Production of high strength bioliquid from municipal solid waste (MSW) using mixed culture

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Abstract. Landfilling is one of the most commonly used methods of municipal solid waste (MSW) disposal. It is necessary because there are residues in all waste management processes that cannot be reused or recovered further and are essentially landfilled. For the degradation of MSW, thermal, mechanical and biological pretreatment techniques are used. MSW could be treated and converted into liquid biomass (bioliquid) by using enzymes. This research focused on the production of high strength bioliquid in MSW. The experiments were conducted in three stages namely: acclimatization process, preliminary study and factorial analysis to determine the production of high strength bioliquid in MSW. The selected factors were ratio of AMC and substrates (1:2 and 1:5), temperature (room temperature and 37 °C), pH (adjusted pH and no pH adjustment), PET plastics size (1cm² and 4cm²) and Hydraulic Retention Time (3 hours and 9 hours). Design Expert software (Version 7) was used to construct an experimental table where all the factors were randomized. Two-level factorial analysis (TLFA) was used to analyze the most contributing factor and interaction between the factors. The results showed that the most significant factor was temperature, while the interaction factors were between temperature and HRT. The best conditions of COD increment were determined at AMC:S ratio 1:5, adjusted pH, PET plastics size of 1cm x 1cm, at room temperature and HRT of 3 hours. The experimental and targeted final increments of COD were 2.19 and 1.99 respectively. Since the error was less than 10%, it is acceptable, and the objectives of this research were successfully achieved.

KEYWORDS: municipal solid waste (MSW); bioliquid; enzyme; factorial analysis; design expert software

1. Introduction
In Malaysia, solid waste generation is estimated at about 26 million tons in 2007. According to Dong Energy, ‘REnescience’ handles unsorted MSW and industrial waste similar to MSW, using enzymes to convert biodegradable waste material into liquid biomass (bioliquid) (Lending, 2016). Hence, the solid and recyclable fractions of the waste are able to separate the biodegradable material. The bioliquid is suitable as feedstock to produce biogas and other chemicals production such as lactic acid and butanol which can be used in the chemical industry or as transportation fuel.

Bioliquid is liquid substrate made from biomass for energy purposes. Bioliquid is usually produced from waste such as municipal solid waste or agricultural waste. The bioliquid is highly suitable as a feedstock for the production of biogas, offering a high biogas yield that makes it possible
to generate energy efficiently. Biogas production produced digestate as a by-product, which contains phosphorous that can act as fertilizer. In power plants that generate electricity and heat, solid fuels are ideal for replacing fossil fuels. High bioliquid intensity means high levels of chemical oxygen demand (COD), which implies a higher amount of organic oxidizable content in the substrate. Therefore, the production of the high strength bioliquid is very important as a substrate for production of high biogas yield.

There are a few factors give contribution to the production of high strength bioliquid such as temperature, oxygen availability, water content, pH, particle size and density (Farquhar and Rovers, 1973). Two-Level Factorial Analysis (TLFA) is employed to determine the most affecting factor to produce the high COD bioliquid. According to Box and Draper (1987), the most important aspect of TLFA is the design of experiments. This research focused on the production of high COD bioliquid in MSW. The purpose of TLFA is to identify the variables that have large effects on the bioliquid production.

2. Materials and Methods

2.1. Sample Collection and Analysis
The samples were organic waste and soil mixed culture (SMC). The samples were collected at Sungai Ikan, Kuala Terengganu. The SMC was collected at the bottom level of the site that was in contact with the organic waste at the site.

2.2. Acclimatization of Mixed Culture
This process was conducted to adapt the soil mixed culture to the biodegradation process. The organic waste and PET plastic were mixed with water at volume ratio of 1:3 (known as substrate). Then the substrate was mixed with the SMC and the COD concentration was determined. Initially one liter SMC was added into acclimatization bioreactor. Then, 500 mL substrate was added daily into the bioreactor for 14 days. The reactor was agitated manually to make sure the SMC was well mixed with substrate. The working volume for acclimatization bioreactor was eight liters. COD concentration was measured daily within this acclimatization process. After the acclimatization process, the SMC was known as acclimatized mixed culture (AMC).

2.3. Sample analysis using DR/2400 HACH Spectrophotometer
2 mL of filtered sample was poured into a chemical oxygen demand (COD) reagent vial. Then, the vial was inverted gently several times to mix. The vial was left in the COD digestion reactor for 2 hours. After 2 hours, the vials were put in tube rack to be cooled to room temperature. COD of samples was determined by using HACH Spectrophotometer.

