

THESIS

BIOGAS PRODUCTION USING ANAEROBIC BIODIGESTER FROM CASSAVA STARCH EFFLUENT WITH RUMINANT BACTERIA AS BIOCATALYST



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by:

Audra Ayu O.

L2C006023

Vincensia Dyan Aryati

L2C006106

**CHEMICAL ENGINEERING DEPARTMENT
TECHNICAL FACULTY DIPONEGORO UNIVERSITY
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PAGE OF APPROVAL
THESIS

Name/NIM : Audra Ayu O./L2C006023
Name/NIM : Vincensia Dyan Aryati/L2C006106
Tittle of Research : Biogas Production using Anaerobic Biodigester from Cassava Starch
Effluent with Ruminant Bacteria as Biocatalyst.
Advisor : Dr. nat.techn. Siswo Sumardiono, ST, MT

Semarang, January 19th 2010

Has approved,

Advisor,

Dr. nat.techn. Siswo Sumardiono, ST, MT

NIP 19750915 200012 1 001

SUMMARY

IKMs' factory activity in Margoyoso produces liquid and solid wastes. The possible alternative was to use the liquid effluent as biogas raw material. This study focuses on the used of urea, ruminant, yeast, microalgae, the treatment of gelled and ungelled feed for biogas production, pH control during biogas production using buffer Na_2CO_3 , and feeding management in the semi-continuous process of biogas production that perform at ambient temperature for 30 days. Ruminant bacteria, yeast, urea, and microalgae was added 10 % (v/v), 0.08% (w/v), 0.04 % (w/v), 50 % (v/v) of mixing solution volume, respectively. The pH of slurry was adjusted with range 6.8-7.2 and was measured daily and corrected when necessary with Na_2CO_3 . The total biogas production was measured daily by the water displacement technique.

Biogas production from the ungelling and gelling mixture of cassava starch effluent, yeast, ruminant bacteria, and urea were 22,701 ml and 6,198 ml. Biogas production from ungelling mixture without yeast was 1,830 ml. Biogas production from ungelling mixture added by microalgae without yeast was 1,835 ml and that with yeast was 5,904 ml. Biogas production from ungelling mixture of cassava starch effluent, yeast, ruminant bacteria, and urea in semi-continuous process was 18,161 ml. Adding of microalgae as nitrogen source did not give significant effect to biogas production. Adding of yeast was very helpful to accelerate biogas production. The biogas production increased after adding of cassava starch effluent and yeast. Requirement of Na_2CO_3 to increase alkalinity of fermenting slurry depends on pH-value.

Key Words: *biogas cassava; C/N ratio; ruminant bacteria; semi-continuous biodigester; yeast*

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CHAPTER I

INTRODUCTION

1.1 BACKGROUND OF STUDY

Indonesia is the third country of cassava producer after Brazil and Thailand, and has 1,205,440 hectares planted area and 21,990,381 tons cassava production/year (BPS, 2009). Mostly, cassava root is produced for tapioca starch. One of the tapioca starch central producer is in Margoyoso, Pati, Central Java, Indonesia. There are 399 small-medium scale industries (IKMs) which have average production capacity 10 tons cassava/IKM-day. In Margoyoso, demand of cassava root is approximately 3,990 tons/day with total water consumption 15,960 m³/day. Consequently, the IKMs' factory activities produce liquid and solid wastes. Concerning liquid waste, there are the washed cassava water and extracted water from the roots by squeezing (Cereda, 1994).

Water consumption of each IKM's factory is 40 m³/day. The problem is that the effluent from tapioca starch factories is released directly into the river before properly treatment. It has been a source of pollution and has caused environmental problems to the nearby rural population. Therefore, the effluent is the most important to be treated before discharging to the river. To treat the effluent, we can conduct coagulation and flocculation process (Malhotra, et.al., 1994). But the recovery result of the product can not be used for food product because of chemical residue. The possible alternative is to use the effluent as raw material of biogas.

Biogas originates from bacteria in the process of bio-degradation of organic material under anaerobic (without oxygen) conditions. Biogas is a mixture of gases that is composed chiefly of methane (CH₄): 55-70 vol. %, carbon dioxide (CO₂): 30-45 vol. %, other gases: 1-5 vol. % (Deublein *et al.*, 2008).

Biogas technology of cassava wastewater was used anaerobic biodigester (Barana, 2000). Biogas production technology development nowadays is dominated by how can increase the concentration and the retention time of microbe in biodigester to increase biogas production rate. Biogas production with biodigester using cassava effluent with ruminant bacteria as biocatalyst is a new breakthrough in this project.

Biodigester is biogas production process from many organic materials anaerobically in anaerobic biodigester using some bacteria simultaneously in biochemical reaction

steps, such as Hydrolysis, Acidogenesis, Acetogenesis and Methanogenesis (Speece, 1996). One of the four bacteria, methano bacteria (the main component of biogas) has slowest growth rate. So, this bacteria is the most considerable factor that control the overall rate of biogas production. Biogas conversion from the effluent then not only results in a production of a useful form of energy but also deals with the problem of environmental pollution caused by the tapioca starch processing plants.

1.2 STATEMENT OF THE PROBLEM

As one of the tapioca starch producer, IKMs' factory activities in Margoyoso produces liquid, solid, and gas wastes. The problem is that the effluent from tapioca starch factories is released directly into the river before proper treatment. It has been a source of pollution and has caused environmental problems to the nearby rural population. Therefore, the effluent is the most important to be treated before discharging to the river. To treat the effluent, we can conduct coagulation and flocculation process (Malhotra, et.al., 1994). But the recovery result of the product can not be used for food product. The possible alternative is to use the cassava effluent as raw material of biogas.

The main problem of the biogas production of cassava waste is acid forming-bacteria quickly produced acid resulting significantly in declining pH below the neutral pH and diminishing growth of methane bacteria when the biodigester initially fed (Ribas, 2003, Rodtong, et.al., 2004). So far, a biogas production of cassava effluent has not been much investigated in Indonesia yet. The things to be considered of biogas production of cassava effluent are nitrogen source to support the growth of the methane bacteria (Manilal, 1990); microbiology as biocatalyst (Werner Kossmann, *et al*, 2008); pH control during biogas production to keep methane bacteria alive (Werner Kossmann, *et al*, 2008); and feeding management in the semi-continuous process of biogas production. So, the problem was focusing on how to convert cassava effluent to biogas using anaerobic biodigester by adding urea and microalgae as nitrogen source, yeast as substrate activator, ruminant bacteria as biocatalyst, the treatment of gelled and ungelled feed, and feeding management in the semi-continuous process of biogas production, with pH control during biogas production.

1.3 THE OBJECTIVES OF THE STUDY

Generally, the aim of this study is developing the experimental method of biogas production from cassava effluent in biodigester. The result of the study can give contribution to motivate small and middle industries to convert cassava effluent to biogas.

The specific objectives of this study can be defined as follows:

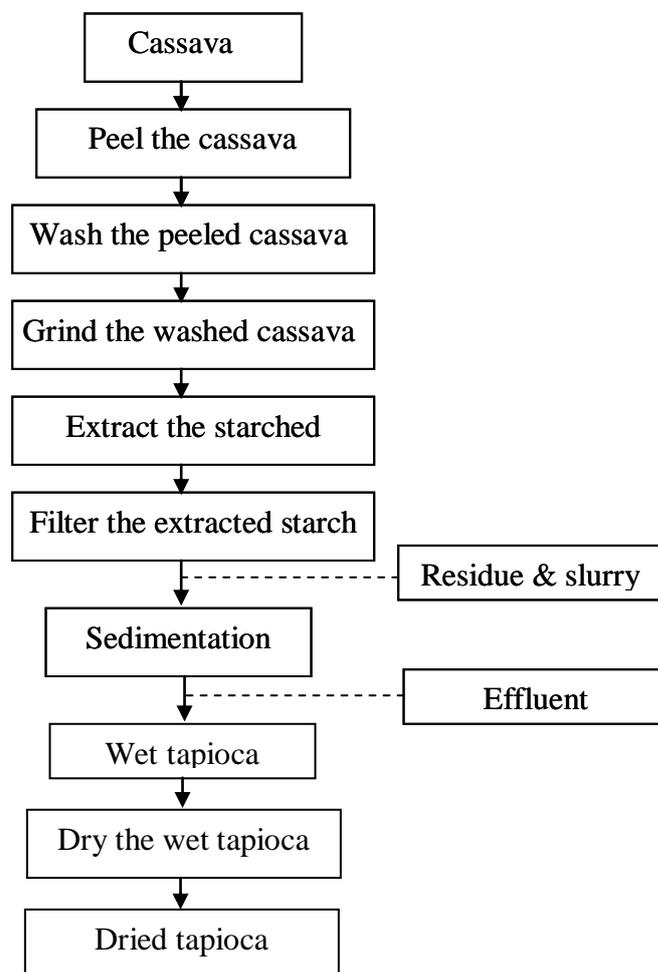
- a. Study of biogas production from cassava starch effluent by adding microalgae as nitrogen source and yeast as substrate activator.
- b. Study of biogas production from cassava starch effluent with the treatment of gelled and ungelled feed.
- c. Study of pH control during biogas production using buffer Na_2CO_3 .
- d. Study of feeding management in the semi-continuous process of biogas production.

CHAPTER II

THEORITICAL REVIEW

2.1 TAPIOCA AND EFFLUENT

Indonesia has 1,205,440 hectares planted area and 21,990,381 tons cassava production/year (BPS, 2009). Tapioca is one of product made from cassava. The flow diagram process of tapioca production process can be seen in the figure 2.1.



*) Mulyanto, 2005

Figure 2.1 Flow Diagram Tapioca Production Process

From this process it can be identified that there is liquid and solid tapioca waste in the level of extraction. The tapioca effluent is potential raw material to convert to biogas (Anunputtikul, *et al*, 2004).

2.2 BIOGAS

Biogas originates from bacteria in the process of bio-degradation of organic material under anaerobic (without O₂) conditions. The natural generation of biogas is an important part of the biogeochemical carbon cycle. Methanogens (methane producing bacteria) are the last link in a chain of micro-organisms which degrade organic material and return the decomposition products to the environment. In this process biogas is generated, a source of renewable energy (Werner Kossmann, *et al*, 2008).

Biogas consists mainly of methane and carbon dioxide, but also contains several impurities. It has specific properties which are listed in Table 2.1. Biogas with methane content higher than 45% is flammable (Dieter Deublein, 2008).

Table 2.1 General Features of Biogas

Composition	55 - 70% Methane (CH ₄) 30 - 45% Carbon Dioxide (CO ₂) Traces of Other Gases
Energy content	6.0 – 6.5 kWh/m ³
Fuel equivalent	0.60 – 0.65 L oil/m ³ biogas
Critical pressure	75 – 89 bar
Critical temperature	– 82.5° C
Normal density	1.2 kg/m ³
Smell	Bad eggs (the smell of desulfurized biogas is hardly noticeable)
Molar Mass	16.043 kg/kmol

*) Dieter Deublein, 2008

2.3 BIOGAS PRODUCTION PROCESS

Bacteria decompose the organic matter in anaerobic environment. Biogas is an intermediate product of their metabolism. The decomposition process can be divided into 4 steps. Each of those are accompanied by different bacteria groups:

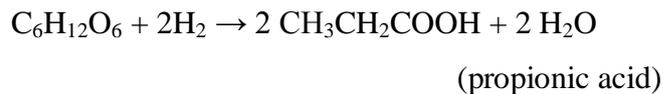
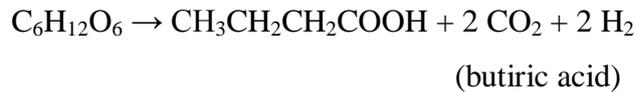
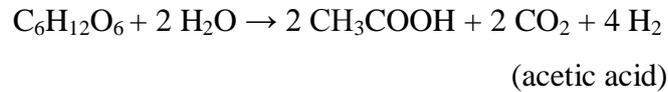
2.3.1 Hydrolysis

In the first stage aerobic bacteria reconstructs high - molecular substances (protein, carbohydrates, fats, cellulose) by means of enzymes to low-molecular compounds like monosaccharide, amino acids, fatty acids and water. Enzymes assigned by hydrolysis bacteria decompose substrate components to small water-soluble molecules. Polymers turn into monomers (separate molecules). This process called hydrolysis.

