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Optimization of Cattle Manure and Food Waste Co-Digestion for Biohydrogen Production in a Mesophilic Semi-Continuous Process

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Received: 18 June 2020; Accepted: 27 July 2020; Published: 28 July 2020

Abstract: Biohydrogen production from organic solid waste has shown particular advantages over other methods owing to the combination of waste reduction and bioenergy production. In this study, biohydrogen production from the co-digestion of cattle manure and food waste was optimized in a mesophilic semi-continuous process. To maximize hydrogen production, the effects of the mixing ratio (the proportion of food waste in the substrate), substrate concentration, and hydraulic retention time (HRT) on the co-digestion were systematically analyzed using a Box–Behnken design. The results showed that strong interactive effects existed between the three factors, and they had a direct effect on the responses. Hydrogen was primarily produced via the butyrate pathway, which was accompanied by the competing heterolactic fermentation pathway. Propionate and valerate produced from lipids and proteins, respectively, were obtained along with butyrate. The optimal process parameters included a mixing ratio of 47% to 51%, a substrate concentration of 76 to 86 g L^{-1}, and an HRT of 2 d. Under these optimal conditions, the hydrogen production rate and hydrogen yield were higher than 1.00 L L^{-1} d^{-1} and 30.00 mL g^{-1} VS, respectively, and the predicted results were consistent with the experimental data. The results indicate that the co-digestion of cattle manure and food waste is a practical and economically promising approach for biohydrogen production.

Keywords: biohydrogen; cattle manure; co-digestion; food waste; response surface methodology

1. Introduction

In the past decade, owing to the limited sources of fossil fuels and their related environmental problems, renewable energy production has been a research focus [1–3]. Meanwhile, the global production of organic solid waste (OSW), such as crop residue, food waste, and livestock waste, has continued to increase, owing to a rapidly growing population and improvements in economic status [4,5]. OSW is usually disposed in landfills, which utilize land and waste resources. Therefore, it is necessary to identify sustainable ways by which OSW can be treated for energy recovery [6]. Dark fermentation, which is a process by which microbes produce hydrogen based on the biological conversion of organic matter, is a promising alternative method to manage OSW because biohydrogen has the highest energy content per unit weight, and it does not emit any harmful substances [7–10].

Dark fermentation with a single substrate, such as crop residue, food waste, or livestock waste, has attracted considerable attention [11–14]. However, the main disadvantage is that the fermentation

equilibrium can be disturbed by the accumulation of volatile fatty acids (VFAs), high ammonia nitrogen concentrations, or trace elements shortage [15–17]. To maintain optimal fermentation conditions, a process adjustment method by which exogenous chemical regulators are added to the substrate has often been applied under lab-scale conditions [18]. However, this approach may not be feasible for full-scale application [19]. Recently, the co-digestion process, which is performed by adding complementary materials to the substrate, has attracted attention because it offers the advantages of better buffer conditions, more balanced nutrients, and the dilution of inhibitors. The co-digestion process has been considered as an economical and feasible approach that can be employed to improve the yield and stability of the hydrogen production process [12,20–23].

For example, cattle manure is rich in alkali and nutrients, and it has been reported to be suitable for co-digestion with carbohydrate-rich and rapidly degradable substrates, such as food waste [24,25]. However, studies on the co-digestion of cattle manure and food waste have been primarily focused on biomethane production [26–29]. Less attention has been given to biohydrogen production via the co-digestion of food waste and cattle manure; thus, available data in this regard are still limited [30]. Compared with methane, hydrogen is a carbon-free clean fuel, and it can be directly used in fuel cells for producing electricity and in the chemical industry for producing fertilizers. Thus, hydrogen has been considered to be the base of future sustainable energy systems [2,8]. However, different from biomethane processes where the VFAs are consumed by acetogenic bacteria and methanogens, dark fermentation processes are unstable because of the continuous production of VFAs [13,14]. For continuous co-digestion application, the equilibrium of biohydrogen production is typically influenced by the mixing ratio as well as by other direct influencing factors, such as the substrate concentration and the hydraulic retention time (HRT). Furthermore, there may be interactions among these factors, and limited information is available on the effect of such factors on the continuous co-digestion process, as related systematic studies are scarce [31–33].

The objective of this work was to investigate the individual and interactive effects of the mixing ratio, substrate concentration, and HRT on the mesophilic semi-continuous co-digestion of cattle manure and food waste. Then, the active metabolic pathways were evaluated based on the Pearson correlation coefficients of the relationship between liquid metabolites and hydrogen production. Finally, the fermentation process was optimized using a Box–Behnken design based on response surface methodology [34,35].