2.4. Experimental Setup: Factorial Analysis
There were five selected factors in this study as shown in Table 1. The factors were ratio of AMC/S ratio, pH, temperature, PET plastics size and Hydraulic Retention Time (HRT). Experimental design set-up was performed by Design Expert software. Analysis factors were performed in this study to identify the design factors that have large effects for the experiment. The experiment was conducted based on Table 2. Next, experimental data were analyzed to determine the most contributing factor and interaction between factors by using Design Expert software.
### Table 1. List of selected factors and their ranges.

| Factors          | Low Level         | High Level        |
|------------------|-------------------|-------------------|
| AMC/S ratio      | 1:2               | 1:5               |
| pH               | Initial           | Adjusted (7)      |
| Temperature      | Room Temperature (27 °C) | 37 °C          |
| Specimen Size    | 1cm²              | 4cm²              |
| HRT              | 3 hours           | 9 hours           |

### 3. Result and Discussion

#### 3.1. Screening of factors affecting on the COD increment

Two-level factorial analysis was used to determine the effective effect on COD increment from MSW. Table 2 shows 16 runs of experiments and the result of COD increment. The most contributing factor and interaction between the factors that have effect on COD increment were analyzed using ANOVA, at more than 95% of confidence level. The ranges for response of COD increment (fold) from 0.31 to 2.02. Table 3 shows the percentage contribution of each factor on COD increment which is temperature (Factor C) has the highest percentage with value 37.75%, followed by HRT (Factor E), AMC/S Ratio (Factor A), pH (Factor B) and lastly PET Plastic Size (Factor D) with the least value of 0.034%.

### Table 2. Experimental setup for factorial analysis.

| Std Run | A: Ratio of AMC and organic waste | B: pH | C: Temperature (°C) | D: Specimen Size (cm²) | E: HRT (hour) | Initial COD (%) | Final COD (%) | Fold (%) |
|---------|------------------------------------|-------|---------------------|------------------------|--------------|----------------|--------------|---------|
| 1 2     | 1:2 No pH adjustment               | Room Temperature | Room Temperature | 1 | 9.00 | 82630 | 71560 | 0.87 |
| 2 11    | 1:5 No pH adjustment               | Room Temperature | Room Temperature | 1 | 3.00 | 191860 | 47630 | 0.25 |
| 3 16    | 1:2 Adjusted pH                    | Room Temperature | Room Temperature | 1 | 3.00 | 81860 | 40660 | 0.5  |
| 4 1     | 1:5 Adjusted pH                    | Room Temperature | Room Temperature | 1 | 9.00 | 81860 | 81200 | 0.99 |
| 5 13    | 1:2 No pH adjustment               | 37.00  |                      | 1 | 3.00 | 74830 | 84330 | 1.13 |
| 6 5     | 1:5 No pH adjustment               | 37.00  |                      | 1 | 9.00 | 89060 | 99160 | 1.11 |
| 7 10    | 1:2 Adjusted pH                    | 37.00  |                      | 1 | 9.00 | 56800 | 67330 | 1.19 |
| 8 8     | 1:5 Adjusted pH                    | 37.00  |                      | 1 | 3.00 | 55730 | 112560 | 2.02 |
| 9 9     | 1:2 No pH adjustment               | Room Temperature | Room Temperature | 4 | 3.00 | 201530 | 61530 | 0.31 |
| 10 4    | 1:5 No pH adjustment               | Room Temperature | Room Temperature | 4 | 9.00 | 58300 | 85200 | 1.46 |
Table 3. Contribution of factors in bioliquid production.

| Term               | % contribution |
|--------------------|----------------|
| A: AMC/S Ratio     | 3.28           |
| B: pH              | 0.66           |
| C: Temperature     | 37.75          |
| D: PET Plastic Size| 0.034          |
| E: HRT             | 6.66           |

3.2. Analysis of variance (ANOVA) for COD increment

Table 4 shows the ANOVA summary for COD increment to estimate the coefficient of the model, check the significance of each parameter and indicate the interaction strength of each parameter. The ANOVA study revealed an effective model with a p-value below 0.05. The main factors, AMC/S ratio (Factor A), pH (Factor B), temperature (Factor C) and HRT (Factor E) are significant model terms. For interactions between factors, pH and PET Plastic Size (Factor BD), pH and HRT (Factor BE) and temperature and HRT (Factor CE) are significant model terms. Meanwhile, the model terms of PET Plastic Size (Factor D) and AMC/S ratio and pH (Factor AE) are not significant with p-value greater than 0.1. The determination coefficient (R^2) and adjusted determination coefficient (R^2 adj) were 0.9985 and 0.9926 respectively (Table 5). Olmez (2009) indicates that at least 0.80 should be a good match for a model R^2, indicating that the regression models clarified the process well. The final empirical model (actual factors) was determined according to Equation 1 to express the COD increment as a function of independent variables.

