Anaerobic biodegradation of polylactic acid under mesophilic condition using thermal-alkaline pretreatment

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Abstract. The propose of this study was to investigate the effect of thermal-alkaline pretreatment with emphasis on sodium hydroxide concentration (NaOH), temperature and reaction time, on enhancement of polylactic acid (PLA) films degradation and biogas production. The results found that NaOH concentration and reaction time were two main parameters influencing on PLA degradation. While, less significant was found for temperature. From the Response Surface Methodology (RSM), it was concluded that the optimum pretreatment conditions were at 0.5 M of NaOH, temperature of 60\degree C and 24 hr of reaction time. This was generated about 3.7 times (215.47 ml/gVS\textsubscript{added}) higher gas production comparing to non-pretreated PLA films which was 58.28 ml/gVS\textsubscript{added}. The maximum biodegradability of PLA film was 20.14\%. This was estimated to be 4.7 times higher than non-pretreated PLA (4.32\%). This finding demonstrated the benefit of thermal-alkaline pretreatment on surface of PLA films destruction. Consequently, the microbial enzymes could degrade PLA more easily, resulting in an increase of biogas production.

1. Introduction
In order to reduce the consumption of petroleum based plastics, bioplastic mainly PLA is widely utilized in many countries. Due to the environmental friendly and renewable resources while its processing, the releases of greenhouse gases is lower than other petroleum based plastics. Moreover, the biodegradable plastics are completely degraded under the appropriate environmental conditions. The life cycle in degradation of bioplastic is the same as a natural based materials [1].

PLA is aliphatic polyester synthesized from lactic acid in sugar and starch based crop fermentations such as corn, sugarcane, and cassava. PLA is categorized in thermoplastic which is useful for glass, film, and food packaging. For this type of bioplastic, landfill is considering as the waste disposal method employing biological enzymes, oxidation, hydrolysis, photolysis, and radiolysis in natural degradation [2]. At the end of process, carbon dioxide, water (aerobic condition), and methane (anaerobic condition) are generated as a product. Methane content in produced mixed gases called biogas from anaerobic process is useful for renewable energy source. However, in practical, PLA was highly crystalline structures that were not easily degraded under aerobic and anaerobic mesophilic conditions [3]. From previous studies, it was reported that thermophillic digestion condition could increase the biodegradation rate. However, the energy consumption of digestion process in long term operation should be carefully considered [4].
Therefore, the short time experimental period of thermal pretreatment is tempting. This could be used to enhance the biogas production and biodegradation of PLA by increasing the accessibility of micro-organisms in biodegradation process. Moreover, alkaline pretreatment invoked in changing of PLA to lactic acid. Thereby, lactic acid is converted into methane. Moreover, the rising of temperature also increase the hydrolysis [5]. However, the combination effect of thermal and alkaline solution on PLA degradation is limited. In this study, an attempt is focused to investigate the effect of alkaline concentration, pretreatment temperature and reaction time on the degradation and biogas production from PLA film under mesophilic condition.

2. Materials and Methods

2.1. Raw materials
In this work, the degradation PLA films was study. It was prepared from commercial PLA pellets by blown film process. The PLA film was 80 µm thickness. The glass transition temperature (T_g) was 55-60 °C and melt temperature (T_m) was 145-160 °C. The PLA films were first cut into 3-5 mm with a grinder. Milled samples were analyzed for major chemical components, such as total solids (99.78%) and total volatile solid (99.78%).

2.2. Experimental designed conditions
The PLA pretreatment conditions were optimized by using Central Composite Design (CCD) under Response Surface Methodology (RSM) to determine the influence of several variables. That was combined with mathematical and statistical based theory wherein interactions between variable can be recognized with fewer experimental runs. The RSM also provides an optimization of pretreatment condition to offer an economic side. The CCD contained 20 experiment runs. In practical, the NaOH concentration, temperature and reaction time were used as the independent variables on biogas production, sCOD removal (%) and biodegradability (%). Within the experimental, NaOH concentration, temperature and reaction time were ranged of 0-0.5 M, 20-60 °C and 0-24 hr, respectively. The real values from CCD used in the experiment design are presented in Table 1.

