Optimization of hydrogen production from agricultural wastes using mixture design

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Abstract: Hydrogen production from food waste, cattle manure, potato pulp and pig manure was optimized through using mixture design in this study. The synergic and antagonistic effects of the four substrates on hydrogen yield, substrate conversion efficiency and pH were evaluated. The results showed that the optimal proportion of food waste, cattle manure, potato pulp and pig manure were 61.6%, 38.4%, 0, and 0, respectively. Under the optimal condition, hydrogen yield of 21.0 mL/g VS with VS reduction of 29.4% and pH of 5 could be obtained. The interaction between food waste and cattle manure had strongest synergistic effects. Hydrogen was mainly produced by acetic-butyric metabolic pathway, and ammonification of protein played an important role in the maintenance of pH.

Keywords: hydrogen, biohydrogen production, agricultural waste, dark fermentation, mixture design

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1 Introduction

Biohydrogen production from dark fermentation of renewable organic matters has been considered as one of the most promising solutions to both energy crisis and environmental pollution challenges[1-4].

Many previous studies have demonstrated that the dark fermentation process is influenced by many factors, such as temperature, influent substrate concentration, reactor configuration, pH and nutritional requirements[5,6]. Among these factors, pH has been found to play a most critical role in microbial activity regulation. The pH of 5 is often considered optimal for biohydrogen production[7-9]. Usually on-line pH adjustment with addition of acid or base is proposed in lab; however, this approach is challenging in full scale application due to cost. According to previous reports, the nutrient composition has a significant influence on pH of hydrogen fermentation. To maintain the pH of dark fermentation process in suitable range, the co-fermentation of different type substrates may be an alternate approach. Studies have been conducted to evaluate the effect of two components co-fermentation on hydrogen production[10-13]. However, there was no comprehensive study available to understand the effect of composition on the dark fermentation process in order to optimize the response variables.

Mixture design is a special experiment design focusing on composition optimization for multi-component

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system. In a mixture experimental design, the total amount of substrates is held constant; the response depends on the relative proportions of the components in the mixture. It can be used to evaluate interactive effects of substrates and offers a great advantage over conventional one-factor-at-a-time methods[14-17].

Therefore, the main objective of this study was to explore the interactive effects of typical agricultural wastes such as food waste (FW), cattle manure (CM), potato pulp (PP) and pig manure (PM) on hydrogen production, and to optimize the composition of the substrates through using mixture design.

2 Materials and methods

2.1 Materials

CM and PM were collected from experimental farm of northeast agricultural university (NEAU), Harbin, China; FW and PP were collected from a dining hall at NEAU. Long grass and gravel were removed from CM and PM. Plastic cups, egg shell and animal bones were removed from the FW. The FW and PP were homogenized with an electronic blender (HR2006, Philips Inc., Zhuhai, China), and then the substrates were packed into zip-lock bags and stored at –20°C until use. The characteristics of the FW, PP, PM and CM are summarized in Table 1.

Table 1 Characteristics of substrates

Items	FW	PM	PP	CM
TS (% of fresh waste)	19.53±0.33	31.40±0.06	20.85±2.12	22.15±0.29
VS (% of fresh waste)	18.20±0.31	23.87± 0.05	16.46±0.56	18.21±0.28
pH	5.28±0.54	7.70±0.12	5.59±0.03	7.22±0.04
Protein (% of TS)	15.72±0.24	9.13±0	13.08±1.01	11.86±0.78
Lipid (% of TS)	29.38±0.13	10.53 ±0.16	0.08±0.09	2.38±0.29
Starch (% of TS)	25.51±0.53	1.67±0.14	37.19±0.74	0.51±0.11
Lignocellulose (% of TS)	13.16±0.12	35.07±0.17	15.61±0.09	51.35±0.11
Ash(% of TS)	6.23±0.23	23.62±0.13	14.04±2.35	17.90±0.94
C/N	21.98±0.22	16.92±0.19	26.50±0.35	18.81±0.18
Ammonia/mg N·L^{-1}	47.78±1.02	119.34±2.16	326.05±5.34	487.17±8.67
Alkalinit/mg $CaCO_3$·L^{-1}	225±31	6539±43	1725±22	5171±57

Note: FW, CM, PP and PM are the abbreviations of food waste, cattle manure, potato pulp and pig manure respectively.

