

# Effects of Volatile Solid Concentration and Mixing Ratio on Hydrogen Production by Co-Digesting Molasses Wastewater and Sewage Sludge

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Received: February 17, 2014  
Revised: July 15, 2014  
Accepted: July 19, 2014

First published online  
July 22, 2014

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pISSN 1017-7825, eISSN 1738-8872

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Co-digesting molasses wastewater and sewage sludge was evaluated for hydrogen production by response surface methodology (RSM). Batch experiments in accordance with various dilution ratios (40- to 5-fold) and waste mixing composition ratios (100:0, 80:20, 60:40, 40:60, 20:80, and 0:100, on a volume basis) were conducted. Volatile solid (VS) concentration strongly affected the hydrogen production rate and yield compared with the waste mixing ratio. The specific hydrogen production rate was predicted to be optimal when the VS concentration ranged from 10 to 12 g/l at all the mixing ratios of molasses wastewater and sewage sludge. A hydrogen yield of over 50 ml H<sub>2</sub>/g VS<sub>removed</sub> was obtained from mixed waste of 10% sewage sludge and 10 g/l VS (about 10-fold dilution ratio). The optimal chemical oxygen demand/total nitrogen ratio for co-digesting molasses wastewater and sewage sludge was between 250 and 300 with a hydrogen yield above 20 ml H<sub>2</sub>/g VS<sub>removed</sub>.

**Keywords:** Co-digestion, hydrogen production, mixing ratio, molasses wastewater, response surface methodology, sewage sludge

## Introduction

Enhanced hydrogen-energy production has been achieved in the co-digestion of different organic wastes. Co-digestion is advantageous owing to the dilution of toxic materials, the balanced nutrients, and other synergistic effects on bacteria during the biological reactions [21, 23]. Supplementary micronutrients that exist in sewage sludge are crucial to improve the growth of hydrogen-producing bacteria during anaerobic digestion of tofu waste [16]. Co-digesting municipal food waste and sewage sludge is effective for improving the pH buffering capacity [35]. In addition, a balanced C/N ratio mainly enhances hydrogen production from dairy wastewater [10].

Inexpensive substrates are needed to produce hydrogen commercially. Therefore, the use of waste-activated sludge has been a focus owing to its high carbohydrate, protein, and other nutrient content [26, 31]. However, direct use of raw sludge has its limitations because bacteria cells are mainly composed of hemicellulose and lignin that are not easily biodegradable. Thus, various physical and chemical

pretreatment methods to accelerate the hydrolysis rate, including alkaline, ozonation, and ultrasound, have been studied [2, 3, 12]. There should be an acceptable compromise between energy consumption and energy recovery in hydrogen production from sewage sludge.

Molasses wastewater is preferred for hydrogen production owing to the high concentration of carbohydrates, mainly composed of glucose and fructose. However, synthetic fertilizer to supply microorganisms with adequate nitrogen and phosphorus has been added during hydrogen production, so that the chemical oxygen demand (COD): N: P is adjusted to a moderate ratio of 100–1,000:5:1 [10, 11, 22, 29]. A long-term hydrogen-producing system from molasses wastewater is not sustainable owing to a lack of those nutrients, which may result in a low bacterial growth rate in the system, furthermore deteriorating the hydrogen production performance. Thus, a fundamental reason for a co-substrate such as sewage sludge is to offer nutrients (nitrogen or phosphorus), including microelements, when carbohydrate-rich wastes are digested for hydrogen fermentation [16, 17, 34]. In addition, a co-substrate is also

expected to provide hydrolytic bacteria with a continuous hydrogen-producing process to which various bacteria from sewage sludge might be fed continually.

The main purpose of this study was to determine the practical optimal conditions during co-digesting molasses wastewater and sewage sludge as complementary energy resources. Thus, response surface methodology (RSM) was implemented through reproducible batch experiments with regard to various dilution ratios and waste mixing compositions. Here, performance indicators, including hydrogen production rate and yield based on the removal of volatile solids (VS), were considered due to waste reduction.

