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Statistical key factors optimization of conditions for hydrogen production from S-TE (solubilization by thermophilic enzyme) waste sludge

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HIGHLIGHTS

- Hydrogen production from S-TE sludge could be affected by different factors.
- Statistical key factors for S-TE sludge H2 production were optimized using uniform design.
- Maximum hydrogen yield and optimum conditions could be predicted from regression model.
- Comprehensive effect of factors on nutrients and metabolites changing was investigated.

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ABSTRACT

Waste sludge can be solubilized after S-TE (solubilization by thermophilic enzyme) pretreatment as the cryptic growth occurs at the expense of the cell lysate. The hydrogen production from S-TE sludge is greatly influenced by many factors. In this study, factors including pH, C/N, C/P, and Fe^{2+} affecting hydrogen production from S-TE sludge were optimized using uniform design. The optimum condition for maximum hydrogen yield of 68.4 ml H_2/g VSS (volatile suspended solid) could be predicted from regression model, and the optimum conditions were pH of 6.4, C/N ratio of 38, C/P ratio of 265, and Fe^{2+} concentration of 85 mg/L. There was interaction effect of factors on hydrogen production from S-TE sludge. Different pH, C/N, C/P and Fe^{2+} conditions could influence the VSS removal rate, carbohydrate and protein utilization. When the highest compositions of acetate and ethanol and lowest propionate were observed in metabolites, effective hydrogen production was also achieved.

1. Introduction

The thermophilic aerobic treatment can be applied for sludge reduction integrated in the wastewater handling units. When thermophilic aerobic bacteria are subjected to optimal conditions for their growth, such as in the thermophilic aerobic reactor, they perform enzymatic hydrolysis at expenses of other microbial cells. Using the theory of lysis enzyme secrete by thermopile bacteria to hydrolysis the waster sludge, it developed the S-TE (solubilization by thermophilic enzyme) technology (Song and Hu, 2006). The scheme of the S-TE process was proposed by Kobelco-solutions Co. (Japan) and applied at full-scale in a small WWTPs in Japan Sawatari water quality control center (Sakai et al., 2000; Shiota and Hasegawa, 2002). The sludge was solubilized after S-TE pretreatment both by the thermal effect and the thermophilic enzyme, where cryptic growth occurs at the expense of the cell lysate (Foladori et al., 2010). Cryptic growth is termed that some organic substance which was released after waste sludge solubilization could be reused by sludge microbial. In the temperature range from 60 to 75 °C, the biodegradation of the macromolecules making of the sludge organic matter is generally improved in relation to the flocs structure disintegration and its partial solubilization (Camacho et al., 2003). Microbial cells in sludge might be break down or hydrolyze into micro-molecules which could be used as substrate for hydrogen production. In our previous study, methanogen in the waste sludge could be restrained after S-TE pretreatment, and the S-TE pretreated sludge was fit for hydrogen production (Guo et al., 2008, 2010a,b).

The biological H_2 generation is greatly influenced by many factors, including C/N and C/P ratios (Argun et al., 2008), strain type, substrate concentration, cell concentration, metal ions,

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temperature and pH, etc. (Das and Veziroglu, 2008; Lee et al., 2008; Skonieczny and Yargeau, 2009; Yasin et al., 2011; Zhang and Shen, 2006; Zhao et al., 2012). Thus, it is of great significance to find appropriate levels of different factors in order to increase the ability of anaerobic sludge to produce H_2. Several researchers have reported the optimization of fermentation H_2 production by statistical method to investigate the effects of different factors on fermentative H_2 production (Shi et al., 2010; Sreela-or et al., 2011).

If there are S factors of which each factor has Q levels in an experiment, where $Q > 1$, then the orthogonal array is often used; if a design of Q level experiments is proposed by the theory of uniform distribution which will be called the uniform design (Wang and Fang, 1981). Some applications to material, architecture, medicine and military, chemical industry showed the advantages of the uniform design as compared with the orthogonal array and other methodology (Fang, 1994; Wang and Fang, 1981). Uniform design could decrease the number of experimental times, automated classification the experimental factors and order rank according to the significance, through the discussion of results to prediction the affecting factors (Lv and Ding, 2012; Zhang et al., 2009).

Although many studies have been done on the effect of environmental factors on hydrogen production from various kinds of synthetic substrates and wastes, but the information on the statistically optimization of environmental factors on bio-hydrogen production from S-TE pretreated sludge are still lacking. Therefore, the main objective of this work was to explore the effects of pH, C/N, C/P and Fe^{2+} on H_2 fermentative using the uniform design method. The information obtained from this study could find the optimum H_2 production conditions from S-TE pretreated sludge.