\[
\text{COD increment (Fold)} = 1.02 + 0.079A + 0.036B + 0.27C + 8.125E - 0.03D + 0.11E + 0.017AB - 0.063AE - 0.13BD - 0.11BE - 0.086CD - 0.23CE + 0.081DE
\]

(1)

Where A is AMC/S Ratio, B is pH, C is temperature, D is PET plastics size and E is HRT.

| Table 4. ANOVA of COD increment. |
|----------------------------------|
| **ANOVA for selected factorial model** |
| Source | Sum of Square | Df | Mean Square | F value | P-value Prob > F |
|--------|---------------|----|-------------|---------|----------------|
| Model  | 3.07          | 12 | 0.26        | 168.06  | 0.0007         |
| A-AMC/S ratio | 0.10  | 1  | 0.10        | 66.19   | 0.0039         |
| B-pH   | 0.02          | 1  | 0.02        | 13.33   | 0.0355         |

Where A is AMC/S Ratio, B is pH, C is temperature, D is PET plastics size and E is HRT.
### Table 5. Values for coefficient of determination (R-Squared).

|                | Std. Dev. | R-Squared | Pred R-Squared | Adeq Precision |
|----------------|-----------|-----------|----------------|----------------|
| Std. Dev.      | 0.039     |           |                |                |
| Mean           | 1.02      |           |                |                |
| C. V. %        | 3.84      |           |                |                |
| PRESS          | 0.13      |           |                |                |

#### 3.3. Analysis of main effect and interaction effect between factors on COD increment

Figure 1 illustrated the main effects and interaction effects of the factors on the COD increment. Main effects show two main factors that contribute to COD increment. Factor C (Temperature) has shown the highest effects followed by Factor E (HRT). In Table 4, Factor C does give the highest contribution among other factors. Based on Table 2, for Factor C, room temperature (27 °C) was set as low level and 37 °C was set as high level. Factor A, B, C, D and E gave a positive effect toward the COD increment. It means the response is increasing when the factor is increasing. To obtain higher COD increment, all these positive effect factors should be increasing too. This means that to increase the COD increment the temperature should be set at high level (37 °C). It demonstrates that PET Plastic Size and HRT (Factor DE) had a positive influence on the COD increment for interaction effects. The other interaction gave negative effect on the response. The negative effect arises when the factor is not proportional to the response value. Therefore, as the negative impact factor grows, the COD increment decreases.
3.4. Effect of independent processing parameters on COD increment

Figure 2 shows the effect of independent variables on the COD increment. Factor C gave the highest contribution to COD increment. Figure 2a shows the slope which indicates the COD increment value in MSW increase as the temperature increase. COD increment was higher when temperature at 37 °C with value 1.19, meanwhile, it decreased to 0.65 when temperature was at room temperature (27 °C). It showed that higher COD increment was achieved by using temperature at 37 °C. During the biological process, low temperatures decreased the rates of microbial growth and substrate usage (Kim et al., 2006; Stuckey, 2010). Beside, a study by Kashyap et al. (2003) claimed that low temperatures may also result in an exhaustion of cell energy, leakage of intracellular substances and complete lysis. This high difference of COD increment gives the temperature is the major independent factor that affects COD production. From Figure 2b, the COD increment increase as the HRT increase. At HRT is lower (3 hours), the COD increment is 0.14 lower than HRT higher (9 hours) which is 1.16. It also showed that higher COD increment achieved at HRT higher (9 hours). Figure 2(c) showed that the slope slightly increases when the ratio increases from 1:2 to 1:5. Thus it can conclude from Figure 2(c) where there is no significant difference in ratio to the COD increment. The benefits of acclimatized culture are easy to adapt for various toxic wastes, simplicity of operation and maintenance. Compared to pure culture, acclimatized mixed culture has a better advantage where the substrate must be sterile and more factors affect or retard its growth and results (Swaminathan and Ramanujam., 1999). It was proved that the amount of microbe does not give a big difference in COD increment.
3.5. Interaction effects between factors on COD increment