Anaerobic digester sludge was obtained as seed sludge from local CH_4 fermentation pilot plant for treating cattle manure located in NEAU. To inactivate hydrogen-consuming bacteria, the inoculum was pretreated at 100°C in boiling water bath for 30 minimmediately before using[18].

2.2 Methods

TS, VS and pH were measured according to standard methods[19].

Total kjeldahl nitrogen (TKN) and ammonia nitrogen (AN) were analyzed by an automatic kjeldahl nitrogen analyzer (Kjeltec 2300, Foss Inc., Hagfors, Sweden) according to the instruction of manufacturer.

The measured biogas was adjusted to the standard condition of temperature (0°C) and pressure (760 mm Hg). The percentage of H_2, CH_4, CO_2 and N_2 was analyzed by a gas chromatograph (GC 6890N, Agilent Inc., Santa Clara, USA) equipped with TDX-01 column and a thermal conductivity detector (TCD). The injector temperature was not controlled. Oven and detector temperatures were 170°C and 220°C, respectively. Argon was used as carrier gas at a flow rate of 40 mL/min.

Both the VFAs (acetic acid, propionic acid, n-butyric acid, i-butyric acid, valeric acid and i-valeric acid) and ethanol were analyzed by the same GC equipped with infused-silica capillary column and a flame ionization detector (FID). The temperatures of the injector and detector were 220°C and 250°C, respectively. The oven temperature was initially kept at 60°C, and followed with a ramp of 15°C/min for 5.33 min, then held at final temperature of 140°C for 1.2 min. Nitrogen was used as the carrier gas with constant pressure of 187 kPa and makeup gas at 30 mL/min. Lactic acid was analyzed by a high performance liquid chromatograph (HPLC 600E-2487, WATERS Inc., Milford, USA) equipped with an ultraviolet (210 nm) detector and 250×4.6 mm C18 column using acetonitrile and phosphoric acid (volume ratio of 2.5/97.5) as mobile phase.

2.3 Experimental design

Mixture design is special class of response surface design where the response is considered a function only of the proportions of the components present in the mixture and not a function of the amount of the mixture[20]. The proportions of all the components must be nonnegative and sum to 100%. In general, linear, quadratic and special cubic models were used for analysis of the mixture design using Equations (1), (2) and (3).

Linear model: $$Y=\sum_{i=1}^{n}\beta_i x_i \quad (1)$$

Quadratic model: $$Y=\sum_{i=1}^{n}\beta_i x_i+\sum\sum_{i<j}^{n}\beta_{ij}x_i x_j \quad (2)$$

Special cubic model:

$$Y=\sum_{i=1}^{n}\beta_i x_i+\sum\sum_{i<j}^{n}\beta_{ij}x_i x_j+\sum\sum_{i<j<k}^{n}\beta_{ijk}x_i x_j x_k \quad (3)$$

where, Y presents the response to proportions of components. The linear term $\beta_i x_i$ represents the effect of linear combination of single component. The quadratic term $\beta_{ij}x_i x_j$ represents the synergic or antagonistic interaction between two components. The cubic term $\beta_{ijk}x_i x_j x_k$ accounts for the interaction effect of three components.

To evaluate mixture effects of the four substrates (CM, PM, FW and PP) on biohydrogen production, an augmented simplex lattice mixture design was used. The design consisted of 20 combinations including five replicates (Table 2).