2.3.2 Acidogenesis

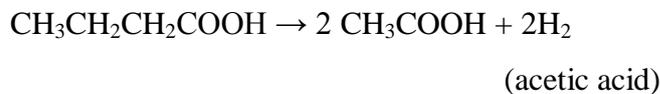
Then decomposition is made by acid-forming bacteria. Separate molecules penetrate into bacteria cells where further transformation takes place. This process

is partially accompanied by anaerobic bacteria that consume rest of oxygen hence providing suitable anaerobic environment for methane bacteria. The step is called acidogenesis.



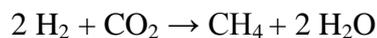
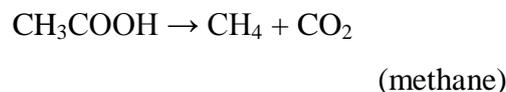
2.3.3 Acetogenesis

Afterwards acid-forming bacteria forms initial products for methane formation: acetic acid, carbon dioxide and hydrogen. These products are formed from organic acids. For vital functions of these bacteria that consume hydrogen, stable temperature mode is very important.



2.3.4 Methanogenesis

The last step is methane, carbon dioxide and water formation. Methane producing microorganisms occurs to the extent that anaerobic conditions are provided. 90% of methane yield takes place at this stage, 70% from acetic acid. Thus acetic acid formation (3rd step) is the factor that defines the speed of methane formations.



(ZORG Ukraine Biogas Plants, 2009, Hargono, 2007)

The above equations show that many products, by-products and intermediate products are produced in the process of digestion of inputs in an anaerobic condition before the final product (methane) is produced.

2.4 FACTORS AFFECTING BIOGAS PRODUCTION

2.4.1 Nutrients

Organic matters, which are broken down by microorganisms without oxygen, will produce some quantities of methane. All biological process requires sufficient supply of nutrients particularly carbon and nitrogen as well as other elements are also required in trace quantities. The lack of specific elements required for microorganism growth will limit the production of biogas. Nutrients are assigned by the ratio of carbon and nitrogen (approximate 20-30:1) (Anunputtikul, 2004).

2.4.2 pH

Hydrocarbons are easier to acidify, and no pH-buffering ions are released as with the degradation of proteins. Therefore the pH-value decreases more easily. With the degradation of carbohydrates, the partial pressure of hydrogen increases more easily, as with other substances. This happens in combination with the formation of reduced acidic intermediate products. The pH optimum of the methane-forming microorganism is at pH = 6.8-7.2. Therefore, it is important to adjust the pH-value. Only *Methanosarcina* is able to withstand lower pH values (pH = 6.5 and below). With the other bacteria, the metabolism is considerably suppressed at pH <6.7 (Dieter Deublein, 2008).

2.4.3 Temperature

Two optimum temperature levels have been established the mesophilic level (25-40°C) and thermophilic level (50-65°C) (Bitton, 1994; Busby *et al.*, 1977; Mackie and Bryant, 1995). Most of the methanogenic microorganisms belong to the mesophiles. Only a few are thermophilic. Methanogenics are sensitive to rapid changes of temperature. Thermophilic methanogens are more temperature-sensitive than mesophiles. Even small variations in temperature cause a substantial decrease in activity. Therefore, the temperature should be kept exactly within a range of +/- 2°C. Under mesophilic operating conditions, the inhibition of ammonium is reduced because of the lower content of inhibiting free ammonia. In general, it has to be mentioned that the energy balance is better in the mesophilic range than in the thermophilic range (Dieter Deublein, 2008).

2.5 PREVIOUS EXPERIMENT

Biogas can be made from substrates that contain carbon elements. So far, studies on biogas productions used raw materials from tapioca, tapioca effluent, cassava roots, tomatoes wastes, cow dung, chicken manure, etc. Several experiments have been conducted to get optimum yield of biogas, either by using different C/N ratio, different biodigester, existing bacteria, or nutrient addition. The results of previous study are as follow:

Table 2.2 Previous Experiments

No.	Researcher	Substrate	Method	Result
1.	M. Saev, B. Koumanova, Iv. Simeonov (2009)	Wasted tomatoes, Cow dung	Anaerobic co-digestion, Semi-continuous, Mesophilic condition, CD/WT = 80:20 & 90:10	Gas yield 220 dm ³ /kg of wasted tomatoes
2.	Adi Mulyanto and Titiresmi (2005)	Cow dung, Tapioca wastewater	Anaerobic fixed bed polyethylene bioreactor	Biogas productivity 1.2 m ³ /m ³ of wastewater
3.	Sureelak Rodtong and Wantanee Anunputtikul (2004)	Cassava roots 100 g/L, Chicken manure	Single state digester, Temperature 29-31 ⁰ C Urea (46% N ₂) 0.4 gr/L	1.95 L/day from 5 L cassava slurry

CHAPTER III

RESEARCH METHOD

3.1 RESEARCH ROADMAP

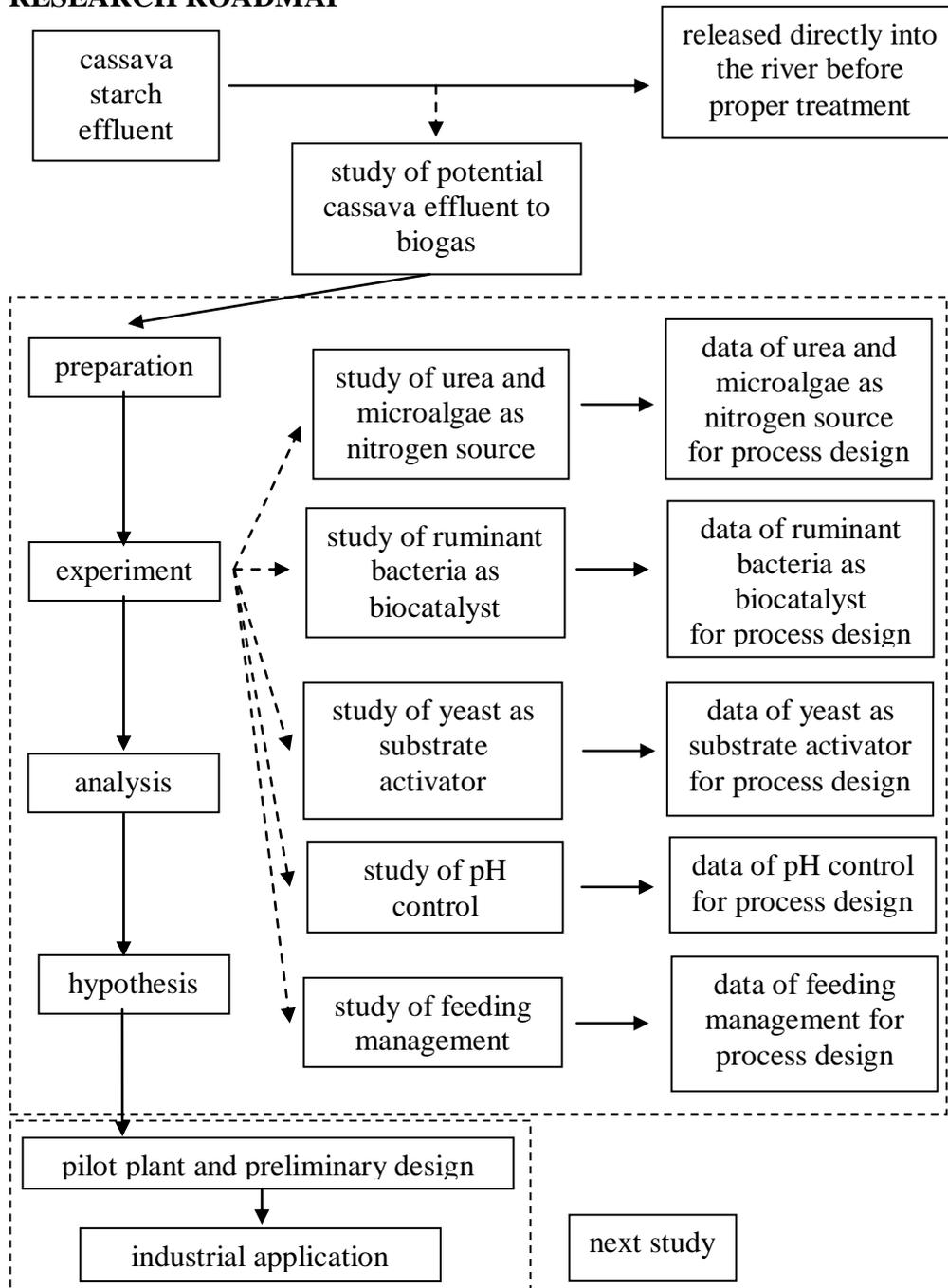


Figure 3.1 Research Roadmap and Inter-relation All Research Aspect

3.2 RESEARCH ACTIVITIES

The research was focused on study of urea and microalgae as nitrogen source, ruminant bacteria as biocatalyst, yeast as substrate activator, pH control, and feeding management. In the end of research part, the application of the potential of effluent from tapioca starch factories convert to biogas was used as new alternative energy and environmental safety. The composition of biogas produced should be tested. Furthermore, the pilot plant test, preliminary design and economic evaluation on industrial scale will be investigated as further study of this research as industrial application.

3.2.1 Location

The research experiment was conducted in the Waste Treatment Laboratory, Chemical Engineering Department, Diponegoro University.

3.2.2 Material Preparation

Cassava starch effluent was made synthetically with total solid 1% (w/v). 25 gr of cassava starch was dissolved in 2500 ml of mixing solution. Prepare urea as much as 0.04% (w/v) of mixing solution volume, ruminant bacteria as much as 10% (v/v) of mixing solution volume, yeast as much as 0.08 % (w/v) of mixing solution volume, and microalgae as much as 50% (v/v) of mixing solution volume. Mixing solution was heated and agitated for making the gelling solution. Na_2CO_3 was also prepared if necessary for rising the alkalinity of pH solution.

3.2.3 Variable

Urea and ruminant bacteria were used as dependent variable, while yeast, microalgae, and the treatment of gelled and ungelled feed were used as independent variable.

3.2.4 Experiment Procedures

- a. Study of biogas production from cassava starch effluent by adding microalgae as nitrogen source and yeast as substrate activator with the treatment of gelled and ungelled feed

The production of biogas from cassava effluent was performed using anaerobic biodigester of 5 L digestion volume (Figure 3.2), but the volume of mixing solution was half of biodigester reactor. On batch process, Tank 1 was fed ungelling mixture of cassava starch effluent, yeast, ruminant bacteria, and urea, Tank 2 was fed ungelling mixture of cassava starch effluent, ruminant bacteria, and urea, Tank 3 was fed gelling mixture of cassava starch effluent, yeast, ruminant bacteria, and urea, Tank 4 was fed ungelling mixture of cassava starch

effluent, ruminant bacteria, urea, and microalgae, Tank 5 was fed ungerminating mixture of cassava starch effluent, ruminant bacteria, urea, yeast, and microalgae. While on semi-continuous process, the tank was fed ungerminating mixture of cassava starch effluent, yeast, ruminant bacteria, and urea. This study was conducted in duplicate sample at ambient temperature for 30 days to obtain the volume of biogas production. The total biogas production was measured daily by the water displacement technique. The pH was measured daily and corrected when necessary with an alkaline buffer solution (Na_2CO_3). The equipment components to measure the total biogas were fabricated as in Figure 3.2.

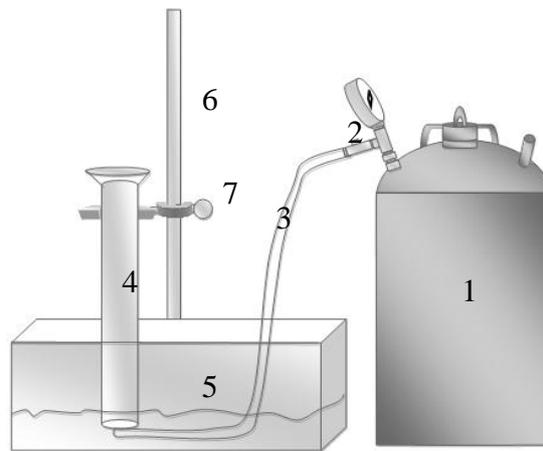


Figure 3.2 Water Displacement Technique

Notes:

1. anaerobic biodigester 5L
2. valve
3. hose
4. measure glass
5. wash basin
6. statif
7. clamp

b. Study of pH control during biogas production using buffer Na_2CO_3

The pH was measured daily and corrected when necessary with an alkaline buffer solution (Na_2CO_3).

c. Study of feeding management in the semi-continuous process of biogas production

Cassava starch effluent was fermented to achieve maximum output of biogas and reduction of total solids with minimum retention time under ambient conditions. In semi-continuous process, fresh feed (2 grams of total solid) was added every two days in order to displace the same amount of digested material to maintain the constant biogas production volume. Fresh yeast (0.08 % (w/v)) was also added every five days to stimulate biogas production.