## Materials and Methods

### Substrate and Seed Sludge

Molasses wastewater and sewage sludge were used as substrates. The molasses wastewater was obtained from a domestic sugarcane-processing factory (Incheon, South Korea). The sewage sludge was provided from a gravity thickener at a domestic wastewater treatment plant (Seoul, South Korea) that was a mixture of primary and secondary sludge at a mixing ratio of 8:2 (v/v). The characteristics of the substrates are shown in Table 1. Dilution was conducted as follows. Each substrate of 100, 200, 400, and 800 ml was placed in a 4 L bottle, and the remaining space was filled with tap water (40-, 20-, 10-, and 5-fold) after purging with nitrogen for 20 min. The seed sludge was sampled from a continual hydrogen-producing reactor operated by co-digesting molasses wastewater and sewage sludge in our laboratory (unpublished data). Briefly, 1 L of harvested hydrogenic sludge, after heat treatment of the digested sludge at 90°C for 30 min, was inoculated into the system. The ratio of molasses wastewater to sewage sludge was 2:1 with a 42 h hydraulic retention time. The system temperature and pH were maintained at 35°C and 5.5.

**Table 1.** Average characteristics of molasses wastewater and sewage sludge.

Parameter	Unit	Molasses wastewater	Sewage sludge
pH	-	3.45	6.30
TCOD	mg/l	128,400	17,800
SCOD	mg/l	119,200	120
TSS	mg/l	4,267	13,667
VSS	mg/l	4,267	11,667
TS	mg/l	89,700	44,000
VS	mg/l	86,200	32,200
T-N	mg/l	30	1,800
Glucose	mg/l	11,414	ND

ND, not detected.

### Experimental Procedure

Ten milliliters of seed sludge and 50 ml of diluted substrate were placed in serum bottles having an effective working volume of 100 ml. The mixing ratios of molasses wastewater to sewage sludge were 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100 based on a volume basis and various dilution ratios. Each diluted substrate was placed in four serum bottles with effective volume of 500 ml in accordance with different mixing ratios of molasses wastewater to sewage sludge (500:0, 400:100, 300:200, 200:300, 100:400, and 0:500 ml/ml). Total VS concentrations were controlled to range from 0.81 to 17.2 g/l (Table 2). Therefore, the total COD concentrations and COD/total nitrogen (T-N) ratios ranged from 1.78 to 39.7 g COD/l and 31 to 17,705, respectively. The initial pH of the substrates was controlled at  $5.7 \pm 0.1$  with either NaOH ranging from 0.25 to 1.2 N or HCl ranging from 0.3 to 1.2 N. The headspaces of the bottles were flushed with nitrogen gas for 15 min. The bottles were sealed using rubber caps and placed in a shaking incubator at 35°C and 120 rpm for 3 or 4 days. The amount of biogas produced was determined using several 30 ml gas syringes. Liquid samples from the bulk solution were taken to periodically analyze the volatile fatty acids (VFAs) concentration using a 10 ml syringe. Batch runs were scheduled to collect samples at every time interval between 3 and 6 h on the first day. Then, the gas was sampled every 24 h, except for the first day when the samples were collected at every time interval between 3 and 6 h. The batch experiments were conducted in duplicate.

### Analytical Methods

The hydrogen content in the biogas was analyzed using a gas chromatograph (Agilent Technologies 7890, Palo Alto, CA, USA) equipped with a capillary column (CP-Molsieve 5A 30 m  $\times$  0.32 ID mm  $\times$  0.45 OD mm, Agilent) and a thermal conductivity detector. The operating temperatures of the oven, injector, and detector were 50°C, 80°C, and 120°C, respectively. Helium was used as the carrier gas at a flow rate of 3 ml/min. VFAs were analyzed by a high-performance liquid chromatograph (Waters Binary HPLC Pump, Milford, MA, USA) with an ultraviolet (210 nm) detector and a 300 mm  $\times$  7.7 mm, 8  $\mu$ m Agilent Hi-Plex H PL1170-6830 column, using 0.005 M sulfuric acid as the mobile phase. The liquid samples for VFA analysis were prepared after filtration with a 0.45  $\mu$ m membrane filter. Total COD and T-N were determined with a digestion solution from HACH Co. (Loveland, CO, USA) and spectrophotometers (Thermo Orion AQ2040 and DR6000 UV VIS, Rockford, IL, USA). Glucose was analyzed as a reduced sugar with reference to a modified dinitrosalicylic acid method [15, 24]. VS was determined according to a standard method [1].