2. Materials and Methods

2.1. Inoculum

Inoculated sludge was obtained from a biogas pilot plant for treating cattle manure located at the Northeast Agricultural University, Harbin, China. To deactivate hydrogen-consuming bacteria, the inoculum was pretreated at 100 °C in a boiling water bath for 30 min immediately before use. The total solid (TS) and volatile solid (VS) contents of the inoculum were 5.19 ± 0.14% (w/w) and 3.51 ± 0.06% (w/w), respectively.

2.2. Substrates

Cattle manure was collected from the experimental farm at the Northeast Agricultural University. Food waste was obtained from the university dining room. The substrate composition is presented in Table 1.

Cattle manure and food waste were homogenized using a blender and stored at −20 °C until use. Before digestion, the cattle manure and food waste were diluted and mixed in accordance with the substrate concentration and mixing ratio defined by the experimental design.

Table 1. Substrate composition. TS: total solid, VS: volatile solid.

Items	Solid Contents (% w/w)		pH	Component (% of TS)				C/N	Total Alkalinity (mg $CaCO_3$ L^{-1})
	TS	VS		Proteins	Lipids	Starch	Lignocellulose		
Food waste	19.53 ± 0.33	18.20 ± 0.31	5.28 ± 0.54	15.72 ± 0.24	29.38 ± 0.13	25.51 ± 0.53	13.16 ± 0.12	21.98 ± 0.22	225 ± 31
Cattle manure	22.15 ± 0.29	18.21 ± 0.28	7.22 ± 0.04	11.86 ± 0.78	2.38 ± 0.29	0.51 ± 0.11	51.35 ± 0.11	18.81 ± 0.18	5171 ± 57

2.3. Experimental Design and Statistical Analysis

A three-factor Box–Behnken design was used to evaluate the effects of the operating parameters as well as those resulting from their interactions, on the experimental responses [34]. The three operating parameters chosen as independent variables were as follows: (x_1) the mixing ratio (the proportion of food waste in the substrate, as a percentage), (x_2) the substrate concentration (the total solid per liter of fermentation broth, g L^{-1}), and (x_3) the HRT (d). The hydrogen production performance was characterized by (y_1) the hydrogen production rate (HPR) and (y_2) the hydrogen yield (HY). The HPR, which is indicative of the specific production efficiency of the reactor, was expressed in L L^{-1} d^{-1}, and HY, which represents the efficiency of the transformation of the substrate, was expressed in mL g^{-1} VS.

In this experimental design, 15 trials, comprising 12 factorial points and 3 center points, were run. According to the Tenca et al. [23], Cuetos et al. [36], and pre-experiments [37,38], the variation range of the three variables was selected. A summary of the experimental design matrix is presented in Table 2. Design Expert software (6.0.10, Stat-Ease Inc., Minneapolis, MN, USA) was used to analyze the experimental data.

Table 2. Experimental design matrix. HRT: hydraulic retention time.

Run	Coded Values			Actual Values		
	x_1	x_2	x_3	Mixing Ratio (%)	Substrate Concentration (g L^{-1})	HRT (d)
1	−1	−1	0	40	60	2
2	1	−1	0	60	60	2
3	−1	1	0	40	100	2
4	1	1	0	60	100	2
5	−1	0	−1	40	80	1
6	1	0	−1	60	80	1
7	−1	0	1	40	80	3
8	1	0	1	60	80	3
9	0	−1	−1	50	60	1
10	0	1	−1	50	100	1
11	0	−1	1	50	60	3
12	0	1	1	50	100	3
13	0	0	0	50	80	2
14	0	0	0	50	80	2
15	0	0	0	50	80	2

2.4. Experimental Operating Procedure

The co-digestion experiments were conducted using 500-mL Erlenmeyer flasks in an oscillating incubator maintained at a temperature of 35 ± 1 °C and with an oscillation speed of 150 rpm. To conduct the experiments, 80 mL of pretreated inoculum and 320 mL of substrate were mixed in accordance with the experimental design matrix and poured into the reactor; thus, the operating volume was 400 mL. Reactors were purged with pure nitrogen gas for 60 s and sealed with rubber stoppers before the digestion process. At the beginning of the experiment, the reactors were operated in batch mode for 36 h. After hydrogen production had peaked, the reactor mode was converted into a semi-continuous

system for 15 d, during which the substrate was fed every 12 h, and biogas and effluents were sampled and measured simultaneously. The average values of the experimental indexes were used to evaluate the hydrogen production performance of the system.