CHAPTER IV

RESULT AND DISCUSSION

4.1 PHYSICAL AND CHEMICAL COMPOSITIONS OF CASSAVA STARCH EFFLUENT

Cassava starch effluent was taken from Margoyoso, Pati, Central Java, Indonesia. There were 399 small-medium scale industries (IKMs) which had average production capacity 10 tons cassava/IKM-day. Water consumption of each IKM's factory was 40 m^3/day , so the total water consumption was 15,960 m^3/day . Some physical and chemical compositions of cassava starch effluent is shown in the Table 4.1.

Table 4.1 Physical and Chemical Compositions of Cassava Starch Effluent

No.	Sample	Amylose (ppm)	Amylopectine (%)	Total Solid (%)
1.	Sutiyo	381.109	0.35638	0.62650
		383.35	0.35816	0.60505
2.	Bambang	104.965	0.33740	1.1287
		107.203	0.33717	1.17900
3.	Harno	428.099	0.31409	0.79520
		425.86	0.314331	0.75930

*) *Analysys Certificate No. PS/106/IV/08 Food and Nutrient Study Center Gajah Mada University*

Table 4.1 shows that physical and chemical compositions of cassava starch effluent from some IKMs vary in range. They contain 104.965-428.099 ppm of amylose, 0.314331-0.35816% of amylopectine, and 0.60505-1.179% of total solid. In this study, cassava starch effluent was made synthetically with total solid 1% (w/v). Total solid is used as the base for performing gas production rate.

4.2 BIOGAS PRODUCTION FROM CASSAVA STARCH EFFLUENT USING ANAEROBIC BIODIGESTER OF 5 L DIGESTION VOLUME

In this study, cassava starch effluent synthetic with 1% (w/v) of total solid as the substrate for biogas production was conducted in laboratory scale. The production was performed in the anaerobic biodigester of 5 L digestion volume. The anaerobic digestion was performed at ambient temperature (29.5-33 °C) for approximately 30 days.

4.3 STUDY OF PARAMETERS ADJUSTMENT FOR PRODUCING OPTIMUM BIOGAS

4.3.1 The Concentration of Nitrogen Source

Nutrients have been considered to be the main factor affecting microorganisms in the biogas production. Like any other biological process, methanogenesis involves consortia of microorganisms that convert organic matters into methane, carbon dioxide and traces of other gases. The overall rates of organic matter utilization and methane production depend on the extent to which the nutritional requirements of the methanogenic bacteria and non-methanogenic bacteria could be met by constituents of the organic matters and by primary or secondary metabolites produced by one species (Bardiya and Gaur, 1999). Main nutrients is necessary for microorganisms in biogas production including carbon and nitrogen. Microorganisms in the anaerobic digestion commonly use carbon as an energy source for growth and nitrogen to built cell structure.

Cassava starch effluent prepare as substrate for biogas production contained 0.46 % of nitrogen and 39.58 % of carbon, which has a carbon to nitrogen ratio of around 86 : 1. The high carbon to nitrogen ratio (86) of cassava starch effluent induce excess acid production and nitrogen deficiency. The lack of specific elements required for bacterial growth would also limit the biogas production. The proper carbon to nitrogen ratio for biogas production is 20 to 30 : 1 (Sanders and Bloodgood, 1965; Polprasert, 1989). In addition, Pohland and Bloodgood (1963) state that if the carbon to nitrogen ratio exceeded 16 : 1, the capacity of microorganisms for organic digestion would not

increase. The gas generation fails whenever the carbon to nitrogen ratio is higher than 52 : 1 (Sanders and Bloodgood, 1965). The carbon to nitrogen ratio employed in this study is 20-30:1.

Thus nitrogen supplement should be considered to enhance the biogas production from raw cassava starch effluent. Nitrogen can be added in inorganic form (e. g. ammonia) or in organic form (e. g. urea, animal manure or food waste) (Sterling *et al.*, 2001). In this study, urea was selected as nitrogen source due to it was easily digested to ammonia by variety of microorganisms. Additionally, urea which contained the nitrogen became nutrition source for the growth of microorganism. The concentration of urea 0.04% (w/v) was employed. Anunputtikul (2004) recommend for the biogas production from raw cassava starch effluent, concentration of urea should be 0.04 % (w/v). The overload of urea can be inhibited the methane production.

4.3.2 pH

Basicity or acidity of fermenting solution is indicated by pH. Most of methanogenic bacteria have pH optimal near neutrality (Jones *et al.*, 1987). In this study, as Anunputtikul (2004) recommend, the optimum pH for biogas production was in the range of 6.8-7.2 with the limitation of the range for operation without significant inhibition being 6.5 to 7.6. Methanogenic bacteria can occasionally grow at pH ranges which defined as 6.5-8.2. As the reason for the methanogenic bacteria keeps alive and the production of biogas is optimum, the pH range should be 6.8-7.2.

4.3.3 Temperature

Methane production has been documented under a wide range of temperature ranging between 0 and 97⁰C (Bitton, 1994). Two optimum temperature levels have been established the mesophilic level (25-40⁰C) and thermophilic level (50-65⁰C) (Bitton, 1994; Busby *et al.*, 1977; Mackie and Bryant, 1995). Time required for the anaerobic digestion depended on the temperature of digestion (Bunchueydee, 1984). The biogas production rate could be stimulated at high temperature more than low temperature levels but thermopilic digestion requires energy for maintaining a temperature in the biogas production. In this study, biogas production was performed at ambient temperature (mesophilic level: 25-40⁰C). This level was used because high energy for maintaining a temperature was not required.

4.4 DETERMINATION OF CHARACTERISTICS OF BIOGAS PRODUCTION

The biogas components and biogas yield depend on a feed materials due to the difference of material characteristics in each raw material (Anunputtikul, 2004). Many kinds of feed composition were investigated to determine characteristics of biogas production. In this study, the experiment had been done in batch and semi-continuous process. The characteristics of biogas production using batch and semi-continuous process are discussed as follow.

4.4.1 Batch Process

Tank 1 contained ungelling mixture of cassava starch effluent, yeast, ruminant bacteria, and urea. Tank 2 contained ungelling mixture of cassava starch effluent, ruminant bacteria, and urea. Tank 3 contained gelling mixture of cassava starch effluent, yeast, ruminant bacteria, and urea. Tank 4 contained ungelling mixture of cassava starch effluent, ruminant bacteria, urea, and microalgae. Tank 5 contained ungelling mixture of cassava starch effluent, ruminant bacteria, urea, yeast, and microalgae. The anaerobik biodigester with working volume of 5 L was performed at ambient temperature (29.5-33 °C) for 30 days.

a. The characteristics of the experiment result of each tank.

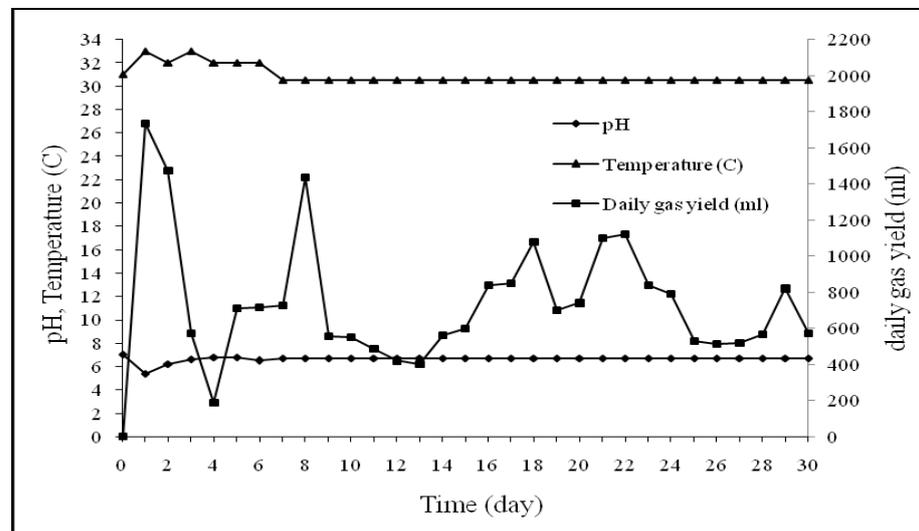


Figure 4.1 The Characteristics of The Experiment Result of Tank 1

Figure 4.1 shows that the biogas production from ungelling mixture of cassava starch effluent, yeast, ruminant bacteria, and urea at the beginning fermentation is very high. At the first day, it is 1,735 ml and the second day, it is 1,475 ml, with operation temperature at 32-33°C and pH substrate 5.38-7.05. But after the second day, the biogas yields was lower than the previous day. On third

and fourth days, the production of biogas decreased significantly, only 570 ml and 186 ml of which pH conditions at 6.64-6.79 and temperature 32-33⁰C. Biogas production on fifth to seventh days tended to be equal to the average production of 715 ml per day at temperature 31-32⁰C and pH 6.48-6.79. Whereas on eighth day, there was an significant increased of biogas production reached 1.435 ml at temperature 31⁰C and pH 6.54. Biogas production after eighth day remained fluctuative but lower than previous days, while the pH and temperature on eighth day to thirtieth remain stable at pH 6.9 and temperature 31⁰C.

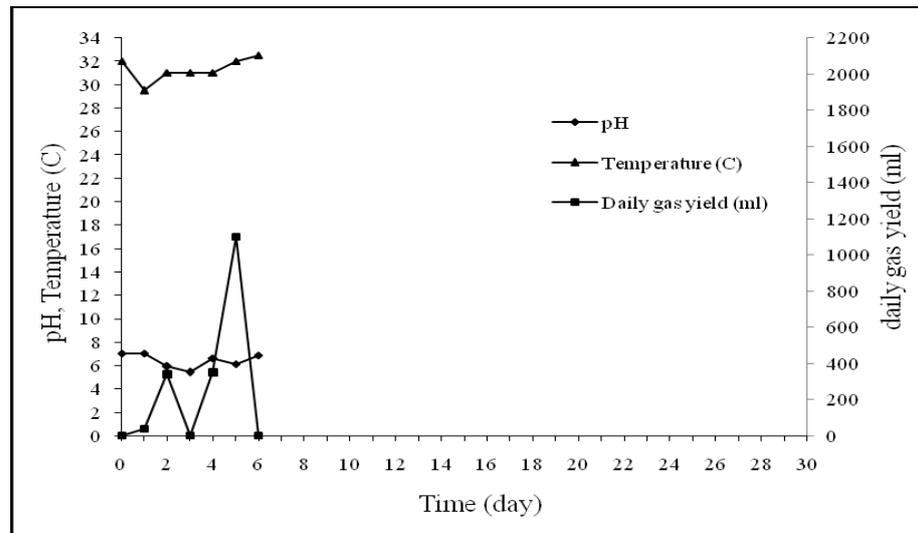


Figure 4.2 The Characteristics of The Experiment Result of Tank 2

Figure 4.2 shows that biogas production from ungerling mixture of cassava starch effluent, ruminant bacteria, and urea significantly increase on fifth day of 1,100 ml at pH 6.1 and the temperature at 32⁰C. Biogas production on the first day up to the second day was 0-340 ml. However, on third day, there is no production of biogas. The bacteria produces biogas again on fourth day and continued rising until fifth day. But starting but on sixth day biogas production stopped. pH at the beginning of the experiment was 7 and decreased on the third day. On fourth day to sixth day pH range from 6.1-6.9. For the temperature profile in Tank 2, the temperature performed at ambient temperature of 29.5-32⁰C.

