Effect of inoculums content and screening of significant variables for simultaneous COD removal and H_2 production from tapioca wastewater using Plackett-Burman Design

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ABSTRACT

The effect of four selected variables on Chemical Oxygen Demand (COD) removal and H_2 production by anaerobic mixed cultures from tapioca wastewater in batch mode (viz. ferrous sulphate ($FeSO_4$), initial pH, sodium bicarbonate ($NaHCO_3$) and nutrient solution with two inoculums (3,750 mgVSS/L and 7,500 mgVSS/L) were sought. Identification and screening of significant variables were conducted using the Plackett-Burman Design. An independent sample t-test was applied using 12 trials to evaluate inoculums content to determine the optimum level of the main variables and inoculum content at the steepest ascent. $FeSO_4$ and initial pH both had a statistically significant ($P<0.05$) influence on COD removal and H_2 production. COD removal and H_2 production was greater at 7,500 mgVSS/L inoculums content than at 3,750 mgVSS/L ($P< 0.05$). An initial pH of 10 and $FeSO_4$ at 2.5 g/L yielded the maximum H_2 production potential (443.37 mL H_2/L) and COD removal (61.54 %).

Keywords: Initial pH, ferrous sulphate ($FeSO_4$), sodium bicarbonate ($NaHCO_3$), nutrient solution, Plackett-Burman Design, inoculums content

INTRODUCTION

The world is burning fossil fuels at an unprecedented rate, belching 34 billion tons of CO_2 into the atmosphere in 2011, accelerating global warming (Olivier, 2012). Biogas technology from fermentative hydrogen production (Kim & Kim, 2013) derived from animal waste (Sirirote et al., 2010), food production (Zhu et al., 2011), cassava detoxification (Wang et al., 2012) and corn processing (Cheng et al., 2012) is an alternative source of energy. It has a high heating value of 142 KJ/g and does not release greenhouse gasses during combustion (Singh et al., 2013).

Fermentative hydrogen production is influenced by many factors such as inoculum, substrate, alkalinity, reactor type, organic

loading rate, pH and temperature (Mohammadi et al., 2012). When microorganisms degrade, organic substrates electrons (COD removal), which need to be disposed of to maintain electrical neutrality, are produced. Tapioca is grown in almost every tropical country; its biodegradable starch is an important source of carbohydrates for livestock (Blagbrough, Bayoumi, Rowan, & Beeching, 2010). The tapioca starch-processing industry in Thailand is the world's largest (DAO, 2015). Tapioca's highly organic wastewater is an effective substrate for H_2 production through dark fermentation (Chavalparit & Ongwandee, 2009; Show, Lee, Tay, Lin, & Chang, 2012).

Iron is an important nutrient element needed to form hydrogenase and other enzymes, and a small additional amount of $FeSO_4$ at high cell concentration is sufficient to enhance H_2 production (Sinha & Pandey, 2011). $NaHCO_3$ can maintain pH at a favourable range for hydrogenesis (Li, Jiang, Xu, & Zhang, 2008; Mohammadi, Ibrahim, & Mohamad Annuar, 2012). The Plackett-Burman experimental design has had the greatest impact on screening variables (Kevin & Dennis, 2015). The lack of information on tapioca wastewater vis-à-vis H_2 production required us to statistically screen for significant variables for simultaneous COD removal and H_2 production.

The main objectives of the current study were to assess the effect of (a) iron (II) sulphate ($FeSO_4$); (b) initial pH; (c) sodium bicarbonate ($NaHCO_3$); and (d) nutrient amendments on COD removal and H_2 production efficiency using tapioca wastewater as the substrate with the Plackett-Burman Design. Low (3,750 mgVSS/L) and high (7,500 mgVSS/L high) inocula content was assessed for its effect on COD removal and H_2 production.