Table 2 Four-component augmented simplex lattice mixture design matrix

Run	Point type	Mixture composition (% of substrate weight)			
		FW	PM	PP	CM
1	Vertex	100	0	0	0
2	Vertex	0	100	0	0
3	Vertex	0	0	100	0
4	Vertex	0	0	0	100
5	Vertex	100	0	0	0
6	Vertex	0	100	0	0
7	Vertex	0	0	100	0
8	Vertex	0	0	0	100
9	Edge centroid	50	50	0	0
10	Edge centroid	50	0	50	0
11	Edge centroid	50	0	0	50
12	Edge centroid	0	50	50	0
13	Edge centroid	0	50	0	50
14	Edge centroid	0	0	50	50
15	Check blend	62.5	12.5	12.5	12.5
16	Check blend	12.5	62.5	12.5	12.5
17	Check blend	12.5	12.5	62.5	12.5
18	Check blend	12.5	12.5	12.5	62.5
19	Overall centroid	25	25	25	25
20	Overall centroid	25	25	25	25

The response values were hydrogen yield (HY), mL/g Vs; VS reduction (VSR), % and pH. The HY and VSR were determined by Equations (4) and (5).

$$HY=\frac{\mathit{Cumulative\ hydrogen\ production\ volume}\ (\mathrm{mL})}{\mathit{weight\ of\ VS\ added}\ (\mathrm{g})} \quad (4)$$

$$VSR=\frac{VS_{initial}-VS_{final}}{VS_{initial}}\times 100\% \quad (5)$$

The TS of substrates were kept at 8% by diluting raw substrates with tap water before using. Batch experiments were performed using 500 mL erlenmeyer flask contain 80 g pretreated inoculum and 320 g substrate. These flasks were stripped with pure nitrogen gas and sealed off with rubber stoppers. These flasks were incubated at (35±1)°C in an orbital shaker with a speed of 120 r/min. The experiment lasted 136 h until hydrogen production of all flasks reached plateau. The biogas was collected by sample bags, and volume of biogas was measured by water displacing method at 12 h intervals. The statistical analysis was performed using the software Design Expert (6.0.10, Stat-Ease Inc., Minneapolis, USA).

3 Results and discussion

3.1 Model fitting and regression analysis

The liquid metabolite concentrations for all the mixtures were analyzed at the end of fermentation, and the HY, VSR, and pH, were also obtained.

As shown in Table 3, the liquid metabolites consist of ammonia, ethanol, lactic acid and VFAs (viz. acetic acid, propionic acid and n-butyric acid). Valeric acid, i-butyric acid and i-valeric acid were not detected in any of the runs.

For individual substrate, total amount of liquid metabolites varied from 525.15 mmol/L to 110.19 mmol/L according to substrate types. Sole food waste treatments (runs 1 and 5) obtained the largest total concentration of liquid metabolites production of 525.15 mmol/L. Lactic acid and ethanol were main products, indicating that the heterolactic fermentation described by Equation (6) was dominant metabolic pathway[21]. This zero-hydrogen-balance metabolic pathway is in good agreement with the low HY in food waste based runs.

$$C_6H_{12}O_6\rightarrow CH_3CH(OH)COOH+CH_3CH_2OH+CO_2 \quad (6)$$

In treatments with sole PP (runs 3 and 7), though less total amount of liquid products than FW was obtained, the distribution became more balanced. Ammonia, ethanol, lactic acid, acetic acid, and n-butyric acid made up the majority of liquid products, averaging 11.61%, 15.11%, 27.50%, 28.22% and 13.95%, respectively. Average HY of 27.35 mL/g VS was obtained with acetic acid and butyric acid as the dominant constituents of the VFAs. The molar ratio of acetic acid and butyric acid was approximately 2, showing that in addition to heterolactic fermentation, the acetic-butyric hydrogen fermentation pathway expressed by Equation (7) was also active metabolic pathway[22,23].

$$C_6H_{12}O_6 \rightarrow CH_3COOH + 1/2CH_3(CH_2)COOH + 3H_2 + 2CO_2 \quad (7)$$

The sole PM treatments (runs 2 and 4) produced 113.01 mmol/L liquid products in average, while for CM (runs 6 and 8) the value was 181.79 mmol/L. Both of them were obviously less than FW and PP. Ammonia, lactic acid and acetic acid were main constituents, and almost no hydrogen was produced. Compared with PM, the sole CM treatments produced equal amount of acetic acid and twice amount of ammonia and lactic acid.