2.5. Analytic Methods

TS, VS, total alkalinity, and pH were determined using the standard methods outlined in American Public Health Association (APHA) [39]. Biogas was collected using sample bags that were connected to the reactor. Biogas volume was measured using the water displacement method and adjusted to the standard conditions (0 °C and 760 mm Hg) [36]. The percentages of H_2, CH_4, CO_2, and N_2 were analyzed using a gas chromatograph (6890N, Agilent Inc., Santa Clara, CA, USA) equipped with a TDX-01 column and a thermal conductivity detector. Argon was the carrier gas, and it flowed at 40 mL/min. The oven and detector temperatures were 170 °C and 220 °C, respectively. The ethanol and VFA (e.g., acetate, propionate, n-butyrate, and n-valerate) contents of the effluent were also analyzed using a gas chromatograph equipped with an infused-silica capillary column and a flame ionization detector. Nitrogen was the carrier gas, and it flowed at 30 mL/min. The temperatures of the injector and detector were 220 °C and 250 °C, respectively. The initial oven temperature was 60 °C. It was increased at 15 °C/min for 5.33 min, after which it was kept at 140 °C for 1.2 min. Acetonitrile and phosphoric acid (at a volume ratio of 2.5:97.5) were employed as the mobile phase to analyze lactate using a high-performance liquid chromatograph (600E-2487, WATERS Inc., Milford, CT, USA) equipped with an ultraviolet detector (210 nm) and a C18 column (250 × 4.6 mm) [38].

3. Results and Discussion

3.1. Overall Performance

The experimental results are summarized in Table 3. Generally, the HPR was in the range of 0–1.13 L L^{-1} d^{-1} and the HY was in the range of 0–31.70 mL g^{-1} VS. The considerable variations in HPR and HY indicate that the metabolic pathways in the co-digestion of cattle manure and food waste were complex. To study the metabolic pathway, the pH and liquid metabolites in the effluent were also analyzed. As shown in Table 3, the pH was in the range of 3.62–5.28, and the liquid metabolites were primarily ethanol, acetate, propionate, n-butyrate, n-valerate, and lactate. The distribution of the liquid metabolites showed that multiple metabolic pathways were active in all the runs.

Table 3. Experimental design matrix and results.

Run	Hydrogen Production Rate (L L^{-1} d^{-1})	Hydrogen Yield (mL g^{-1} vs)	pH	Liquid Metabolites (mmol·L^{-1})					
				Ethanol	Acetate	Propionate	Butyrate	Valerate	Lactate
1	0.09	3.59	5.34	23.65	24.50	7.61	21.62	8.07	38.03
2	0.05	2.00	4.02	85.02	23.05	3.43	2.37	1.47	119.91
3	0.49	10.99	5.42	25.71	47.56	15.97	27.12	8.07	72.62
4	0	0	3.79	135.02	27.05	3.43	3.37	1.47	215.67
5	0.67	9.70	5.21	60.02	27.41	20.96	39.26	12.52	44.50
6	0	0.03	3.88	97.82	46.63	10.28	3.33	0.93	143.59
7	0.01	0.61	5.37	14.40	47.11	9.10	18.78	5.08	85.74
8	0	0	3.85	93.24	24.97	2.53	5.49	1.04	168.83
9	0.58	10.95	5.06	52.29	18.68	8.18	29.47	6.68	155.81
10	1.13	12.83	5.06	80.66	28.77	22.64	63.37	20.10	51.48
11	0.20	11.41	5.29	29.14	33.45	6.12	26.20	8.79	31.92
12	0.24	8.29	5.2	36.78	30.58	1.51	22.03	3.71	83.24
13	1.10	31.20	5.14	39.62	31.44	19.94	33.51	13.82	49.03
14	1.09	31.70	5.19	73.75	41.75	15.85	29.33	11.50	40.78
15	1.00	28.53	5.20	29.00	40.01	13.04	20.56	9.47	50.23

3.2. Hydrogen Production Rate

A quadratic regression model was established to determine the influence of independent variables on the HPR. Analysis of variance (ANOVA) was used to ensure the accuracy of the model. As shown

in Table 4, the "Model F value" was 25.18, and the corresponding p value was <0.05, indicating that the model was significant. The "Lack of Fit F value" was 6.47, and the corresponding p value was >0.1, demonstrating that the lack of fit was insignificant.