MATERIALS AND METHOD

Seed Mixed Culture Inoculum

Anaerobic seed sludge was collected from a tapioca starch factory's full-scale, up-flow anaerobic sludge blanket (UASB) reactor. The factory was in Kalasin Province, Thailand. Normally, this UASB produces methane. To inactivate methanogenic microbes, the sludge was heated to 105ºC for 2 hr, after which it was cooled in a desiccator at room temperature. Inoculum preparation followed the method of Thanwised, Wirojanagud and Reungsang (2012).

Tapioca Wastewater

In the current study, tapioca wastewater was obtained from the tapioca factory as recommended by Thanwised, Wirojanagud and Reungsang (2012). It was immediately transferred to the laboratory and stored at 4ºC until needed. The characteristics of the tapioca wastewater was as follows: pH 4.58±0.29, COD 9,277±414 mg/L, BOD_5 5,800±256 mg/L, TS 13,430±1018 mg/L and TSS 1,524±581 mg/L.

Biohydrogen Production and COD Removal

A working volume of 70 mL in 120 mL serum bottles was used for the H_2-production experiment. The H_2 production medium contained a respective 3,750 mgVSS/L and 7,500 mgVSS/L of inoculum. Different concentrations of $FeSO_4$, $NaHCO_3$ and nutrient amendments were added and the initial pH adjusted according to the experimental design.

Analytical Methods

Biogas composition was measured via gas chromatograph (GC-2014, Shimadzu) as per Thanwised, Wirojanagud and Reungsang (2012). Standard methods (APHA, 21st Ed., 2005) were used for measuring COD and hydrogen gas production calculated as per Zheng and Yu (2005).

Kinetic Modelling

A modified Gompertz Eq. [1] was used as per Zheng and Yu (2005):

$$H(t) = P \exp\{ -\exp[(R_m e/P)(\lambda\text{-t})+1]\} \tag{1}$$

where, H represented the cumulative volume of hydrogen produced (mL); P_s the hydrogen production potential (mL); R_m the maximum production rate (mL/h); λ the lag-phase time (h); t the incubation time (h), and; e equalled 2.718281828.

Screening and Identifying Procedure

The current study used the Plackett–Burman Design to identify and screen for significant variables vis-à-vis COD removal and H_2 production by mixed cultures in tapioca wastewater. The parameters investigated included nutrient addition, initial pH, $FeSO_4$ and $NaHCO_3$ concentration. Composition of nutrient solution modified from Lin and Lay (2004) as recommended by Thanwised, Wirojanagud and Reungsang (2012). The Plackett-Burman experimental design based on the first-order model followed Plackett and Burman (1946) Eq. 2:

$$Y= \beta_o+ \sum\beta_i X_i \tag{2}$$

where, Y was the response (hydrogen production); β_o the model intercept; β_i the linear coefficient, and; x_i the level of the independent variable. The initial pH (X_1), nutrient addition (X_2), iron (II) sulphate ($FeSO_4$) (X_3) and sodium bicarbonate ($NaHCO_3$) (X_4) were examined to determine if they had any effect on hydrogen production and/or COD removal. Based on the Plackett–Burman Design, each factor was prepared in two levels: -1 for low levels and +1 for high levels (Table 1). A centre point was run to evaluate the linear and curvature effects of the variables (Plackett & Burman, 1946). In the present study, four assigned variables were screened in 12 experimental runs in addition to three runs at their centre points. Hydrogen production was carried out in triplicate and the average value was used to represent the response. The factors significant at the 95% level ($P<0.05$) were considered to have a significant effect on hydrogen production and COD removal.

