Anaerobic Co-digestion of Food Waste for Biohydrogen Production

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Abstract— **The objective of this study was to find the optimum conditions for anaerobic co-digestion of food waste and sewage sludge and hydrogen (H_2) production. The selected parameters for optimization of H_2 production (e.g. temperature, initial pH, inoculum size) were analysed using Response Surface Methodology with Full Factorial Design. Two types of substrates were tested; food waste as a sole substrate and food waste mixed with palm oil mill effluent (POME) at volume ratio1:1. The optimized conditions for food waste as a sole substrate were pH 4.5, temperature of 35°C and inoculum size of 20% (v/v), with maximum predicted cumulative hydrogen production (MPCHP) of 0.22 ml H_2/ml substrate. On the other hand, for food waste mixed with POME, pH 4.5, temperature of 35°C and inoculum size of 20% were the optimum conditions with MPCHP of 0.26 ml H_2/ml substrate. Subsequently, verification experiments at optimal parameter values yielded cumulative H_2 of 0.28 ml H_2 /ml substrate for food waste only, and 0.33 ml H_2/ml substrate for food waste mixed with POME.**

Keywords— Biohydrogen; Anaerobic digestion; Food waste; Anaerobic sewage sludge; Response surface methodology

I. INTRODUCTION

The energy crisis and environmental pollution are the two pressing issues for sustainable development. Hydrogen (H_2) is a sustainable energy source with minimal use of hydrocarbon. In addition, the high energy yield of 142 kJ/g makes H_2 an attractive alternative to fossil fuels [1].

Conventional hydrogen gas production methods are steam reforming of methane (SRM) and other hydrocarbons (SRH), non-catalytic partial oxidation of fossil fuels (POX) and autothermal reforming which combines SRM and POX. Those energy-intensive methods require high temperature (more than 850°C). Among other methods that have been developed for improvement are the membrane processes, selective oxidation of methane and oxidative dehydrogenation [2].

Biological H_2 production is a viable alternative to the other methods for H_2 gas production, and biohydrogen gas production from renewable sources has received a lot of attention in recent years. Some of the major bioprocess for hydrogen production are biophotolysis of water by algae, dark-

fermentative hydrogen production and two stage dark fermentation and photofermentative production of hydrogen.

Biohydrogen can be used directly in combustion engines for transportation and also to produce electricity in fuel cells [3]. For the time being, H_2 is widely used in production of fertilizers, diesel refinery and industrial synthesis of ammonia rather being used as an energy source.

Simple sugars such as glucose and sucrose are preferred substrate for biohydrogen production however, they can be expensive. When choosing a substrate for biohydrogen production, factors such as abundance, cost, carbohydrate content and its biodegradability are primary consideration. Few examples of biomass that have been studied for hydrogen productions are industrial effluent, dairy wastewater, starch residues, palm oil mill effluent (POME) and food waste [4-5].

Food waste constitutes a major fraction of the municipal solid wastes. However, food waste poses a problem of variations in carbohydrate and protein types and concentrations in the mixture [2]. POME generated from palm oil milling process is a major pollutant from agro-industry. POME is rich in organic carbon and has been studied as substrate for biohydrogen production [6-7]. The feasibility of combining two types of carbohydrate-rich substrate is not fully explored.

The objective of this study was to find the optimum condition for anaerobic dark fermentation of food waste and sewage sludge for H_2 production, with the supplement of POME. The selected parameters for optimization conditions of H_2 production were

TABLE I. CHEMICAL CHARACTERIZATION OF SUBSTRATES AND SEWAGE SLUDGE

Parameter	Food waste	POME	Sewage sludge
TSS (mg/l)	762.7	833.2	284
VSS (mg/l)	526.7	567.5	244
COD mg/l)	194625	320040	242000
TKN (mg/l)	260.4	554.4	492.8
pH	4.0 – 4.3	4.2 – 4.5	7.2 – 7.5

TABLE II. LEVELS AND VARIABLES FOR FULL FACTORIAL DESIGN

Symbols	Variables	Levels		
A	Initial pH	4.5	5.5	6.5
B	Temperature (°C)	35	45	55
C	Inoculum size (%vol/vol)	2	10	20

analysed using Response Surface Methodology with Full Factorial Design. Individual and interactive effects of these process parameters were evaluated.

II. MATERIALS AND METHODS

A. *Seed sludge*

A mixed culture of anaerobic bacteria was used as inoculum, obtained from anaerobic sludge digester of Indah Water Konsortium in Kuala Lumpur. The sludge was collected from the gravity thickener part of the sludge holding tank and kept in cold room at 4°C. Prior to use, the sludge was sieved and heat treated at 100°C to inactivate the methanogenic bacteria [8].

