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Research paper

Performance enhancement by rumen cultures in anaerobic co-digestion of corn straw with pig manure

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ABSTRACT

Anaerobic co-digestion of corn straw with pig manure by rumen cultures was investigated in this study. Both Box-Behnken design and response surface methodology (RSM) were conducted for parametric optimization in batch tests, and later evaluated the performance enhancement in biogas production for continuous anaerobic digestion test. The optimal pretreatment time and mixing ratio (C/N ratio) were obtained at 3 days and 1:1 (C/N ratio, 19.15:1), corresponding total VFA yield, acetate concentration and $VS_{degradation}$ at 0.76 g g^{-1}·$VS_{degraded}{}^{-1}$, 4014.43 mg L^{-1} and 79.44%, respectively. Subsequently, after a start-up time of 23 days, the continuous co-digestion reactor was successfully operated with the rumen microorganisms. The performance of co-digestion was not affected by retention time and dry matter content, resulting in a stable concentration of reducing sugar, TVFA and biogas yield at about 150 mg L^{-1}, 100 mg L^{-1} and 430 mL g^{-1}·VS^{-1}. The role of rumen microorganisms was confirmed by FPase, CMCase and xylanase activities, up to 0.31 U mL^{-1}·min^{-1}, 0.35 U mL^{-1}·min^{-1}, and 0.45 U mL^{-1}·min^{-1}. A methane content of 60% was found, together with the carbon dioxide and hydrogen content of 40% and 0%. These results demonstrated that there was a remarkable improvement on anaerobic acidification and co-digestion of corn straw with pig manure using rumen microorganisms as inoculum. Co-metabolism maybe play a more important role in this process than pretreatment not only to increase the efficiency of saccharification and acidogenesis but also further provide tremendous potential in biogas production.

1. Introduction

China is one of the largest agricultural countries in the world. There were more than 841 million tons of waste straw every year by 2005, in which only 686 million tons could be collectable and utilizable quantity especially for approximately 23% were open-field burned and about three-fourths of collectable and utilizable straw including straw residue were returned to the field directly [1]. The amount of animal manure reached 2.121 billion tons in 2011, and the yield of animal manure in 2020 and 2030 will attain 2.875 and 3.743 billion tons based on the calculation results, respectively [2].

The methods that can be applied to treat such large-scale waste have been widely studied, for example, anaerobic digestion, composting, gasification, and pyrolysis [3]. Compared with other treatment technologies, anaerobic digestion is suggested as a sustainable and environmentally friendly method for converting a high organic content waste into a renewable energy [4]. The digestion process can be divided into four phases: the first three phases including hydrolysis, acidogenesis, and acetylation conducted by a distinct bacterial consortia, while the fourth step of methanogenesis is carried out by other specialized groups of methanogenic archaea [5]. Evaluation of the acidification phases can reveal the degradation and conversion process of substrates definitely, while the whole digestion process for biogas production can detect the adaptability and stability of the system comprehensively [6].

Nonetheless, the efficiency and stability of individual digestion for manure are inferior due to the low C/N ratio contrast with applicable range from 20 ~ 30 [7]. The bacteria which generally uses nitrogen-rich materials such as animal manures and carbon-rich materials such as straws as substrates, is identified that nitrogen-rich substrates could provide a high buffering capacity against failure caused by a drop in pH and a wide range of nutrients for bacterial growth, and carbon-rich substrates could improve the C/N ratio of the feedstock, thereby increasing stability and creating a suitable culture for digestion [8]. Additionally, a previous study reported that ammonification had a significant improvement on the digestibility of maize straw by rumen microorganisms [9]. Pig manure has a high concentration of $NH_4{}^+$, thus it might provide a source of ammonia to not only destroy the structure of the straw but also disrupt crystalline cellulose for

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enhancement of degradation.

In a co-digestion system, carbon sources are mainly composed of agricultural waste such as straws, binds, etc. The chemical and physical structure of these substrates, which are mainly composed of lignin, cellulose and hemicellulose, is difficult for bacterial degradation during digestion phases [10]. In recent years, several research works revealed that rumen microorganisms with an abundant fungus for extensive colonization and potent cellulolytic activities had strong ability to degrade lignocellulosic biomass [11,12]. Compared with other inoculums, the system inoculated rumen microorganisms had a higher degradable efficiency and conversion rate for both lignocellulosic and high organic content wastes. Most of the researches focused on the improvement of acidogenesis phase by rumen cultures in vitro [11,13], while it was neglected that the rumen microorganisms comprised about $10^8 \sim 10^{10}\,g^{-1}$ of methanogens [14] indicating that the anaerobic digestion system inoculated with rumen microorganisms probably has a great potential in biogas production.

Furthermore, co-metabolism is considered as a complementary process which plays significant roles in microbes digesting substrates especially for improving degradation efficiency attributed to: (i) providing energy or carbon source by resolving readily biodegradable substances; (ii) producing specific enzyme systems induced by the degrading process [15–17]. In this context, it could be inferred that anaerobic conversion of corn straw and pig manure by rumen cultures might be a prospective way.

