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Second Generation Bioethanol from Lignocellulosic Biomass Using Worm Tea as Pretreatment

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Abstract. Production of ethanol from mixed fruit waste (biomass) to represent lignocellulosic biomass was investigated. Worm tea was used as an alternative for the pretreatment of fruit waste. A 2^k fractional factorial experimental design was used to analyze the five factors use in this study (pH, temperature, biomass loading, worm tea ratio and heating time). Results from the analysis revealed loading and ratio had the strongest effect on the bioethanol yield with the highest reading of 0.501mg/l.

Keywords: mixed fruit waste, lignocellulosic biomass, bioethanol, pretreatment, worm tea, factorial design

1. Introduction

Bioethanol is considered as a sustainable alternative to gasoline to mitigate the global energy problem due to depletion of fossil fuel and also to reduce greenhouse gas emissions. The food and fuel conflict due to the production of first generation bioethanol from sugar and starchy food materials is an important issue from the food security point of view (Guragain et al., 2010). In order to avoid the competition with food, use of abundantly available and non-edible parts of plants including agricultural wastes, fast growing aquatic plants as feedstock is being attempted to produce second generation bioethanol (Hu et al., 2008). Lignocellulose is considered an attractive feedstock for the production of fuel ethanol because of its availability in large quantities at low cost (Cardona and Sánchez, 2007; Cheng et al., 2009). Lignocellulosic materials are mainly comprised of cellulose, which is a glucose polymer, hemicelluloses, a mixture of polysaccharides composed mainly by glucose, manose, xylose, arabinose, and lignin (Saxena et al., 2009).

Bioethanol production processes involve three important steps-mechanical processing and pretreatment of the material; hydrolysis of the pretreated material; and finally fermentation of the hydrolyzed material by a suitable microorganism to produce ethanol (Mishra et al., 2011). A pretreatment process is required in order to separate the lignin and hemicelluloses from the cellulose, reduce crystallinity and increase the porosity, thus improving cellulose hydrolysis (Kuo and Lee, 2009). Some pretreatment methods including acid pretreatment, wet oxidation and lime pretreatment seem to be economically more feasible than others such as biological pretreatment. However due to environmental concern and production of furfurals which inhibit the yeast fermentation during these pretreatment processes are known to be a major hurdle that needs to be overcome for commercial production of bioethanol (Hu et al., 2009; Hendriks et al., 2009; Mosier et al., 2005). Therefore, the development of efficient, cost effective and environmentally friendly pretreatment method is important (Hendriks et al., 2009).

In this experiment worm tea is use throughout the bioethanol production process which includes pretreatment, enzymatic hydrolysis and fermentation. Vermicomposting produces a leachate as micro-organisms release water during the decomposition of the organic material. Leachate derived from

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vermicomposting often called “worm tea”, is regarded as beneficial in the sense that it contains large amount of nutrients (Federico Antonio et al., 2008).

Factorial designs are widely used in experiments involving several factors where it is necessary to study the effect of the factors on a response. The 2k design is particularly useful in the early stages of experimental work, when they are likely to be many factors to investigate. As the number of factors increase, the number of runs required for a complete replicate of the design rapidly outgrows the resources, hence fractional factorial is use in the screening experiment. These designs are widely used in factor screening experiments (Montgomery, 2009).

This study investigates the effects of process variables such as mixed fruit waste loading, worm tea ratio, pH, incubation temperature and heating time on the yield of bioethanol from mixed fruit waste. 2k fractional factorial design is employed to identify the critical process factors.

2. Materials and methods

2.1. Materials

Worm tea was obtained from an agricultural company in Penang. Mixed fruit waste was obtained from a fruit stall within Universiti Sains Malaysia.

2.2. Methods

The amount of mixed fruit waste (biomass) loading, worm tea ratio, heating time, pH, incubation temperature are base on Table 1, there are 2 levels for each factors. To enhance the breakdown of the complex polymer, the biomass was pre-heated at 90^oC with distilled water first. The mixture was then cooled before adding worm tea. Instead of the conventional method of using dilute acid, worm tea was used to further breakdown the lignin, cellulose and hemicelluloses into fermentable sugars. After worm tea was added the total working volume is 200ml. The samples were then shaken at 120rpm in ambient condition for four days. After four days the samples were transferred in an incubator shaker at 120rpm for 48 hours. Samples were filtered after the two days incubation and the filtrate was fermented at ambient temperature for two days. To obtain ethanol the fermented samples were distilled.

Table 1. Experimental parameters

Factors	Low Level(-1)	High Level(1)
Mixed Fruit Waste (biomass) Loading	5	10
Worm tea	40ml	160ml
pH	7	9
Temperature (incubated)	35^oC	50^oC
Time (preheated)	45min	90min

2.3. Experimental Design

A 2^{5-1} fractional factorial design consisting of 18 experimental runs is used for screening process in bioethanol yield from fruit waste. The parameters in this study (Table 1) are loading of biomass, ratio, worm tea, pH, temperature and time. The results from the experimental work were analyzed using Minitab 14 at 95% confidence interval to generate the analysis of variance (ANOVA) and effect of each parameter.

3. Results and discussion

3.1. Yield of bioethanol

Table 2 shows yield of ethanol concentrations (mg/L) for 16 samples. The ethanol concentrations was ranged from 0.003 to 0.501 mg/L. Response reveal the possibility of worm tea as an alternative in replacing the usual dilute acid pretreatment method and also assist in the enzymatic and fermentation process. A benign alternative to harsh chemicals is microbial pretreatment, which employs microorganisms especially fungi and their enzyme systems to breakdown lignin present in lignocellulosic biomass (Shi et al., 2009).Worm tea is considered a microbial consortium where different species of microorganism act together as a community. In a microbial consortium one might find any number of organisms with different metabolic capabilities.