B. *Substrate*

Food waste was collected from various cafeterias around University of Malaya. It was mainly made up of rice, vegetables and chicken. After removing the bones and unnecessary waste, the food waste was grounded using an electrical blender. The food waste was mixed with tap water to facilitate blending. After blending, the food waste was filtered using domestic sieve (pore size 2 mm) to remove excess water. The blended wastes were then packed into small plastic bags, and kept in freezer at -20^0C and thawed overnight prior use. The POME used in this study was collected from a local palm oil mill. The collected POME was stored at 4°C. The characteristics of both substrates (food waste and POME) are summarized in Table I.

C. *Experimental design and procedure*

Batch experiment was conducted in 120 ml serum bottles with a working volume of 50 ml, while varying the initial pH, inoculum size and temperature (Table II). The bottles were capped with rubber stopper and aluminium cap. The first experiment's substrate comprised solely of food waste, and the second one comprised of

food waste mixed with POME on 1:1 volume ratio and will henceforth be identified as Production 1 (P1) and Production 2 (P2), respectively.

All experiments were conducted in an incubator shaker at 150 rpm for 72 hours. Samplings were done at eight hours interval. The biogas collected using syringes were kept in acidic water (pH 2) using water displacement method. The acidic pH helps to prevent the gas from dissolving into the water.

The biogas composition was determined using a gas chromatograph (Perkin Elmer, AutoSystem GC) equipped with thermal conductivity detectors (TCD) and digital data acquisition system. H_2 content was analyzed by GC-TCD fitted with a 1.5 m stainless steel column packed with a molecular sieve (80/100 mesh). The temperatures of injection port, oven and detector were 80°C, 200°C and 200°C respectively. Argon was used as the carrier gas at a flow rate of 30 ml/min. One ml gas sample was injected in replicates.

Chemical oxygen demand (COD) and volatile suspended solids (VSS) analysis were done before and after each run following the procedures in the standard method.

H_2 gas production was calculated from headspace measurement of gas composition and the total volume of hydrogen produced using the mass balance equation in (1) [6]:

$$V_{Hi} = V_{Hi-1} + C_{Hi}(V_{Gi} - V_{Gi-1}) + V_{H0}(C_{Hi} - C_{Hi-1}) \quad (1)$$

where V_{Hi} is the cumulative hydrogen gas volumes at the current time (i), V_{Hi-1} is the previous time interval (i-1). V_{Gi} is the total biogas volume at the current time interval, V_{Gi-1} is the total biogas volume at the previous time interval, C_{Hi} is the fraction of hydrogen gas in the headspace at the current time interval and V_H is the headspace volume of the serum bottle (70 ml).

III. Results and Discussions

For food waste alone as substrate, the multiple regression analysis using Minitab® 16.1 software showed the importance of main effect and interaction effects of the three variables based on the response of cumulative H_2 production.

The important effects of the variables on the cumulative hydrogen production are dependent on their *P*-values and *t*-values, as shown in Table III for P1. The predicted value of the response was obtained from full quadratic model fitting which includes the main and interaction effects. The regression equation generated is given in (2):

$$Y = 29.3333 - 3.2927A - 0.4967B - 0.0514C + 0.0693AB + 0.0072AC + 0.0014B \quad (2)$$

The ANOVA analysis for (2) is shown in Table III. The temperature and inoculum size showed significant effect towards the cumulative hydrogen production ($p < 0.05$).

The initial pH chosen for this study, ranging from 4.5 to 6.5 did not show significance; probably due to the small range. However, the

TABLE III. Analysis of Variance for Hydrogen Produced from Food Waste, P1 (Coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	3	29.5414	29.5414	9.8471	10.08	0.000
pH	1	0.2225	0.2225	0.22	0.23	0.638
Temp	1	23.9518	23.9518	23.9518	24.52	0.000
Inoculum	1	5.3671	5.3671	5.3671	5.49	0.030
2-Way Interactions	3	12.0181	12.0181	4.0060	4.10	0.020
pH*Temp	1	11.5189	11.5189	11.5189	11.79	0.003
pH*Inoculum	1	0.1000	0.1000	0.1000	0.10	0.752
Temp*Inoculum	1	0.3992	0.3992	0.3992	0.41	0.530
Residual Error	20	19.5404	19.5404	0.9770		
Curvature	1	3.7451	3.7451	3.7451	4.50	0.047
Lack of Fit	1	0.0045	0.0045	0.0045	0.01	0.944
Pure Error	18	15.7908	15.7908	0.8773		
Total	26	61.0999				

two-way interaction effects between initial pH and temperature did show significance (p = 0.003).

The coefficient of multiple determinations, R^2 of the model was 0.6802. This means that the model could explain 68% of the total variation in the system.