2. Methods

2.1. Preparation of feedstocks

The fed sludge was taken from the secondary treatment stage of waste water treatment plants (WWTPs) in Qingdao. Prior to use, the sludge was sieved by grid size 2.0 mm to remove coarse matters and stored at 4 °C prior the usage. Then, the sludge was pretreated by S-TE (solubilization by thermophlic enzyme) method (Guo et al., 2010a,b, 2012). The characteristics of raw sludge and S-TE pretreated sludge are shown in Table 1. It was found that S-TE pretreatment could enhance the releasing of SCOD (soluble chemical oxygen demand), protein, carbohydrate and NH_4^+–N into the liquid phase by 20.3, 6.0, 3.3, 3.0 times, respectively. SS (suspended solid) and VSS (volatile suspended solid) were all decrease as solubilization and reduction effect of S-TE on waste sludge.

2.2. Bio-hydrogen fermentation

Substrate (100 ml) was anaerobically (blow N_2 for 10 min) incubated at 35 °C in 250 ml serum bottles with stirring of 125 r/min and was not added other nutrients. The bottles were capped with silica gel stoppers. Under identical condition, three fermentation bottles were measured and their average of data was reported to prevent any possible errors introduced by sampling procedure. Different conditions of pH (5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9), C/N (21, 24, 27, 30, 33, 36, 39, 42, 45), C/P (100, 125, 150, 175, 200, 225, 250, 275, 300) and Fe^{2+} (20, 30, 40, 50, 60, 70, 80, 90, 100 mg/L) were investigated in S-TE sludge fermentation. The initial conditions of pH, C/N, C/P and Fe^{2+} could be adjusted through dosing. The concentration of H_2 was tested frequently during fermentation and the H_2 production was also recorded during the whole examination.

Table 2
Results of $U_9{}^*(9^4)$ uniform design.

Run	X_1	X_2	X_3	X_4 (mg/L)	Y (ml H_2/g VSS)
1	5.0	27	250	100	1.3
2	5.5	36	175	90	55.8
3	6.0	45	100	80	56.6
4	6.5	24	275	70	41.6
5	7.0	33	200	60	50.3
6	7.5	42	125	50	23.6
7	8.0	21	300	40	38.0
8	8.5	30	225	30	35.6
9	9.0	39	150	20	29.0

2.3. Experimental design and data analysis

C/N, C/P, pH, and Fe^{2+} were chosen as four independent factors and the maximum H_2 production rate (R_m) were selected the dependent output variable. A four-parameter nine-level experimental design of uniform design method was used to find the optimum H_2 production condition and was given in Table 2. The experiments were performed at random order and the Y values are response, such as R_m to the independent variables X_1, X_2, X_3 and X_4, which are pH, C/N, C/P, and Fe^{2+}, respectively. Regression analysis was performed with the data obtained to estimate the response function as below:

$$Y = b + \Sigma a_i X_i + \Sigma a_{ii} X_i^2 + \Sigma a_{ij} X_i X_j \quad (1)$$

where Y is the response of hydrogen yield, b is the constant coefficient, a_i, a_{ii} and a_{ij} are the coefficients estimated by the model. They represent the liner, quadratic and cross-product effects of the X_1, X_2, X_3 and X_4, respectively. The parameters of the response equation and analysis of variance (ANOVA) were evaluated using SPSS 17.0.

The accumulative volume of hydrogen produced (H) over the time course during the batch tests was fitted with the Gompertz equation (Lay et al., 1999):

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

where P is the hydrogen potential (ml), R_m is the maximum hydrogen production rate (ml/h), λ is the lag phase time (h), and e is 2.718281828.

2.4. Analytical methods

The volume of produced biogas was determined by draining saturated salt water method and the biogas contents were analyzed with a gas chromatograph (SP-6890, Lunan Ruihong, Shandong) equipped with a thermal conductivity detector (TCD) with a 2 m column packed with Porapak Q. The soluble metabolites of VFAs (volatile fatty acids) and ethanol were measured with a gas chromatograph (Shimadzu GC2010, Japan) equipped with a flame ionization detector (FID) and a capillary column (DB-FFAP, 30 m × 0.25 mm × 0.25 μm). The pH was measured with a digital pH-meter (PHB-5, Aolilong, Hangzhou). The concentration of COD

Table 1
Characteristics of raw sludge and S-TE pretreated sludge.

	pH	SS (g/L)	VSS (g/L)	TCOD (mg/L)	SCOD (mg/L)	Protein (mg/L)	Carbohydrate (mg/L)	NH_4^+–N (mg/L)	TN (mg/L)	TP (mg/L)
Raw	7.3 ± 0.1	21.8 ± 0.4	8.8 ± 0.2	12,896 ± 25	293 ± 13	74.8 ± 6.3	37.7 ± 5.8	43.8 ± 3.7	228.3 ± 17.2	2.1 ± 0.1
S-TE	6.9 ± 0.1	17.8 ± 0.3	7.6 ± 0.2	11,924 ± 28	6230 ± 22	520.3 ± 25.1	162.6 ± 12.3	174.3 ± 13.9	267.9 ± 14.5	3.2 ± 0.1