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Enhanced degradation efficiency of grass pulp mill excess sludge by inoculate rumen microorganisms

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ABSTRACT

The objective of this research was to develop new cultures that is prior to usual anaerobic sludge to improve removal rates of organic materials consisted in grass pulp mill excess sludge (GPES). Two groups of laboratory-scale experiments were carried out at the same time in two completely mixed bioreactors whose capacity was 1,200 mL with 1,000 mL working volume. The GPES lignin structure was well disrupted and reducing sugars, volatile fatty acids (VFAs) and soluble chemical oxygen demand (SCOD) produced from organic materials were greater by inoculate rumen microorganism than that by usual anaerobic sludge. The peak values of reducing sugars, VFAs and SCOD reached 3,829, 3,008 and 4,874.5 mg/L, respectively. By inoculated rumen microorganism, the degradation efficiencies of cellulose, hemicellulose and lignin attained 61.97%, 51.19% and 24.07%, which were enhanced by 26.76%, 26.32% and 15.55%, respectively. Our previous investigation suggests that anaerobic digestion by inoculated rumen microorganism could provide an effective method for improving degradation efficiency with GPES.

Keywords: Grass pulp mill excess sludge; Anaerobic digestion; Rumen microorganism; Degradation efficiencies

1. Introduction

The broader application of the activated sludge process in pulp and paper mills together with increased production has amplified sludge management problems [1]. In China, the yield of pulp mill was 2,628 Mt at a water content of 800 g/kg of sludge in 2007, and an estimated increase to 3,088 Mt with the same water content is expected in 2020 [2]. In addition, the major sludge is from grass pulp mill and contaminants of the grass pulp mill excess sludge (GPES) are more complex than that of the wood pulp mill excess sludge, especially the high lignin content.

Anaerobic digestion has been applied in sewage sludge treatment for over one hundred years [3]. However, the anaerobic systems have not become increasingly common in the treatment of GPES, because long retention times (20–30 d) and low overall degradation efficiency are typical in GPES digestion [4,5]. Those limiting factors are generally associated with the hydrolysis stage which is a main step in the anaerobic digestion process [6]. The reason is that the main compositions of the GPES, lignocellulosic materials (cellulose, hemicellulose and lignin), hardly be degraded by common hydrolysis bacteria [7]. In nature, the rumen ecosystem of ruminant animals is the most elegant and highly evolved lignocellulosic digesting system [8–10]. Degradation of lignocelluloses by rumen microorganisms has been studied extensively in recent years [11–14]. The conversion rate and degradation efficiency for lignocellulosic waste of rumen ecosystem were higher than that of conventional anaerobic

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waste disposal [15,16]. However, little effort has been made to study the anaerobic digestion efficiency of high lignin content GPES by rumen microorganisms.

The main objective of this study was to investigate the degradation efficiency of GPES during anaerobic degradation seeded with rumen microorganisms, compared with seeded with anaerobic sludge from usual sources. Reducing sugar, volatile fatty acids (VFAs), soluble chemical oxygen demand (SCOD) and degradation efficiencies of cellulose, hemicellulose and lignin were examined.

2. Experimental setup

2.1. Inoculum and media

GPES samples were collected from the primary and secondary settling tanks of a pulp and paper plant using reed as the raw material in Shandong province, China. The composition of the sludge was shown in Table 1.

Rumen liquid was taken from a fresh stomach of cattle from Dalian Bangchuidao Meat-Packing Plant and put into a bottle purged with N_2 gas. Before the fresh rumen liquid was used in the experiment, the rumen liquid was first filtered through a four layer muslin cloth, then it was centrifuged at low speed (125×G) in a Secali SS-1 centrifuge tube for 5 min to remove the nonbacterial matters as much as possible [17]. The supernatant liquid is rumen microorganisms and the characteristics are shown in Table 2. Meanwhile, the normal anaerobic seed sludge for the control test was taken from a municipal sludge anaerobic digester, which stored in the refrigerator at –4°C.