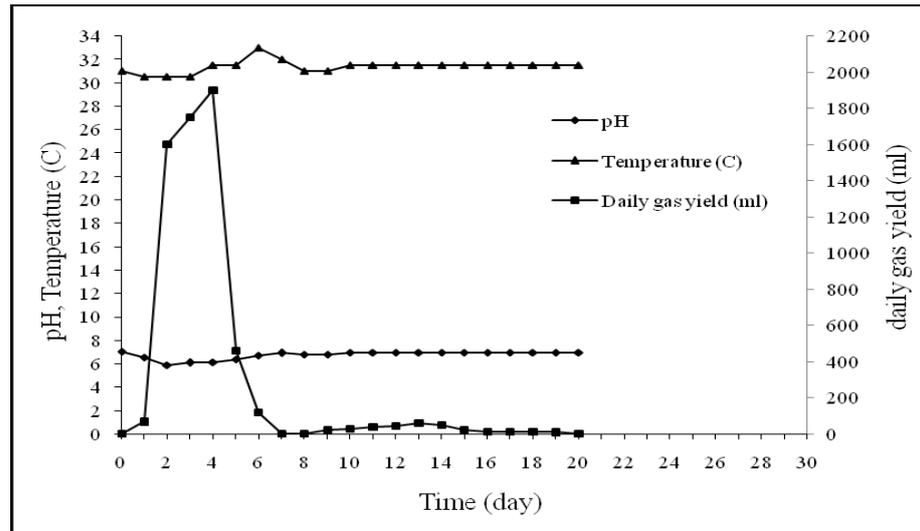


Figure 4.3 The Characteristics of The Experiment Result of Tank 3

Figure 4.3 shows that biogas production from gelling mixture of cassava starch effluent, yeast, ruminant bacteria, and urea was very significant at the beginning of fermentation up to fourth day. Biogas production from fifth day decrease and began running out on seventh day. However, on day nine to day nineteen, biogas production is very low, between 9-58 ml. Biogas production was completely discharged at twentieth day. pH of the substrate decreased at the beginning of the experiment until second day, but the pH increased and was stable from tenth day to twentieth day. At the beginning fermentation, the production of biogas increased at day ten and the temperature fluctuations was between 30.5-33⁰C. Temperature stabilized on day eleven to day twenty in the temperature at 31.5⁰C.

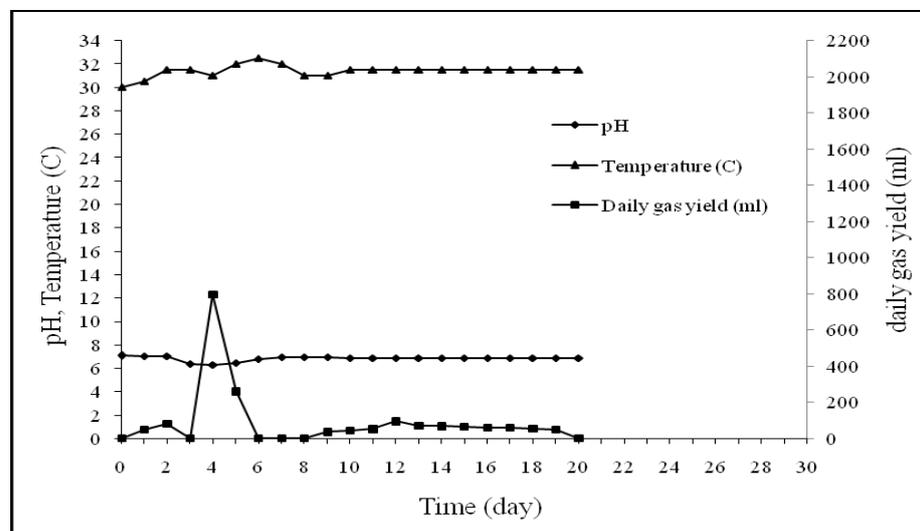


Figure 4.4 The Characteristics of The Experiment Result of Tank 4

Figure 4.4 shows that the production of biogas with ungelling mixture cassava starch effluent, ruminant bacteria, microalgae, and urea at the beginning of the experiment until second day, the biogas production was between 50-80 ml. On third day, there is no biogas production. However, on fourth day biogas production was very significantly increased at 795ml. The decline occurred on day five and biogas did not produce on day six to day eight. However, on day nine to day nineteen biogas still produced but in small number (very low), between 36-94ml. Biogas productions was completely discharged on day twenty. pH of the substrate decreased on day three and day four. The pH was increased and stable from day ten to day twenty. At the beginning of fermentation, it increased up to day ten, and the temperature fluctuated between 30-31⁰C. Temperature remained stable on the day eleven to the day twenty at 31.5⁰C.

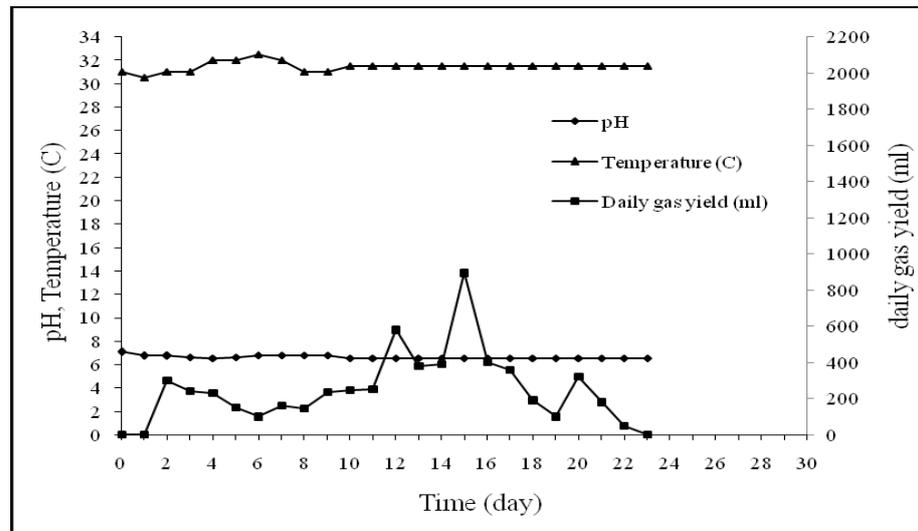


Figure 4.5 The Characteristics of The Experiment Result of Tank 5

Figure 4.5 shows that the maximum biogas production from ungelling mixture of cassava starch effluent, ruminant bacteria, urea, yeast, and microalgae occurred on the day fifteen for 895 ml. On the first day, the biogas production has not happened. Biogas production was be available on day two for 300 ml. On third until sixth days, the production of biogas decrease from 240-144 ml. On day seven to day twelve biogas production increase, but on day thirteen and fourteen biogas

production decrease again. Biogas yield fluctuations still occur on day sixteen to day twenty-two. On twenty-third day, the biogas was not produced anymore. The decreasing of pH occurred until the day four of the experiment. On the day five to day eight, there was an increasing of pH (6.64-6.8). pH was relatively stable from day nine to day twenty-three at pH 6.83. At the beginning of fermentation up to day ten, the temperature fluctuated between 30.5-32.5⁰C. Temperature remained stable on day eleven to day twenty-two in the temperature at 31.5⁰C.

b. Biogas production from various feed compositions

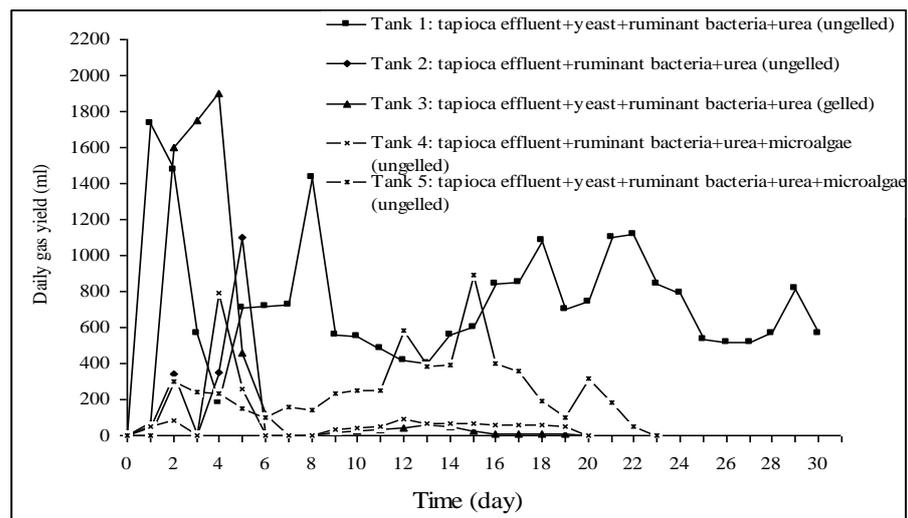


Figure 4.6 Daily Biogas Production (ml) from Various Feed Compositions Performed in Anaerobic Biodigester of 5 L Digestion Volume.

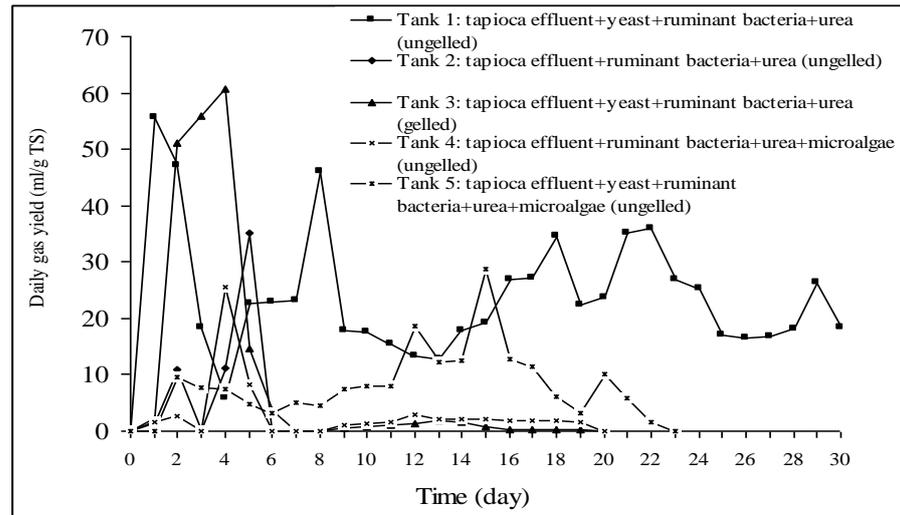


Figure 4.7 Daily Biogas Production per Gram Total Solid (ml/g total solid) from Various Feed Compositions Performed in Anaerobic Biodigester of 5 L Digestion Volume.

Figure 4.6 shows biogas production in ml and Figure 4.7 shows biogas production in ml per gram total solid.

A significant increasing in biogas production per day was found when the feed was gelled (Tank 3). At the beginning of fermentation, the biogas production per day from the ungelled feed of Tank 2, 4, and 5 were relatively low but the biogas production per day from the ungelled feed of Tank 1 was high from the beginning. The maximum of biogas production per day from Tank 1 was 1,735 ml or 55.52 ml/g total solid at the first day retention time. The maximum of biogas production per day from Tank 2 was 1,100 ml or 35.2 ml/g total solid at fifth day retention times. The maximum biogas production per day of Tank 3 and 4 were 1,900 ml and 795 ml or 60.8 ml/g total solid and 25.44 ml/g total solid, respectively at fourth day retention times. The maximum of biogas production per day from Tank 5 was 895 ml or 28.6 ml/g total solid at day twelve retention times. The fermentation reactions of Tank 1, 2, 3, 4 and 5 were ceased after operating for 40, 6, 20, 20, and 23 days, respectively.

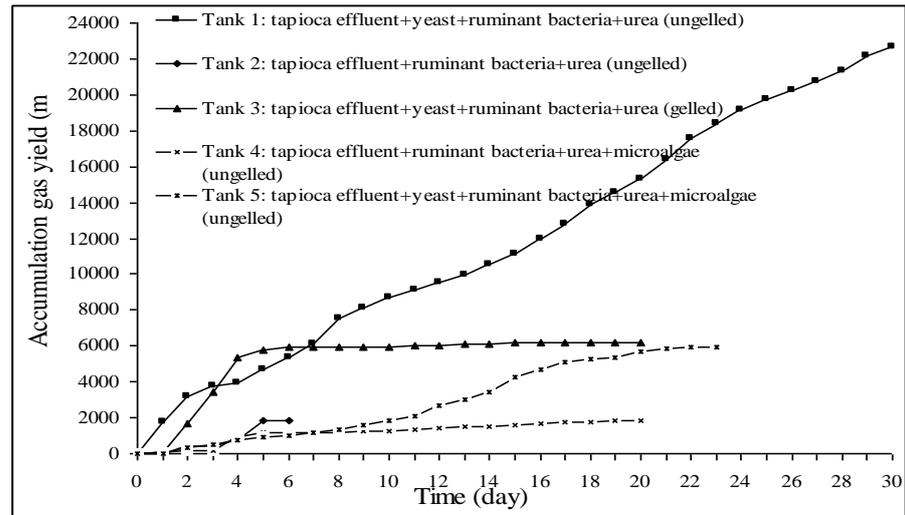


Figure 4.8 Accumulation Biogas Production (ml) from Various Feed Compositions Performed in Anaerobik Biodigester of 5 L Digestion Volume.

