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Optimization of Media Compositions for Hydrogen Production from Newspaper Hydrolysate by Anaerobic Mixed Cultures in Elephant Dung

องค์ประกอบอาหารที่เหมาะสมเพื่อผลิตไฮโดรเจนจากไฮโดรไลเสทกระดาษหนังสือพิมพ์โดยกลุ่มจุลินทรีย์ไม่ใช้อากาศในมูลช้าง

Ontan Attajak (อ่อนตาล อัฒจักร)* Dr.Khanittha Fiala (ดร.ขนิษฐา เฟียล่า)**,***

ABSTRACT

The Plackett-Burman method was used to screen the variables that affect the hydrogen production from newspaper hydrolysate by anaerobic mixed cultures in elephant dung. The media compositions in this study including $FeSO_4$, $NiCl_2{\cdot}6H_2O$, $MgCl_2{\cdot}6H_2O$, $CaCl_2{\cdot}6H_2O$, $NaHCO_3$ and N in Aji-L. Two significant variables affecting hydrogen production including $FeSO_4$ and $NiCl_2{\cdot}6H_2O$ were further optimized by response surface methodology (RSM) with central composite design (CCD). The optimization of media compositions was 39.32 mg/L of $FeSO_4$ and 0.08 mg/L of $NiCl_2{\cdot}6H_2O$ with hydrogen production potential of 1,683 mLH_2/L and hydrogen yield of 472 mmolH_2/mol substrate consumed. The results suggest that newspaper hydrolysate can be used for hydrogen production.

บทคัดย่อ

การออกแบบการทดลองโดยวิธี Plackett-Burman ถูกนำมาใช้ในการคัดกรองปัจจัยที่มีผลต่อการผลิตไฮโดรเจนจากไฮโดรไลเสทกระดาษหนังสือพิมพ์โดยกลุ่มจุลินทรีย์ไม่ใช้อากาศในมูลช้าง องค์ประกอบอาหารที่ศึกษาได้แก่ $FeSO_4$, $NiCl_2{\cdot}6H_2O$, $MgCl_2{\cdot}6H_2O$, $CaCl_2{\cdot}6H_2O$, $NaHCO_3$ และ N ใน Aji-L โดยพบว่ามีสองปัจจัยที่มีผลต่อการผลิตไฮโดรเจนอย่างมีนัยสำคัญ ได้แก่ $FeSO_4$ และ $NiCl_2{\cdot}6H_2O$ ซึ่งทั้งสองปัจจัยนี้ได้ถูกนำไปศึกษาสภาวะที่เหมาะสมโดยใช้การออกแบบการทดลองวิธีพื้นผิวตอบสนองร่วมกับการออกแบบการทดลองแบบส่วนประสมกลาง โดยองค์ประกอบอาหารที่เหมาะสมในการผลิตไฮโดรเจน คือ $FeSO_4$ 39.32 มิลลิกรัมต่อลิตร และ $NiCl_2{\cdot}6H_2O$ 0.08 มิลลิกรัมต่อลิตร ได้ปริมาณไฮโดรเจน 1,683 มิลลิลิตรไฮโดรเจนต่อลิตร และได้ผลได้ 472 มิลลิโมลไฮโดรเจนต่อโมลสารตั้งต้นที่ใช้ ผลการทดลองแสดงให้เห็นว่า ไฮโดรไลเสทกระดาษหนังสือพิมพ์สามารถใช้ผลิตไฮโดรเจนได้

Key Words: Hydrogen production, Newspaper hydrolysate, Elephant dung

คำสำคัญ: การผลิตไฮโดรเจน ไฮโดรไลเสทกระดาษหนังสือพิมพ์ มูลช้าง

**Student, Master of Science Program in Biotechnology, Faculty of Technology, Khon Kaen University*

***Assistant Professor, Department of Biotechnology, Faculty of Technology, Khon Kaen University*

****Assistant Professor, Fermentation Research Center for Value Added Agricultural Products (FerVAAP), Faculty of Technology, Khon Kaen University*

Introduction

Environmental pollution due to the use of fossil fuels and their shortage require the search for alternative energy sources that are environmentally friendly and renewable. Hydrogen satisfies the above requirements because it produces only water after the combustion with oxygen (Wang and Wan, 2008). Hydrogen has a high energy yield of 122 kJ/g, which is 2.75 times greater than fuel from hydrocarbon (Kapolan and Kargi, 2006). Hydrogen can be produced via several methods, including electrolysis of water, thermo-catalytic reformation of hydrogen and biological processes (Kim et al., 2009).