### Assay Methods

Hydrogen and methane gas production amounts were calculated from the gas composition of the headspace and the total volume of biogas produced, using the following mass balance equation:

**Table 2.** Characteristics of mixed wastes and kinetic parameters of hydrogen production calculated from Gompertz equations.

Dilution ratios	MW/SS	COD (g/l)	VS (g/l)	COD/T-N	P (H <sub>2</sub> ) (ml)	R <sub>m</sub> (H <sub>2</sub> production rate) (ml/h)	λ (Lag phase) (h)	H <sub>2</sub> yield (ml/g VS <sub>removed</sub> )	Total VFAs (mg/l)
Forty fold	100:0	4.96	2.16	17,705	11.0	3.37	2.1	47.7	1766
	80:20	4.32	1.89	368	0.73	0.84	2.28	3.9	2477
	60:40	3.69	1.62	159	1.06	0.76	2.18	5.9	1103
	40:60	3.05	1.35	88	0.54	0.88	2.37	3.6	842
	20:80	2.41	1.08	52	0.17	0.78	2.69	0.8	522
	0:100	1.78	0.81	31	0	-	-	0	248
Twenty fold	100:0	9.92	4.31	17,705	15.4	1.24	3.73	53.0	1884
	80:20	8.64	3.77	368	4.55	0.47	3.32	17.7	2233
	60:40	7.37	3.23	159	2.47	2.06	2.82	13.1	1523
	40:60	6.10	2.69	88	1.3	0.9	2.68	7.2	1102
	20:80	4.83	2.15	52	0.43	0.24	2.14	5.0	1060
	0:100	3.55	1.61	31	0	-	-	0	882
Ten fold	100:0	19.8	8.62	17,705	31.5	2.36	7.28	73.6	3243
	80:20	17.3	7.54	368	14.3	5.88	8.77	35.8	3337
	60:40	14.7	6.46	159	7.74	6.88	8.65	23.3	3119
	40:60	12.2	5.38	88	2.48	2.07	8.86	16.3	3057
	20:80	9.65	4.30	52	0.15	0.14	7.06	0.9	1696
	0:100	7.11	3.22	31	0	-	-	0	1418
Five fold	100:0	39.7	17.2	17,705	36.9	1.29	3.89	49.9	4946
	80:20	34.6	15.1	368	15.5	0.85	18.1	22.1	5489
	60:40	29.5	12.9	159	14.3	5.79	8.95	27.4	4583
	40:60	24.4	10.8	88	7.66	4.06	9.03	19.6	2997
	20:80	19.3	8.60	52	3.64	4.19	8.80	12.3	570
	0:100	14.2	6.44	31	-	-	-	0	157

MW, molasses wastewater; SS, sewage sludge.

P, ultimate hydrogen production.

R<sub>m</sub>, hydrogen production rate.

λ, lag-phase time.

$$V_{H,i} = V_{H,i-1} + C_{H,i}(V_{G,i} - V_{G,i-1}) + V_H(C_{H,i} - C_{H,i-1}) \quad (1)$$

where  $V_{H,i}$  and  $V_{H,i-1}$  are cumulative hydrogen gas volumes at the current (i) and previous (i-1) time intervals,  $V_{G,i}$  and  $V_{G,i-1}$  are the total biogas volumes at the current and previous time intervals, and  $C_{H,i}$  and  $C_{H,i-1}$  are the fractions of hydrogen gas at the current and previous intervals, respectively.  $V_H$  is the total volume of headspace in the reactor.