Regarding to multi substrates treatments, most of the mixtures showed good performance of hydrogen production, and similar liquid metabolites distribution pattern to sole PP treatments. The highest HY of 36.7 mL/g VS was observed with a slight addition of FW, CM and PM to PP (run 17 with PP:FW:CM:PM= 50:10:10:10 on TS basis).

For exploring the synergistic or antagonistic effects of substrates on responses variables, all the response variables were fitted to linear, quadratic and special cubic models. To make the residuals be normally distributed with a constant variance, transformations y=log10 (y_{HY}+0.009) and y=log10 (y_{VSR}) were applied to response value of hydrogen yield and VS reduction respectively. The results of ANOVA on the experimental data were shown in Table 4.

After applying the criteria of adjusted R^2, predicted R^2, model p-value and lack of fit p-value, quadratic model was found to suitable for all of HY, VSR and pH. The regression coefficients for responses were shown in Table 5. The positive quadratic coefficients reflect synergistic effects for response, and negative quadratic coefficients mean antagonistic effects.

Table 3 Liquid metabolites and response variables

Run	Liquid metabolites/mmol·L^{-1}							Response variables		
	AN	Et	HLa	HAc	HPr	n-HBu	Total	HY/mL·(g VS)$^{-1}$	VSR/%	pH
1	17.8	224.3	270.3	12.5	0.3	0.0	525.2	0.1	12.8	3.8
2	37.5	3.4	33.6	32.6	1.4	1.7	110.2	0.0	3.7	6.7
3	40.3	59.5	102.7	103.3	12.2	53.0	371.0	30.5	26.9	4.6
4	71.0	1.0	62.9	31.3	9.7	2.3	178.2	0.1	2.9	6.5
5	18.2	172.9	280.3	19.4	5.6	0.8	497.1	0.1	11.4	3.8
6	38.6	7.9	34.6	29.6	3.5	1.6	115.8	0.0	3.6	6.6
7	42.6	48.5	93.7	98.3	13.6	46.6	343.2	24.2	24.9	4.8
8	70.4	2.1	62.9	34.8	9.1	6.1	185.4	0.0	2.3	6.5
9	22.7	126.0	87.6	1.9	3.3	59.2	300.7	8.3	14.4	5.2
10	26.7	149.8	223.7	20.2	0.2	2.2	422.8	0.1	6.0	4.1
11	59.6	25.7	86.6	72.0	1.3	50.1	295.3	21.3	23.3	5.3
12	26.7	42.2	117.9	106.3	2.3	51.8	347.2	16.7	17.0	4.8
13	52.8	6.9	49.3	40.5	0.2	2.6	152.3	0.0	3.8	6.5
14	40.3	54.9	120.4	98.1	1.6	62.6	377.9	16.0	25.9	5
15	27.3	198.2	53.7	3.1	2.4	59.6	344.2	7.9	23.1	4.6
16	28.4	0.8	62.3	62.5	0.6	21.6	176.3	0.5	9.6	5.5
17	35.8	109.8	82.2	93.3	3.1	76.3	400.5	36.7	31.9	4.7
18	58.5	1.7	95.2	62.5	5.8	21.6	245.3	0.3	18.2	5.7
19	35.2	126.5	93.1	64.7	9.4	54.7	383.5	25.8	28.1	5.1
20	38.6	99.0	96.1	65.9	6.5	57.9	363.9	25.2	27.4	5

Note: AN, Et, Hla, HAc, HPr and n-HBu are the abbreviations of ammonia nitrogen, ethanol, lactic acid, acetic acid, propionic acid and n-butyric acid respectively; total means sum of the liquid metabolites; HY and VSR are the abbreviations of hydrogen yield and VS reduction respectively.

