Month 2011, Volume x, No. x
International Journal of Chemical and Environmental Engineering

Factorial Analysis on Acetic Acid Production Using Banana Stem Waste

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Abstract

The performance of acetic acid production using banana stem waste as substrate in anaerobic system was investigated under the conditions of various OLR (1.0 g TS/l.d–7.0 g TS/l.d) and HRT (3 d–15 d). Conditions for OLR and HRT in this study were based on the best range obtained from literature review. Mixed culture for this study was from banana plantation soil and acclimatized in anaerobic condition. Factorial analysis was applied allowing a screening of the experimental variables organic loading rates (OLR) and hydraulic retention times (HRT). In this work the estimation of the regression coefficients of all models were done using Matlab programming which operates by minimizing the sum of squared differences between the actual and predicted yields. Results from factorial analysis have shown that both variables which were OLR and HRT have significant effect to acetic acid production. The acetic yield is ranging from 487 mg/l to 811 mg/l depending on HRT and OLR value. Acetic acid production was found to be strongly influenced by HRT and OLR. Besides, the types of inoculums and waste used can affect the range of HRT and OLR in production of acetic acid. Increased in HRT and OLR could increased the acetic acid concentration as shown in this study and other researches. The longer period of time and the higher organic loading rate in the reactor may promote microbe growth and high yield of acetic acid.

Keywords: acetic acid, banana stem waste, factorial analysis, HRT, OLR.

1. Introduction

Acetic acid is an important chemical reagent and industrial chemical, used in the production of soft drink bottles, photographic film as well as synthetic fibers and fabrics. In households, diluted acetic acid is often used in descaling agents. In the food industry, acetic acid is used as an acidity regulator and as a condiment. The global demand of acetic acid is around 6.5 Mt/a of which approximately 1.5 Mt/a is met by recycling; the remainder is manufactured from petrochemical feedstock or from biological sources. Agricultural waste is an excellent energy source for acetic acid production since it does not result in a net increase of carbon dioxide in the atmosphere because biomass growth removes from the atmosphere the same amount of carbon dioxide that biomass combustion generates [1]. Generation and accumulation of the agricultural wastes is another environmental problem in Malaysia. In

Peninsular Malaysia approximately 4.2 million tons of crop residues were generated in 2006. These wastes contain very high concentrations of organic material, suspended solids, nitrogen and phosphorus. From 1986 to 1990, agricultural waste contributed 13 percent of the total BOD pollution load. In this research, banana stem waste is used as the substrate to a mixed culture of anaerobic microorganisms to produce acetic acid. The advantages of this process include the use of low-value substrates without the use of a sterile environment or the need for enzyme addition. Banana stem waste is known to contain a high content of cellulose (cellulosic fiber), which can be the substrate for microorganisms producing acetic acid. Agricultural activity involving banana generates large amounts of residues, because each plant produces only one bunch of bananas. After harvesting the fruits, banana stem (also known as the bare psuedostems) are cut and usually left in the soil plantation to be used as organic material. It has been estimated that for every 60 kg of banana grown, 200 kg of waste stem is thrown away. In Malaysia alone, the area for banana plantation is estimated to be 34, 000 ha [2]. Therefore utilization of these wastes is considered a way of minimizing the agricultural waste disposal. Moreover using banana stem waste can significantly reduce the cost of acetic acid production as it is cheap and widely available in Malaysia.

This paper deals with the factorial analysis of the acetic acid production of banana stem waste (BSW). There were two variables to be analyzed namely organic loading rate (OLR), biomass volume and the hydraulic retention time (HRT). The method of factor analysis will be used to screen the experimental variables which are most relevant to the acetic acid production.

1.1 Theory

The method of factorial analysis enables us to describe the various experimental variables in terms of mutually orthogonal factors which are uncorrelated to each other but which have the same mean and the same variance as the standardized form of the experimental variables. Mutually orthogonal factors are important in that only such factors may be used to construct linear models, where the interactions between factors are not taken in to account. Empirical models are constructed to describe the yields in terms of these mutually orthogonal factors. The significance of each actor in its effect on the yield is then determined by removing the particular factor from the model involving all the factors and comparing the mean square difference between the actual data and the prediction of the resulting model with the mean square difference between the actual data and the predictions of the model involving all the factors using the statistical F-test. These factors can then be classified into categories according to how significantly each of them affects the yield. If an experimental variable contributes only to factors which do not affect the yields significantly then it can be concluded that it is not relevant to the yield and can be dropped from subsequent experiments. If an experimental variable contributes to one or more factors which have significant effects on the yield then the experimental variable is relevant to the yield and should be retained for further investigation and optimization [3].

