Consistency of batch anaerobic digestion process of high and low activated sludge concentrations to the interference of sodium benzoate as preservative material

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Abstract. Anaerobic digestion resulted best performance in degradation of organic materials. Degradation of the organic materials was stated as complete by the formation of methane, carbon dioxide etc. (or biogas) as the final product of degradation. Food or beverages products utilized preservative agents in order to prolong the expired date. Sodium benzoate is the most common of preservative agent which can be used for both food and beverages. This experiments were pointed out to observe the effect of high and low activated sludge to the formation of biogas. Research was conducted in some batch mode reactor systems employing high and low of activated sludge (80% and 20% of volume) and solution concentration of sodium benzoate volume. The activated sludge used was 12 g MLSS/L. Concentrations of sodium benzoate used were 400, 600, and 800 mg/L. Product of biogas samples was measured every two days for 60 days of experiment. The results showed that the volume ratio of activated sludge and sodium benzoate of 80% able to reduce the interference of sodium benzoate and for 20% was not able to produce biogas where the load of sodium benzoate influenced the production of biogas.

1. Introduction.
Anaerobic digestion (AD) is a biological process through activated sludge by utilized the anaerobic microorganisms to degrade organic substances [1]. Degradation of the organic substances in AD through four steps distinction: 1. Dissolution of organic substances (hydrolysis), 2. Formation of acids (acidogenesis), 3. Formation of acetic acid (acetogenesis) and, 4. Formation of biogas (methanogenesis) [2]. Recently, the AD process is most popular in treated wastewater both of its high removal efficiency of the pollutants and produce renewable energy (biogas) [3]. Other advantages of AD process is suitable for wastewater with high content of Chemical Oxygen Demands (COD) which is greater than 1500 mg COD/L, less produce of sludge, convenient for tropical countries, etc [4].

Most of food and beverage products have longer expired date, it can be done by addition of preservative materials [5, 6]. This materials able to control the microorganism activity which can destroy the food and beverage products. The most common preservative material applied in the food and beverage staffs is sodium benzoate (SB) [7]. Treatment of wastewater contains preservative materials is predicted to reduce the performance of AD process. It is because the function of preservative materials is to eliminate or reduce the microorganism growth [8].
Total of microorganisms in activated sludge has played an important role in the degradation of organic substances. The high content of microorganisms stated as Mixed Liquor Suspended Solid (MLSS) is easier to degrade the organic substances. In order to observe the performance of removal efficiency of the activated sludge in AD process with the interference of sodium benzoate, this research was pointed to apply high and low concentration of MLSS to the various load of SB. The parameter of the completion AD process is the formation of biogas. Formation of biogas indicated that the process of AD has been completed. As the comparison, the control of the research has been conducted by set up for 100 % activated sludge (without SB solution) and 100 % SB solution (no activated sludge).

2. Material And Methods

2.1. Materials
Research material was obtained from chemical store in Semarang City, Sodium Benzoate (> 98% purity of product of Sigma-Aldrich Corp) was used for preservative material, sodium hydroxide and hydrochloric acid for adjusting the pH also Sucrose. The source of activated sludge obtained from centralized wastewater treatment plant of tofu and tempe’s small-scale industry of Lamper Tengah, Semarang, Central Java, Indonesia.

2.2. Acclimatization of activated sludge.
Sample of activated sludge was taken by water sampler in the middle of anaerobic reactor. Furthermore, the sludge is concentrated through Imhoff cone by separating the water content until achieved the solid concentration of 12 g/L. Adjustment of the pH of sludge was set up 7.0-7.5, this can be done by addition of aqueous solution of sodium hydroxide or hydrochloric acid. The activated sludge is further acclimated by addition of sucrose of 5 g/L every day and this set up for 60 days etc [4].

2.3. Experimental set up
2 L volume polyethylene bottles series were used as anaerobic reactors. These bottles were plugged with rubber plug and hermetically equipped with valve to measure biogas production. The system of the anaerobic digesters was designed for batch system and operated at room temperature. Production of biogas during the digestion was measured using water displacement method which also has been used by previous researchers [9]. To measure the biogas production, each of the digester was connected to a gas collector to reserve gradual glass cylindrical. The connection of the digester and cylindrical glass was facilitated by a connecting plastic tube. The gas collector was immersed in through of water in order to complete sealing. Biogas production resulted from digestion process was collected by reading of the downward displacement of water.

2.4. Experimental design
The batch system of 2 L volume polyethylene bottles for experimental laboratory was conducted for the anaerobic digestion. The ratio of the substrate in the system was composed with various mixture of the activated sludge and aqueous SB solution. The composition of the mixture based on the proportional volume of the activated sludge and SB solution of 0%, 20% (low), 80% (high) and 100% of the activated sludge. Adjustment for the mixture of pH condition of 7.0 by addition of NaOH aqueous solution. NaOH crystal (technical grade) was used to prepare the NaOH aqueous solution. The design of experiments can be seen in Table 1.

| % activated sludge : SB solution | Concentration of SB solution mg/L |
|---------------------------------|----------------------------------|
| activated sludge              | SB solution                     |
| 0% (control)                  | 100%                             |
| 20%                            | 80%                              |
|                                | 400, 600, 800                    |
2.5. Experimental procedures
The design of degradation duration of digestion process was conducted for 60 days. This degradation duration was suggested from the literature of biogas production [10]. Production of biogas was measured the volume for every two days, it’s to quantify the biogas production using water displacement method. Each digester was gently manually mixed for one minute once a day.