Table 4 Models for pH, HY and VSR

Item	Source	R^2	Adjusted R^2	Predicted R^2	Model p-value	Lack of Fit p-value
Hydrogen yield[a]	Linear	0.410	0.299	0.123	0.0339	0.0006
	Quadratic	0.953	0.912	0.693	<0.0001	0.0517
	Special Cubic	0.985	0.954	–3.415	0.0951	0.0906
VS reduction[b]	Linear	0.653	0.588	0.453	0.0006	0.0001
	Quadratic	0.992	0.985	0.922	<0.0001	0.2357
	Special Cubic	0.997	0.989	0.538	0.2096	0.2997
pH	Linear	0.931	0.919	0.889	<0.0001	0.0003
	Quadratic	0.996	0.993	0.980	<0.0001	0.0746
	Special Cubic	0.999	0.997	0.919	0.0402	0.4033

Note: a: transformation of y=log10(*HY*+0.009) was applied; b: transformation of y=log10(*VSR*) was applied.

Table 5 Regression coefficients for quadratic models for hydrogen yield, VS reduction and pH

Item	Log10(*HY* + 0.01)		Log10(*VSR*)		pH	
	coefficient	p-value	coefficient	p-value	coefficient	p-value
FW	–0.89	<0.0001	1.07	<0.0001	3.79	<0.0001
PM	–1.77	<0.0001	0.39	<0.0001	6.62	<0.0001
PP	1.44	<0.0001	1.44	<0.0001	4.7	<0.0001
CM	–1.53	<0.0001	0.97	<0.0001	6.45	<0.0001
FW×PM	9.64	0.0002	1.78	<0.0001	–0.01	0.9681
FW×PP	–3.65	0.0517	0.26	0.1763	–0.42	0.2177
FW×CM	10.54	<0.0001	1.81	<0.0001	0.91	0.0174
PM×PP	6.24	0.0036	2.20	<0.0001	–3.54	<0.0001
PM×CM	–1.82	0.2957	–0.30	0.1230	–0.49	0.1549
PP×CM	5.42	0.0083	0.93	0.0004	–2.06	<0.0001

ANOVA is applied to determine the significance of the regression coefficients of the substrates. Smaller p-value, which indicates more significance for the corresponding coefficient, was preferred. Based on the results of ANOVA, the insignificant model terms with p-values greater than 0.05 were eliminated, the reduced models were presented by Equations (8), (9) and (10).

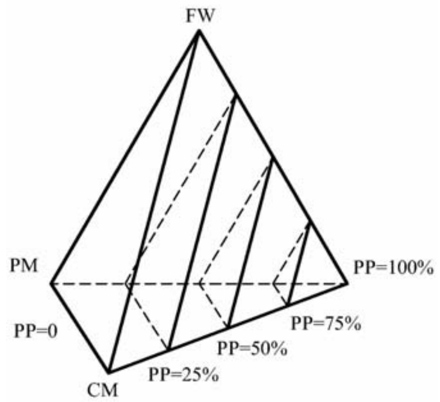
$$\log(Y_{HY}+0.99)=-0.88\times FW-1.87\times PM+1.45\times PP-1.63\times CM+9.62\times FW\times PM-3.83\times FW\times PP+10.52\times FW\times CM+6.225\times PM\times PP+5.40\times PP\times CM \tag{8}$$

$$\log(Y_{VSR})=1.09\times FW+0.37\times PM+1.46\times PP+0.96\times CM+1.77\times FW\times PM+1.81\times FW\times PP+2.20\times PM\times PP+0.93\times PP\times CM \tag{9}$$

$$Y_{pH}=3.77\times FW+6.60\times PM+4.67\times PP+6.42\times CM+0.90\times FW\times CM-3.55\times PM\times PP-2.07\times PP\times CM \tag{10}$$