To construct the models, a new table of the experimental results consisting of the yields and the factors has to be evaluated by calculating the experimental values of each factor. A linear regression between these factors and the yields is then constructed. Other models are constructed in the same manner but employing successively less factors. The evaluation of the significance of each factor is then done by the statistical F-test [4].

In this work the estimation of the regression coefficients of all models were done by using Matlab programming which operates by minimizing the sum of squared differences between the actual and predicted yields. The results of the regression analysis were tested for R^2 value. R^2 is a statistic that will give some information about the goodness of fit of a model. In regression, the R^2 coefficient of determination is a statistical measure of how well the regression line approximates the real data points. An R^2 of 1.0 indicates that the regression line perfectly fits the data [5].

2 Materials and methods

Feedstock Materials

Banana stems waste was used as feedstock in the experiments. The banana stem waste (BSW) first has to be washed to remove the soil. After it is washed clean, the banana stem waste is then dried at ambient temperature between one to two weeks. These were cut into small pieces (about 5 cm) and mixed with distilled water**.**

Mixed Culture and Inoculums

Banana stem sludge (BSS) was the mixed culture inoculums source in this study. Both BSS and BSW for this study were collected from banana plantation in Kuantan, Pahang. About 30 kg BSS was collected from banana plantation soil and placed in 10 l container. 1000 g/l of BSW was then added into the container as substrate for the BSS. The acclimatization process between the BSS and the BSW takes about three months period at HRT of 5 d. .

Experimental Set-up

Experiment was done at ambient temperature in 2 liter anaerobic batch reactor with gas outlet. The process performance was tested under the conditions of various organic loading rates OLR (1.0 g TS/l.d–7.0 g TS/l.d) and HRT (3 d–15 d). Mixed culture inoculums for this study was from banana plantation soil and acclimatized with banana stem waste as substrate in anaerobic condition. Initial mixed culture inoculums concentration was 5000 mg/l. Reactor equipped with agitator to ensure the mixed culture inoculums and BSW mixed evenly. Influent and effluent from reactor were done manually. To maintain anaerobic condition in the reactor nitrogen was purged every time the inlet and outlet were done

Analytical Method

Samples was periodically taken by syringe, oxidized by exposure to air to stop the reaction, and stored at 4°C for 48 h prior to analysis by HPLC. Acetic acid concentration was measured by HPLC. HPLC system equipped with a refractive index detector and a Biorad Aminex HPX-87H analytical column with corresponding guard column. The column temperature was maintained at 55^o C. Sulphuric acid was used as mobile phase at a flow rate of 0.6 ml/min. The volume of the injection loop was 20 µl [6]..

Experimental Values

In this work the value of each experimental variable was varied over as wide a range as possible so as to gain the maximum knowledge of the behavior of the system over wide-ranging conditions. In most cases practical difficulties prevented the use of too large variation in the values of the experimental variables. In cases of zero yields the experiment could not be treated statistically since a zero yield is not sensitive to further changes in the values of the variables which would otherwise have decreased the yield. It was assumed that the response surface did posses a maximum. If the area that has been examined did not cover the maximum, a linear model might be sufficient to represent since there would not be as much curvature as there would be if it had contained the maximum. Therefore, a linear model approach was tried first. If it did not fit the data of the orthogonal factors and yields, a non-linear model would have to be fitted instead so as to account for the curvature that is likely to occur near the maximum yield [7].

3 Results and Discussion

The experimental variables that characterize a factor are defined as those that have absolute values of coefficients greater than 0.4 (thus allowing for 40% error) in the equation describing that factor in terms of the experimental variables. The following conclusions can be made from Table 4.