Table 1. The real values used in the experimental composite design

| A : NaOH conc. (M) | B : Temperature (°C) | C : Reaction time (hr) |
|-------------------|----------------------|----------------------|
| 0*                | 20                   | 0*                   |
| 0                 | 30                   | 0                    |
| 0.25              | 45                   | 12                   |
| 0.50              | 60                   | 24                   |
| 0.67              | 70                   | 32.2                 |

* the value was analyzed from program is negative then assigned to zero.

2.3. Thermal-alkaline pretreatment
Thermal-alkaline pretreatment was conducted by soaking the grinded PLA films in sodium hydroxide solution at 10% (w/v) of PLA to solution. The individual variable effects of NaOH solution (0-0.67 M), temperature (20-70 °C) and reaction time (0-32.2 hr) were used according to a CCD setup. For temperature effect, the sample was incubated in the controlled temperature water batch.

2.4. Biochemical methane potential (BMP) assay
An assay called BMP was conducted. This experiment was used to determine the amount of accumulated produced biogas and sCOD removal (%) under 90 days incubation at mesophilic
temperature (30±2 °C). BMP tests were conducted in duplicate. Then, the pretreated PLA were added to the serum bottles with inoculums, obtaining a substrate: inoculum ratio was set to 60:40 on working volume and inoculum only (blank). Each bottles were added with PLA at a concentration of 2% VS based and 2.5% VS of inoculum. Fresh anaerobic sludge from UASB digester treating soft-drink was applied. The bottles were shacked with 165 rpm for 90 days at room temperature at 30±2 °C. Produced biogas was routinely monitored.

2.5. Analysis
Soluble COD (sCOD) was determined by closed reflux method according to the standard procedure. Gas measuring syringe used for determination of gas volume produced. The biodegradation (%) was calculated comparing to the theory as indicated by equation (1) and (2) [3].

\[
C_nH_{3a}O_b + \left( n - \frac{a}{4} - \frac{b}{2} \right)H_2O \rightarrow \left( \frac{n}{2} + \frac{a}{8} - \frac{b}{4} \right)CH_4 + \left( \frac{n}{2} - \frac{a}{8} + \frac{b}{4} \right)CO_2 \quad (1)
\]

\[
\text{Biodegradation (\%)} = \frac{\Sigma V(s) - \Sigma V(b)}{\Sigma V(\text{max})} \times 100 \quad (2)
\]

\(\Sigma V(s)\) is the CO\(_2\) and CH\(_4\) volume from the sample bottles under ambient conditions (at 30 °C, 1 atm) in milliliters. \(\Sigma V(b)\) is the CO\(_2\) and CH\(_4\) volume from the blank under ambient conditions in milliliters. \(\Sigma V(\text{max})\) is the maximum theoretical volume of biogas (CO\(_2\) and CH\(_4\)) evolved after complete biodegradation of the test material under ambient conditions in milliliters (calculated from equation (1)).

3. Results and discussion

3.1. Effect of NaOH pretreatment
The results clearly demonstrated that NaOH was an important parameter for PLA degradation enhancement. At the same temperature of 45 °C and 60 °C, it was found that biogas production were increased with the concentration of NaOH increase. At 0.5 M of NaOH pretreated PLA, the produced biogas was 1.3 times (164.74 ml/gVS\(_{\text{added}}\)) and 1.7 times (212.86 ml/gVS\(_{\text{added}}\)) higher than gas production from non-pretreated condition at 0 M of NaOH. Similar result was also observed for sCOD removal (%) and biodegradability (%), which were 1.3 times (51 and 67.4%) and 1.3 times (11.27 and 15.03%), respectively. Therefore, the alkaline pretreatment process can positively affect on biogas production and biodegradation. As reported in previous literature, alkaline pretreating PLA would be a beneficial for changing PLA to lactic acid by increasing rate of hydrolysis process [5]. Lactic acid can be subsequently fermented into biogas. In addition, the alkaline pretreatment of PLA can be an efficient way to increase biogas production and biodegradation because it can increase the accessibility of microorganisms.