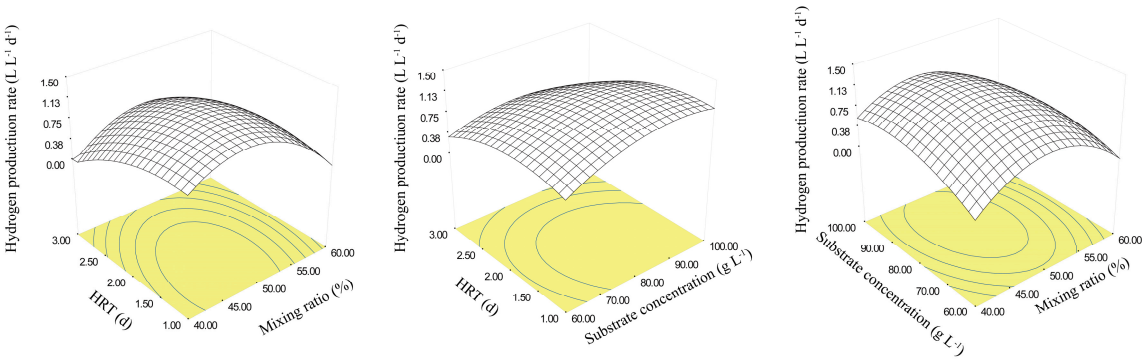
Table 4. Analysis of variance (ANOVA) for the regression model of hydrogen production rate.

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p Value
Model	2.78	9	0.31	25.18	0.0012
x_1	0.18	1	0.18	14.99	0.0117
x_2	0.11	1	0.11	8.91	0.0306
x_3	0.46	1	0.46	37.54	0.0017
$x_{1\times2}$	0.05	1	0.05	4.13	0.0978
$x_{1\times3}$	0.11	1	0.11	8.74	0.0317
$x_{2\times3}$	0.06	1	0.06	5.24	0.0708
${x_1}^2$	1.48	1	1.48	121.02	0.0001
${x_2}^2$	0.27	1	0.27	21.91	0.0054
${x_3}^2$	0.24	1	0.24	19.86	0.0067
Residual	0.06	5	0.01	-	-
Lack of Fit	0.06	3	0.02	6.47	0.1368
Pure Error	0.01	2	0.00	-	-
Cor Total	2.84	14	-	-	-

According to the ANOVA results, Equation (1), with respect to coded factors, had a determinant coefficient of 0.9784, showing that there was a significant correlation between the experimental and predicted values. The optimal conditions for the maximum HPR were a mixing ratio of 47.33%, a substrate concentration of 88.89 g L^{-1}, and an HRT of 1.73 d.

$$y_1 = 1.06 - 0.15x_1 + 0.12x_2 - 0.24x_3 - 0.11x_{1\times2} + 0.16x_{1\times3} - 0.13x_{2\times3} - 0.63{x_1}^2 - 0.27{x_2}^2 - 0.26{x_3}^2 \quad (1)$$

The regression coefficients of the linear effect terms were compared, and the HRT was identified as the factor that contributed the most to the HPR, followed by the mixing ratio, and then the substrate concentration. To investigate the effects of the interaction of these three contributing factors, three-dimensional (3D) response surface and two-dimensional (2D) contour plots were generated based on the experimental data and regression equations. Additionally, the intensities of the interactive effects were reflected by the shape of the contour plots. The effect of the interaction between the mixing ratio and HRT on HPR (at a constant substrate concentration of 80 g L^{-1}) is illustrated in Figure 1a, which shows that the contour plot was elliptical, indicating a strong interaction between the two factors.



(**a**) (**b**) (**c**)

Figure 1. Three-dimensional (3D) response surface and two-dimensional (2D) contour plots for hydrogen production rate. (**a**) Mixing ratio and hydraulic retention time (HRT), (**b**) substrate concentration and HRT, and (**c**) mixing ratio and substrate concentration.

When the HRT was fixed, the HPR first increased slowly, and thereafter decreased rapidly as the mixing ratio increased. When the mixing ratio increased to 50%, the proportion of starch, which constituted a greater fraction of the food waste than it did the cattle manure, increased. Reportedly, readily biodegradable carbohydrates, such as starch, are associated with higher HPR than other substrates [40]. When the mixing ratio exceeded 50%, the alkalinity, which was lower in the food waste than it was in the cattle manure, decreased. The readily biodegradable carbohydrates and the lower alkalinity in the digestion system caused the rapid accumulation of undissolved VFAs, which reduced the pH to below the optimal range [18,23].

When the mixing ratio was fixed, the HPR increased slowly at first and thereafter decreased rapidly as the HRT increased. This phenomenon could possibly be attributed to the coupling of the sludge retention time and the HRT in the fully mixed reactor. A longer HRT meant that less substrate was fed into the reactor. However, a longer sludge retention time meant that more hydrogen-producing bacteria were present in the reactor. When the HRT increased from 1 to 2 d, the two effects almost canceled each other out, causing the HPR to increase at a slower pace. Nevertheless, a longer HRT and sludge retention time are beneficial to the growth of hydrogen consumers, such as methanogens [41]. Consequently, when the HRT increased from 2 to 3 d, the HPR decreased rapidly.