Data analysis from Design Expert software indicate the interaction gives positive effect with the highest contribution is interaction between Factor D (PET Plastic Size) and Factor E (HRT) with a percentage of 3.38%. Figure 3(a) shows the interaction between Factor D and E. The COD increment was highest when HRT at 9 hours and PET Plastic Size at 4 cm² with value of 1.47. For both HRT which are at 3 hours and 9 hours, when PET Plastics Size increase, the COD increment also increased. The COD increment was 0.85 when the PET Plastic Size is 1 cm² at the HRT of 9 hours. The COD increment increased till 1.46 at the same HRT but different in PET Plastic Size at 4 cm². For both factors, the COD increment was increasing when PET Plastic Size and HRT were increasing. Figure 3(b) shows the interaction between Factor C (temperature) and E (HRT). For Factor C and E, room temperature (27 °C) was considered as low level, and 37 °C were as high level. The COD increment was highest when HRT at 9 hours and temperature at 37 °C with value of 1.24. The COD increment
slightly increased at from room temperature to 37 °C at same HRT which is at 9 hours. On the other hand, the COD increment was lowest when HRT and temperature at 3 hours and room temperature respectively with value of 0.14. However, the COD increment increased till 1.13 as temperature increasing to 37 °C. For both factors, the COD increment was increasing when temperature and HRT were increasing. According to Luostarinen et al. (2007), high temperature result in higher conversion rates of organic matter in the anaerobic process. Meanwhile, as a result of slow hydrolysis of volatile solids at a given HRT, more organic matter typically stays undegraded at low temperatures (Seghezzo, 2004).

Figure 3(c) shows the interaction between Factor B (pH) and D (PET Plastic Size). From the graph, it shows a big difference in COD increment at no pH adjustment with different PET Plastic Size. For PET Plastic Size of 1 cm² and 4 cm², the COD increment was at 0.42 and 0.87 respectively. Meanwhile, for the adjusted pH, it shows there is a small difference in COD increment. For PET Plastic Size of 1 cm² and 4 cm², the COD increment was at 0.72 and 0.65 respectively. It shows that at adjusted pH, the PET Plastic Size did not give a big effect on COD increment but slightly different between 1 cm² and 4 cm².

Figure 3. Most effective interaction factors in COD increment

(a) (b) (c)
3.6. Validation experiment
The COD increment values were predicted using the suggested best conditions from Design Expert Software. Table 6 shows the criteria for best conditions determination.

| Criteria           | Goal       | Value                           |
|--------------------|------------|---------------------------------|
| AMC/S Ratio        | In range   | 1:2 – 1:5                       |
| pH                 | In range   | No pH adjustment – Adjusted pH  |
| Temperature        | In range   | Room Temperature – 37°C         |
| PET Plastics Size  | In range   | 1 cm² – 4 cm²                   |
| HRT                | In range   | 3 hour – 9 hours                |
| Fold               | Maximize   | -                               |

Table 6. Best conditions criteria.

Table 7 shows the suggested best conditions and their predicted COD increment. Triplicate experiments were conducted to validate the predicted and experimental COD increment. The error from this experiment was 9.9%, 20.18% and 35.74% for Run 1, 2 and 3 respectively, calculated by using Equation 2.

\[
\text{Error (\%) = 100\% \times \frac{\text{predicted} - \text{experimental}}{\text{predicted}}} \quad (2)
\]

Table 7. Validation experiment data.

| Factor 1 (A): AMC/S Ratio | Factor 2 (B): pH Adjusted | Factor 3 (C): Temperature 37°C | Factor 4 (D): PET Plastics Size 1 cm x 1 cm | Factor 5 (E): HRT 3 hours | Predicted COD Increment 1.99 | Experiment COD Increment (%) |
|---------------------------|---------------------------|---------------------------------|-----------------------------------------------|--------------------------|----------------------------|--------------------------------|
| Run 1                     | Run 2                     | Run 3                           |
| 1.99                      | 2.19                      | 1.59                            | 1.28                                          |

4. Conclusion
The purpose of this research was to establish the most important factors in COD increment. Design Expert software (Version 7) was used to construct the experimental design and analyzed experimental data through TLFA to analyze the most contributing factors and interaction factors. Five selected factors were AMC/S ratio, pH, temperature, PET plastics size and Hydraulic Retention Time (HRT). The contribution factor rank of COD increment were temperature > HRT > AMC/S ratio > pH > PET Plastic Size. This comes to the conclusion only temperature and HRT were the significant factors. The targeted final increment of COD 1.99 was achieved from Design Expert software. The final increment of COD value from experimental data was 2.19. Since the error was below than 10%, it is acceptable. Thus, the objective of this research was successfully achieved.

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