3.2 Interpretation of contour plots

For clarifying the synergistic effects of four components on HY, VSR and pH, ternary contour plots should be drawn based on the fitted model Equations (8), (9) and (10). However, ternary contours for systems with four components graphically cannot be plotted in two dimensions. Thus one of the components must be fixed. As shown in Figure 1, the four-component tetrahedron used in present study was divided by fixing proportion of PP at 0, 25%, 50% and 75%.



Note: FW, CM, PP and PM are the abbreviations of the proportion of food waste, cattle manure, potato pulp and pig manure respectively.

Figure 1 Division of four-component tetrahedron

Effects of FW, PM, PP and CM on HY were showed in Figure 2. Compared to individual fermentation of FW or CM, the co-fermentation of CM and FW greatly enhanced hydrogen production (Figures 2a and 2b), HY of 21.3 mL/g VS was obtained with substrates ratio of

FW:CM:PP:PM=50:50:0:0. Though PM has similar characteristics to CM, its interaction with FW was weaker than CM. HY of only 8.3 mL/g VS was obtained with substrates ratio of FW: PM: PP: CM=50:50:0:0. With the increase of PP proportion (Figures 2c and 2d), the synergic effect of PP with CM and PM become more significant than other mixtures, and higher HY were observed.

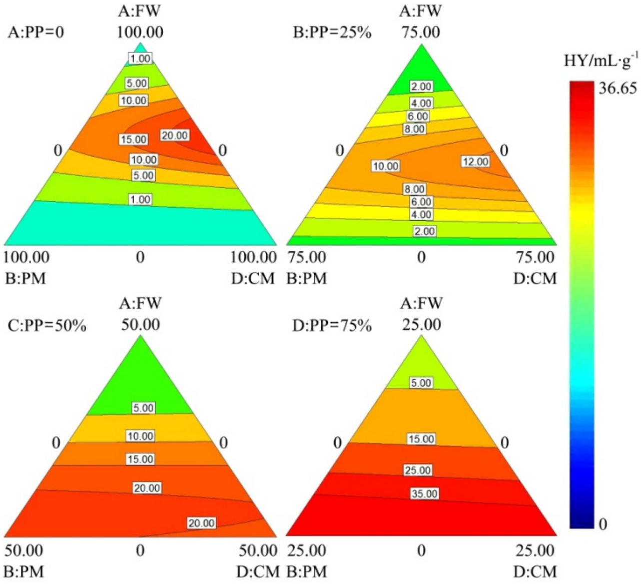


Figure 2 Contour plots for effects of FW, CM, PP and PM on hydrogen yield

Composition of the four components influences VSR in similar way with that of hydrogen yield (Figure 3). It was found that the most significant VSR was observed at high levels PP proportion (Figures 3c and 3d). PM and CM showed very low VSR at individual substrate level, indicating that the livestock manures were almost not degraded during the hydrogen fermentation. However, the synergic effect between CM and FW played a major role in VSR at low levels of PP proportion (Figures 3a and 3b). This result demonstrated that to certain extent, co-fermentation of FW and CM could enhance hydrogen production and simultaneously improve substrate conversion.

Effects of FW, PM, PP, and CM on pH were presented in Figure 4. The lowest pH of 3.8 was observed at the vertex of FW, while the highest pH of 6.7 was found at vertex of PM. The pH of around 5 occurred in the middle area of the ternary contour, where FW were mixed with equivalent CM or PM approximately (Figure 4a). With the increase of PP, the pH increased at the side of FW, and decreased at sides of CM and PM, at last the pH stabilized at 4.6 with PP as individual substrate (Figures 4b, 4c and 4d).