The research was conducted in accordance with the Declaration of Helsinki and with the Guide for Care and Use of Laboratory Animals as adopted and promulgated by the United National Institutes of Health. All experimental protocols were approved by the Review Committee for the Use of Human or Animal Subjects of Dalian Polytechnic University.

Table 1
Physical and chemical characteristics of excess sludge

Item	Values
SCOD (mg/L)	1,161
COD (mg/L)	11,448
pH	6.8
MLSS (mg/L)	8,480
MLVSS (mg/L)	6,440
SDI (g/100mL)	2.12
SVI (mL/g)	47.17
TKN (mg/L)	231
NH_4^+–N (mg/L)	23
Ash (%)	24.06
Water content (%)	73.6

Note: TKN means total Kjeldahl nitrogen, MLSS means mixed liquor suspended solids, MLVSS means mixed liquor volatile suspended solids, SDI means sludge density index, SVI means sludge volume index.

The synthetic media (pH 6.8) was added in the system. The composition was as follows: 450 mg/L K_2HPO_4, 450 mg/L KH_2PO_4, 90 mg/L NaCl, 90 mg/L $(NH_4)_2SO_4$, 90 mg/L $MgSO_4{\cdot}7H_2O$, 90 mg/L $CaCl_2$, 500 mg/L $CO(NH_2)_2$ and 250 mg/L l-cysteine HCl. The media was saturated with CO_2 to remove the sedimentation.

2.2. Experimental design

The rumen fermentation experiment was operated for 12 d, two groups of tests (each group include three parallel tests) were operated at the same time. In this experiment, the volume of the reaction bottles which filled with nitrogen gas was 1,200 mL. All the bottles were filled with the same weight of feedstock at the amount of 1,000 g, in which 250 mL inoculated sludge (three bottles are rumen liquid and another three bottles are usual anaerobic sludge) and media were added in the end to keep the total amount up to 1,000 g. The initial condition was that the total suspended solids was 10% and the total volatile suspended solids of the inoculated microorganism was 2,000 mg/L. The bottles were put into a thermostatic incubator with the temperature of 39°C ± 1°C and the stirring speed of 80 rpm. Every day, samples of the mixture in the bottles were taken by a needle tubing sampler to monitor the VFAs, SCOD and reducing sugar.

2.3. Analytical methods

Chemical oxygen demand (COD), SCOD, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and ash were measured according to the standard methods [18]. The pH value was determined by pH meter (Model 20, Denver Instruments Ltd., US). Reducing sugar was measured by the dinitrosalicylic acid method [19]. VFAs were quantified and differentiated using a gas chromatograph (GC-2010, Shimadzu Inc., Japan) equipped with a flame ionization detector and a 30 m × 0.1 μm × 0.53 mm HP-FFAP column. The oven temperature was initially at 70°C for 3 min, followed by a ramp-up of 20°C/min for 6 min and held at a final temperature of 180°C for 3 min. Nitrogen was used as a carrier gas with a flow rate of 1 mL/min. Total Kjeldahl nitrogen (TKN) was measured by Kjeldahl instrument (SKD-800, Shanghai Peiou Co., China). All the methods mentioned above were pretreated by centrifuging the samples of 10,000 rpm for 15 min, and then passed through a 0.45 μm membrane filter. The contents of the cellulose, hemicellulose and lignin were measured by modified method of Wang and Xu [20].

Table 2
Physical and chemical characteristics of rumen liquid

Item	Values
Reducing sugar (mg/L)	313
pH	7.2
MLSS (mg/L)	7,476
MLVSS (mg/L)	6,905
SDI (g/100mL)	2.49
SVI (mL/g)	40.12

2.4. Calculation methods

The degradation efficiency of sludge was calculated with the following equations [21]:

$$VS_{degradation} = \frac{VS_{ini} - VS_{end}}{VS_{ini}} \times 100\% \quad (1)$$

where $VS_{degradation}$ is the degradation efficiency of the lignin and cellulose and hemicellulose in the GPES; the VS_{ini} and VS_{end} are the initial and final concentration of lignin and cellulose and hemicellulose in the GPES.