Mixed cultures from various natural sources, such as compost (Cheong and Hansen, 2006), activated sludge (Kim et al., 2009), cow dung (Fan et al., 2006) and cattle manures (Gilroyed et al., 2008), have been used as an inoculum for hydrogen production by dark fermentation. In this study, the mixed cultures presence in the elephant dung are of interest to be used as the seed inoculums for biohydrogen production from newspaper hydrolysate. Elephant dung is expected to be abundant with cellulase producing microorganisms due to the elephant's diet which is mainly plant materials (Fangkum and Reungsang, 2011).

Composition of media is one of the most important environmental factors affecting the hydrogen production. Nutrients, micronutrients and vitamins in the media are necessary for the growth of microorganisms and the fermentation of hydrogen (Angelidaki and Sanders, 2004). Nitrogen are most abundant after carbon and major element in nucleic acids and amino acids. The microelements such as iron and nickel are important for cytochromes and these are usually necessary for specific enzymes. Magnesium stabilizes ribosomes, cell membranes and nucleic acids. Calcium is important to stabilize the bacterial cell wall and is important for stabilizing endospores (Madigan et al., 2000). $NaHCO_3$ functions as a buffer preventing the inhibition effect on hydrogen producers caused by a rapid drop of pH due to volatile fatty acids (VFAs) accumulation in the fermentation broth during hydrogen fermentation (Khamtib et al., 2011; Oh et al., 2003). In this study, a response surface methodology (RSM) was used to design the experiments and to evaluate the significance of independent variables, and simultaneously examining the optimum conditions for the desired response (Fan et al., 2006; Gilroyed et al., 2008). Therefore, the aim of this research was to optimize the compositions of media for hydrogen production from newspaper hydrolysate by anaerobic mixed cultures in elephant dung using RSM

Objective

To optimize the media compositions for hydrogen production from newspaper hydrolysate by anaerobic mixed cultures in elephant dung using statistical method.

Methodology

Materials

Waste newspaper was collected from Statistical Office, Buriram province, Thailand. The cellulose content in the waste newspaper was 40-55% (Sun and Cheng, 2002).

Elephant dung was collected from elephant village, Surin province, Thailand

Optimization of media compositions

1. Plackette-Burman Design (PBD)

PBD was used to screen the important factors that significantly affect hydrogen production. The investigated parameters were $FeSO_4$, $NiCl_2{\cdot}6H_2O$, $MgCl_2{\cdot}6H_2O$, $CaCl_2{\cdot}6H_2O$, $NaHCO_3$ and N in Aji-L. Levels of each factor used in the experimental design and the design matrix were shown in Tables 1 and 2, respectively. PBD is based on the first order polynomial model:

$$Y = \beta_0 + \Sigma\, \beta_i X_i \qquad (1)$$

where Y is response (hydrogen production potential: P_s), β_0 is the model intercept, β_i is the linear coefficient and X_i is the level of the independent variable. The variables were examined at two levels: -1 for low level and +1 for high level. The factors that were significant at the 95% level ($P \leq 0.05$) were considered to have a significant effect on P_s and were used in the optimization step by central composite design (CCD)

Table 1 Level of variables, estimated effect, coefficient, and corresponding F and P values for P_s in the Plackett-Burman Design.

Variable (mg/L)	Low level (-1)	High level (+1)	Coefficient	Effect (E_{Xi})	F-value	P-value Prob > F
N in Aji-L	1000	3000	67.25	134	0.89	0.3889
$NiCl_2{\cdot}6H_2O$	0.1	2.5	-306.65	-613	18.50	0.0077
$MgCl_2{\cdot}6H_2O$	100	200	-89.50	-179	1.58	0.2648
$FeSO_4$	50	200	-266.04	-532	13.92	0.0136
$CaCl_2{\cdot}6H_2O$	400	1000	-56.46	-113	0.63	0.4643
$NaHCO_3$	2000	6000	15.66	31	0.048	0.8348

Table 2 Plackett-Burman experimental design matrix with P_s from newspaper hydrolysate by anaerobic mixed cultures.

Run	P_s (mLH_2/L)	
	Observed	Predicted
1	1712	1479
2	857	978
3	858	605
4	718	790
5	965	1218
6	1493	1501
7	779	821
8	849	1032
9	1155	934
10	47	155
11	470	387
12	217	221

Table 3 CCD experimental design with two independent variables and results of P_s.