Figure 1b illustrates the effects of the interaction between the HRT and substrate concentration on the HPR (at a 50% constant mixing ratio). The contour plot was elliptical, indicating that there is a significant interaction between these two factors. Similar to Figure 1a, as the HRT increased, the HPR initially increased slowly, and thereafter, it decreased rapidly. However, as the substrate concentration increased, the HPR first increased rapidly and thereafter decreased slowly. This can primarily be attributed to the higher substrate concentration, which resulted in a higher organic loading rate. Within an optimum range, the increased organic loading rate enhanced the HPR of the dark fermentation process. However, when it was too high, the accumulation of VFAs and dissolved hydrogen in the digestion broth could potentially inhibit the activity of the hydrogen-producing bacteria [31,42].

Figure 1c shows the effects of the interaction between the mixing ratio and the substrate concentration on the HPR (at 2 d constant HRT). The HPR first increased, and thereafter, it decreased as the mixing ratio and substrate concentration increased. The contour plot was elliptical, indicating a strong interaction between the two factors.

3.3. Hydrogen Yield

ANOVA was also used to evaluate the effects of the mixing ratio, substrate concentration, and HRT on the HY. As shown in Table 5, the quadratic regression model was significant, and the lack of fit was insignificant.

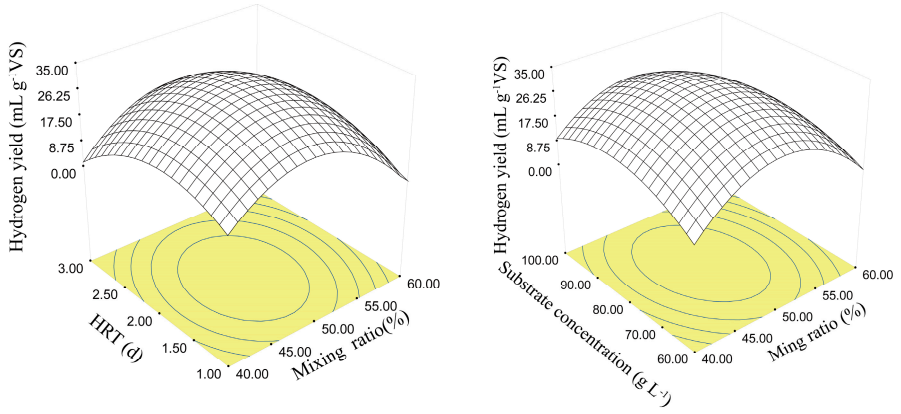
Table 5. Analysis of variance (ANOVA) for the regression model of hydrogen yield.

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	*p* Value
Model	1740.11	8	217.51	61.00	<0.0001
x_1	65.25	1	65.25	18.30	0.0052
x_2	2.17	1	2.17	0.61	0.4652
x_3	21.77	1	21.77	6.11	0.0484
$x_{1\times2}$	22.10	1	22.10	6.20	0.0472
$x_{1\times3}$	20.47	1	20.47	5.74	0.0536
${x_1}^2$	1106.18	1	1106.18	310.23	<0.0001
${x_2}^2$	300.51	1	300.51	84.28	<0.0001
${x_3}^2$	413.29	1	413.29	115.91	<0.0001
Residual	21.39	6	3.57	-	-
Lack of Fit	15.59	4	3.90	1.34	0.4693
Pure Error	5.81	2	2.90	-	-
Cor Total	1761.50	14	-	-	-

Based on the ANOVA results, the quadratic regression equation was derived using the regression fitting (Equation (2)) with a regression coefficient of 0.9879. The insignificant interaction effect terms of substrate concentration and HRT were removed. However, to enhance the regression model hierarchy, the insignificant linear model term of the HRT was retained. Based on the regression coefficients of the individual factors, the mixing ratio had the most significant impact on the HY, followed by the substrate concentration, and then the HRT.

$$y_2 = 30.47 - 2.86x_1 + 0.52x_2 - 1.65x_3 - 2.35x_{1\times2} + 2.26x_{1\times3} - 17.31{x_1}^2 - 9.02{x_2}^2 - 10.58{x_3}^2 \quad (2)$$

The 3D response surface and 2D contour plots of the HY regression model are shown in Figure 2. With the increasing mixing ratio, HRT, and substrate concentration, the HY increased to an optimal value, and thereafter, it decreased as these three controlling factors increased. The 2D contour plot of HY was elliptical with respect to the mixing ratio and the HRT (Figure 2a) and the substrate concentration and the mixing ratio (Figure 2b), indicating that both have significant interactive effects on HY.