3. Results and discussion

3.1. Reducing sugar

The first step for searching for the GPES degraded pattern was to identify the variables of the reducing sugar. Fig. 1 shows the concentration of reducing sugars in the system ferment by rumen microorganisms and usual anaerobic sludge.

The level of the reducing sugar appeared to have a significant rise during the first day in rumen microorganism system and the concentration of the reducing sugar reached about 3,829 mg/L at the second day, which is a maximum value during the operation. The concentration of reducing sugar in the bottles which inoculated usual anaerobic sludge reached maximum at the fourth day and the concentration obviously decreased. Generally speaking, the organic carbon of GPES mainly involved cellulose, hemicellulose and lignin, which are macromolecule and have complex structure and difficult to be degraded directly by microorganism. This study demonstrated that GPES could be effectively converted into reducing sugars by the rumen microorganism. The rapid increase in reducing sugar production was attributed to the fast conversion of some organics in the sludge to reducing sugar by the rumen microorganisms. More cellulose hydrolysis bacteria in the rumen microorganisms could produce large amount of reducing sugar by rapid hydrolysis of cellulose.

After the concentrations reached maximum, they both declined rapidly kept pseudo-stable with slight fluctuation at about 1,000 and 500 mg/L, respectively. The following decrease was attributed to (1) the soluble and easily digestible portions of the sludge were consumed completely and the conversion rate declined and (2) reducing sugar, as a middle production during fermentation, transform to VFA continually. At the end of the fermentation, the concentration of reducing sugar restricted to stabilize indicating the hydrolytic stage was the restrict step for the fermentation of pulp and paper excess sludge by rumen microorganisms [22].

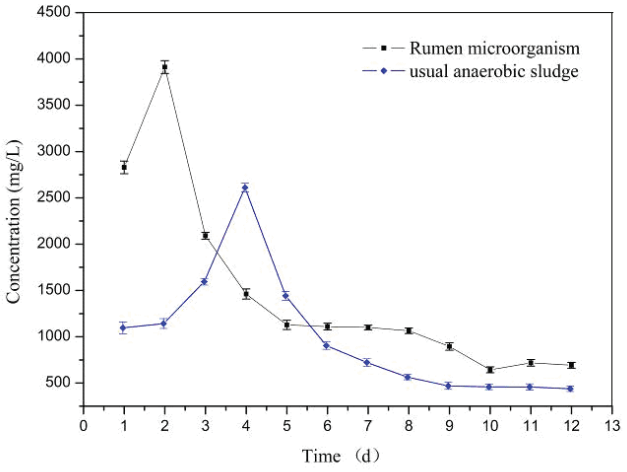

Fig. 1. Reducing sugar concentration in the bottles by different inoculated sludge.

3.2. Volatile fatty acid

In anaerobic digestion process, the concentration of VFAs is an important performance indicator. VFAs production is always associated with the conversion of organic fraction to acid intermediates in anaerobic microenvironments with the help of specific group of bacteria. Acidogens grow faster and are less sensitive to pH variation than methanogens [23]. This usually results in the accumulation of organic acids and the decrease of pH, leading to the suppression of methanogenic activities, and in some cases, even process failure [24]. The evolutions of the VFAs (Fig. 2(a)), acetate (Fig. 2(b)) and propionate (Fig. 2(c)) during the experiment are illustrated in Fig. 2. Acetate and propionate were found to be the two