Table 1
Regression Coefficient, Estimated Effect and Corresponding F and P Values

Table 1A
H₂ Production

Code	Variable	Unit	Low Level (-1)	High Level (+1)	Coefficient Low	Coefficient High	Effect (E_{xi}) Low	Effect (E_{xi}) High	F-value Low	F-value High	P-value Prob>F Low	High
X_1	Initial pH	-	5	7	39.64	49.42	79.28	98.83	13.17	15.9979	0.0084	0.0052
X_2	Nutrient	ml/L	1	10	-11.32	-17.44	-22.63	-34.89	1.07	1.9937	0.3346	0.2008
X_3	FeSO₄	g/L	0.5	1	17.94	27.48	35.88	54.96	2.70	4.9478	0.1445	0.0615
X_4	NaHCO₃	g/L	1	5	-4.51	-4.40	-9.01	-8.79	0.17	0.1267	0.6923	0.7324

Table 1B
COD Removal

Code	Variable	Unit	Low Level (-1)	High Level (+1)	Coefficient Low	Coefficient High	Effect (E_{xi}) Low	Effect (E_{xi}) High	F-value Low	F-value High	P-value Prob>F Low	High
X_1	Initial pH	-	5	7	2.78	2.68	5.56	5.37	15.49	13.8836	0.0056	0.0074
X_2	Nutrient	ml/L	1	10	-0.67	-0.40	-1.34	-0.79	0.89	0.3025	0.3758	0.5994
X_3	FeSO₄	g/L	0.5	1	2.24	1.33	4.48	2.66	10.05	3.4146	0.0157	0.1071
X_4	NaHCO₃	g/L	1	5	-1.29	-0.92	-2.59	-1.83	3.35	1.6146	0.1099	0.2444

Note. Low = low inoculum = 3,750 mg-VSS/L and High = high inoculum = 7,500 mg-VSS/L

The effect of each variable was determined as per Eq. [3] and a tool for statistical analysis by Saraphirom and Reungsang (2010):

$$E_{(Xi)} = 2(\Sigma M_{i+} - M_{i-})/N \qquad [3]$$

where, E_{Xi} was the concentration effect of the tested variable; M_{i+} and M_{i-} were P_s from runs where the variable (X_i) measured was present at the high and low concentration, respectively, and; N was the number of runs (12).

Comparison of Inoculums Content

SPSS version 22 was used to calculate the independent samples t-test for 12 trials so as to evaluate the varying significance levels of inoculums content on COD removal and H_2 production (Table 2).

Table 2
Observed and Predicted H$_2$ Production and COD Removal

| Run | X_1 | X_2 | X_3 | X_4 | H$_2$ Production (mL H$_2$/L) | | | | COD removal (%) | | | |
| # | Initial pH | Nutrient | FeSO$_4$ | NaHCO$_3$ | Low inoculum | | High inoculum | | Low inoculum | | High inoculum | |
		(ml/L)	(g/L)	(g/L)	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted
1	7	1	0.5	1	241.35±1.71	191.94±2.07	351.06±1.57	299.84±1.80	50.00±0.62	48.84±0.59	53.88±0.35	53.21±0.33
2	5	10	0.5	5	113.14±1.11	81.02±1.13	201.01±1.34	157.32±1.43	40.87±0.81	39.36±0.79	46.83±0.85	45.21±0.83
3	7	10	0.5	5	126.19±1.18	160.30±1.22	230.70±0.78	256.15±0.75	43.64±0.68	44.92±0.65	50.00±0.31	50.58±0.28
4	5	1	0.5	1	88.50±0.84	112.66±0.82	171.75±0.90	201.01±0.88	40.79±0.33	43.28±0.43	45.30±0.33	47.84±0.43
5	7	1	1.0	5	181.63±1.29	218.81±1.36	311.35±1.06	346.01±1.07	46.83±0.19	50.73±0.16	50.00±0.12	54.04±0.27
6	7	10	1.0	1	205.70±0.02	205.19±0.02	334.61±0.45	319.91±0.42	51.85±0.07	51.98±0.06	55.56±0.25	55.08±0.23
7	5	10	1.0	1	147.99±0.76	125.91±0.74	253.09±0.98	221.08±0.98	46.34±0.04	46.42±0.04	49.59±0.06	49.71±0.06
8	5	1	1.0	1	121.26±0.94	148.54±0.94	229.75±0.80	255.97±0.78	48.78±0.55	47.76±0.52	52.03±0.80	50.50±0.78
9	5	10	1.0	5	116.85±0.00	116.90±0.00	189.81±0.69	212.29±0.66	43.29±0.29	43.84±0.27	46.51±0.72	47.88±0.69
10	5	1	0.5	5	100.94±0.09	103.65±0.09	194.47±0.07	192.21±0.06	41.27±0.31	40.69±0.29	46.88±0.46	46.01±0.43
11	7	10	0.5	1	148.75±0.71	169.31±0.68	222.49±1.30	264.95±1.38	48.02±0.27	47.50±0.26	52.38±0.02	52.41±0.02
12	7	1	1.0	5	260.73±1.45	218.81±1.61	382.66±1.12	346.01±1.15	54.37±0.95	50.73±0.66	57.54±0.84	54.04±0.36
Determination coefficient (R^2)					0.97		0.96		0.98		0.98	
Adjusted determination coefficient (Adjusted R^2)					0.95		0.96		0.97		0.97	