3.2. Effect of reaction time
The increase of reaction time was also affected the biogas production, sCOD removal (%) and biodegradability (%). The maximum reaction time of 32.2 hr (0.25 M of NaOH, 45 °C) were produced the maximum biogas production, sCOD removal (%) and biodegradability (%) which were 230.21 ml/gVS\(_{\text{added}}\), 74.68% and 21.62%, respectively. This result showed that reaction time was related to the increasing degradation of PLA. Similarly, Chauliac (2013) [5] found that the highest yield of lactic acid was obtain when increasing of reaction time due to the NaOH can degraded PLA more completely.
3.3. Effect of thermal pretreatment

From the result, it was found that the temperature was less significant affect to PLA degradation. The biogas production, sCOD removal and biodegradability were slightly increased when increasing of the temperature. For example, the different temperature (20, 45, 70 °C) at NaOH concentration of 0.25 M and reaction time of 12 h, the biogas production were 126.15, 132.42 and 147.14 ml/gVS\textsubscript{added}, respectively. However, the high temperature condition has been reported to enhance PLA degradation [4]. At 60 °C with NaOH solution can increase the reaction and the amount of lactic acid released from PLA. Since 60°C is not high enough to completely melt the PLA beads within the four hour, NaOH access is restricted to the amorphous layer on the bead surface [5]. The RSM analysis was presented in figure 1 and 2, that calculated for the optimum condition with providing maximum biogas production, sCOD removal (%) and biodegradability which were 0.5 M of NaOH, 60 °C and a 24 h of reaction time.

In discussion, the optimization pretreatment condition by using RSM analysis were estimated. RSM method with quadratic regression equation for describing the effects of the three independent variables was applied. The result showed that the highest biogas production was achieved at 215.47 ml/gVS\textsubscript{added}, while sCOD remove efficiency and biodegradability of 79.67% and 20.14%, respectively, at the highest desirability of 0.93, 0.5 M of NaOH, 60 °C and a 24 hr of reaction time. From the ANOVA result of quadratic regression model for 2 responses (NaOH concentration and reaction time) demonstrated that the models were significant (P-values< 0.05). For effect of temperature shown that it did not significantly affect on biogas production (P-values > 0.05), sCOD removal, and
biodegradability. Final equation of independent and dependent variables in terms of coded factors (Equation (3)-(5)):

\[
\begin{align*}
\text{Biogas production (ml/gVS}_{\text{added}}) &= 134.69 + 14.23A + 6.03B + 32.93C + 1.48AB + 22.15AC + 3.94BC - 3.99A^2 - 3.18B^2 + 7.20C^2 \\
\text{sCOD removal (%) } &= 60.12 + 6.27A + 0.96B + 3.21C - 4.42AB + 9.06 AC + 7.65BC - 3.29A^2 \\
\text{Biodegradability (%) } &= 12.01 + 1.43A + 0.61B + 3.31C + 0.15AB + 2.23AC + 0.40BC - 0.40A^2 - 0.32B^2 + 0.72C^2 
\end{align*}
\] (3) (4) (5)

A result from scanning electron microscopy (SEM) of non-pretreated PLA films and pretreated PLA films shows in Figure 3 and 4. It was shown that using the thermal-alkaline pretreatment, the surface of PLA films were significantly destroyed [5].

![Figure 3. Surface of non-pretreated PLA films (A) and pretreated PLA films with 0.5 M NaOH at 60°C for 24 hr (B)](image)

![Figure 4. Surface of non-pretreated PLA film after BMP test (A) and pretreated PLA films with 0.5 M of NaOH at 60 °C for 24 hr after BMP test (B)](image)

4. Conclusion
In this study, the RSM was applied to optimize individual variables for maximizing response. PLA is potentially used as biogas substance in anaerobic biodegradation. This research shown that reducing of hydrolytic retention time in anaerobic digestion by thermal-alkaline pretreatment. This process used 0.5 M of NaOH at 60 °C for 24 hr. The highest biogas production was 215.47 ml/gVS_{added} which was 3.7 times higher than the non-pretreated PLA films (58.28 ml/gVS_{added}) in 90 days.

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