Figure 4.3 shows that accumulation biogas production from the gelling mixture (Tank 3) is high enough at the beginning of fermentation rather than the ungel mixture (Tank 2,4,5). It is caused by the formation of shorter carbon chains and yeast. Shorter carbon chains are more easily degraded by external enzymes produced by fermentative microorganism to lower molecular weight molecules (Milono, *et al*, 1981) so that in the next stage the bacteria would be easier to convert these organic materials into biogas. Yeast added also simplifies the hydrolysis processes. Unfortunately, biogas productions using the gelling mixture can not long lasting because the substrates digest faster by hydrolysis bacteria which accelerate with availability of yeast and form of shorter carbon chains. It ceases at sixth day retention time. If the substrates completely hydrolyzed and there is no fresh feeding into biodigester, biogas production will be finished. Better yield comes from Tank 1 which contains cassava starch effluent, ruminant bacteria, yeast, and urea without gelling. From the beginning, Tank 1 increase more significant than the other and still produce biogas until more than 30 days. Yeast as substrate activator and ruminant bacteria accelerates biogas production, but the substrates in biodigester do not hydrolyzed completely. It keeps biodigester produce biogas for long lasting.

Biogas production processes from cassava starch effluent was started with hydrolysis of cassava starch effluent as the complex organic materials by fermentative bacteria to simple organic material. Then, the acid forming bacteria

used these simple organic materials as substrates to produce volatile acids, hydrogen, and carbon dioxide. Finally the methanogenic bacteria became established in the biodigester, and used end product from the acid forming bacteria digestion to produce methane. In this stage, methanogenic bacteria were predominant (Anunputtikul, 2004). Thus, the methane content of the biogas was increased.

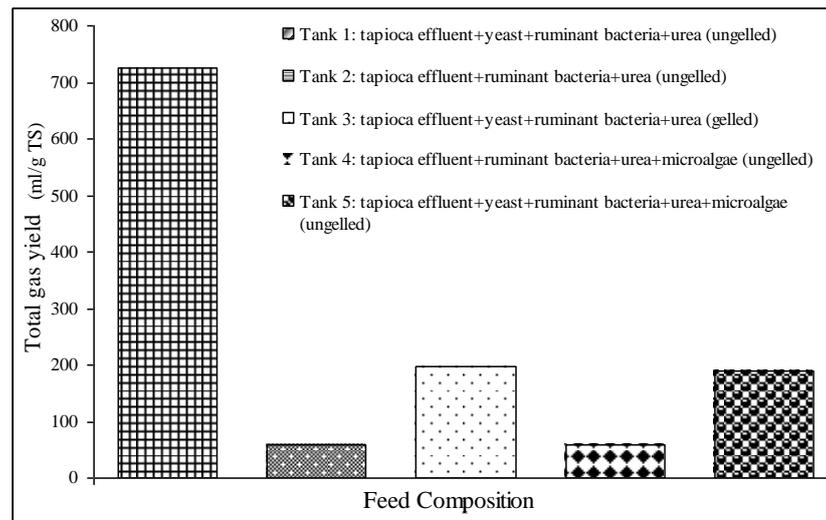


Figure 4.9 Total Gas Yield per Gram Total Solid (ml/g total solid) from Various Feed Compositions Performed in Anaerobik Biodigester of 5 L Digestion Volume.

The total biogas yield from Tank 1, 2, 3, 4 and 5 were 726.43, 58.6, 198, 58.72 and 189 ml/g total solid, respectively (Figure 4.9). The maximum of total biogas yield of this experiment was obtained from un-gelling mixture of cassava starch effluent, yeast, bacteria ruminant, and urea (726.43 ml/g total solid).

If total biogas yield from Tank 2 and 4 were compared, adding microalgae as nitrogen source did not give significant effect to biogas production. Microalgae did not influence biogas production exceedingly. At Tank 1, 3, and 5 which used yeast as substrate activator, adding of yeast was very helpful to accelerate biogas production, so it also was used for semi-continuous process.

The steps employed in this study were as Milono, et al (1981). In the first stage, which was the fermentative stage, organic materials (protein, cellulose, lipid, and starch) were broken down by fermentative microorganism to lower molecular weight molecules. The second stage was the acid-forming stage. In this stage,

products from the first stage were converted by acetogenic bacteria (acetate and H₂-producing bacteria) into acetate, hydrogen gas, carbon dioxide, and few other VFA such as propionic and butyric acid. The third stage was the methanogenic stage. The methanogenic bacteria or methane-forming bacteria produce methane, carbon dioxide, trace gases (e.g., H₂S), and water. It was almost that 70% of methane was formed from acetate, and the rest was formed from carbon dioxide and hydrogen.

c. pH from various feed compositions

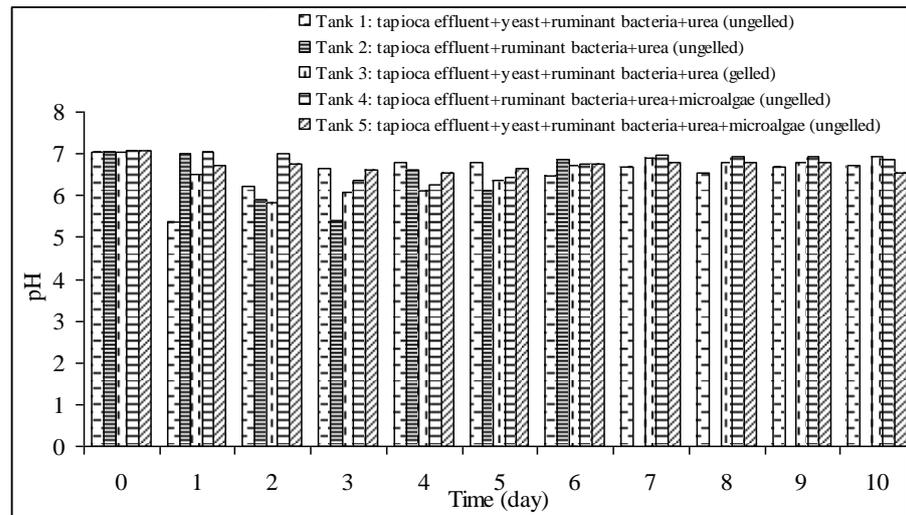


Figure 4.10 pH from Various Feed Composition Performed in Anaerobik Biodigester of 5 L Digestion Volume.

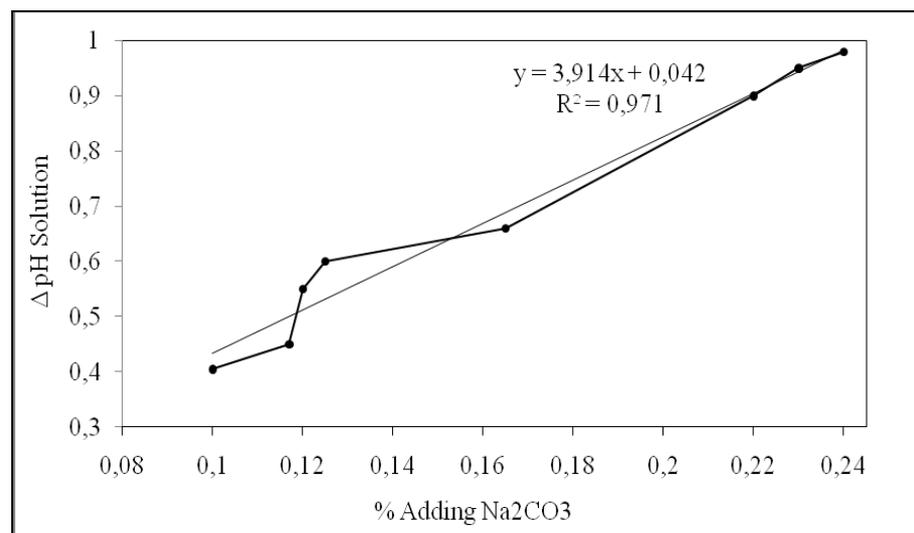


Figure 4.11 Buffer Na₂CO₃ Required for Rising Alkalinity Performed in Anaerobik Biodigester of 5 L Digestion Volume.

Figure 4.10 shows that the pH ranges of 5.84-6.94; 5.38-7.07; 5.38-6.9; 6.25-7.03; and 6.53-7.07 were found in the Tank 1, 2, 3, 4 and 5, respectively. Figure 4.11 shows Na_2CO_3 required for rising alkalinity based on delta pH solution. % adding of Na_2CO_3 to delta pH solution was expressed by equation $y = 3.914x + 0.042$, so 0.39% of Na_2CO_3 was added in Tank 1 because the pH decrease from 7.1 to 5.4 at the first day fermentation. At the second, third, and sixth days, the pH was 6.2, 6.6, and 6.5 as a result 0.19%, 0.09%, 0.117% of Na_2CO_3 was added, respectively. In Tank 2, at the second, third, fourth, and fifth days, the pH was 5.9, 5.41, 6.6, and 6.12 therefore of 0.27%, 0.395%, 0.09%, and 0.21% of Na_2CO_3 was added, respectively. In Tank 3, 0.28%, 0.22%, 0.21%, 0.15% of Na_2CO_3 was added at second, third, fourth, and fifth days. In Tank 4, 0.18% of Na_2CO_3 was added at fourth day. In Tank 5, 0.1% of Na_2CO_3 was added at fourth day.

When the digester was initially heavily fed, acid forming bacteria quickly produced acids. The drop of pH was caused acid forming bacteria produce acetate, hydrogen gas, carbon dioxide, and few other VFA such as propionic and butyric acid. A low pH value inactivated microorganisms involved in the biogas production especially methanogenic bacteria (Vicenta et al., 1984).

In order to allow the methanogenic bacteria to grow, digester should be properly fed and buffered to rising alkalinity. In this study, Sodium carbonate (Na_2CO_3) was used to increase alkalinity or buffering capacity of fermenting slurry. It was added to the digester whenever the pH below 6.8.

Since the reaction rate involving acid-forming bacteria proceeded much faster than the reaction involving methanogenic bacteria, a larger population of methanogenic bacteria must be fed and maintained. The methanogenic bacteria population might not be adequate to consume the acids produced and maintain a neutral pH resulting in declining pH below the neutral pH and diminishing growth of methanogenic bacteria and methanogenesis. The pH could be maintaining by adding Na_2CO_3 to increase alkalinity. The pH was the key indicator of operational stability (Tanticaroen et al., 1984). Methanogenic bacteria could occasionally grow at the pH range of 6.5-8.2 (Anunputtikul, 2004). Viswanath et al. (1992) mentioned that there was a perfect link of the acidogenic and methanogenic phases when the pH was remained at 7 and there was no drastic increase in acidity or alkalinity.

d. Temperature from various feed compositions

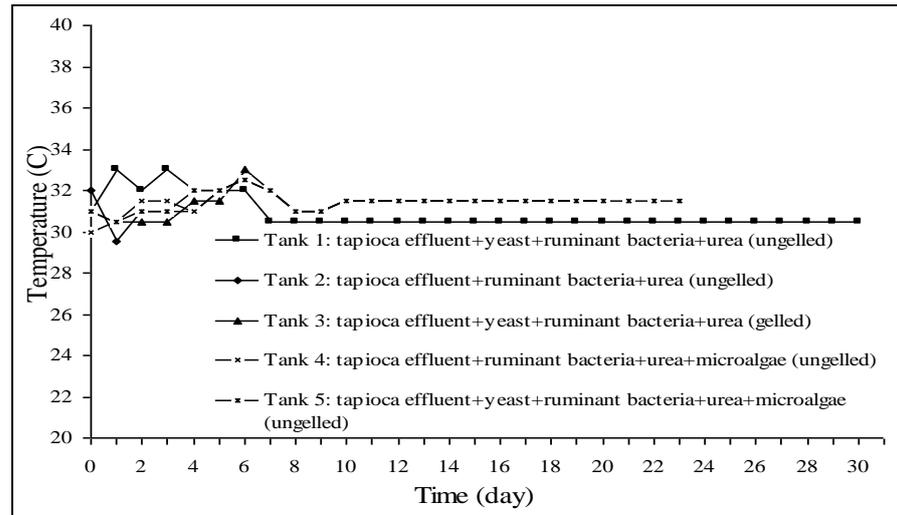


Figure 4.12 Temperature from Various Feed Composition Performed in Anaerobic Biodigester of 5 L Digestion Volume.

During 30 days of operation, the temperature ranges of 30.5-33⁰C, 29.5-33⁰C, 31-33⁰C, 30-32.5⁰C, and 30.5-32.5⁰C were found in the digester 1, 2, 3, 4 and 5, respectively (Figure 4.12). At the initial retention time, the temperature was fluctuating from 29.5⁰C until 33⁰C, but since the day thirteenth the temperature of mixtures stabilized. A slight difference of temperature between the gelling feed and ungelting feed was observed but they still in the range of mesophilic temperature (29.5-33⁰C).