Note. Low inoculum = 3,750 mg-VSS/L and high inoculum = 7,500 mg-VSS/L

Path of Steepest Ascent

This step was used to determine the optimum level for the main variable. In the current study, this was done by increasing the initial pH (from 1 to 11) and $FeSO_4$ concentrations (from 1.0 to 3.0 g/L) based on the high level (as per positive signs, Table 1) for improving COD removal and H_2 production potential.

RESULTS AND DISCUSSION

Diagnostic Checking of the Fitted Model

The multiple regression analysis was applied to the data in Table 2 and the attained second-order polynomial equation could well explain the COD removal and hydrogen production as per Eq. [4] to [7]:

$$Y_1 = 50.54 + 2.68\ X_1 - 0.40\ X_2 + 1.33\ X_3 - 0.92 X_4 \tag{4}$$

$$Y_2 = 46.34 + 2.78 X_1 - 0.67 X_2 + 2.24 X_3 - 1.29 X_4 \tag{5}$$

$$Y_3 = 256.06 + 49.42\ X_1 - 17.44\ X_2 + 27.48\ X_3 - 4.40\ X_4 \tag{6}$$

$$Y_4 = +154.42 + 39.64\ X_1 - 11.32\ X_2 + 17.94\ X_3 - 4.51\ X_4 \tag{7}$$

where, Y_1 and Y_2 were the predicted COD removal; Y_3 and Y_4 were the predicted H_2 production of high and low inocula content, and; X_1, X_2, X_3 and X_1 were the coded values of initial pH, nutrient, $FeSO_4$ and $NaHCO_3$, respectively.

The R^2 value of 0.97, 0.96 and 0.98 (Table 2) indicated good agreement between the experimental and predicted values and implied that the mathematical model predicted the hydrogen production rate (Saraphirom & Reungsang, 2010; Zhang, Liu, & Shen, 2005), while a high value of the adjusted determination coefficient of 0.95, 0.96 and 0.97 suggested the significance of the model (Saraphirom & Reungsang, 2010).

Effect of Main Variables on COD Removal and H_2 Production

The P-value (Table 1) indicated the relative importance of the initial pH, nutrient addition, $FeSO_4$ and $NaHCO_3$ concentration on COD removal and H_2 production. The P-value of the initial pH (both low and high inoculums content) was less than 0.05 ($P < 0.05$) (Table 1A); this means that initial pH had a significant effect on H_2 production. This was not surprising since pH is the most important factor in hydrogen production due to its effects on Fe-hydrogenase activity, metabolic pathways and the duration of the lag phase (Liu & Shen, 2004).

