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Anaerobic degradation of pulping midcourse wastewater by rumen microorganisms in batch reactor

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ABSTRACT

Batch experiments were performed to investigate the anaerobic degradation of pulping midcourse wastewater (PMW) by rumen microorganisms (RM). The operation included two stages. In stage one, 400 mL of PMW and 100 mL of RM were fed into a stirred batch-test reactor (1.2 L) which was kept at 39 ± 1°C for 14 days. The pH of the reactor was maintained at 6.7–7.0 to accommodate rumen organisms. Declined volatile fatty acids production was in line with the trend of the soluble chemical oxygen demand (SCOD) concentration profile. The SCOD removal efficiency was 38.0% at the end of stage one. The results showed that rumen organisms could accommodate PMW. The treatment effect of PMW by RM was investigated in stage two. About 200 mL of fresh PMW was added to the reactor. The maximum SCOD removal efficiency was 53.6% in stage two. Acetate and propionate were the major aqueous fermentation products, while butyrate and valerate were also found in smaller quantities. Generated biogas was composed of carbon dioxide, methane and hydrogen. This research provides useful information for the application of rumen cultures to the treatment of PMW.

Keywords: Pulping midcourse wastewater; Anaerobic degradation; Rumen microorganisms; VFA; SCOD

1. Introduction

The pulp and paper industry is a water-intensive industry that consumes as much as 60 m^3 of freshwater for producing one ton of paper [1]. In China, the water pollution from pulp and papermaking industry represents an actual and severe problem. The pulping midcourse wastewater (PMW) was obtained from the process of pulp washing and bleaching [2]. As a major water pollution source, PMW contains high concentrations of inorganic and organic matters and is highly colored. Thus, the generated effluents contains a wide range of contaminants, such as salts, lignin, cellulose, semicellulose, etc., [3] which will result in high biological oxygen demand (BOD) and chemical oxygen demand (COD).

PMW needs to be treated. Physical, chemical, electrochemical, and biological methods have been developed to remove these pollutants from PMW [1].

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Conventional aerobic and anaerobic treatments are the most widely used methods to remove COD, BOD, chlorophenols and color [4–6]. The effective degradation of lignocellulosic materials is the key process for these biological treatments.

Rumen ecosystem consists mostly of obligate anaerobic micro-organisms such as anaerobic bacteria, fungi, protozoa, methanogenic archaea, and methane-forming bacteria of the genus methanobrevibacter [7]. The mixed rumen microorganisms (RM) have complete enzyme components and high enzyme activities for the degradation of cellulosic materials [8]. It shows that they are primary colonizers of fibrous plant materials in the rumen and are able to degrade lignin-containing plant cell walls [9,10]. The potential application of rumen cultures for anaerobic digestion of lignocellulosic materials has been investigated [11]. Recently, culture resources and RM were applied to degrade lignocellulosic materials [12,13]. The results showed that RM are superior over other microbes for the degradation of lignocellulosic materials, which was attributed to higher cellulolytic activities [12]. Thus, anaerobic digestion of lignocellulosic materials by rumen cultures might be an alternative to the current methods. However, there is no report on anaerobic digestion of lignocellulosic materials in the PMW employing rumen cultures. Therefore, in this paper, the technical feasibility of anaerobic digestion of PMW by RM was investigated.

2. Materials and methods

2.1. Feed water and rumen bacteria

The wastewater was collected from the midcourse wastewater treatment plant equalization tank of a paper mill in Liaoning, China. The composition of PMW was shown in Table 1.

The rumen fluids were taken from Dalian Bangchuidao Meat-packing Plant. The samples were strained through a fourfold muslin cloth, and the vials with rumen fluid were purged with N_2 gas. The strained liquid was centrifuged at low speed ($125 \times G$) in a Servali SS-1 centrifuge tube for 5 min to remove the nonbacterial matter as much as possible [13]. The supernatant liquid was the RM and the characteristics are shown in Table 2.

2.2. Experimental conditions

The reactor was operated for 24 days. During stage one a 1.2 L reactor with a working volume of 1 L received 100 ml of RM and 400 ml of PMW. The pH of the PMW was adjusted to 6.8–7.0 with a 0.5 M H_3PO_4 solution before the PMW was feed in the reactor. The reactor temperature was maintained at 39 ± 1℃ and stirred at 80 rpm. RM were grown in the anaerobic reactor with PMW as the sole carbon and energy source. Samples were taken to monitor the volatile fatty acids (VFA), COD, and reducing sugar every day. This stage lasted for 14 days with a neglectable COD concentration change at the end. In stage two, to investigate the treatment effect of the RM, 200 mL of fresh PMW was added to the reactor, and the amount of VFA, COD and biogas volume were monitored daily. This stage was kept for 10 days.