1. Factor F1 being characterised by HRT
2. Factor F2 being characterised by OLR

At 90% confidence level, the best model for acetic acid concentration is the model involving the factor F1 (Table 8). Table 4 shows that these factors consisted of contribution from experimental HRT

Table 1 : Experimental Results

	HRT (d)	**OLR (gTS/l.d)**	
	X1	**X2**	**Acetic acid (mg/l)**
1	5.4	2.2	707
2	12.6	2.2	811
3	5.4	5.8	655
4	12.6	5.8	805
5	5.4	2.2	588
6	12.6	2.2	799
7	5.4	5.8	610
8	12.6	5.8	778
9	3.0	4.0	487
10	15.0	4.0	740
11	9.0	1.0	741
12	9.0	7.0	708
13	9.0	4.0	792
14	9.0	4.0	700
15	9.0	4.0	600
16	9.0	4.0	600
17	9.0	4.0	615
18	9.0	4.0	599
19	9.0	4.0	605

Table 2: Calculation of W

	W1	W2
1	-1.18	-1.18
2	1.18	-1.18
3	-1.18	1.18
4	1.18	1.18
5	-1.18	-1.18
6	1.18	-1.18
7	-1.18	1.18
8	1.18	1.18
9	-1.98	0.00
10	1.98	0.00
11	0.00	-1.98
12	0.00	1.98
13	0.00	0.00
14	0.00	0.00
15	0.00	0.00
16	0.00	0.00
17	0.00	0.00
18	0.00	0.00
19	0.00	0.00

Table 3: The Correlation Matrix of the Standardized Variables

	HRT	OLR
HRT	1	0
OLR	0	1

Table 4: The Eigen Vector Values

	F1	F2
HRT	1	0
OLR	0	1

Table 5: The Values of the Factors

	F1	F2
1	-1.18	-1.18
2	1.18	-1.18
3	-1.18	1.18
4	1.18	1.18
5	-1.18	-1.18
6	1.18	-1.18
7	-1.18	1.18
8	1.18	1.18
9	-1.98	0.00
10	1.98	0.00
11	0.00	-1.98
12	0.00	1.98
13	0.00	0.00
14	0.00	0.00
15	0.00	0.00
16	0.00	0.00
17	0.00	0.00
18	0.00	0.00
19	0.00	0.00

Table 6: The Coefficients of the Linear Model

	Value
Coefficient	**Acetic acid concentration**
a0	681.1
a1	65.7
a2	-6.98

Table 7: The Evaluation of the Linear Model

No	Actual yield	Predicted yield	Squared error
1	707	611.8	9061.1
2	811	766.9	1948.1
3	655	595.3	3559.6
4	805	750.4	2982.3
5	588	611.8	566.9
6	799	766.9	1032.8
7	610	595.3	215.0
8	778	750.4	762.3
9	487	551.0	4097.8
10	740	811.2	5067.4
11	741	694.9	2123.3
12	708	667.3	1658.2
13	792	681.1	12298.8
14	700	681.1	357.2
15	600	681.1	6577.2
16	600	681.1	6577.2
17	615	681.1	4369.2
18	599	681.1	6740.4
19	605	681.1	5791.2
		SSE	75786.2
		MSE	3988.7
		RMSE	63.2
		R^2	0.5226

Table 8: The Evaluation of the Linear Model

Model	MSE	MSEm/MSEfm
12	3988.9	1
1	4037.7	1.01
2	8306.9	2.08

Table 9: The Coefficients and Constants of the Linear Models

Model	a0	a1	a2
12	681.1	65.7	-6.98
1	681.1	65.7	
2	681.1		-6.98

Table 10: The Coefficients of the Quadratic Model

	Value
Coefficient	**Acetic acid concentration**
b0	653.0
b1	77.8
b2	-8.26
b11	-0.33
b22	39.6
b12	0.38

Table 11: The Evaluation of the Quadratic Model

No	Actual yield	Predicted yield	Squared error
1	707	626.2	6536.5
2	811	808.7	5.3
3	655	605.6	2440.4
4	805	790.3	217.1
5	588	626.2	1455.5
6	799	808.7	94.1
7	610	605.6	19.4
8	778	790.3	150.5
9	487	497.7	113.7
10	740	805.8	4323.1
11	741	824.6	6989.4
12	708	791.9	7038.0
13	792	653.0	19321.0
14	700	653.0	2209.0
15	600	653.0	2809.0
16	600	653.0	2809.0
17	615	653.0	1444.0
18	599	653.0	2916.0
19	605	653.0	2304.0
		SSE	63194.9
		MSE	3326.0
		RMSE	57.7
		R^2	0.6019

