pulp reaches 52.55 ml / gr TS and 28.13 ml / gr TS.

Biogas production in tea dregs with the addition of microbial consortium is better than biogas production in tea dregs without the addition of microbial consortium. This is due to microbial consortium activity consisting of eight major microbes, consisting of genera of Clostridium anaerobic bacteria and Thermoan aerobacterium together with Rhodo aerobic / facultative anaerobic cyclaceae of bacilli bacteria, and uncultured bacteria [28]. Microorganisms also perform the function of delignification, reduce the degree of cellulose polymerization, and hemicellulose hydrolysis. The addition of microbial consortium accelerates the degradation of cellulose, hemicellulose and lignin into compounds needed by biogas-producing microorganisms, so that biogas production increases [3]. Biological pretreatment using microbial consortium results in higher biogas yield with a minimum energy process and minimum chemical compared to other methods [29].

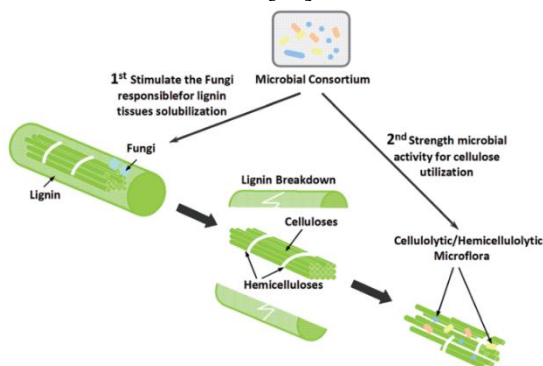


Figure 8. Mechanism of lignin breakdown with microbial consortium [24]

The general structure of lignocellulose can be explained as shown in Figure 8 where cellulose is wrapped with hemicellulose and lignin as the main barrier for cellulose, and destruction of the structure by lignin removal is advantageous for cellulose utilization [24]. Strong bonding of lignocellulose can be disturbed by destroying most of the lignin and cellulose accessibility can be improved properly after pretreatment using a microbial consortium. In Figure 8, it can be seen that structural damage by enlarging specific surface areas can be achieved through this pretreatment and structural disruption increases the accessibility of cellulose residues (hemicellulose) [24].

### 3.3 Effect of Size of Tea Leaves on Tea Leaves on Biogas Production

Pretreatment can increase the digestibility of lignocellulose which is inhibited by several factors, such as: lignin content and composition, cellulose crystallinity, degree of polymerization, pore volume, acetyl groups bound to hemicellulose, surface area and biomass particle size [30]. In Figure 9 and Figure 10 we can see a comparison of the production of biogas from the pulp with a C / N ratio of 25 and 30 the ratio of the size of the tea pulp (coarse and fine).

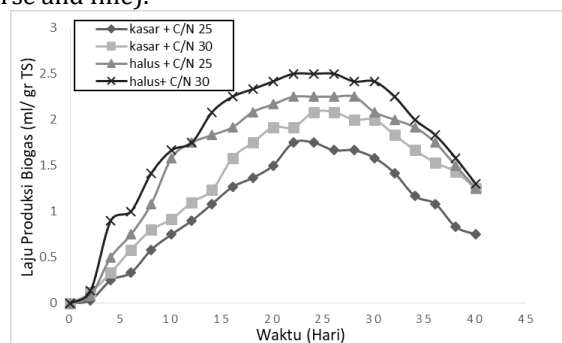


Figure 9. Daily biogas production from C: N 25 and 30 ratio tea grounds with a ratio of tea pulp sizes (rough and smooth).

In Figure 9 shows the daily production rate of tea pulp with a C / N ratio of 25 and 30 the ratio of the size of tea pulp (coarse and fine). Biogas production began on day 2 on all variables. In crude tea pulp C / N ratio of 25 biogas production occurs until the 23rd day, then decreases until the 37th day. In crude tea pulp C / N 30 biogas production occurred until the 22nd day then fluctuated until the 28th day, then decreased until the 40th day. In fine tea dregs the C / N ratio of 25 biogas production occurs until the 28th day, then decreases until the 40th day. In fine tea dregs the C / N 30 ratio of biogas production occurs until the 30th day, subsequently decreases until the 40th day. Highest biogas production Fine tea pulp with C / N 30.

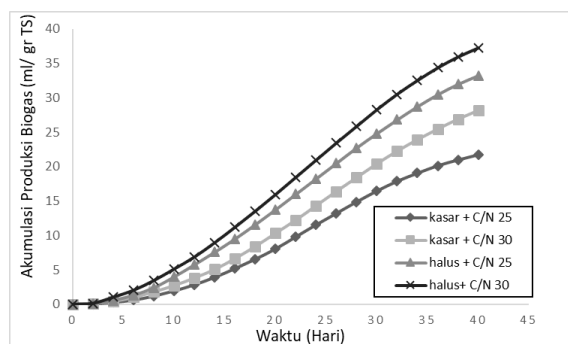


Figure 10. Accumulation of biogas production from C: N 25 and 30 ratio of tea pulp with comparison of tea bag size (coarse and fine).

In Figure 10 shows the total biogas production from tea grounds C / N ratio of 25 and 30 with a ratio of the size of tea pulp (rough and smooth). In crude tea pulp C / N ratio of 25 total biogas volume of 24.97 mL / gr TS. Coarse tea pulp C / N ratio 30 total biogas volume 28.2167 mL / gr TS. In fine tea pulp C / N ratio 25 total volume of biogas 34.2167 mL / gr TS. In coarse tea pulp C / N ratio 30, total volume of biogas 37,383 mL / gr TS.

Physical pretreatment is done by grinding and making parts of the substrate smaller or compressing them to destroy the cell structure, increasing the specific surface area of the biomass. This pretreatment may be better for enzymatic destruction, especially in the lignocellulosic substrate. Particle reduction not only increases the rate of enzymatic decomposition, but also reduces the viscosity in the digester (which makes it easier to stir) and can reduce problems in the floating layer [31].

The sample size can affect porosity which then influences the contact with the delignifier [32]. In addition, decreasing the sample size will break the long polymer chains into shorter polymer chains, making it easier to separate lignin from cellulose bonds [33]. This is because the reduction in particle size can reduce the crystallinity of cellulose and disrupt the defense of lignin. Thus, it will facilitate the hydrolysis process so as to result in increased bioenergy production [34]. In addition, reducing the size of tea pulp can increase the surface area and pore size, and reduce the crystallinity and degree of polymerization of cellulose [25].

#### 4. Conclusion

Biogas production in tea dregs that were given pretreatment was better than that without pretreatment. Optimum biogas production is obtained at a C: N 30 ratio. Comparison of C: N substrate will affect the growth of microorganisms, microbes that play an anaerobic process need nutrients to grow and develop, in the form of carbon and nitrogen. The highest biogas accumulation produced was 73,167 ml / gr TS on variable C / N 30 ratio, NaOH pretreatment, microbial consortium and fine tea pulp size. Continuous research needs to be done to determine the length of fermentation in biogas production, test the gas

content produced to determine the methane gas content and other gases, test the fuel value of the biogas produced.

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