Table 1
Physical and chemical characteristics of PMW used in this study

Parameters	Values
$SCOD_{Cr}$ (mg/L)	4,230
BOD_5 (mg/L)	1,426
Chromaticity (PCU)	1,309
Turbidity (NTU)	22.6
Reducing sugar (mg/L)	98
pH	11.4
SS (mg/L)	612
T.N (mg/L)	3.2
T.P (mg/L)	0.9

Note: BOD means biological oxygen demand; T.N means total nitrogen; T.P means total phosphorus.

Table 2
Physical and chemical characteristics of the RM

Parameters	Values
Reducing sugar (g/L)	0.3130
pH	7.2
MLSS (mg/L)	15,083
MLVSS (mg/L)	7,270

2.3. Analytical methods

Determinations of COD, BOD, T.N, T.P, chromaticity, turbidity, mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS) were carried out according to the standard analytical procedures as described in standard methods [14]. PH values were determined by a pH meter (Model 20, Denver instruments Ltd). The RM suspension was subjected to scanning electron microscopy (SEM, Hitachi S-3000N) after fixed in glutaraldehyde (2.5% in 0.05 M phosphate buffer, pH = 7.2, 24 h, 4℃) followed by post fixation in aqueous osmium tetroxide (2%) in the same

buffer solution for 2 h. After the post fixation, samples were dehydrated in series of graded alcohol and dried prior to scanning. Biogas was measured using the water displacement method and analyzed by another gas chromatograph (SP-6800, Lulan Co., China). GC was equipped with a thermal conductivity detector and a 1.5 m × 2 mm stainless-steel column packed with 5 Å molecular sieves. The temperatures of the injector, detector, and column were kept at 100˚C, 105˚C, and 60˚C, respectively. Argon was used as the carrier gas at a flow rate of 30 ml/min.

About 25 mL of liquid samples taken from the reactor was centrifuged at 10,000 rpm for 15 min, and the supernatant was passed through a 0.45-μm membrane filter for the analysis of soluble COD (SCOD) and VFA. VFA was determined by gas chromatography (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.

3. Results and discussion

3.1. Stage 1

VFA is an important performance indicator for the anaerobic digestion process. VFA 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 [15]. This usually results in the accumulation of organic acids and lowered pH, leading to the suppression of methanogenic activities, and in some cases, even process failure [16]. Fig. 1 illustrates the variation trends of the VFA, acetate and propionate during the reactor operation. Acetate and propionate were found to be the two major composition of the VFA in this experiment. In addition, butyrate and valerate were also detected, but in low levels.

The VFA concentration showed an increase from 103.7 mg/L to 3161.61 mg/L and VFA production varied consistently with the SCOD (Fig. 2) concentration during first three days. It might be attributed to the high availability of organic substrate resulting in cumulative VFA production. VFA varied drastically during day 4 to 9 and the concentration showed a steady decrease from 2048 to 787.4 mg/L. From day 10 to 14, after reaching steady-state condition, variation in VFA was restricted to about

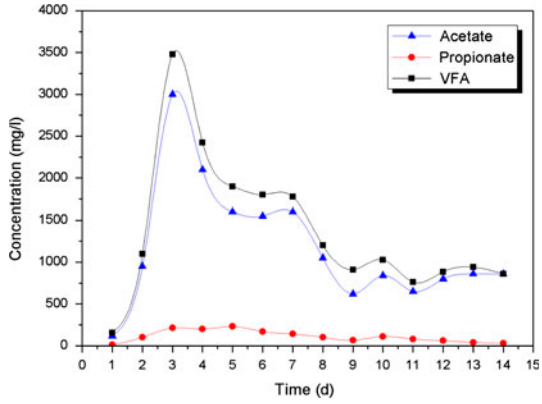

Fig. 1. VFA concentration in the reactor during the stage 1 operation.

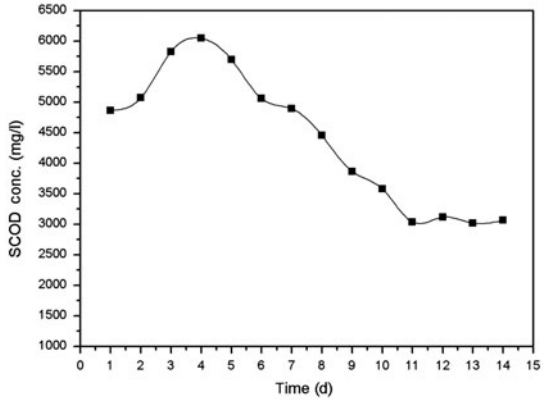

Fig. 2. SCOD concentration in the reactor during stage 1 operation.

700 mg/L indicating the acid production was in a low situation.

The variation trend of the acetate was similar to that of the VFA, which indicated that the variation of VFA is due to the accumulation and transformation of acetate. In contrast to acetate, the propionate had a slight increase in the early days and decreased slowly at the end of fermentation, it may due to the propionate transform to acetate.