(**a**) (**b**)

Figure 2. Three-dimensional (3D) response surface and two-dimensional (2D) contour plots for hydrogen yield. (**a**) Mixing ratio and hydraulic retention time (HRT), and (**b**) substrate concentration and mixing ratio.

Additionally, optimal HY conditions were determined as a mixing ratio of 49.09%, substrate concentration of 80.82 g L^{-1}, and HRT of 1.91 d. The effects of substrate concentration and HRT on HPR and HY were different. Higher substrate concentrations and lower HRTs enhanced the HPR, but not HY. However, the effects of the mixing ratio on HPR and HY were similar, resulting in the same optimal mixing ratio for both indicators.

3.4. pH and Liquid Metabolites

HPR and HY as functions of pH are presented in Figure 3, which shows that the HPR and HY were approximately zero when the pH was <4.02. However, as the pH increased, both the HPR and HY peaked within a pH range of 5.06–5.20, and they decreased thereafter.

Generally, independent of substrate concentration and HRT, the mixing ratio played a crucial role in determining the pH. When the mixing ratio was 60% (i.e., runs 2, 4, 6, and 8), almost all the pH values observed were below 4. Under such conditions, hydrogen production was low or absent. Additionally, when the mixing ratio was 40% (i.e., runs 1, 3, 5, and 7), the pH was greater than 5.20, and the HPR and HY were less than 0.49 L L^{-1} d^{-1} and 10.99 mL g^{-1} VS, respectively. However, when the mixing ratio was 50%, the pH was maintained within range of 5.06–5.20, and maximum HPR and HY values of 1.10 L L^{-1} d^{-1} and 31.70 mL g^{-1} VS were obtained. Notably, significant deviations in HPR and HY were also observed within a pH range of 5.06–5.20. These results indicate that even within the optimal pH range, HPR and HY are sensitive to HRT and substrate concentration.

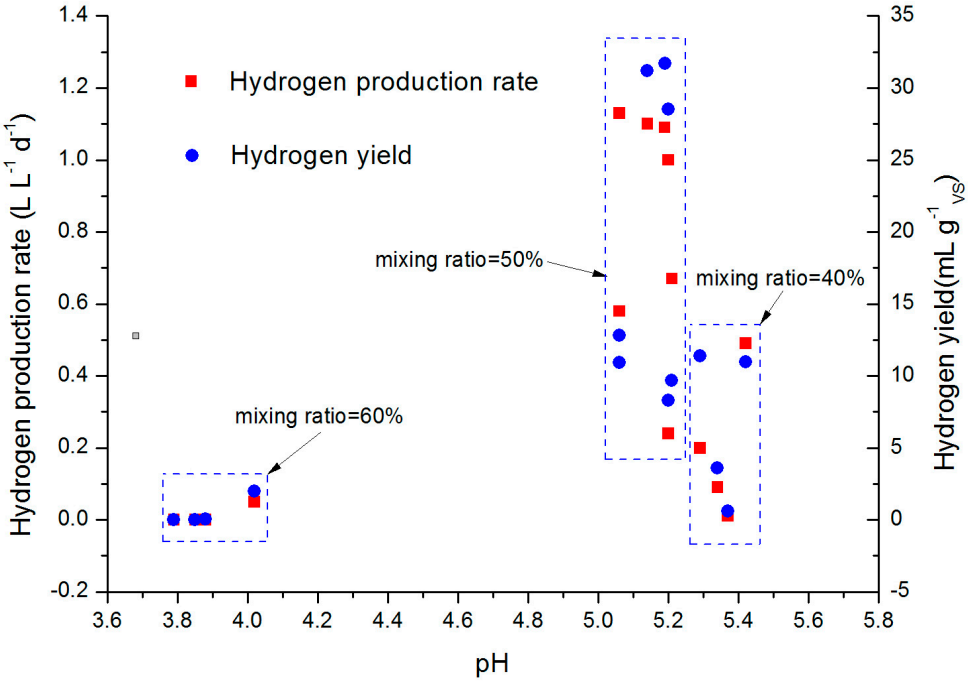


Figure 3. Hydrogen production vs. pH.

Furthermore, to estimate the contribution of different liquid metabolites to hydrogen production, Pearson correlation coefficients between liquid metabolites and responses were calculated (Table 6).

Table 6. Pearson correlation coefficients between liquid metabolites and responses.