As the temperature falls, microbial activity decreases and the biogas production decreases. As the temperature increases some microorganisms begin to die, once again the production of biogas decreases.

4.4.2 Semi-continuous Process

Based on previous batch experiment, it was known that the adding of microalgae was not give significant effect to biogas production. So, in the semi-continuous experiment, microalga was not added. As nitrogen source, adding of urea was well to do.

In semi-continuous experiment, 1 % (w/v) of total solid of cassava starch effluent, 0.08% (w/v) of yeast, 10%(v/v) of ruminant bacteria, and 0.04%(w/v) of urea were mixed in the tank. According to Sitohang (2000), adding of fresh feed was conducted when biogas production achieve the maximum yield condition. In previous batch experiment, the maximum yield condition was occurred at second days, consequently adding of fresh feed was conducted every two days with constant

amount of feeding (2 grams per two days). The aim of this feeding management was increasing of biogas production (Sitohang, 2000).

a. Biogas production

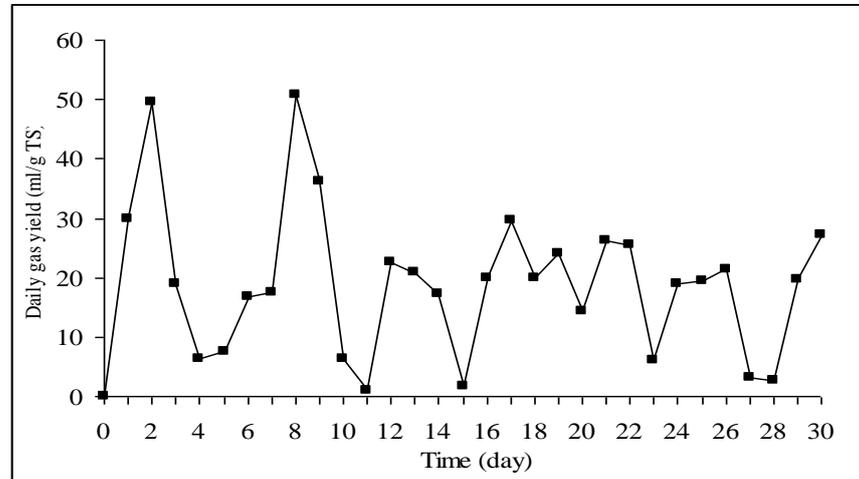


Figure 4.13 Daily and Accumulation Biogas Production from Cassava starch Effluent using Yeast, Ruminant Bacteria and Urea in Anaerobik Biodigester of 5 L Digestion Volume.

Figure 4.13 shows that at the beginning of fermentation, the biogas production per day was relatively high. On the third day, the biogas production per day decreased. The addition of cassava starch effluent did not influence the activities of microorganisms to produce biogas directly. This can be seen on the fourth and fifth days, the biogas production per day still decreased. Yeast was added on fifth day and the biogas production per day increased on sixth and seventh days. It was occurred because the yeast as substrate activator accelerates hydrolysis of substrates by microorganisms, then converts it into biogas. A significant increase in biogas production was occurred on the eighth day retention time (50.69 ml/g total solid/day), after the addition of cassava starch effluent and yeast. On the next day, the biogas production increased and decreased fluctuatively. Overall, a high biogas production was occurred during the early days (1-10 days). The biogas production normally increased after cassava starch effluent and yeast was added. But the addition of cassava starch effluent has been effect in increasing the biogas production after 3-4 days. A significant effect in increasing the biogas production was occurred after yeast was added every 5 days. The maximum of biogas production per day 50.69 ml/g total solid/day was obtained at eighth day retention times (Figure 4.13) and the total biogas production was 581.15 ml/g total solid.

In this semi-continuous process, fresh feed replace the fermented substrate. However, the increasing of feeding-value will increase biogas production until certain value, then biogas production will be decrease exactly. It is based on substrate retention time in biodigester. More adding feeding-value, retention time in biodigester will be shorter, consequently also shorter in reaction time because substrate will be push out before the reaction complete.

b. pH and Temperature

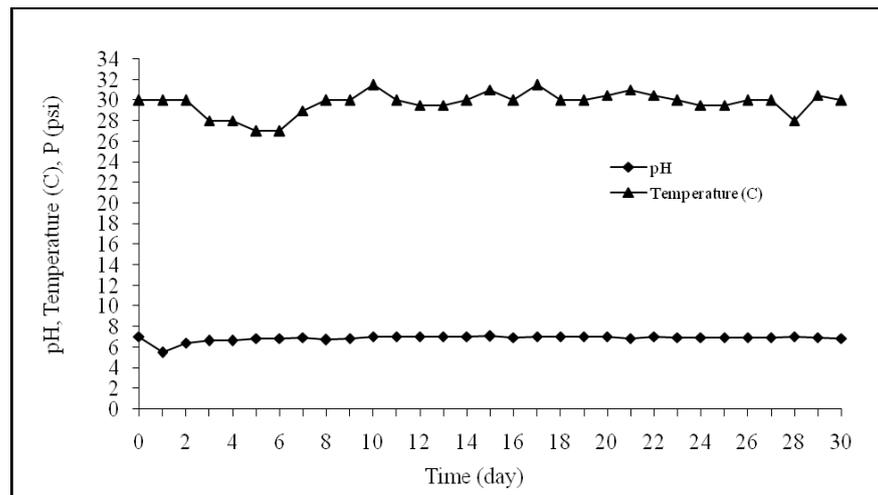


Figure 4.14 pH and Temperature Performed in Anaerobic Biodigester of 5 L Digestion Volume.

During 30 days of operation, the pH ranges of 5.52-7.06 was found (Figure 4.14). The initial drops in pH from 7.04 to 5.52 was observed at first day retention time. At that time, a low pH value inactivated microorganisms involved in the biogas production especially methanogenic bacteria (Vicenta *et al.*, 1984), so adding of Na_2CO_3 (0.2%) is needed to raising alkalinity in order to keep microorganisms alive. On the second day, the pH stabilized at neutral pH. pH stability stayed awake until the 30-day because there were the addition of cassava starch effluent and yeast, so the activities of microorganisms remained stable.

Biogas production The simple anaerobic semi-continuous biodigester volume of 5 L digestion volume was performed at ambient temperature (27-33 °C) for 30 days at mesophilic level. There are three kinds of bacteria which have response with alteration temperature:

- *Psychophilic bacteria*, these bacteria grow under 25°C with the optimum temperature is 15-20°C.

- *Mesophilic bacteria*, these bacteria live at 20-40⁰C with the optimum growth temperature is 35-40⁰C.
- *Thermophilic bacteria*, these bacteria grow at 40-65⁰C with the optimum temperature between 55-60⁰C.

Figure 4.7 and 4.9 show that most of the biogas-producing bacteria are mesophilic bacteria with the temperature production about 29.5-33⁰C. From figure 4.9, there is maximum temperature for maximum biogas production: 30⁰C for 50.69 ml biogas/g total solid/day.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

Anaerobic fermentation of biogas production is very complex reactions which involve many intermediate compound and microorganisms that play important role in anaerobic fermentation. This study focuses on the used of urea, ruminant, yeast, microalgae, the treatment of gelled and ungelled feed for biogas production, pH control during biogas production using buffer Na_2CO_3 , and feeding management in the semi-continuous process of biogas production. The result can be concluded as follows:

- a. Adding of microalgae as nitrogen source did not give significant effect to biogas production. But adding of yeast as substrate activator was very helpful to accelerate biogas production.
- b. The biogas production increased after cassava starch effluent and yeast was added.
- c. Biogas production from the ungelling and gelling mixture of cassava starch effluent, yeast, ruminant bacteria, and urea were 726.43 ml/g total solid and 198 ml/g total solid.
- d. Biogas production from ungelling mixture without yeast was 58.6 ml/g total solid.
- e. Biogas production from ungelling mixture added by microalgae without yeast was 58.72 ml/g total solid and that with yeast was 189 ml/g total solid.
- f. Biogas production from ungelling mixture of cassava starch effluent, yeast, ruminant bacteria, and urea in semi-continuous process was 581.15 ml/g total solid.
- g. Requirement of sodium carbonate (Na_2CO_3) to increase alkalinity or buffering capacity of fermenting solution depends on pH-value.

5.2 RECOMMENDATIONS

This study deals only with the composition of the feed, biogas production rate, the operating temperature, and the operating pH. For further research, it can be recommended as follows:

- a. To find out how to control the stability of pH.
- b. The composition of produced gas should be measured. Biogas composition measurements can be done with Gas Chromatography (GC).

- c. To determine the appropriate time for feeding.

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APPENDIX A

REAGENTS PREPARATION

1. Cassava Starch Effluent Synthetic

$$1\% \text{ (w/v) of mixing solution volume} = \frac{1}{100} \times 2,500 = 25 \text{ gr}$$

2. Urea

$$0.04\% \text{ (w/v) of mixing solution volume} = \frac{0.04}{100} \times 2,500 = 1 \text{ gr}$$

3. Ruminant Bacteria

$$10\% \text{ (v/v) of mixing solution volume} = \frac{10}{100} \times 2,500 = 250 \text{ ml}$$

4. Yeast

$$0.08\% \text{ (w/v) of mixing solution volume} = \frac{0.08}{100} \times 2,500 = 2 \text{ gr}$$

5. Microalgae

$$50\% \text{ (v/v) of mixing solution volume} = \frac{50}{100} \times 2,500 = 1,250 \text{ ml}$$

6. Water

$$\begin{aligned} \text{For Tank 1, 2 and 3, water} &= \text{mixing solution volume} - \text{ruminant bacteria} \\ &= 2,500 - 250 \\ &= 2,000 \text{ ml} \end{aligned}$$

$$\begin{aligned} \text{For Tank 4 and 5, water} &= \text{mixing solution volume} - \text{ruminant bacteria} - \text{microalgae} \\ &= 2,500 - 250 - 1,250 \\ &= 750 \text{ ml} \end{aligned}$$

APPENDIX B

PARAMETERS AND RESULT OF BIOGAS PRODUCTION

USING ANAEROBIC BIODIGESTER FROM CASSAVA

STARCH EFFLUENT

Table 1 B. Biogas Production from Cassava Starch Effluent with Batch Process

Day	Tank1			Tank2		
	cassava starch effluent + yeast + ruminant bacteria + urea (ungelled)			cassava starch effluent + ruminant bacteria + urea (ungelled)		
	V(ml)	pH	T °C	V(ml)	pH	T °C
0	0	7.05	31	0	7.05	32
1	1,735	5.38	33	40	7	29.5
2	1,475	6.22	32	340	5.9	31
3	570	6.64	33	0	5.41	31
4	186	6.79	32	350	6.6	31
5	710	6.79	32	1,100	6.12	32
6	715	6.48	32	0	6.88	32.5
7	725	6.69	31			
8	1,435	6.54	31			
9	555	6.69	31			
10	550	6.71	31			
11	485	6.9	31			
12	420	6.9	31			
13	400	6.9	31			
14	560	6.9	31			
15	600	6.9	31			
16	840	6.9	31			
17	850	6.9	31			
18	1,080	6.9	31			
19	700	6.9	31			
20	740	6.9	31			
21	1,100	6.9	31			
22	1,120	6.9	31			
23	840	6.9	31			
24	790	6.9	31			
25	530	6.9	31			
26	515	6.9	31			
27	520	6.9	31			
28	565	6.9	31			
29	820	6.9	31			
30	570	6.9	31			

Total	22,701			1,830		
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Table 1 B. (Continued)

Day	Tank3			Tank4			Tank5		
	cassava starch effluent + yeast + ruminant bacteria + urea (gelled)			cassava starch effluent + ruminant bacteria + urea + microalgae (ungelled)			cassava starch effluent + yeast + ruminant bacteria + urea + microalgae (ungelled)		
	V(ml)	pH	T °C	V(ml)	pH	T °C	V(ml)	pH	T °C
0	0	7.05	31	0	7.06	30	0	7.07	31
1	68	6.52	30.5	50	7.03	30.5	0	6.73	30.5
2	1,600	5.84	30.5	80	7.02	31.5	300	6.74	31
3	1,750	6.07	30.5	0	6.37	31.5	240	6.6	31
4	1,900	6.12	31.5	795	6.25	31	230	6.54	32
5	460	6.35	31.5	260	6.45	32	150	6.64	32
6	120	6.71	33	0	6.76	32.5	100	6.77	32.5
7	0	6.9	32	0	6.97	32	160	6.78	32
8	0	6.8	31	0	6.92	31	144	6.8	31
9	20	6.8	31	36	6.92	31	237	6.8	31
10	28	6.94	31.5	43	6.87	31.5	248	6.53	31.5
11	37	6.94	31.5	51	6.87	31.5	250	6.53	31.5
12	45	6.94	31.5	94	6.87	31.5	580	6.53	31.5
13	58	6.94	31.5	69	6.87	31.5	380	6.53	31.5
14	50	6.94	31.5	67	6.87	31.5	390	6.53	31.5
15	23	6.94	31.5	66	6.87	31.5	895	6.53	31.5
16	10	6.94	31.5	60	6.87	31.5	400	6.53	31.5
17	10	6.94	31.5	58	6.87	31.5	360	6.53	31.5
18	10	6.94	31.5	56	6.87	31.5	190	6.53	31.5
19	9	6.94	31.5	50	6.87	31.5	100	6.53	31.5
20	0	6.94	31.5	0	6.87	31.5	320	6.53	31.5
21							180	6.53	31.5
22							50	6.53	31.5
23							0	6.53	31.5
24									
25									
26									
27									
28									
29									
30									
Total	6,198			1,835			5,904		