Run	$NiCl_2{\cdot}6H_2O$ (mg/L)		$FeSO_4$ (mg/L)		P_s (mLH_2/L)	
	X_1	Code X_1	X_2	Code X_2	Observed	Predicted
1	0.08	0	40	0	1901	1700
2	0.08	0	40	0	1761	1700
3	0.08	0	40	0	1854	1700
4	0.04	-2	40	0	413	388
5	0.12	+2	40	0	593	290
6	0.06	-1	35	-1	1227	1300
7	0.10	+1	35	-1	493	843
8	0.08	0	50	+2	314	32
9	0.06	-1	45	+1	413	720
10	0.10	+1	45	+1	494	1079
11	0.08	0	40	0	1825	1700
12	0.08	0	40	0	1815	1700
13	0.08	0	30	-2	423	376

2. Central Composite Design (CCD)

CCD was used to optimize the effect of each significant variable on P_s. The levels of the variables and the experimental design are shown in Table 3. The response i.e., P_s was fitted using a predicted polynomial quadratic equation in order to correlate the response variable to the independent variables. The general form of the predictive polynomial quadratic equation:

$$Y = ß_0 + \Sigma ß_i X_i + ß_{ii} X_i^2 + \Sigma ß_{ij} X_i X_j \quad (2)$$

where Y is the predicted response (P_s), $ß_0$ is a constant, $ß_i$ is the linear coefficient, $ß_{ii}$ is the squared coefficient, $ß_{ij}$ is the interaction coefficient, and X_i is the variable. The statistical software Design-Expert (Demo version 7.0, Stat-Ease Inc., Minneapolis, MN, USA) was used for regression and graphical analysis of the experimental data. The P_s (mLH_2/L) was calculated by dividing the amount of hydrogen production (mLH_2) by the volume of substrate (L).

Biohydrogen production

Biohydrogen production from the hydrolysate was conducted in 120-mL serum bottles with a working volume of 70 mL comprising of 21 mL of inoculums (equivalent to 70.32 mg of inoculum as measured by volatile suspended solid (VSS)), and 49 mL of newspaper hydrolysate. The fermentation medium contained newspaper hydrolysate at a reducing sugar concentration of 22.45 g/L, $FeSO_4$, $NiCl_2{\cdot}6H_2O$, $MgCl_2$, $CaCl_2$, $NaHCO_3$, and N in Aji-L (by product of monosodium glutamate process). The concentrations of $FeSO_4$, $NiCl_2$, $MgCl_2{\cdot}6H_2O$, $CaCl_2{\cdot}6H_2O$, $NaHCO_3$ and N in Aji-L were set according to the design (Tables 1 and 3). The medium was adjusted to the initial pH of 6.5. The serum bottles were sealed and capped with rubber stopper and aluminum cap then flushed with nitrogen gas for 5 min in order to create anaerobic condition. During the incubation time, the volume of biogas was measured by using an appropriate size wetted glass tight syringe. At the end of the fermentation, a liquid sample was taken for the analysis concentrations of sugar, and volatile fatty acids (VFAs) by HPLC.

Analytical methods

Biogas was analyzed by a gas chromatograph (GC-17A Shimadzu, Japan) equipped with a thermal conductivity detector (TCD) and a Molecular Sieve 5A column (3 mm x 3 m). The operating temperature of the injection port, column oven and the detector were 100 50 and 100 °C, respectively. Helium was used as the carrier gas at a flow rate of 20 mL/min. The volatile fatty acid (VFAs) in the liquids were analyzed by HPLC (HPLC LC-10AD Shimadzu, Japan) equipped with a ultraviolet (UV) (210 nm) and sugar concentration was determined by a HPLC (HPLC LC-10AD Shimadzu, Japan) with a Refractive Index Detector (RID) and aminex HPX-87H Ion Exclusion (300 mm x 7.8 mm) column. The column oven temperature was 40 °C and 5 mM was used as a mobile phase at the flow rate of 0.6 mL/min.

Kinetic analysis

The cumulative volume of hydrogen produced in the batch experiments is described by following the modified Gompertz equation (Zwietering et al., 1990)

$$H = P\exp\left\{-\exp\left[\frac{R_m e}{P}(\lambda - t) + 1\right]\right\} \quad (3)$$

where H is the cumulative hydrogen production (mL), λ is the lag time (h), P is the hydrogen production potential (mL), R_m is the maximum hydrogen production rate (mL/h), t is incubation time (h) and e is 2.718281828.

Results and discussion

Screening of significant variables for hydrogen production by anaerobic mixed culture in elephant dung.

Table 1 shows the main effect of each variable for hydrogen production using Plackett–Burman. The main effect of each variable upon P_s was estimated as the difference between both averages of measurements made at the high level (+1) and at the low level (-1) of that factor. If the sign of the effect E_{xi} of the tested variable is positive, the influence of the variable on P_s is greater at a high level; if it is negative, the influence of the variable is greater at a low level. From the results analysis in Table 1, indicated that low level of X_2 ($NiCl_2 \cdot 6H_2O$), X_3 ($MgCl_2 \cdot 6H_2O$), X_4 ($FeSO_4$) and X_5 ($CaCl_2 \cdot 6H_2O$) enhanced hydrogen production, respectively, and that high level of X_1 (N in Aji-L) and X_6 ($NaHCO_3$) resulted in high P_s. The probability value of less than 0.05 was found with X_2 ($P<0.0077$) and X_4 ($P<0.0136$) (Table 1), which suggested a significant effect of these variable on hydrogen production. The Plackett–Burman design proved to be a powerful tool for screening significant process parameters. The optimal levels of the individual factors are still unknown but can be determined by the following optimization steps. Therefore, two variable were selected for future optimization experiment through RSM.