Response	Ethanol	Acetate	Propionate	Butyrate	Valerate	Lactate
Hydrogen production rate	−0.18	0.07	0.81 **	0.76 **	0.85 **	−0.55 *
Hydrogen yield	−0.31	0.19	0.59 *	0.48	0.64 *	−0.58 *

Note: Asterisks indicate significant correlations between parameters, * $p < 0.05$, ** $p < 0.01$.

As shown in Table 6, the correlation coefficients between the liquid metabolites and HY and HPR coincided. For ethanol and lactate, the correlation coefficients were negative, indicating that the heterolactic fermentation described by Equation (3) was a competing pathway for hydrogen fermentation. Reportedly, zero-hydrogen heterolactic fermentation becomes the dominant metabolic pathway in continuous anaerobic acidogenesis under low pH conditions [43].

$$C_6H_{12}O_6 \rightarrow CH_3CH(OH)COOH + CH_3CH_2OH + CO_2 \quad (3)$$

For acetate, even though high yields were observed in all runs, correlation coefficients of 0.07 and 0.19 for HPR and HY, respectively, were indicative of a weak correlation between acetate and hydrogen production. Additionally, correlation coefficients of 0.76 and 0.48 for HPR and HY, respectively, demonstrated that the main hydrogen production pathway was the butyratepathway (Equation (4)). However, a large number of by-products, such as propionate and n-valerate, were produced, and they were strongly correlated with hydrogen production. This was possibly due to the food waste having not only a high starch content, but also high lipid and protein contents. Starch digestion tends to produce hydrogen and butyrate, whereas lipid and protein digestions tend to produce propionate and valerate, respectively. These digestion processes occur simultaneously under most conditions [11,44].

$$C_6H_{12}O_6 \rightarrow CH_3COOH + 1/2CH_3(CH_2)COOH + 3H_2 + 2CO_2 \quad (4)$$

3.5. Process Optimization and Verification of the Model

In hydrogen-producing reactors, both HPR and HY should be maximized, because high HPRs offer the possibility of reducing the reactor volume and high HYs offer the possibility of improving the energy conversion efficiency. However, it is difficult to optimize these two responses under the same conditions, because their interest regions differ. Therefore, the optimization region was determined by overlaying the responses according to the defined constraints (Figure 4). The constraints used in the overlaying method were to set the HPR and HY in the range of 1.00 L L^{-1} d^{-1} to its upper amount and 30.00 mL g^{-1} VS to its upper amount, respectively. The optimized processing parameters included a mixing ratio of 47% to 51%, a substrate concentration of 76 to 86 g L^{-1}, and an HRT of 2 d. A mixing ratio of 50%, substrate concentration of 80 g L^{-1}, and HRT of 2 d were selected as the optimal parameters to verify the models. The optimal responses were predicted as 1.08 L L^{-1} d^{-1} and 30.90 mL g^{-1} VS for the HPR and the HY, respectively. Under the optimum conditions, the verification experiment achieved an HPR of 1.09 L L^{-1} d^{-1} and a HY of 30.22 mL g^{-1} VS, respectively, which agrees well with the experimental data. This indicates that the models can effectively predict the hydrogen fermentation process, and the optimum conditions obtained using the response surface methodology were conducive to the growth and activity of hydrogen-producing microbes.

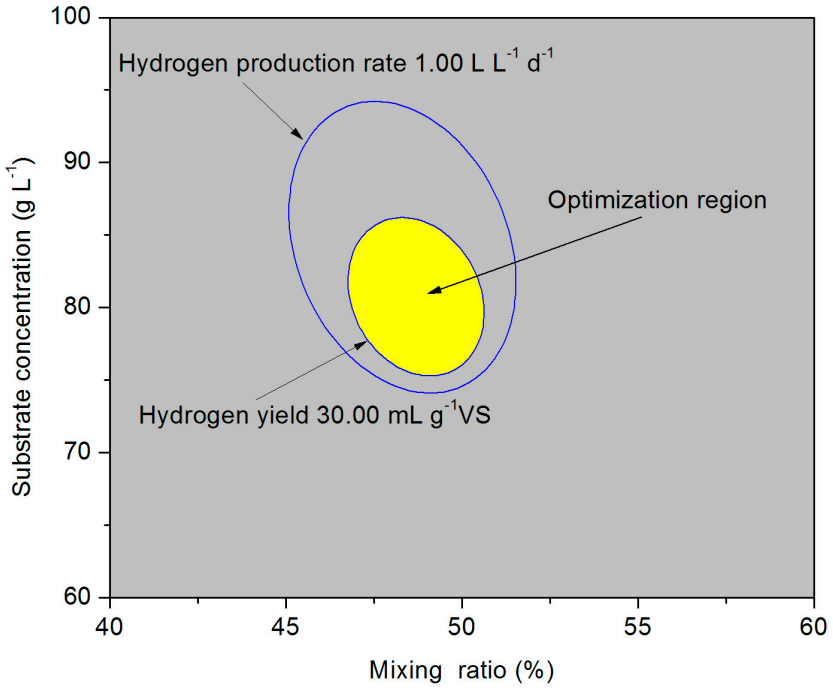


Figure 4. Process optimization (the hydraulic retention time is 2 d).