Time-dependent hydrogen production was fitted to a modified Gompertz Eq. (2). This equation is useful for describing hydrogen production in batch tests [4, 19, 20].

$$H = \exp \left[ -\exp \left\{ \frac{R_m}{P} (\lambda - t) e + 1 \right\} \right] \quad (2)$$

where H is the cumulative hydrogen production (ml H<sub>2</sub>), P is the

ultimate hydrogen production (ml H<sub>2</sub>), R<sub>m</sub> is the hydrogen production rate (ml H<sub>2</sub>/day), λ is the lag-phase time (h), and e is the exponent 1. All parameters in Eq. (2) were calculated using the Solver function in Microsoft Excel 2010. Fitted curves were obtained by minimizing the sum-of-square errors between the experimental data and the estimates.

Response surface methodology is a mathematical and statistical technique used to analyze mutual relationships between the response (dependent) and predicted (independent) variables and to identify optimal operating conditions for a system [32]. In addition, this technique is used to describe all impacts of the parameters on a biological hydrogen production system [13]. In this study, the effects of VS concentrations (mainly related to dilution ratios) and mixing ratios of molasses wastewater and sewage sludge on hydrogen production were analyzed using the second-order quadratic model, as shown in Eq. (3).

**Table 3.** Variables and results of the regression analysis on the quadratic equations.

Eq. (3)				Eq. (4)							
$f = \beta_0 + \beta_1x + \beta_2y + \beta_{11}x^2 + \beta_{22}y^2 + \beta_{12}xy$				$f = \beta_0 + ax + bx^2$							
Independent variable											
x; Molasses wastewater (MW) composition (%)				x; COD/T-N ratio							
y; VS concentration (g/l)											
Dependent variable											
Specific hydrogen production rate (ml H <sub>2</sub> /g VSS/day)			Hydrogen yield (ml H <sub>2</sub> /g VS <sub>rem</sub> <sup>-1</sup> )			Hydrogen yield (ml H <sub>2</sub> /g VS <sub>rem</sub> <sup>-1</sup> )					
Test results of regression analysis											
R <sub>Sqr</sub> : 0.927; $\alpha$ : 0.05 $p < 0.0001$			R <sub>Sqr</sub> : 0.885; $\alpha$ : 0.05 $p < 0.0001$			R <sub>Sqr</sub> : 0.507; $\alpha$ : 0.05 $p = 0.002$					
Coefficient	t	p	Coefficient	t	p	Coefficient	t	p			
$\beta_0$	-3.776	-0.639	0.531	$\beta_0$	-6.069	-1.288	0.214	$\beta_0$	-5.329	-1.101	0.286
$\beta_1$	-0.719	-3.359	0.003	$\beta_1$	-0.321	-1.883	0.076	a	0.210	2.866	0.011
$\beta_2$	5.298	3.228	0.005	$\beta_2$	4.822	3.686	0.002	b	-0.0004	-2.206	0.041
$\beta_{11}$	0.008	3.472	0.003	$\beta_{11}$	0.006	3.344	0.004				
$\beta_{22}$	-0.633	-4.336	0.000	$\beta_{22}$	-0.405	-3.480	0.003				
$\beta_{12}$	0.113	4.623	0.000	$\beta_{12}$	0.038	1.971	0.064				

$$f = \beta_0 + \beta_1x + \beta_2y + \beta_{11}x^2 + \beta_{22}y^2 + \beta_{12}xy \quad (3)$$

where  $f$  is the predicted response,  $x$  and  $y$  are independent variables,  $\beta_0$  is the offset term,  $\beta_1$  and  $\beta_2$  are linear coefficients,  $\beta_{11}$  and  $\beta_{12}$  are squared coefficients, and  $\beta_{12}$  is the interaction coefficient. The effects of COD/T-N ratios on hydrogen production yield were applied to the primary quadratic model as a polynomial expression (4).

$$f = \beta_0 + ax + bx^2 \quad (4)$$

where  $f$  is the predicted response,  $x$  is the independent variable, and  $a$  and  $b$  are linear squared coefficients, respectively. Specific information on the dependent and independent factors in relation to the two quadratic models is summarized in the upper part of Table 3. All parameters in Eqs. (3) and (4) were evaluated using the fit curve function in Sigmaplot 2010 (Systat Software Inc., San Jose, CA, USA).