3. Results And Discussions
3.1. Organic Loading Rate (OLR)
Organic Loading Rate (OLR) = Volume of SB (mL) x concentration (mg COD/L)/total volume (mL). The Table 1 can be converted into Table 2 to set up the relation for common discussion of the organic substances stated as OLR and cumulative biogas production during digestion.

Table 2. Cumulative biogas production during 60 days digestion.

| SB conc. (mg/L) | Concentration of MLSS (g/L) | Volume activated sludge (mL) | Volume of SB solution (mL) | OLR (mg SB/L) | Cumulative biogas (mL) |
|----------------|-----------------------------|-----------------------------|---------------------------|--------------|------------------------|
| 400            | 0 (control)                 | 0                           | 2000                      | 400          | 0                      |
|                | 2400                        | 400                         | 1600                      | 320          | 25.2                   |
|                | 9600                        | 1600                        | 400                       | 80           | 177.2                  |
|                | 12000 (control)             | 2000                        | 0                         | 0            | 394.2                  |
| 600            | 0 (control)                 | 0                           | 2000                      | 600          | 0                      |
|                | 2400                        | 400                         | 1600                      | 480          | 22.0                   |
|                | 9600                        | 1600                        | 400                       | 120          | 65.4                   |
|                | 12000 (control)             | 2000                        | 0                         | 0            | 394.2                  |
| 800            | 0 (control)                 | 0                           | 2000                      | 800          | 0                      |
|                | 2400                        | 400                         | 1600                      | 640          | 8.2                    |
|                | 9600                        | 1600                        | 400                       | 160          | 43.4                   |
|                | 12000 (control)             | 2000                        | 0                         | 0            | 394.2                  |

At OLR of zero (control) indicated that only activated sludge in the system, the degradation of sludge and the remaining of the activated solution produced biogas without any interference of SB. The result indicated 394.2 mL biogas produced during 60 days digestion. It can be used as the reference of the study in order to observe the SB substance in the activated sludge. Increasing the SB concentration will linearly make the high of OLR. At high OLR of each concentration with no activated sludge (OLR of 400, 600, and 800 mg SB/L) resulted that no cumulative production of the biogas [11]. System with only SB solution able to eliminate or suppress the growth of anaerobic microorganisms in completion of organic substance degradation [12]. While for the system with activated sludge (2400 and 9600 mg/L) indicated that the total MLSS has important role in organic degradation [2]. Table 2 indicated that system with MLSS of 9600 mg/L has significantly produced cumulative biogas production greater than MLSS of 2400 mg/L. The SB concentration has primary impact to the biogas production. Increasing the SB concentration of 400, 600, and 800 mg/L resulted the decreasing cumulative biogas production of 25.2, 22.0, and 8.2 mL for MLSS of 2400 mg/L and 177.2, 65.4, and 43.4 mL for MLSS of 9600 mg/L. The mechanism of the reduction of the biogas production has not been understood yet, it may be due to of the elimination or kill the microorganisms in the system or to prolong the degradation time of organic substance [13].
3.2. Biogas production

Figure 1. Cumulative biogas production for various MLSS with SB concentration of 400 mg/L.

Figure 2. Cumulative biogas production for various MLSS with SB concentration of 600 mg/L.
Figure 3. Cumulative biogas production for various MLSS with SB concentration of 800 mg/L.

Figure 1, 2 and 3 indicated the results of biogas production for various concentration of activated sludge (0, 2,400, 9,600 and 12,000 mg/L) and SB concentration (400, 600 and 800 mg/L). For activated sludge of 12,000 mg/L and 0 mg/L were used as the control with condition of without and fully of SB solution. For control condition, fully activated sludge resulted cumulative biogas production of 394.2 mL and for fully SB solution resulted no cumulative biogas formation. In the system of fully activated sludge, the activity of microorganisms has able to degrade the remaining organic substances in the sludge and also sludge itself to form biogas while for fully SB solution the formation of the biogas due to no microorganisms existed in the system [14].

For concentration of SB of 400 mg/L, MLSS of 9,600 mg/L resulted cumulative biogas of 177.8 mL during 60 days digestion, while for MLSS of 2,400 mg/L produced only 25.2 mL. It indicated that the total of microorganisms in the system played important role in the digestion process. As the function of SB in the food product is to prevent or eliminate the growth of microorganisms to prolong the live product. The total concentration of microorganisms and SB solution will have significant effect to the formation of biogas [15]. Observation to the MLSS of 9,600 mg/L showed that increasing the SB solution will reduce the biogas formation (Fig. 2 and 3).