Table 1B shows that a P-value for both the initial pH and $FeSO_4$ was less than 0.05 ($P<0.05$), indicating that both variables had a significant effect on COD removal. The effect sign was positive, meaning that the influence of initial pH and $FeSO_4$ on COD removal and H_2 production was greater at the high level. Iron is an important factor for biohydrogen production (Saraphirom & Reungsang, 2010; Zhang, Liu, & Shen, 2005), as microorganisms degrade organic substrates for energy (electrons) (i.e. COD removal), which need to be disposed of in order to maintain electrical neutrality. In anoxic environments, protons can act as electron acceptors to produce molecular H_2 in the presence of hydrogenase enzyme. These two variables were therefore selected for the next path of steepest ascent. Observed and predicted H_2-production and COD removal is recorded in Table 2.

Comparative of Inocula Content

Both COD removal and H_2 production at high inoculums content were greater than at low inoculums content (P=0.022 and 0.001, respectively) (Table 3). Hence, a higher inoculums content was seen to have provided greater microbial activity, leading to increased COD removal and H_2 production. Previous research demonstrated substantially improved performance and stability of an anaerobic reactor by inoculums (O-Thong, Prasertsan, Intrasungkha, Dhamwichukorn, & Birkeland, 2008; Zheng & Yu , 2005). The next steepest ascent experiment should inoculate at 7,500 mgVSS/L. The COD content at low vs. high inoculums content is presented in Figure 1. The respective COD for low vs. high inoculums content was decreased from the initial 9,277±414 mg/L to 4,968±332 mg/L and 4,670±434 mg/L after 120 hours.

Table 3

Independent-Sample t-Test for H_2 Production and COD Removal

Parameter	Inoculum	Mean	Standard Deviation	Sig. (2-tailed)
H_2 Production (mL H2/L)	Low	154.42	6.02	0.001
	High	256.06	7.76	
$COD_{removal}$ (%)	Low	46.34	4.47	0.022
	High	50.54	3.85	

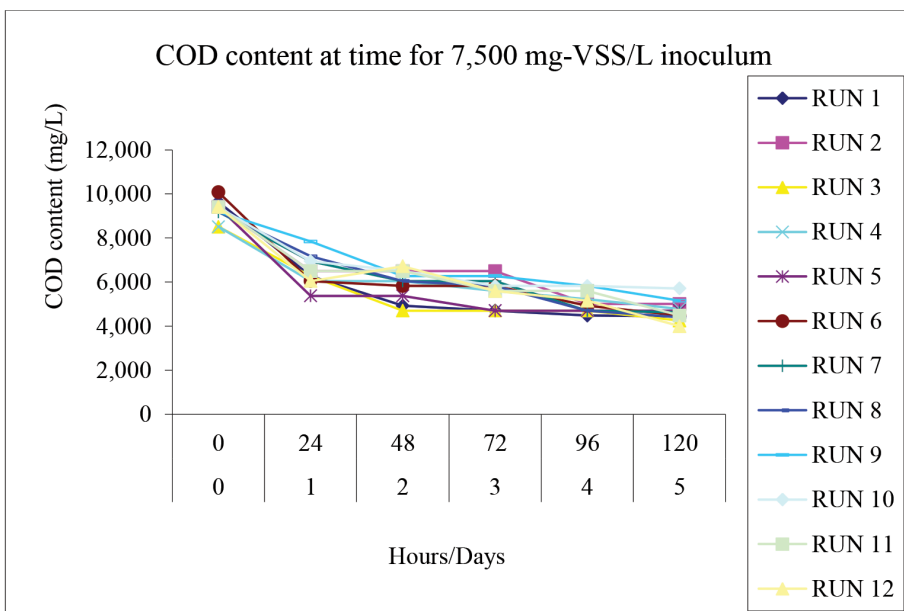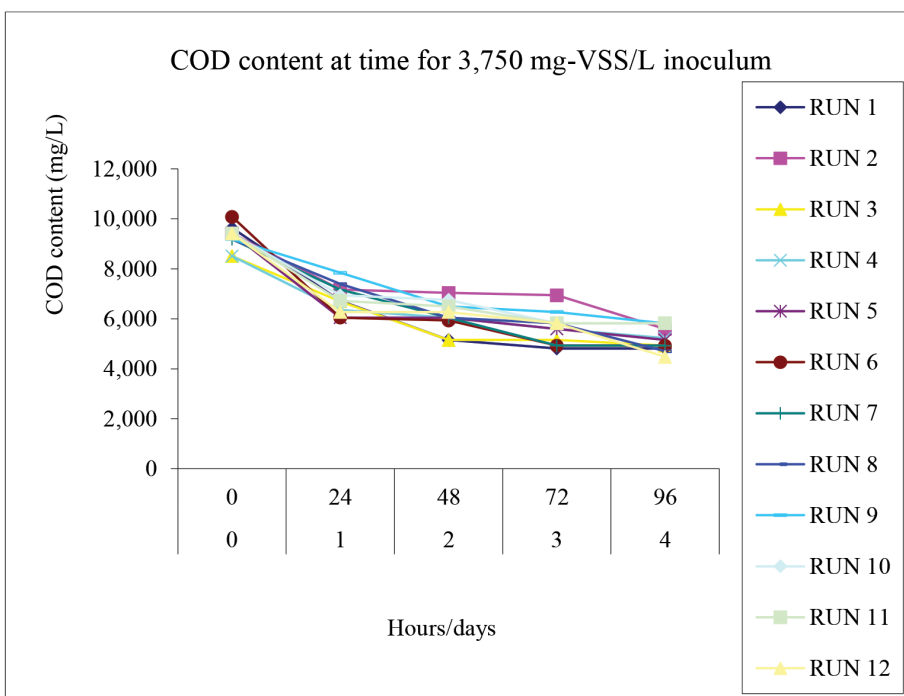*Note*. Low inoculum = 3,750 mg-VSS/L and high inoculum = 7,500 mg-VSS/L