## Results and Discussion

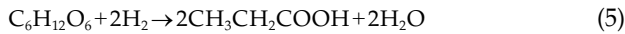
### Effects of VS Concentration and Waste Mixing Ratios of Molasses Wastewater and Sewage Sludge on Hydrogen Production

The hydrogen production kinetic parameters were calculated by Gompertz equations according to the various characteristics of the mixed wastes (Table 2). We used four

dilutions (40-, 20-, 10-, and 5-fold) and six mixing ratios (100:0, 80:20, 60:40, 40:60, 20:80, and 0:100) of molasses wastewater and sewage sludge. The VS concentrations varied from 0.81 to 17.2 g/l, due to the different dilution ratios. The hydrogen production potential ( $P$ ) increased as the dilution ratio decreased and the composition of molasses wastewater increased.

The maximum hydrogen yield based on VS removal was 73.6 ml H<sub>2</sub>/g VS<sub>removed</sub> when only molasses wastewater was fermented at the 10-fold-dilution ratio. However, hydrogen was yielded in a downward trend (49.9 ml H<sub>2</sub>/g VS<sub>removed</sub>) at a high VS concentration (17.2 g/l) and a 5-fold-dilution ratio. Interestingly, hydrogen yield was significantly enhanced during co-digestion with sewage sludge when the dilution ratio was decreased. These results indicate inhibited hydrogen production due to a high organic concentration of molasses wastewater, whereas the hydrogen production yield was enhanced by adding sewage sludge. The hydrogen production potential decreased as the VS concentration was increased further, which may have been due to byproduct inhibition [19, 34]. Various bacteria in the sewage sludge seemed to catabolize organic matters in the molasses wastewater and produced different byproducts. Acetate and butyrate productions were accompanied by hydrogen production. However, the hydrogen production rate was considered to be adversely influenced by

propionate production in the biohydrogen system. This is involved with the following reaction:



In particular, the hydrogen yield increased nearly more than nine times (from 3.9 to 35.8) at a mixing ratio of 80:20 when the dilution ratio was decreased from 40-fold to 10-fold. Unlike co-digestion with sewage sludge, the hydrogen yield increased slightly from 47.7 to 73.6 ml H<sub>2</sub>/g VS<sub>removed</sub> (only 1.5 times) at the waste composition of whole molasses wastewater.

Total VFA concentrations were likely to be high during the co-digestion of molasses wastewater and sewage sludge. The maximum VFA production (5.49 g/l) was found at a waste composition ratio of 80:20 and a VS concentration of 15.1 g/l. Regardless of high VS concentration, the trend in VFA production was proportional to the VS concentration. VFA production can also be a crucial parameter in the design of a two-stage hydrogen and methane production system owing to high energy recovery from waste treatment. VFAs mainly including acetate, propionate, butyrate, and valerate have great potential to be used as substrates for biological nutrient removal reactions in wastewater treatment [33] as well as for methane production [8].

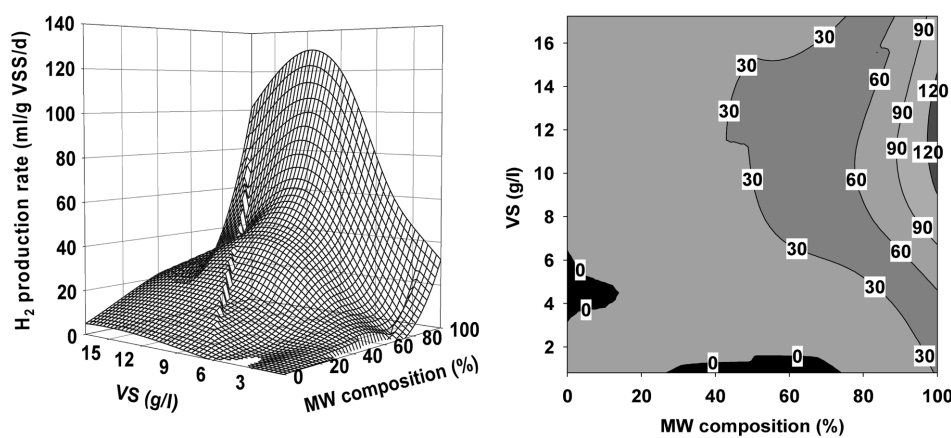
### Regression Analysis on Hydrogen Production by VS Concentration and Mixing Composition of Molasses Wastewater and Sewage Sludge