Table 2. Result of fractional factorial design

RunOrder	Blocks	Biomass Loading	Worm Tea Ratio	pH	Temp	Time	Ethanol Conc.(mg/l)
1	1	5	160	9	35	90	0.049
2	1	10	40	9	50	45	0.021
3	1	5	160	9	50	45	0.004
4	1	5	160	7	50	90	0.003
5	1	5	160	7	35	45	0.019
6	1	10	40	7	50	90	0.027
7	1	10	40	7	35	45	0.044
8	1	10	40	9	35	90	0.023
9	2	10	160	9	35	45	0.501
10	2	10	160	7	35	90	0.291
11	2	10	160	9	50	90	0.316
12	2	5	40	7	50	45	0.074
13	2	10	160	7	50	45	0.372
14	2	5	40	7	35	90	0.080
15	2	5	40	9	35	45	0.069
16	2	5	40	9	50	90	0.030

3.2. Analysis of variance

The ANOVA results from the 2^{5-1} fractional factorial design depicted that the values of the determination of coefficient ($R^2 = 0.94$) and the adjusted determination (Adj. $R^2 = 0.90$) were very high, which indicated the high significance of the experimental design. The most significant variable that was found in this study is biomass loading and worm tea ratio with p-value of less than 0.05.

3.3. Normal Probability of standardize effect

The significance of each factor was evaluated through a normal probability plot of standardized effect with p=0.05 as shown in Figure 1. All of the effect that lies along the line is negligible, whereas the large affects are far from the line. The important effects that emerge from this analysis are loading and ratio.

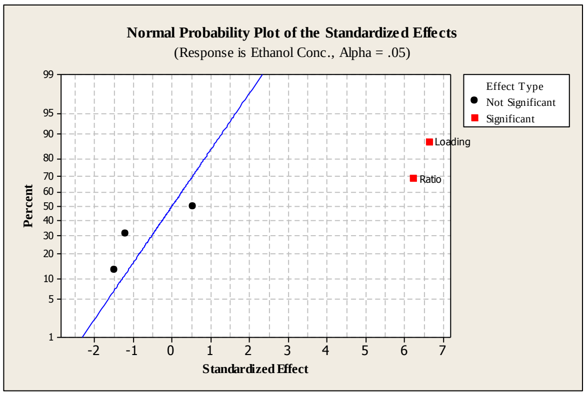

Fig. 1. Normal probability plot

3.4. Effects of the factors

Independent variables which are mixed fruit waste (biomass) loading, worm tea ratio, pH, temperature and time have been taken into consideration to understand their effect on the ethanol yield which is the dependent factor. The main effects and interaction effects are plotted in Figure 2 and Figure 3 respectively. The slope of the plot indicates the relative strength for the effect of the factors.

Figure 3 shows biomass loading and worm tea ratio with a positive effect. Maximum ethanol yield is achieved at biomass loading of 10g and also 160ml worm tea. A positive correlation is seen between biomass loading and worm tea ratio towards the response. As the biomass loading increase, the yield of bioethanol increases, the same goes for the volume of worm tea. Temperature, pH, and time do not show a positive effect which indicates that at any conditions these factors does not give effect to the yield of bioethanol. Various temperature conditions are used in the enzymatic hydrolysis for bioethanol production (Abedinifar et al., 2009; Guragain et al., 2011; Oberoi et al., 2011). The time factor in this experiment is use during the pretreatment for heating the samples. Analysis shows whether 45 or 90 minutes does not give effect to the bioethanol yield.

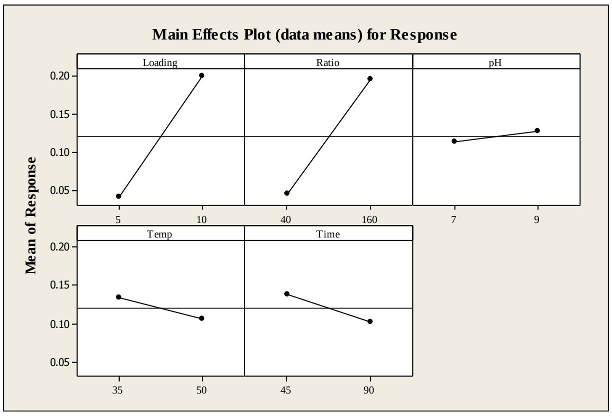

Fig. 2. Main Effect Plot

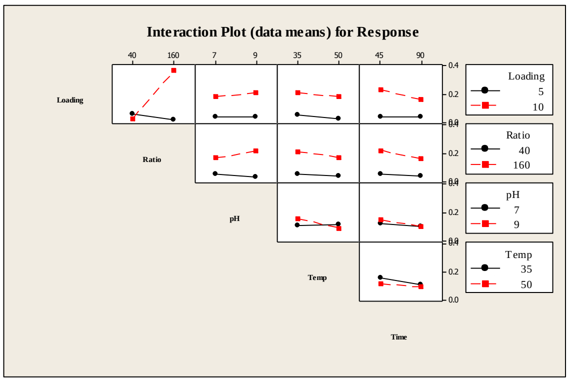

Fig. 3. Interaction Plot

From the interaction plots in Figure 3 the nearly parallel lines indicate only a very weak interaction. However the much steeper loading line shows a stronger effect of bioethanol yield when biomass loading is 10g.

4. Conclusion

The exploitation of worm tea as an alternative pretreatment for mixed fruit waste in the production of bioethanol can be an attractive economic route which indirectly allows us to cut cost of using chemicals. The use of 2^k factorial design helped in showing biomass loading and worm tea ratio as the factors that affect the bioethanol yield while the remaining factors does not give any affect thus for further design of experiment we can exclude the factors that do not affect the response yield.

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