Optimization of factor concentration for hydrogen production from newspaper hydrolysate by anaerobic mixed culture in elephant dung.

The significant independent variables, including $NiCl_2 \cdot 6H_2O$ and $FeSO_4$ were further explored using RSM with CCD. Table 3 Hydrogen ranged from 314-1,901 mLH_2/L, represents the design matrix of the variables together with the experimental results. Experiments 4-10 and 13 were under different conditions and those from 1-3 and 11-12 were under the same conditions. The multiple regression analysis was applied on the data, the following second-order polynomial equation was found to explain the hydrogen production:

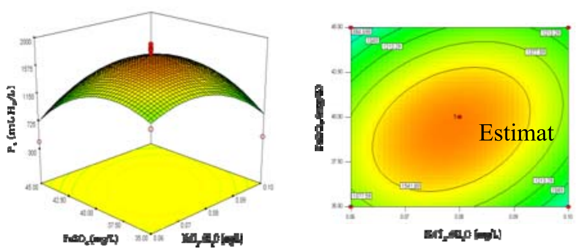
$$Y = 1699.60 - 24.53X_1 - 85.98X_2 + 203.58X_1X_2 - 340.28X_1^2 - 373.90X_2^2 \quad (4)$$

where, Y is the predicted hydrogen production potential; X_1 and X_2 are the coded values of $NiCl_2 \cdot 6H_2O$ and $FeSO_4$, respectively.

The analysis of variance (ANOVA), Table 4 shows the model F-value 8.08 ($P<0.05$) indicated the model is significant, the R^2 value of 0.8523 indicates a good agreement between experimental and predicted values and implies that the mathematical model is very reliable for hydrogen production. Fig. 1 shows the response surface plot and corresponding contour curves based on independent variables $NiCl_2 \cdot 6H_2O$ (X_1) and $FeSO_4$ (X_2). A significant increase on P_s could be achieved when the value of $FeSO_4$ and $NiCl_2 \cdot 6H_2O$ increased. Further increase in $FeSO_4$ and $NiCl_2 \cdot 6H_2O$ caused P_s to decrease slightly, which suggesting that Fe^{2+} and Ni^{2+} are important elements formation of hydrogenase or fundamental enzymes in hydrogen production. Excessive amount of $FeSO_4$ and $NiCl_2 \cdot 6H_2O$ may affect activity of hydrogenase (Junelles et al., 1998; Vallee and Ulmer, 1972). Based on the response surface analysis from Eq. (4), we found the optimal conditions of the model, which was 39.32 mg/L of $FeSO_4$ and 0.08 mg/L of $NiCl_2 \cdot 6H_2O$. In order to confirm the predicted results of the model, the repeated experiments under optimal conditions were carried out and a value of 1,683 mLH_2/L was obtained. The good correlation between these two results verifies the model validation and the existence of an optimal condition.

Table 4 ANOVA for response surface quadratic model

Source	Su of Squares	df	Mean Squares	F-value	P-value
Model	4.831E+006	5	9.662E+005	8.08	0.0080
X_1-$NiCl_2 6H_2O$	7217.71	1	7217.71	0.060	0.8130
X_2-$FeSO_4$	88700.41	1	88700.41	0.74	0.4177
X_1X_2	1.658E+005	1	1.658E+005	1.39	0.2775
X_1^2	2.653E+006	1	2.653E+006	22.18	0.0022
X_2^2	3.203E+006	1	3.203E+006	26.78	0.0013
Residual	8.372E+005	7	1.196E+005		
Lack of Fit	8.266E+005	3	2.755E+005	103.77	0.0003
Pure Error	10620.80	4	2655.20		
Cor Total	5.668E+006	12			



a) b)

Figure 1 a) The response surface plot and b) Corresponding contour plot showing the effects of $NiCl_2 \cdot 6H_2O$ and $FeSO_4$

Conclusions

Two significant variables affecting hydrogen production from newspapre hydrolysate by anaerobic mixed culture, $NiCl_2 \cdot 6H_2O$ and $FeSO_4$ were selected by the Plackett-Burman design experiment. These variable were optimized by RSM with CCD. The optimum condition obtained was 39.32 mg/L of $FeSO_4$ and 0.08 mg/L of $NiCl_2 \cdot 6H_2O$ with hydrogen production potential of 1,683 mLH_2/L and hydrogen yield of 472 $mmolH_2$/mol substrate consumed. The results indicated that microorganisms in the elephant dung could effectively convert the newspaper hydrolysate into hydrogen.

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