The design matrix for the regression analysis and the results obtained based on the experimental data are shown in Table 3. *x* and *y* are independent factors for the

composition of molasses wastewater and VS concentrations, respectively. One-hundred iterations were made to minimize the sum-of-square errors between the experimental data and the estimates. An analysis of variance for the experimental model was conducted to evaluate the significance and adequacy of the quadratic model. For all coefficients of the linear terms (*x* and *y*), quadratic terms (*x*<sup>2</sup> and *y*<sup>2</sup>), and the interactive term (*xy*) in the response variable of hydrogen yield, *p* less than 0.05 values indicated high significance of the corresponding variable at the 95% confidence level. In addition, a high R<sup>2</sup> of 0.927 and 0.885 represented a high degree of correlation between the predicted and actual responses. Thus, the polynomial model obtained was adequate and statistically significant.

Three-dimensional (3D) mesh and two-dimensional (2D) contour curves were plotted to represent the interaction among the independent factors and to consider the optimal conditions of the specific hydrogen production rate (Fig. 1). Fig. 1 shows that a relatively high specific hydrogen production rate was observed when the VS concentration ranged from 6 to 14 g/l. The predicted optimal range indicated that the specific hydrogen production rate would decrease when the VS concentration exceeded from 10 to 12 g/l at all mixing ratios of molasses wastewater and sewage sludge.

Unlike the effects of VS concentration on specific hydrogen production, the specific hydrogen production rate increased as the composition of molasses wastewater increased. The maximum specific hydrogen production rate was 130 ml H<sub>2</sub>/g VSS/day when only molasses wastewater was fermented. Most reported batch fermentation values from organic wastes such as bean plus beverage



**Fig. 1.** Specific hydrogen production rate *vs.* waste mixing composition and volatile solid (VS) concentration depicted from Eq. (3) (left, three-dimensional mesh; right, two-dimensional contour).

wastewater, dehydrated brewery mixture, and food waste are between 406 and 870 ml H<sub>2</sub>/g VSS/day [18, 30]. In our study, the seed sludge from a continuous hydrogen-producing system (our laboratory) was not enriched by co-digestion, and resulted in the growth of methanogenic bacteria (explained in the following sub-section).

The specific hydrogen production rate increased remarkably as the molasses wastewater composition in the waste mixture was increased to over 80%, which was described in the 3D curve. The coefficient of the square term  $\beta_{11}$  was a positive number of 0.008. This may have resulted from the strong impact of the carbohydrate content of molasses wastewater on hydrogen production. The coefficient of the square term  $\beta_{12}$  was  $-0.633$ , which was highly involved in the optimization technique for the specific hydrogen production rate. The effect of VS concentration on the specific hydrogen production rate was more critical than the mixing composition considering the absolute value.