Table 12: Other research show that increase of HRT and OLR will increase Acetic acid yield

Research	HRT (d)	OLR (gTS/l. d)	Acetic yield (mg/l)	Types of Inoculums	Types of Waste
This research	3-15	1.0-7.0	487-811	Mixed culture (soil)	Banana Stem Waste
Hong and Haiyun (2010)	4-12	4-12	382 - 2666	Mixed culture (municipal waste)	Food Waste + Sludge
Vazquez et al. (2009)	0.16 - 0.42	55.4 – 184.4	944 – 2487	*Clostridium sp.*	Food Waste
Zinatiza-deh et al. (2010)	1 - 6	17.6-34.7	158-843.2	Mixed Culture (sludge)	Palm Oil Mill Efflu-ent (POME)
Borja et al. (2005)	10-50	3.8-19.0	215.6-2200	Mixed culture (olive mill waste)	Two Phase Olive Po-mace
Dinsdale et al. (2000)	1-4	3.9 -7.8	598 - 4077	Mixed culture (sludge)	Food Waste + Sludge

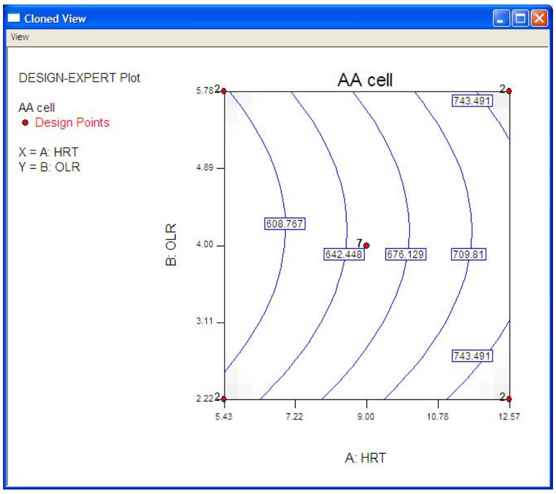

Figure 1. Contour Plot for Acetic Acid Production

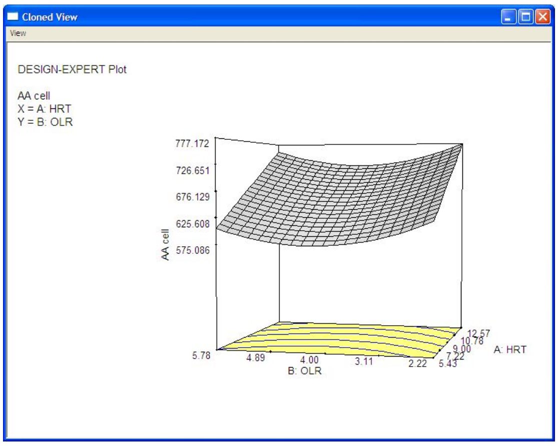

Figure 2. 3D Plot for Acetic Acid Production

The linear regression equation of the response surface of the 2^2 factorial experiments fits satisfactorily with the data points (Table 7) and the coefficients of the variables are small compared with the intercept indicating that the area investigated is a plateau which may contain the maximum point. The evaluation of the regression coefficients of the linear and quadratic equations (Table 7 and Table 11) showed that the R^2 value of quadratic equation is higher than linear equation. This proved that quadratic equation can represent the acetic acid production model. Acetic acid production was explained through the contour and 3D plot in Figure 1 and Figure 2 respectively. Here HRT, OLR and acetic acid concentration were in unit d, gTS/l.d and mg/l respectively. Increased in HRT and OLR could increased the acetic acid concentration. In this study, acetic acid concentration is increase with HRT at (3- 15d) and with increasing OLR from 1.0 g TS/l.d to 7.0g TS/l.d. In Figure 1, contour plot shows the amount of acetic acid production increasing with increasing amount of HRT and OLR simultaneously. The reason for acetic acid concentration increased with HRT and OLR was that more substrate and microbes for fermentation to produce acid was provided at longer HRT and higher loading rate. Higher organic loading rate may increase microbe population, since more organic matter may support large population [8]. There is also other reported where HRT and OLR increasement will increase acetic acid concentration up to 11.1 % [8] and [9]. While in Figure 2, the 3D plot shows more tangible information where the minimum acetic acid production is when OLR is not increase at the same time with HRT. The 3D plot also show the amount of acetic acid is increase with increasing of HRT and OLR concurrently. The reason of minimum acetic acid production shown in 3D plot is that too much increased in organic loading rate will overloading the system if the HRT is not increased. This is because the increase of organic loading rate will provide more substrate for the microbes but there is not enough time for microbes to growth to consume available substrate. Table 12 shows the comparison with other researches done. All the researches done show the HRT and OLR increase the acetic acid production even the waste and inoculums used is totally different. However, there were some significant different in acetic acid yield. The acetic production using food waste is generally high (able to produce more than 2000 mg/l) (Table 12). In the acetic production using food waste, the HRT is much shorter and the OLR is much higher. Lignocellulose waste such as banana stem waste, POME, olive pomace need higher HRT and lower OLR in the process. This is due to the waste structure complexity. Lignocellulose waste contained cellulose, hemicelluloses, and lignin compared to food waste which is much simpler. According to Zhang et al. (2007) the food waste contained high moisture content and balance nutrient content which is suitable and