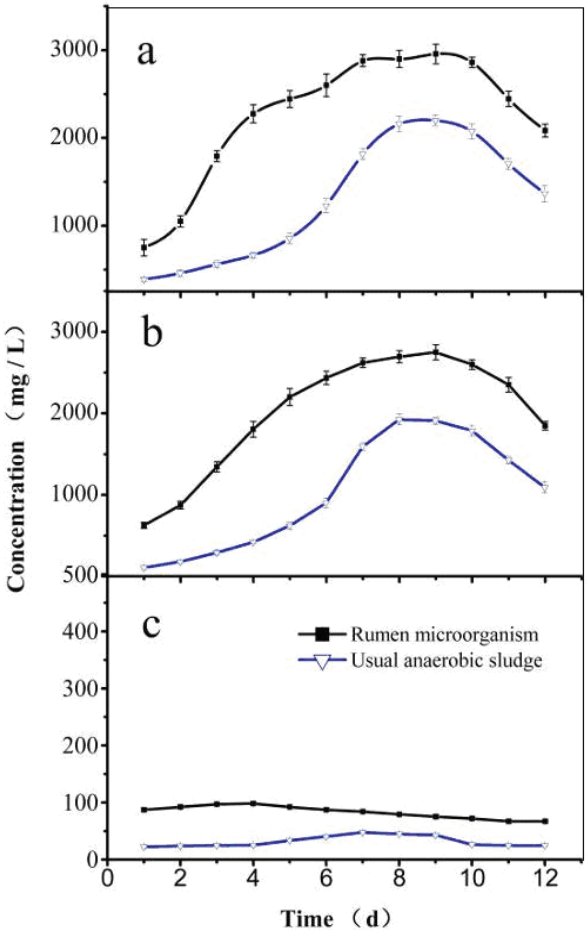

Fig. 2. Concentration of volatile fatty acid (a), acetate (b) and propionate (c) in the bottles by different inoculated sludge.

major compositions of the VFAs in this experiment. In addition, butyrate and valerate were also detected, but in lower levels.

It can be clearly distinguished that the concentration of VFAs increased continuous until the eighth day is shown in Fig. 2(a). This growth might be attributed to the high availability of transformation from reducing sugar to VFAs and resulted in cumulative VFAs production. After the eighth day, the concentration of VFAs varied slightly and stabilized at about 3,000 mg/L until the tenth day and drastically decreased from 3,008 to 2,082 mg/L. The decrease illustrated that the conversion rate of reducing sugar to VFAs was lower than that of VFAs to biogas during this stage. The overall trend of the concentration of VFAs in the bottles inoculated usual anaerobic sludge was similar to that of inoculated rumen microorganism, but the concentration and the yield of the VFAs were lower during early days. The peak value of VFAs yield was 3,008 mg/L, which was higher than that from bottles inoculated usual anaerobic sludge. The results showed rumen microorganism was effective to enhance VFAs yield for GPES anaerobic digestion.

Both of the variation trends of the concentration of acetate (Fig. 2(b)) were similar to that of VFAs. The similar change trends of the acetate and the VFAs indicated that the variation of VFAs is due to the accumulation and transformation of acetate. In contrast to acetate, the propionate (Fig. 2(c)) had a slight increase in the early days (before the fourth day) and constantly slowly decreases until the end of the fermentation, the decline of the propionate may be due to the transformation from propionate to acetate. The major aqueous fermentation products in the VFAs were acetate and propionate. Acetate generated in large amounts indicated that the fermentation pattern in this study were both acetate fermentation.

3.3. SCOD concentration

The variation trend of SCOD concentration was illustrated in Fig. 3. The SCOD concentration reflects the content of soluble organic materials in the liquid. It is clearly found that there was an increase during the early days. This result reflected that some insoluble organic materials in the initial sludge have been hydrolyzed to soluble materials. Otherwise, the value of SCOD in the bottles inoculated rumen microorganism was higher than that inoculated usual anaerobic sludge. Results indicated that the rumen microorganism hydrolyzes most of the organic material immediately in the anaerobic digestion process and the peak value of SCOD was 4,874.5 mg/L. The main organic contents in the GPES were fiber fines (celluloses, hemicelluloses and lignins) generated from the primary sludge and microorganisms in the biological sludge. The rumen ecosystem was the most elegant and highly evolved cellulose digesting system in nature [8,10,25]. The dominant end products of the cellulosic biomass are short chain VFAs (e.g., acetate, propionate and butyrate) [26]. The VFAs dissolved into the fermentation liquid caused the increase of SCOD concentration.

At a later stage, the SCOD concentration gradually decreased until the end of the experiment. This plateau indicated that most of the SCOD, which was degraded by the microorganisms during this phase, was used for cellular reproduction and maintenance in the reactor.