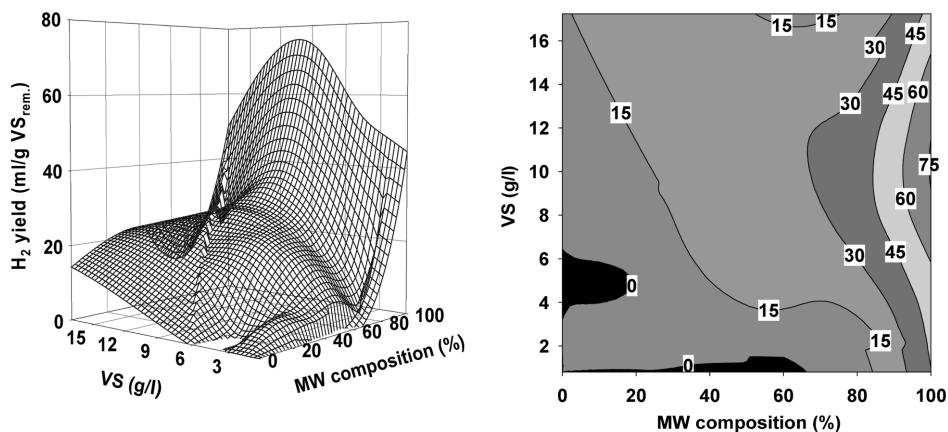
Fig. 2 shows that the hydrogen production yield curves had similar shapes as the specific hydrogen production rate, except the range of molasses wastewater composition was between 20% and 80%. As shown in the 2D contour, the hydrogen production yield increased as the VS concentration increased up to 14 g/l. The high carbohydrate concentration inhibition on the hydrogen production rate was more severe than that on hydrogen yield [19]. The square term of the coefficient ( $\beta_{12}$ :  $-0.405$ ) also demonstrated a weak relationship between hydrogen yield and VS concentration compared with that ( $\beta_{12}$ :  $-0.633$ ) of Eq. (3) with regard to the hydrogen production rate.

The maximum hydrogen yield was 75 ml H<sub>2</sub>/g VS<sub>removed</sub> at a waste composition ratio of 100:0, which was in the high range of previously reported values (37–77 ml H<sub>2</sub>/g VS) for organic waste from food waste and municipal solid waste [5, 7, 28]. A hydrogen yield  $>50$  ml H<sub>2</sub>/g VS<sub>removed</sub> was obtained at a waste composition from 90:10 (10% of sewage sludge content in the mixture) to 100:0 and a VS concentration of 7–12 g/l (about 10-fold dilution ratio). This result indicates that molasses wastewater supplemented with 10% sewage sludge was a compatible resource for hydrogen production.

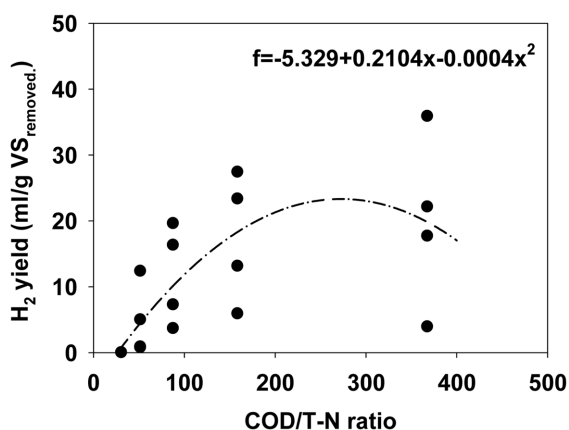
To obtain a hydrogen yield of over 15 ml H<sub>2</sub>/g VS<sub>removed</sub>, the VS concentration was unlikely to affect the co-digestion system at a mixing composition of molasses wastewater from 40% to 80%. However, the VS concentration should be higher than 12 g/l to produce the same amount of hydrogen in mixed waste containing as high as 80% sewage sludge. Thus, appropriately controlling the VS concentration through the dilution and mixing ratios is anticipated to lead to the optimal hydrogen production rate.

#### Relationships Between Hydrogen Yield and COD/T-N Ratios or Methane Production

An adequate combination of sewage sludge and tofuprocessing waste enhances the biohydrogen production yield [16]. Enriched protein from sewage sludge without an external dose of nitrogen explains the enhanced hydrogen production. Proteins such as peptone or yeast extract are a better source than ammonium salts to activate and grow hydrogen-producing bacteria [25]. However, the maximum hydrogen yield could be obtained only when molasses



**Fig. 2.** Hydrogen production yield *vs.* waste mixing composition and volatile solid (VS) concentration depicted from Eq. (3) (left, three-dimensional mesh; right, two-dimensional contour).