Table 2 B. Biogas Production from Cassava Starch Effluent with Semi-continuous Process

Day	cassava starch effluent + yeast + ruminant bacteria + urea (ungelled)		
	V (ml)	pH	T (0C)
0	0	7.04	30
1	934	5.52	30
2	1,550	6.42	30
3	590	6.67	28
4	200	6.67	28
5	233	6.9	27
6	525	6.9	27
7	550	6.94	29
8	1,584	6.74	30
9	1,130	6.9	30
10	200	7.04	31.5
11	33	7.04	30
12	706	7.06	29.5
13	650	7.06	29.5
14	540	7.06	30
15	50	7.1	31
16	620	6.97	30
17	925	7.05	31.5
18	620	7.03	30
19	750	7.03	30
20	450	7.05	30.5
21	819	6.9	31
22	798	7.06	30.5
23	193	6.96	30
24	590	6.96	29.5
25	606	6.96	29.5
26	670	6.96	30
27	100	6.96	30
28	80	7.07	28
29	615	6.94	30.5
30	850	6.89	30
Total	18,161		

APPENDIX C
FIGURES OF RESEARCH MATERIALS AND APPARATUS



Figure 1. Anaerobic bioreactor



Figure 2. Total biogas production measuring by water displacement technique



Figure 3. Temperature measuring



Figure 4. pH measuring



Figure 5. pHmeter



Figure 6. Tapioca starch



Figure 7. Urea



Figure 8. Yeast

BIOGAS PRODUCTION USING ANAEROBIC BIODIGESTER FROM CASSAVA STARCH EFFLUENT WITH RUMINANT BACTERIA AS BIOCATALYST

Audra Ayu O. (L2C006023) and Vincensia Dyan Aryati (L2C006106)
Chemical Engineering Department, Technical Faculty, Diponegoro University
Jalan Prof. Sudharto, Tembalang, Semarang, 50239, Telp/Fax: (024)7460058
Advisor: Dr. nat.techn. Siswo Sumardiono, ST, MT

Abstract

IKMs' factory activity in Margoyoso produces liquid and solid wastes. The possible alternative was to use the liquid effluent as biogas raw material. This study focuses on the used of urea, ruminant, yeast, microalgae, the treatment of gelled and ungelled feed for biogas production, pH control during biogas production using buffer Na₂CO₃, and feeding management in the semi-continuous process of biogas production that perform at ambient temperature for 30 days. Ruminant bacteria, yeast, urea, and microalgae was added 10% (v/v), 0.08% (w/v), 0.04% (w/v), 50% (v/v) of mixing solution volume, respectively. The pH of slurry was adjusted with range 6.8-7.2 and was measured daily and corrected when necessary with Na₂CO₃. The total biogas production was measured daily by the water displacement technique. Biogas production from the ungelting and gelling mixture of cassava starch effluent, yeast, ruminant bacteria, and urea were 726.43 ml/g total solid and 198 ml/g total solid. Biogas production from ungelting mixture without yeast was 58.6 ml/g total solid. Biogas production from ungelting mixture added by microalgae without yeast was 58.72 ml/g total solid and that with yeast was 189 ml/g total solid. Biogas production from ungelting mixture of cassava starch effluent, yeast, ruminant bacteria, and urea in semi-continuous process was 581.15 ml/g total solid. Adding of microalgae as nitrogen source did not give significant effect to biogas production. But adding of yeast as substrate activator was very helpful to accelerate biogas production. The biogas production increased after cassava starch effluent and yeast was added. Requirement of sodium carbonate (Na₂CO₃) to increase alkalinity or buffering capacity of fermenting solution depends on pH-value.

Key Words: *biogas cassava; C/N ratio; ruminant bacteria; semi-continuous biodigester; yeast*

1. Introduction

Indonesia is the third country of cassava producer after Brazil and Thailand, and has 1,205,440 hectares planted area and 21,990,381 tons cassava production/year (BPS, 2009). Mostly, cassava root was produced for tapioca starch. There are 399 small-medium scale industries (IKMs) in Margoyoso, Pati, Central Java, Indonesia, which have average production capacity 10 tons cassava/IKM-day. So, demand of cassava root was approximately 3,990 tons/day with total water consumption 15,960 m³/day. Consequently, the IKMs' factory activities produce liquid and solid wastes. The problem was that the effluent from tapioca starch factories was released directly into the river before properly treatment. To treat the effluent, we can conduct coagulation and flocculation process (Malhotra, et.al., 1994). But the recovery result of the product can not be used for food product because of chemical residue. The possible alternative was to use the effluent as raw material of biogas.

Biogas production was conducted in the semi-continuous biodigester. Urea and microalgae were used as source of nitrogen in the biodigester. In this anaerobic process, ruminant bacteria was added as biocatalyst to enhance biogas production. This study focuses on the used of urea, ruminant, yeast, microalgae, the treatment of gelled and ungelled feed for biogas production, pH control during biogas production using buffer Na₂CO₃, and feeding management in the semi-continuous process of biogas production.

2. Materials and Methods

2.1 Preparation of the Materials for Biogas Production

Cassava starch effluent was made synthetically with total solid 1% (w/v). 25 gr of cassava starch was dissolved in 2,500 ml of mixing solution. Prepare urea as much as 0.04% (w/v) of mixing solution volume, ruminant bacteria as much as 10% (v/v) of mixing solution volume, yeast as much as 0.08% (w/v) of mixing solution volume, and microalgae as much as 50% (v/v) of mixing solution volume. Mixing solution was heated and agitated for making the gelling solution. Na_2CO_3 was also prepared if necessary for rising the alkalinity of pH solution.

2.2 Variable

Urea and ruminant bacteria were used as dependent variable, while yeast, microalgae, and the treatment of gelled and ungelled feed for biogas production as independent variable.

2.3 Experiment Procedures

d. Study of biogas production from cassava starch effluent by adding microalgae as nitrogen source and yeast as substrate activator with the treatment of gelled and ungelled feed

The production of biogas from cassava effluent was performed using anaerobic biodigester of 5 L digestion volume (Figure 1), but the volume of mixing solution was half of biodigester reactor. On batch process, Tank 1 was fed ungelling mixture of cassava starch effluent, yeast, ruminant bacteria, and urea, Tank 2 was fed ungelling mixture of cassava starch effluent, ruminant bacteria, and urea, Tank 3 was fed gelling mixture of cassava starch effluent, yeast, ruminant bacteria, and urea, Tank 4 was fed ungelling mixture of cassava starch effluent, ruminant bacteria, urea, and microalgae, Tank 5 was fed ungelling mixture of cassava starch effluent, ruminant bacteria, urea, yeast, and microalgae. While on semi-continuous process, the tank was fed ungelling of cassava starch effluent, yeast, ruminant bacteria, and urea. This study was conducted in duplicate sample at ambient temperature for 30 days to obtain the volume of biogas production. The total biogas production was measured daily by the water displacement technique. The pH was measured daily and corrected when necessary with an alkaline buffer solution (Na_2CO_3). The equipment components to measure the total biogas were fabricated as in Figure 1.

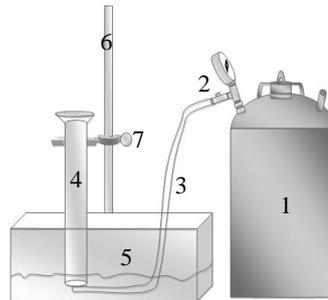


Figure 1. Water Displacement Technique

Notes:

8. anaerobic biodigester 5L
9. valve
10. hose
11. measure glass
12. wash basin
13. statif
14. clamp

e. Study of pH control during biogas production using buffer Na_2CO_3 .

The pH was measured daily and corrected when necessary with an alkaline buffer solution (Na_2CO_3).

f. Study of feeding management in the semi-continuous process of biogas production

Cassava starch effluent was fermented to achieve maximum output of biogas and reduction of total solids with minimum retention time under ambient conditions. In semi-continuous process, fresh feed (2 grams of total solid) was added every two days in order to displace the same amount of digested material to maintain the constant biogas production volume. Fresh yeast (0.08% (w/v)) was also added every five days to stimulate biogas production.

3. Results and Discussion

3.1 Determination of Characteristics of Biogas Production

The biogas components and biogas yield depend on a feed materials due to the difference of material characteristics in each raw material (Anunputtikul, 2004).

4.4.3 Batch Process

a. Biogas production

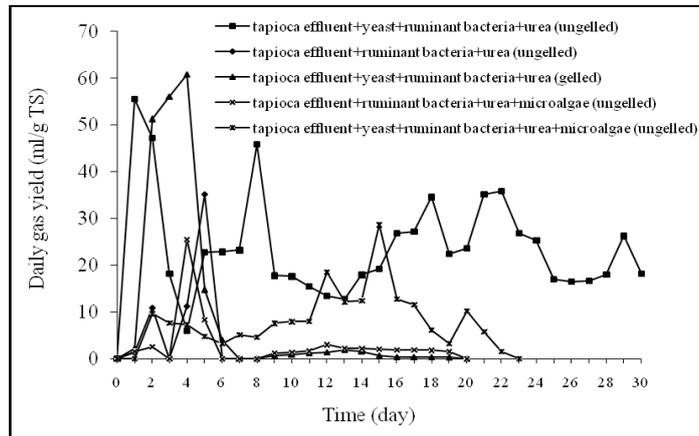


Figure 2. Daily Biogas Production per Gram Total Solid (ml/g total solid) from Various Feed Compositions Performed in Anaerob Biodigester of 5 L Digestion Volume.

Figure 2 shows that a significant increasing in biogas production per day was found when the feed was gelled (Tank 3). At the beginning of fermentation, the biogas production per day from the ungelling feed of Tank 2, 4, and 5 were relatively low but the biogas production per day from the ungelling feed of Tank 1 was high from the beginning. The maximum of biogas production per day from Tank 1 was 55.52 ml/g total solid at the first day retention time. The maximum of biogas production per day from Tank 2 was 35.2 ml/g total solid at fifth day retention times. The maximum biogas production per day of Tank 3 and 4 were 60.8 ml/g total solid and 25.44 ml/g total solid, respectively at fourth day retention times. The maximum of biogas production per day from Tank 5 was 28.6 ml/g total solid at day twelve retention times. The fermentation reactions of Tank 1, 2, 3, 4 and 5 were ceased after operating for 40, 6, 20, 20, and 23 days, respectively.

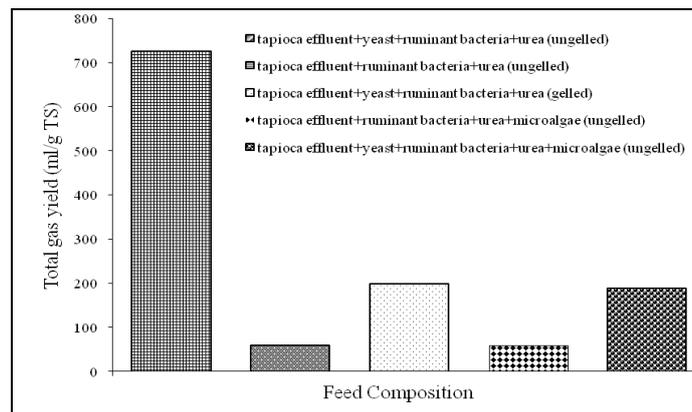


Figure 3. Total Gas Yield per Gram Total Solid (ml/g total solid) from Various Feed Compositions Performed in Anaerob Biodigester of 5 L Digestion Volume.

The total biogas yield from Tank 1, 2, 3, 4 and 5 were 726.43, 58.6, 198, 58.72 and 189 ml/g total solid, respectively (Figure 3). The maximum of total biogas yield of this experiment was obtained from ungelling mixture of cassava starch effluent, yeast, bacteria ruminant, and urea (726.43 ml/g total solid).