The function of SB as preservative material is more extensively appeared when the concentration increased. Increasing SB concentration affected the higher of OLR. From Table 2 and Fig. 1,2, and 3 indicated that the formation of biogas reduced. Biogas production is produced by the microorganisms activity. By increasing OLR, the microorganisms activity reduced. Comparing with the fully activated sludge in biogas production, the influence of the increasing SB reduced biogas production.

3.3. Kinetic study

AD process occurred during 60 days digestion in the batch anaerobic process can be evaluated the kinetic value. This assumed by the AD process has been completed during the time of digestion of 60 days and four anaerobic process of hydrolysis, acidogenesis, acetogenesis, and methanogenesis completed. The advantage of the kinetic study will give understanding of the mechanisms of the degradation of the organic substances in the AD process [16]. The kinetic model introduced in this data is first-order kinetic model [17], it can be used based on the availability of the substrate as limiting factor.

The first-order kinetic is:

\[
dC/dt = -kC \tag{1}
\]

where:
- \( - \) is the reduction of the substrate concentration,
- \( k \) is the rate constant of first-order substrate utilization (time\(^{-1}\)) and
- \( C \) is the biodegradable substrate concentration (mg/L).

If the equation (1) integrated for the substrate concentration will result:

\[
C = C_0 e^{kt} \tag{2}
\]

where:
- \( C \) is the substrate concentration at \( t \) (mg/L)
- \( C_0 \) is the initial substrate concentration (mg/L).

The correlation of the substrate concentration in the system can be indicated by the gas production during the process, then the equation (2) will be:

\[
\frac{G_\infty - G}{G_\infty} = \frac{C}{C_0} \tag{3}
\]

where:
- \( G_\infty \) is the ultimate biogas production (mL)
- \( G \) is the gas production at \( t \) (mL)

When equation (2) and (3) is combined to indicate the relation of the biogas production and time of digestion, the equation become:

\[
G = G_\infty (1 - e^{-kt}) \tag{4}
\]
With: \( k \) is the constant rate of first-order biogas production (day\(^{-1}\)).

The further solution of the equation to be:

\[
\ln \left( \frac{G_x}{G_x - G} \right) = kt \tag{5}
\]

This equation (5) is a linear curve, it can be achieved by plotting the data of \( \ln \left( \frac{G_x}{G_x - G} \right) \) as the ordinate and \( t \) is the abscise, hence the slope is the value of \( k \). The value of \( G_x \) is assumed equal to the cumulative biogas production at the infinity or end of each experiment. The result of this experiment is shown in the Table 3 for the value of \( G_x \), \( k \) and \( R^2 \) and Fig. 4 to 9 introduced the graphs of the prediction of the research data and prediction.

Table 3. Calculation of \( k \) for the AD process with various SB concentrations.

| SB conc. (mg/L) | Concentration of MLSS (g/L) | Volume activated sludge (mL) | Volume of SB solution (mL) | OLR (mg SB/L) | \( G_x \) (mL) | \( k \) (day\(^{-1}\)) | \( R^2 \) |
|-----------------|-----------------------------|-----------------------------|---------------------------|---------------|----------------|----------------|--------|
| 400             | 2400                        | 400                         | 1600                      | 320           | 26             | 0.136          | 0.9700 |
|                 | 9600                        | 1600                        | 400                       | 80            | 183            | 0.0467         | 0.9516 |
| 600             | 400                         | 1600                        | 480                       | 22            | 0.1254         | 0.9795         |        |
| 800             | 2400                        | 1600                        | 120                       | 65.5          | 0.0422         | 0.9057         |        |
|                 | 9600                        |                             | 640                       | 8.2           | 0.0183         | 0.8622         |        |
| 12000 (control) | 2000                        |                             | 0                         | 160           | 46             | 0.0445         | 0.9786 |

Figure 4. Determination of kinetic constant (\( k \)) for activated sludge of 12,000 mg/L (control)

Figure 5. Cumulative biogas production vs time for activated sludge of 12,000 mg/L (control)
From Table 3 resulted that the value of the $G_{\infty}$ was opposite with the OLR, increasing OLR resulted the value $G_{\infty}$ decreased. While for the value of k was proportional with the OLR. The value of k for greater OLR was higher than lower OLR, it can be predicted that the formation of the biogas was occurred in a small range of time at the initial digestion, while for the lower OLR the biogas formation was more flatter but taken place in the long period. The value of $R^2$ with significant effect of 95 % showed most of the data fulfilled the requirement and this indicated that the data obtained from the experiments was satisfied with the first-order reaction [18]. This value of k and $G_{\infty}$ can not be compared with the other researchers because the value of biogas is cumulative.

4. Conclusion
SB was preservative material which effectively prevent or eliminate the growth of the microorganisms, it can be identified by the production of biogas during digestion in batch digester of anaerobic process for 60 days retention time. The drop of the biogas formation can be observed during the digestion with the high-low OLR of SB. The model of kinetic with first-order kinetic introduced to the data was satisfied and has degree of determination of 95% significant value to the data of experiments.
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