Figure 1. COD content at low and high inoculums content

The Path of Steepest Ascent

The results indicated that Run 4, with an initial pH of 10 and $FeSO_4$ of 2.5 g/L (Table 4), yielded the greatest H_2 production potential (443.37 mL H_2/L) and COD removal (61.54 %). A higher hydrogen production rate occurred at a higher initial pH because the latter was sufficient to rapidly buffer the acid production accompanying hydrogen production so that hydrogen production was not inhibited (Li et al., 2008). The *in vivo* activity of hydrogenase in fermentative bacteria was found to decrease with a reduction in Fe. Hydrogen was evolved as the final product of reductant disposal from hydrogenase or nitrogenase activity in which the primary electron donor for both enzymes was ferredoxin (Saraphirom & Reungsang, 2010).

Table 4

H_2 Production and Percentage of COD Removal at Steepest Ascent

Trials	Initial pH	$FeSO_4$ (g/L)	P_{s1} (mL H_2/L)	P_{s2} (% COD removal)
1	7	1.0	119.09	38.46
2	8	1.5	128.00	42.86
3	9	2.0	258.03	50.00
4	10	2.5	443.37	61.54
5	11	3	47.25	16.67

CONCLUSION

Two significant variables affecting COD removal and H_2 production by anaerobic mixed cultures from tapioca wastewater (i.e. $FeSO_4$ and initial pH) were selected though experiments using the Plackett-Bruman Design. COD removal and H_2 production of 7,500 mgVSS/L inoculums content were significantly greater than 3,750 mgVSS/L ($P < 0.05$). An initial pH of 10 and $FeSO_4$ concentration of 2.5 g/L resulted in the maximum H_2 production potential (443.37 mL H_2/L) and COD removal (61.54 %). The next optimisation of COD removal and H_2 production by anaerobic mixed cultures from tapioca wastewater should use $FeSO_4$ and initial pH variables with 7,500 mgVSS/L inoculums content.

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