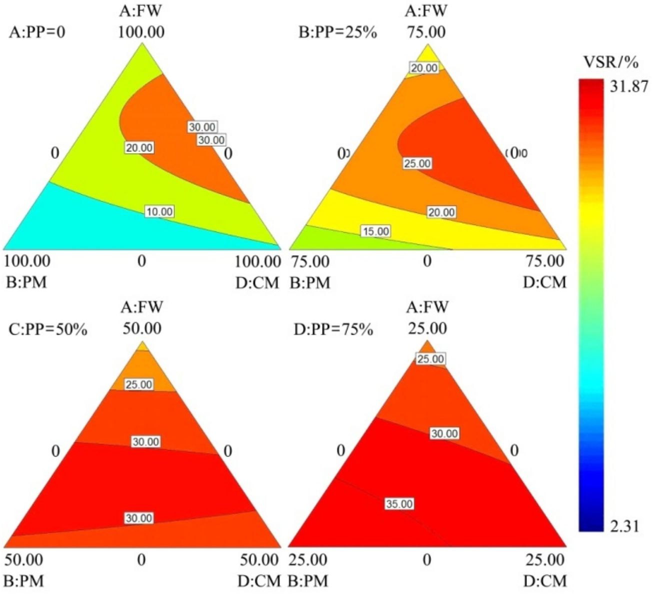


Figure 3 Contour plots for effects of FW, CM, PP and PM on VS reduction

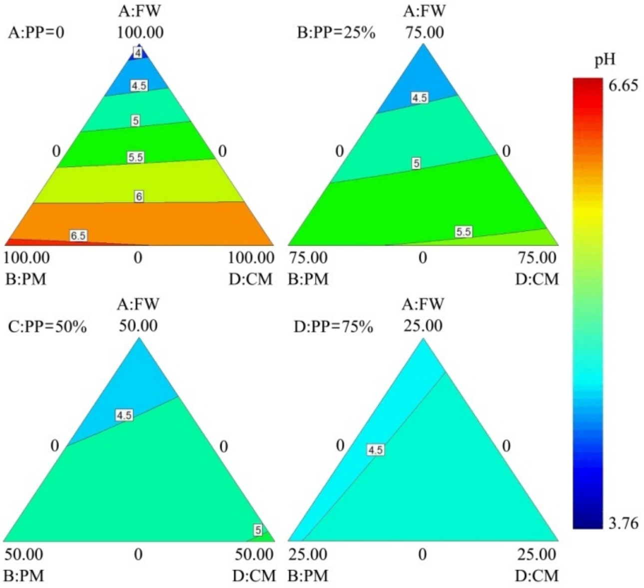


Figure 4 Contour plots for effects of FW, CM, PP and PM on pH

Moreover, to get an insight into the influence of substrate composition on metabolic pathways, the Pearson's correlation coefficients between liquid metabolites and responses were also calculated (Table 6).

As shown in Table 6, only acetic acid and n-butyric acid have positive and significant correlation coefficients with HY, indicating that acetic-butyric metabolic pathway play a major role in the hydrogen production. The correlation between liquid metabolites and VSR were found to coincide essentially with HY, showing that acetic-butyric type fermentation could also promote organic waste hydrolysis.

Table 6 Pearson's correlation coefficients between liquid metabolites and responses

	Ammonia	Ethanol	Lactic acid	Acetic acid	Propionic acid	n-Butyric acid
r_{HY}	–0.04	0.11	–0.13	0.73^{**}	0.38	0.85^{**}
r_{VSR}	–0.19	0.32	0.02	0.61^{**}	0.26	0.89^{**}
r_{pH}	0.69^{**}	-0.81^{**}	-0.78^{**}	–0.09	0.05	–0.34

Note: r_{pH}, r_{HY}, and r_{VSR} are the abbreviations of Pearson's correlation coefficients for liquid metabolites with pH, hydrogen yield, and VS reduction, respectively; **means Significant at α=0.01.