The interaction between the initial pH and temperature on cumulative hydrogen production can be seen in Fig. 1 where strong curvature of the contours depicts their significance. The arrow showed the trajectory of optimization i.e. towards low set of temperature and initial pH. This agreed with optimized conditions obtained i.e. initial pH of 4.5 and 35 °C temperature.

As shown in Fig. 2, the white region in the overlaid contour plot for production of cumulative H_2 and COD removal indicates the possible region for simultaneous optimization of temperature and initial pH.

From the response optimizer function in Minitab®, the maximum predicted H_2 production of 8.65 ml could be obtained with these parameters set; initial pH of 4.5, inoculum size of 20% and temperature of 35°C, with COD removal 68.79%. The composite desirability was 0.8943 i.e. it is likely to get the predicted response 89 times out of 100.

For P2, the predicted value of cumulative H_2 production as a response was obtained from full quadratic model fitting technique which also includes the main and interaction effects. The reduced regression equation generated is given in (3):

$$Y = 57.3948 - 8.9263A - 0.8957B - 3.9011C + 0.1592AB + 0.7544AC + 0.0821BC \quad (3)$$

The ANOVA analysis for (3) is shown in Table IV. All three factors *viz*. initial pH, temperature and inoculum size showed significant effect towards the cumulative hydrogen production ($p < 0.05$). However, the interaction effects between any two factors did not show any significance.

The coefficient of multiple determinations, R^2 shows how well the estimated model fits the data. The value of R^2 for this model was 0.8576. This means that the model could explain 85.76% of the total variation in the system.

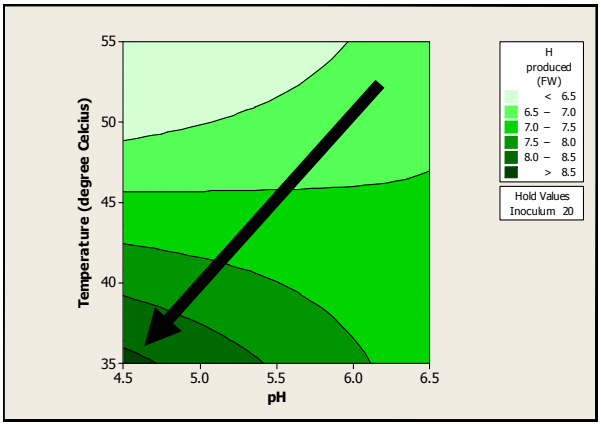

Fig. 1. Contour plot of hydrogen production from food waste (P1) versus pH and temperature.

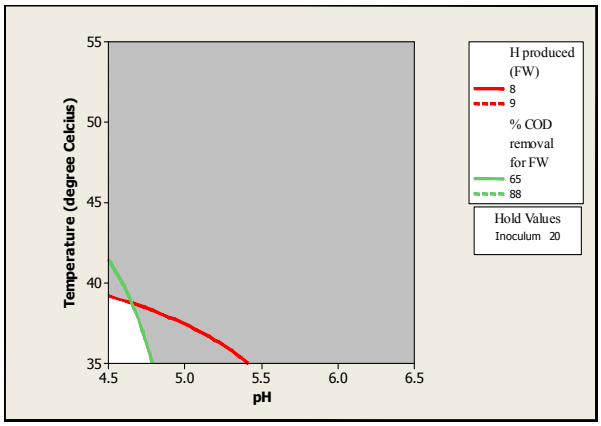

Fig. 2. Overlaid contour plot for cumulative hydrogen production (ml) and COD removal (%) versus pH and temperature for P1.

The contour plot for initial pH and inoculum size on the cumulative hydrogen production is shown in Fig. 3. The arrow showed the direction for optimization is towards lower initial pH and higher inoculum size. This agreed with optimized conditions obtained i.e. initial pH 4.5 and inoculum size 20% (v/v). In Fig. 4, the white region observed in the overlaid contour plot for production of cumulative H_2 and COD removal is the possible region for simultaneous optimization of temperature and initial pH.

TABLE IV. ANALYSIS OF VARIANCE FOR HYDROGEN PRODUCED FROM FOOD WASTE MIXED WITH POME, P2 (CODED UNITS)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	3	54.022	54.0220	18.0073	18.65	0.000
pH	1	37.651	37.6514	37.6514	38.99	0.000
Temp	1	11.240	11.2401	11.2401	11.64	0.003
Inoculum	1	5.131	5.1305	5.1305	5.31	0.033
2-Way Interactions	3	8.391	8.3905	2.7968	2.90	0.062
pH*Temp	1	0.463	0.4628	0.4628	0.48	0.497
pH*Inoculum	1	4.207	4.2074	4.2074	4.36	0.051
Temp*Inoculum	1	3.720	3.7204	3.7204	3.85	0.064
Residual Error	19	18.348	18.3481	0.9657		
Curvature	1	12.384	12.3842	12.3842	37.38	0.000
Pure Error	18	5.964	5.9639	0.3313		
Total	26	128.868				

From the response optimizer function in Minitab®, at initial pH 4.5, inoculum size 20% and temperature 35 °C, the maximum predicted H_2 production of 10.19 ml could be obtained with COD removal 81.62%. The composite desirability was 0.8680, i.e. it is likely to get the predicted response 87 times out of 100.