3.6. Comparison of the Hydrogen Production by Different Substrates

A large number of studies have been conducted on the production of biohydrogen with different substrates. Table 7 compares the HPR and HY observed in this study with those obtained from the existing literature.

Even though stable hydrogen production was achieved via the co-digestion of cattle manure and food waste, the optimal HPR and HY did not show obvious advantages compared with the values reported in the literature. The relatively complex nature of the substrates used in this study could be responsible for this observation. On the one hand, cattle manure has a high lignocellulose content, which makes it resistant to biodegradation [45]. Even under thermophilic conditions, the HY of cattle manure was much lower than that of food waste [12,46]. On the other hand, food waste is rich in lipids. Thus, the accumulation of long-chain fatty acids (LCFAs) generated from the lipid degradation process can inhibit hydrogen production [47].

To enhance the efficiency of the process, pretreatments are often required to promote the disintegration and hydrolysis of complex biomass [14]. However, negative effects such as the formation of inhibitors, high energy consumption, and the necessity to add other chemicals are often associated with such pretreatments [48]. Therefore, increased efforts are required to ensure that such pretreatment processes are more efficient in the long term. From the perspective of a full-scale application, simple operations, such as solid–liquid separation for cattle manure and oil–water separation for food waste would be more practical methods in the near future [49,50]. Although further studies are necessary to improve the process, the co-digestion of cattle manure and food waste is a practical and economically promising candidate for hydrogen production from organic solid waste.

Table 7. Biohydrogen production by different substrates.

Substrates	Fermentation Conditions	Hydrogen Production Rate (L L^{-1} d^{-1})	Hydrogen Yield (mL g^{-1} VS)	References
Food waste	Semi-CSTR (Thermophilic)	0.4–2.1	20–85	[51]
Food waste	Batch (Mesophilic)	-	120	[18]
Cattle manure	Batch (Mesophilic/Thermophilic)	-	0–0.73 [a]	[46]
Cattle manure	Semi-CSTR(Thermophilic)	0.41	10.25	[12]
Humulus scandens	Batch (Mesophilic)	-	65.12 [b]	[52]
Carbohydrates + Proteins	Batch (Mesophilic)	-	350 [c]	[53]
Swine manure + Pineapple waste	CSTR (Mesophilic)	1.49	266.91 [c]	[54]
Food waste + Sewage sludge	Batch (Mesophilic)	-	174.6	[22]
Dog food + Cow manure	Batch (Thermophilic)	-	57.67 [a]	[30]
Food waste + Cattle manure	Semi-CSTR (Mesophilic)	1.09	30.22	This study

Note: [a] calculated from data in literature, [b] mL g^{-1} $_{TS}$, [c] mL g^{-1} $_{COD}$.

4. Conclusions

Biohydrogen production during the mesophilic co-digestion of cattle manure and food waste was optimized using the Box–Behnken design. The optimal process parameters were a mixing ratio of 47% to 51%, a substrate concentration of 76 to 86 g L^{-1}, and an HRT of 2 d. Under these optimal conditions, the HPR and HY were higher than 1.00 L L^{-1} d^{-1} and 30.00 mL g^{-1} VS, respectively, and the predicted results were consistent with the experimental data. Overall, it can be concluded from the current study that biohydrogen production from the co-digestion of cattle manure and food waste is feasible. Additionally, stable biohydrogen production can be obtained using the mixing ratio, substrate concentration, and HRT as the controlling factors. These findings serve as a foundation for improving biohydrogen production from organic solid wastes.

Author Contributions: Conceptualization, S.L. and W.L.; methodology, S.L.; software, S.L. and L.L.; validation, S.L.; formal analysis, S.L.; investigation, S.L.; resources, S.L.; data curation, G.Z. and H.Y.; writing—original draft preparation, S.L.; writing—review and editing, S.L., G.Z., and H.Y.; visualization, S.L. and L.L.; supervision, W.L.; project administration, S.L.; funding acquisition, S.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (grant number 51506027) and the"Young Talents" Project of Northeast Agricultural University (grant number 16QC18).

Conflicts of Interest: The authors declare no conflict of interest.

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