As to correlation between liquid metabolites and pH, ammonia has positive and significant correlation with pH. However, ethanol and lactic acid showed negative and significant correlation. Acetic acid, propionic acid and n-butyric acid, almost have no significant correlation.

3.3 Response optimization and verification of model

Usually, the goal for optimization of hydrogen fermentation is to obtain maximum HY and VSR, the pH was seldom considered. Nevertheless, the performance of hydrogen fermentation is known to be a function of pH. Hence, to comprehensively optimize the substrate composition for hydrogen fermentation, in addition to obtaining maximum HY and VSR, the pH should be restricted at 5. HY, VSR and pH were combined into one desirability function, and regression Equations (8), (9) and (10) were solved by method describe by [24]. The optimal substrates composition with desirability of 0.967 was achieved as follow: FW=61.6%, CM=38.4%, and PP=PM=0. The optimal responses were predicted as HY of 21.0 mL/g VS, VSR of 29.4%, and pH of 5. Under the optimum condition, a verification experiment was carried out and responses as HY of 25.3 mL/g VS, VSR of 28.6%, and pH of 5.2 were achieved. The experimental results were in good agreement with the predicted values, suggesting that the models were effective for prediction of hydrogen fermentation process.

3.4 Discussion

As shown in Table 1, the nutrient composition and chemical properties widely varied among the substrates. To classify the substrates, a cluster analysis was applied based on the squared Euclidean distance between any two objects of the characteristics of substrates. All the four substrates could be classified into two characteristic groups with similarity larger than 94%: (I) easily biodegradable group; (II) hard biodegradable group. Group I include FW and PP which have high C/N and be rich in starch, but lack of alkalinity. Moreover the pHs of FW and PP were found to be slightly acidic. CM and PM belong to group II which have lower C/N, high content of lignocellulose, and almost no starch, but be rich in alkalinity. The pHs of PM and CM were found to be neutral.

Numerous studies showed that the optimal pH for hydrogen fermentation was about 5. Fermentation operated at pH lower than 5 could lead to heterolactic or acetic-propionic metabolic pathway[25-28].

Considering all the results shown in Table 3, for individual substrate, it seems that potato pulp which is rich in starch is the most suitable substrate for hydrogen fermentation. Several studies have suggested the feasibility of hydrogen production from potato[22,23]. However, exogenous buffer may be needed to maintain pH in optimal range, owing to deficiency of alkalinity.

For mixture substrates, it seems that the interactive effect between substrate group I and group II could obviously enhance the hydrogen production. The livestock manure, especially cattle manure, could provide not only the organisms and nutrients but also buffering capacity to maintain optimal pH for hydrogen production. As shown in Table 6, ammonification of protein could increase the alkalinity, and the pH could be determined by balance between ammonification and acidogenesis.

The optimal hydrogen yield is lower than most of those reported[11,12], the difference was attributed to the addition of hard biodegradable CM, which was reported to obtain hydrogen yield of less than 1 mL/g VS at 37°C[29] and 10.25 mL/g VS at 60°C[30]. Thus the hydrolysis of lignocellulose in the CM seems to be a key for improvement of hydrogen production from co-fermentation of FW and CM.

4 Conclusions

In this study, the synergic and antagonistic effects of food waste, cattle manure, potato pulp and pig manure on hydrogen production were evaluated using mixture design. The experimental results indicated that food waste and cattle manure showed the strongest synergistic effect and acetic-butyric metabolic pathway play a major role in the hydrogen production. The optimal hydrogen yield of 21.0 mL/g VS with VS reduction of 29.4% and pH of 5 was obtained using substrates in the ratio of FW:CM:PP:PM=61.6:38.4:0:0. The pH could be determined by balance between ammonification and acidogenesis. In addition, enhancement of hydrolysis of lignocellulose in the CM is needed to improve hydrogen production from co-fermentation of FW and CM.

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