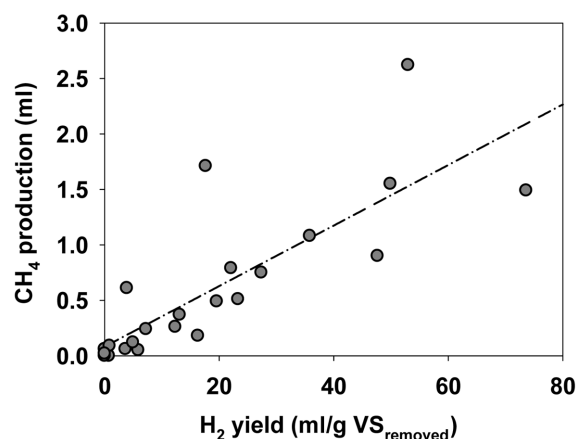


**Fig. 3.** Effects of the chemical oxygen demand/total nitrogen (COD/T-N) ratio on hydrogen production depicted from Eq. (4).

wastewater was digested at all dilution ratios. As shown in Table 1, molasses wastewater is carbohydrate-rich (11.4 g glucose/L, 128 g COD/L), whereas sewage sludge is nitrogen-rich (1.8 g T-N/L). Because of the characteristic difference of the two substrates, an extremely high COD/T-N ratio (17,705) was observed at the mixing composition of 100:0 (Table 2). In contrast, other ratios merely ranged from 31 to 368. Thus, the effects of the COD/T-N ratio on the hydrogen production yield were only depicted at mixing composition ratios of 80:20, 60:40, 40:60, 20:80, and 0:100 (Fig. 3).

As summarized in Table 3, the primary quadratic model (Eq. (4)) was valid because the *p*-value of the squared term (0.002) was less than 0.05, despite a rather low  $R^2$  (0.507). The optimum ratio of COD:N for hydrogen production is 40–200 [11, 22, 29]. However, the hydrogen yield over 20 ml  $H_2$ /g  $VS_{removed}$  was at a COD/T-N ratio of 250–300 in this study (Fig. 3). The hydrogen yield was likely to decrease at a COD/T-N ratio over 280. Most reported COD/N values were obtained from a continuous hydrogen-producing system, whereas we showed an appropriate value in batch experiments.

Methane was slightly evolved in all bottles, mainly due to methanogenic bacteria in the sewage sludge [14]. The methane production increased up to 2.0 ml  $CH_4$  when the hydrogen yield was 75 ml  $H_2$ /g  $VS_{removed}$ . The maximum methane production yield from VS removed was 9.0 ml  $CH_4$ /g  $VS_{removed}$ . This was similar to the value of 8.1 ml  $CH_4$ /g  $VS_{removed}$  reported in batch tests using mixed food waste and sewage sludge [17]. There is reasonable evidence to conclude that methane production had a linear relationship



**Fig. 4.** Relationship between hydrogen yield and methane production.

with hydrogen production yield (Fig. 4). However, methane production was co-related with the waste mixing composition, particularly for sewage sludge content (data not shown).

Methanogens produce methane for either reduction of  $CO_2/H_2$  (hydrogenotrophic) or from fermentation of acetate to  $CH_4$  and  $CO_2$  (acetoclastic) [6]. There are conflicting observations with respect to the relative contribution of these substrates to  $CH_4$  production, but most known methanogenic bacteria have an optimum pH at 7 [27]. Because of the low pH of 5.7 (initial condition) in our experiment and the deep co-relationship between methane production and hydrogen yield, methane production would slightly deteriorate the hydrogen production performance as a result of the following hydrogen-consuming mechanism.



Reducing the partial pressure of hydrogen in a reactor enhances hydrogen production. Using a vacuum or sparging the reactor vessel with nitrogen gas also increases hydrogen yields [9]. Unlike batch tests, which are usually done in a shaking incubator, methane generation should be suppressed to improve the hydrogen production in a continuous co-digesting system with sewage sludge.

## Acknowledgments

This study was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean Government (MISP) (No. NRF-2012R1A2A2A03046724).

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