If total biogas yield from Tank 2 and 4 were compared, adding microalgae as nitrogen source did not give significant effect to biogas production. Microalgae did not influence biogas production exceedingly. At Tank 1, 3, and 5 which used yeast as substrate activator, adding of yeast was very helpful to accelerate biogas production, so it also was used for semi-continuous process.

The steps employed in this study were as Milono, et al (1981). In the first stage, which was the fermentative stage, organic materials (protein, cellulose, lipid, and starch) were broken down by fermentative microorganism to lower molecular weight molecules. The second stage was the acid-forming stage. In this stage, products from the first stage were converted by acetogenic bacteria (acetate and H₂-producing bacteria) into acetate, hydrogen gas, carbon dioxide, and few other VFA such as propionic and butyric acid. The third stage was the methanogenic stage. The methanogenic bacteria or methane-forming bacteria produce methane, carbon dioxide, trace gases (e.g., H₂S), and water. It was almost that 70% of methane was formed from acetate, and the rest was formed from carbon dioxide and hydrogen.

b. pH from various feed compositions

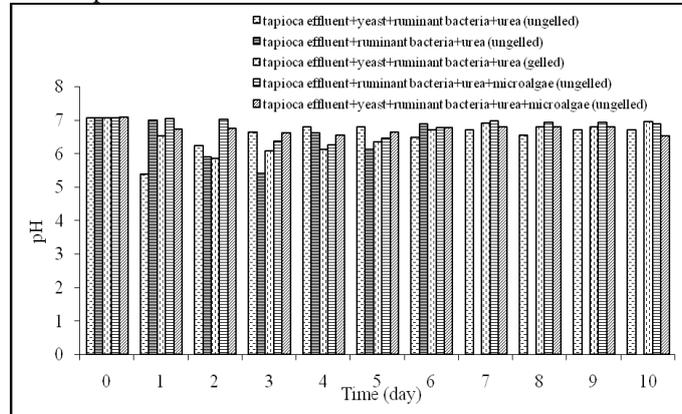


Figure 4. pH from Various Feed Composition Performed in Anaerob Biodigester of 5 L Digestion Volume.

Figure 4 shows that the pH ranges of 5.84-6.94; 5.38-7.07; 5.38-6.9; 6.25-7.03; and 6.53-7.07 were found in the Tank 1, 2, 3, 4 and 5, respectively. The pH was corrected with Na₂CO₃ when the pH below 6.8. Based on previous study that the relation of percentage adding of Na₂CO₃ to delta pH solution was expressed by equation $y = 3.914x + 0.042$, so 0.39% of Na₂CO₃ was added in Tank 1 because the pH decrease from 7.1 to 5.4 at the first day fermentation. At the second, third, and sixth day, the pH was 6.2, 6.6, and 6.5 as a result 0.19%, 0.09%, 0.117% of Na₂CO₃ was added, respectively. In Tank 2, at the second, third, fourth, and fifth day, the pH was 5.9, 5.41, 6.6, and 6.12 therefore of 0.27%, 0.395%, 0.09%, and 0.21% of Na₂CO₃ was added, respectively. In Tank 3, 0.28%, 0.22%, 0.21%, 0.15% of Na₂CO₃ was added at second, third, fourth, and fifth days. In Tank 4, 0.18% of Na₂CO₃ was added at fourth day. In Tank 5, 0.1% of Na₂CO₃ was added at fourth day.

When the digester was initially heavily fed, acid forming bacteria quickly produced acids. The drop of pH was caused acid forming bacteria produce acetate, hydrogen gas, carbon dioxide, and few other VFA such as propionic and butyric acid. A low pH value inactivated microorganisms involved in the biogas production especially methanogenic bacteria (Vicenta et al., 1984).

In order to allow the methanogenic bacteria to grow, digester should be properly fed and buffered to rising alkalinity. In this study, Sodium carbonate (Na₂CO₃) was used to increase alkalinity or buffering capacity of fermenting slurry. It was added to the digester whenever the pH below 6.8.

Since the reaction rate involving acid-forming bacteria proceeded much faster than the reaction involving methanogenic bacteria, a larger population of methanogenic bacteria must be fed and maintained. The methanogenic bacteria population might not be adequate to consume the acids produced and maintain a neutral pH resulting in declining pH below the neutral pH and diminishing growth of methanogenic bacteria and methanogenesis. The pH could be maintained by adding Na₂CO₃ to increase alkalinity. The pH was the key indicator of operational stability (Tanticaroen et al., 1984). Methanogenic bacteria could occasionally grow at the pH range of 6.5-8.2 (Anunputtikul, 2004). Viswanath et al. (1992) mentioned that there was a perfect link of the acidogenic and methanogenic phases when the pH was remained at 7 and there was no drastic increase in acidity or alkalinity.

c. Temperature from various feed compositions

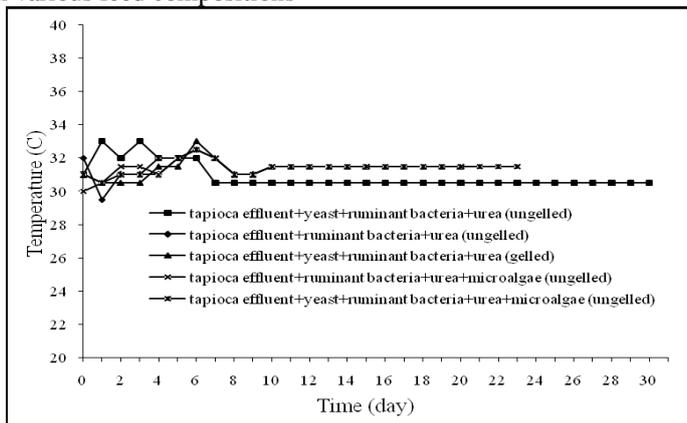


Figure 5. Temperature from Various Feed Composition Performed in Anaerob Biodigester of 5 L Digestion Volume.

During 30 days of operation, the temperature ranges of 30.5-33⁰C, 29.5-33⁰C, 31-33⁰C, 30-32.5⁰C, and 30.5-32.5⁰C were found in the digester 1, 2, 3, 4 and 5, respectively (Figure 5). At the initial retention time, the temperature was fluctuative from 29.5⁰C until 33⁰C, but since the day thirteenth the temperature of mixtures stabilized. A slightly difference of temperature between the gelling feed and ungelling feed was occurred but they still in the range of mesophilic temperature (29.5-33⁰C).

As the temperature falls, microbial activity decreases and the biogas production decreases. As the temperature increases some microorganisms begin to die, once again the production of biogas decreases.

4.4.4 Semi-continuous Process

In semi-continuous experiment, 1% (w/v) of total solid of cassava starch effluent, 0.08% (w/v) of yeast, 10% (v/v) of ruminant bacteria, and 0.04% (w/v) of urea were mixed in the tank. According to Sitohang (2000), adding of fresh feed was conducted when biogas production achieve the maximum yield condition. In previous batch experiment, the maximum yield condition was occurred at second days, consequently adding of fresh feed was conducted every two days with constant ammount of feeding (2 grams per two days). The aim of this feeding management was increasing of biogas production (Sitohang, 2000).

a. Biogas production

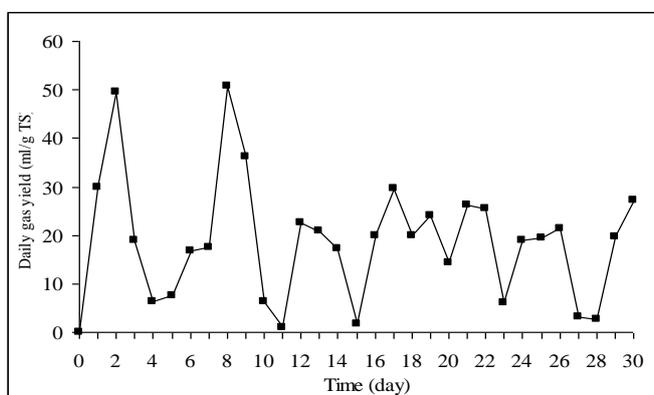


Figure 6. Daily and Accumulation Biogas Production from Cassava starch Effluent using Yeast, Ruminant Bacteria, and Urea in Anaerob Biodigester of 5 L Digestion Volume.

Figure 6 shows that at the beginning of fermentation, the biogas production per day was relatively high. On the third day, the biogas production per day decreased. The addition of cassava starch effluent did not influence the activities of microorganisms to produce biogas directly. This can be seen on the fourth and fifth days, the biogas production per day still decreased. Yeast was

added on fifth day and the biogas production per day increased on sixth and seventh days. It was occurred because the yeast as substrate activator accelerates hydrolysis of substrates by microorganisms, then converts it into biogas. A significant increase in biogas production was occurred on the eighth day retention time (50.69 ml/g total solid/day), after the addition of cassava starch effluent and yeast. On the next day, the biogas production increased and decreased fluctuatively. Overall, a high biogas production was occurred during the early days (1-10 days). The biogas production normally increased after cassava starch effluent and yeast was added. But the addition of cassava starch effluent has been effect in increasing the biogas production after 3-4 days. A significant effect in increasing the biogas production was occurred after yeast was added every 5 days. The maximum of biogas production per day 50.69 ml/g total solid/day was obtained at eighth day retention times (Figure 6) and the total biogas production was 581.15 ml/g total solid.

The rising of biogas production was caused by fresh feed replace the fermented substrate. However, the increasing of feeding-value will increase biogas production until certain value, then biogas production was decrease exactly. It was based on substrate retention time in biodigester. More adding feeding-value, retention time in biodigester was shorter, consequently also shorter in reaction time because substrate was push out before the reaction complete.

b. pH and Temperature

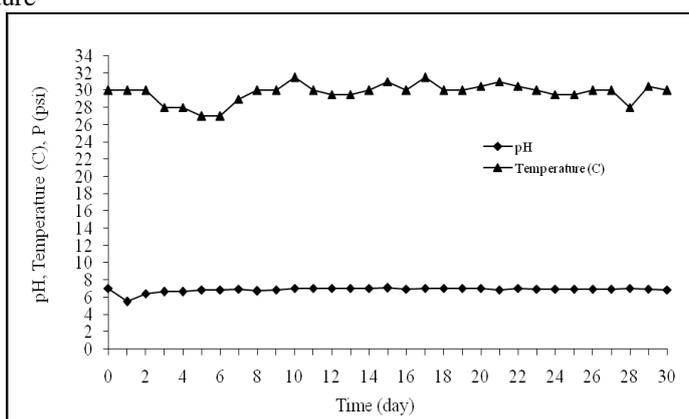


Figure 7. pH and Temperature Performed in Anaerob Biodigester of 5 L Digestion Volume.

During 30 days of operation, the pH ranges of 5.52-7.06 was found (Figure 7). The initial drops in pH from 7.04 to 5.52 was observed at first day retention time. At that time, a low pH value inactivated microorganisms involved in the biogas production especially methanogenic bacteria (Vicenta et al., 1984), so adding of Na_2CO_3 (0.37%) was needed to raising alkalinity in order to keep microorganisms alive. On the second day, the pH stabilized at neutral pH. pH stability stayed awake until the 30-day because there were the addition of cassava starch effluent and yeast, so the activities of microorganisms remained stable.

Biogas production the simple anaerob semi-continuous digester volume of 5 L digestion volume was performed at ambient temperature (27-33 °C) for 30 days at mesophilic level. From Figure 7, there was maximum temperature for maximum biogas production: 30°C for 50.69 ml biogas/g total solid/day.

4. Conclusions

Based on the discussion above, it can be concluded as follow:

- h. Adding of microalgae as nitrogen source did not give significant effect to biogas production. But adding of yeast as substrate activator was very helpful to accelerate biogas production.
- i. The biogas production increased after cassava starch effluent and yeast was added.
- j. Biogas production from the ungelling and gelling mixture of cassava starch effluent, yeast, ruminant bacteria, and urea were 726.43 ml/g total solid and 198 ml/g total solid.
- k. Biogas production from ungelling mixture without yeast was 58.6 ml/g total solid.
- l. Biogas production from ungelling mixture added by microalgae without yeast was 58.72 ml/g total solid and that with yeast was 189 ml/g total solid l.
- m. Biogas production from ungelling mixture of cassava starch effluent, yeast, ruminant bacteria, and urea in semi-continuous process was 581.15 ml/g total solid.

- n. Requirement of sodium carbonate (Na_2CO_3) to increase alkalinity or buffering capacity of fermenting solution depends on pH-value.

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