The main objective of this study is to evaluate: 1) optimal parameters and 2) the performance enhancement in the hydrolysis and digestion by rumen microorganisms. Box-Behnken design and RSM were employed for optimized experimental design in order to investigate the effects of pretreatment time and mixing ratio (C/N ratio) for two substrates on total VFA (TVFA) yield, acetate concentration and $VS_{degradation}$. The chemical properties of mixture, characteristics on optimized and other two contrastive tests were both explored for revealing the role of rumen microorganisms in hydrolysis, acidogenesis and acetylation phases. Based on the results of optimization tests, further exploration of reducing sugar, VFAs and enzyme activities in continuous co-digestion system was conducted to investigate the adaptability of rumen microbes in start-up stage, and then evaluate the influence of retention time and dry matter content on the stability of digestion and biogas yield.

2. Materials and methods

2.1. Seed microorganisms and substrates

The rumen fluid was taken from the fresh stomach of cattle, and then transferred to the laboratory immediately as inoculable microorganisms. Nitrogen gas was used to purge the air to ensure anaerobic environment. Pig manure obtained from a pig farm and corn straw collected from a village were used as the substrates. After being cleaned by deionized water, the corn straw was ground into 5–10 mm particles by a hammer mill (FE130, Staida Co., Tianjing, China) and then oven-dried at 65 °C for 16 h. The selected physicochemical properties of pig manure and corn straw are shown in Table 1.

2.2. Experimental design and conditions

This research composed of two steps. First step is the batch tests for detecting optimal parameter of pretreatment and characteristic of anaerobic acidogenesis. Second step is the continuous CSTR operation for evaluating the performance enhancement in anaerobic digestion of pretreated substrate. The detailed experimental design and conditions are described below.

2.2.1. Optimization tests

First, RSM and Box-Behnken methods were suitable for optimizing the ruminal degradation process [18,19]. It was adopted for the experiment design of optimization tests and developing a statistical model. Fifteen experiments including two control tests, in which contained control A: corn straw only and control B: pig manure only were performed to evaluate the effect of different conditions on TVFA yield (mg L^{-1}), acetate concentration (mg L^{-1}) and $VS_{degradation}$ (%). Pretreatment time and C/N ratio, which was replaced by mixing ratio for accuracy of experiment operation, were the independent variables and the three levels of experimental results mentioned above were selected as the dependent variables.

The values of variables are shown in Table 2. To predict the optimal conditions, a second order polynomial function is employed as:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_{12}x_1x_2 + b_{11}x_1^2 + b_{22}x_2^2 \quad (1)$$

where Y is the predicted response, b_0, b_1, b_2, b_{11}, b_{22} and b_{12} are regression coefficients of the model, and x_1 and x_2 represent pretreatment time and mixing ratio, respectively. The coefficient of determination (R^2) is calculated to detect data variability, thus verifying the quality of fit to the model. The p-values of the parameter estimation are used to validate the model when p-values < 0.05 indicated the significant model terms. This is a square regression model in terms of real values. MATLAB 7.0 is used to estimate the parameters of Eq. (1) according to the results of tests.

Optimization tests included two parts. First, pretreatment processes were performed in 500 mL wide-mouth bottles with fresh pig manure and smashed corn straw for intuitively investigating whether ammonification happened due to high levels of NH_4^+ in the pig manure. The bottles were tightened and maintained at ambient temperature. Secondly, acidification tests were conducted in 600 mL serum vials with 500 mL of working volume consisting of 125 mL of the strained rumen fluid of as a microbial inoculation for obtaining results to predict the

Table 1
Selected physicochemical properties of raw materials.

Parameter	Pig manure[b]	Corn straw[b]
pH	7.89 ± 0.03	–
Moisture (%)	82.66 ± 0.17	0.01
Volatile solid[a] (%)	35.67 ± 0.21	93.60 ± 0.55
Total organic carbon[a] (%)	19.82 ± 0.78	52.16 ± 1.61
NH_4^+-N (mg L^{-1})	1649.21 ± 0.05	–
Total Kjeldahl nitrogen[a] (%)	2.99 ± 0.12	0.70 ± 0.09
C/N ratio	6.63 ± 0.53	74.51 ± 1.08

[a] Dry weight basis.
[b] Means ± S.E. (N = 3).

Table 2
Experimental design and results for optimization tests.

Run	Condition		Results		
	Mix treatment time (days)	Mixing ratio (CS:PM)	TVFA (mg L^{-1})	Acetate concentration (mg L^{-1})	$VS_{degradation}$ (%)
Control A	0	1:0	4475.84	2790.21	71.67
Control B	0	0:1	3414.84	2154.12	74.58
1	7	2	5160.59	3404.73	76.47
2	7	1.5	4550.37	3061.85	72.03
3	3	1.5	4828.99	2947.37	74.83
4	3	1.3	5207.83	3363.08	76.78
5	21	2	5160.36	3422.37	70.83
6	3	1	6039.47	4014.43	79.44
7	15	1.7	4540.67	3150.94	71.64
8	21	1	4627.51	3011.26	75.43
9	21	1.5	4935.53	3296.02	73.68
10	15	1.5	4442.53	3075.46	71.96
11	3	2	4895.36	3333.77	78.61
12	15	1.3	4469.71	3125.34	72.85
13	7	1	5422.36	3774.48	76.84