3.4. Degradation efficiencies

In order to check for the degradation efficiencies of the organic material, the VS in the bottles of the sludge were analyzed. The purpose was to verify the degradation ability of the rumen microorganism through contrast the degradation efficiencies. The percentage of cellulose, hemicellulose and lignin in GPES before and after anaerobic digestion and degradation efficiencies are listed in Table 3.

The cellulose and hemicellulose and lignin contents of GPES in each bottle decreased after anaerobic digestion; the decrease rates of cellulose, hemicellulose and lignin were in detail of 61.97%, 51.19% and 31.17% (inoculated rumen microorganism) and 35.21%, 24.87% and 13.97% (inoculated usual anaerobic sludge). The decrease rates in the bottles inoculated rumen microorganism were obviously higher; this result

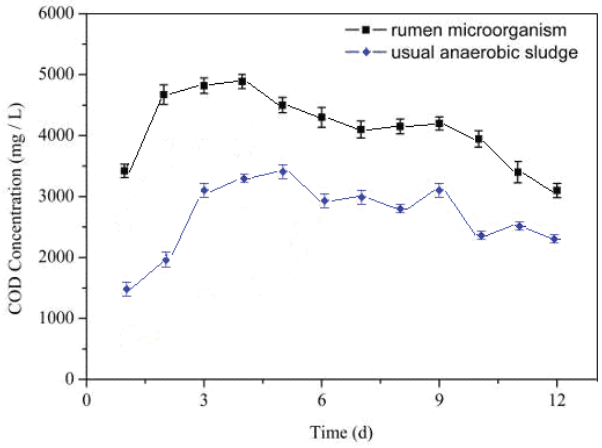

Fig. 3. Concentration of SCOD in the bottle during the operation.

Table 3
Percentage of cellulose, hemicellulose and lignin in GPES before and after anaerobic digestion (% of TS)

Item	Before AD	After AD		Degradation efficiencies	
		R	*U*	*R* (%)	*U* (%)
Cellulose	26.98 ± 0.21	10.26 ± 0.19	17.48 ± 0.16	61.97	35.21
Hemicellulose	9.65 ± 0.09	4.71 ± 0.04	7.25 ± 0.09	51.19	24.87
Lignin	18.32 ± 0.13	13.91 ± 0.10	16.76 ± 0.15	24.07	8.52

Means ± S.E. ($N = 3$); AD means anaerobic digestion, *R* means inoculated rumen microorganism, S.E. means standard error, TS means the total suspended solids, *U* means inoculated usual anaerobic sludge.

showed that applying rumen microorganism would be favorable to degrade organic material in the GPES during anaerobic digestion. Additionally, comparing with an 8.52% lignin removal by the usual anaerobic sludge, the application of rumen microorganism resulted in a 15.55% increase in lignin removal. The same result was obtained in another literature and the loss of lignin could be attributed to its solubilization [16,27]. Furthermore, more cellulose and hemicellulose could be exposed and contacted with the rumen microorganism because of the removal of lignin; maybe this was the reason for the higher removal rate of the cellulose and hemicellulose.

4. Conclusions

Anaerobic fermentation of GPES is a very complex bioprocess. In the present study, two groups of experiments were employed to evaluate the organic material removal rate of GPES when inoculated rumen microorganism prior to usual anaerobic sludge at retention times of 12 d on 39°C ± 1°C. GPES after anaerobic digestion by rumen microorganism had greater reducing sugar, VFAs, SCOD, degradation efficiencies of cellulose, hemicellulose and lignin were enhanced by 26.76%, 26.32% and 15.55%, respectively.

This study demonstrated that GPES could be effectively converted into reducing sugars and VFAs by using rumen microorganism in vitro. The major aqueous fermentation products were acetate and propionate, the highest concentration of acetate was 3,008 mg/L. The fermentation pattern in this study was acetate fermentation.

Because of the recalcitrance of lignin, the low cellulolytic activity, and slow specific growth rates of the anaerobic microorganisms involved, the anaerobic conversion efficiencies of lignocellulosic wastes are usually very low in conventional bioreactors by the usual anaerobic sludge. Our previous investigation suggests that rumen microorganism could provide a valuable model for pulp and papermaking excess sludge degradation.

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