easy for anaerobic microorganism to growth. Meanwhile, lignocellulose contained lignin which is hard to access by microbes and require higher retention time and lower OLR. Higher organic loading rate may increase microbe population in the system. However, because of the complicated lignocellulose structure, higher retention time is needed to ensure the microbe growth. Too much organic loading rate may alter the pH condition in the bioreactor and microbes such as acidogens which are very sensitive to pH changed. Therefore, only suitable amount of loading rate are applicable in certain period of retention time to promote better environment for microbe growth. The inoculums used in the bioreactor to produce acetic acid may affect the yield of acetic acid. In Table 12, there is one pure strains of *Clostridium sp.* are used for anaerobic process to produce acetic acid from food waste. The yield of acetic acid using this is viable and the shorter retention time and high organic loading rate can be applied. Meanwhile, compared to this study and other studies available in the table, the mixed cultures of microbes were used. Mixed culture of microbes contained many species that are able to live syntrophic to each other. Acetic acid producing microbes live in a syntrophic, relationship with other microorganisms that consume the feedstock. Therefore, mixed culture is very suitable for the process but there is a need to find suitable range of HRT and OLR. It is found that acidogenic bacteria are actively growth below 8 days retention times. However, the bacteria that involved in hydrolysis process need time to growth and convert available substrate for acidogens used. In addition, microorganism is very sensitive to pH changed. Besides, the pH interaction between substrate concentration and pH was significant and this is due to substrate composition. Therefore, to create optimum environment for each microorganism in reactor is almost impossible [10]. The objective here is to create a stable working relationship among the mixed culture population in the bioreactor. Higher HRT and OLR may provide stability in the process to promote microbe growth and increase product yield. Conversely, any rapid variations of these conditions will cause the mixed culture population to shift dramatically and possibly upset the overall system balance in the bioreactor. HRT is an important parameter because it influences the efficiency of the biogas digester. According to Beal and Raman (2000) shorter retention times will create the risk of washout, a condition where active bacteria are washed out of the digester at too young an age, making the population of bacteria unstable and potentially inactive. This is because low HRT and OLR triggered a transition in the bacterial community structure resulting decrease in production rate. This is due to maintain specific production from substrate conversion is related to maintaining adequate concentration of microbes or enzymes in bioreactor [11]. Thus, by increased HRT and OLR will increase the concentration of microbes or enzyme in bioreactor and a lot of microbes will be available for substrate utilization. As a result, daily conversion of organic material to acetic acid will continue to increase per unit increase of weight of bacteria up to a certain point [12].

4 Conclusion

This study demonstrates that from factorial analysis have shown that both variables which were OLR and HRT have significant effect to acetic acid production. Acetic acid production was found to be strongly influenced by HRT and OLR. Besides, the types of inoculums and waste used can affect the range of HRT and OLR in production of acetic acid. Increased in HRT and OLR may increase the acetic acid concentration. The longer period of time and the higher organic loading rate in the reactor may promote microbe growth and high yield of acetic acid.

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