Normally the optimum pH for hydrogen production in dark fermentation process is within the range of 5 to 7, with the common optimum pH at 5.5 [9]. In this study, pH 4.5 was the most suitable pH for the simultaneous production of H_2 and COD reduction. A study by Fang et al [11] showed that at low pH of 4.5, hydrogen production process is most effective. The microbial DNA profiling data indicated that the uncultured microbes were mainly made up of *Clostridium* sp. (data not shown).

Temperature has a great influence on the activity of hydrogen producing bacteria. Different studies showed different optimum temperature but fermentative hydrogen production most commonly fell into the mesophilic range; around 37°C [10-11], which agreed well with the temperature obtained in the study.

Table V summarizes the expected cumulative hydrogen production as obtained from the response optimizer on Minitab® and also the actual result obtained from verification experiment. At given optimum set of parameters namely initial pH 4.5, inoculum size 20% and temperature 35 °C, both P1 and P2 produced cumulative hydrogen slightly higher than expected value. The COD removal for both P1 and P2 however were lower than expected. It is possible that the increase in the value of one response may be achieved at the expense of the other response i.e. in this case H_2 production and COD removal, respectively.

The P2 will definitely produce more hydrogen than food waste as the only substrate since POME itself contain a number of hydrogen producing microbes. Ismail et al [7] has demonstrated how POME can be used as inoculum. Hence the addition of microbe-rich sewage sludge will only enhance the hydrogen producing capability.

TABLE V. RESULTS FOR VERIFICATION EXPERIMENT

P	Expected		Observed	
	H_2 *(*ml H_2/ ml substrate)	***COD removal (%)***	***H_2*** *(*ml H_2/ml substrate)	***COD removal (%)***
P1	0.22	68.79	0.28	41.73
P2	0.26	81.62	0.33	75.59

IV. CONCLUSIONS

In this study, it is feasible to use food waste with or without further supplementation by POME as a substrate for microbial H_2 gas production. Both systems exhibited optimal yield for simultaneous microbial H_2 gas production and COD removal at pH 4.5, 35 °C and 20% (v/v) inoculum.

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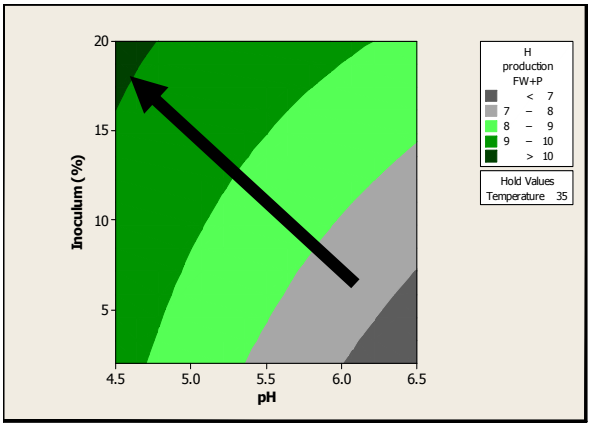

Fig 3. Contour plot of hydrogen production from food waste mixed with POME (P2) versus pH and inoculum (%).

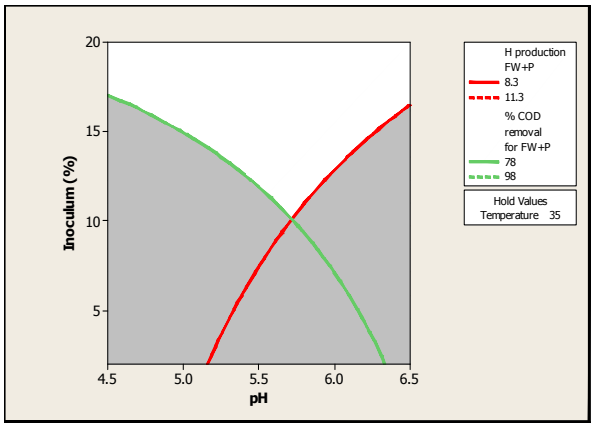

Fig 4. Overlaid contour plot for cumulative hydrogen production (ml) and COD removal (%) versus pH and inoculum (%) for P2**.**

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