During this stage, the SCOD concentration in the reactor is illustrated in Fig. 2, unexpected results are shown. Although the SCOD concentration was anticipated to decline steadily, it increased from day 1 to day 4 and reached a maximum of 6,010 mg/L on day 4. This can possibly be ascribed to the acclimatization phase, the environmental changes for the microorganisms' consortium that has been taken from the original environment (the cow rumen fluid) then fed to the new environment (the anaerobic reactor)

[17]. Under the conditions of reactor's environment, partial RM died, hydrolyzed, and dissolved in PMW which caused the rise of the SCOD concentration. After day 4, the SCOD in the reactor gradually decreased and stabilized for 7 days. This can be ascribed to the stabilization phase. 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. From day 11 to 14, the SCOD concentration has almost no change. This indicates that the organic matter in the reactor was difficult to be degraded, and this trend was in line with the trend of VFA production profile. The maximum SCOD removal efficiency was 38.0%.

Actually, the principal biogases that are produced in the rumen are CO_2 (60%), CH_4 (30–40%) and a variable amount of N_2 with traces of H_2S, H_2 and O_2. All these biogases are the final products of fermentation [18]. Fig. 3 represents the biogas production. The biogas produced during the fermentation was composed of carbon dioxide and lesser hydrogen and methane. This could be attributed to the presence of sufficient amount of organic compounds that are needed for the biogas genesis process. It is obvious from the experimental data in Fig. 3 that biogas production was observed in conjugation with SCOD removal. It indicates that PMW participated as the primary carbon source in metabolic reactions for molecular biogas generation.

3.2. Stage 2

After the stage 1 operated for 14 days, 200 mL of fresh PMW was added to the reactor to investigate the treatment effect of the stage 1.

As shown in Fig. 4, the acetate production is much higher than propionate production, and the variation trend of the acetate and VFA are similar. It is same to the found in stage 1.

According to the dilute effect of the fresh PMW, the initial VFA concentration was 385.4 mg/L, and it showed a gradual fluctuation between 251 mg/L and 437.9 mg/L during the whole operation stage as shown in Fig. 4. The lower concentration of VFA in stage 2 can be illustrated as the production of biogas. Generally, biogas production is accompanied by the consumption of VFA due to the aerogenic bacterium metabolism and it accorded with the high biogas production phenomenon in stage 2 as shown in Fig. 6.

The SCOD trend in the stage 2 is shown in Fig. 5. The SCOD concentration declined continuously during the first 7 days. This probably occurred due to the acclimatization of RM to PMW and the organic matter in PMW degraded continuously. At the end of the stage 2, the SCOD was 2031 mg/L, and it indicates that the PMW contains certain amount of hard biodegradable component such as lignin. The maximum SCOD removal efficiency was 53.6% in this stage.

The biogas production trend after the addition of fresh PMW is shown in Fig. 6. The amount of biogas production kept increasing through the stage 2. Different from the stage 1, the lag phase was not observed in this stage. The sharp change of biogas was due to the presence of aerogenic bacterium which accumulated in stage 1. Similar to the stage 1, the biogas production increased along with the SCOD degradation. It indicates that the organic matters in PMW were converted and removed as biogases. In stage 2, the yields of hydrogen and methane are higher than in stage 1, especially methane. This could be attributed to the low proliferation rate of methanogens and the

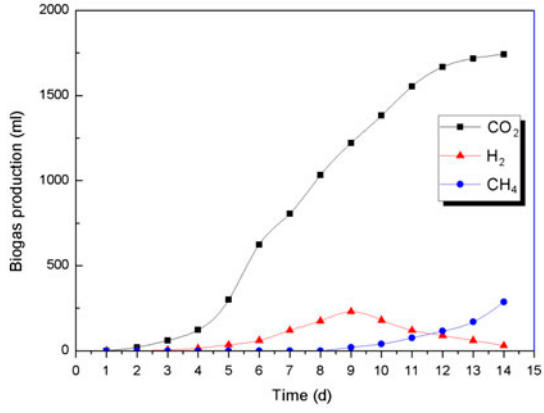

Fig. 3. Cumulative biogas volume during stage 1 operation.

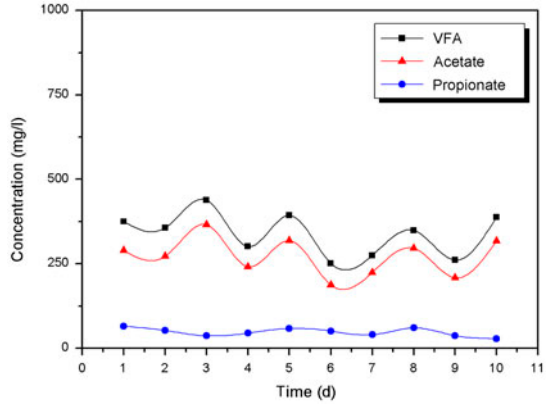

Fig. 4. VFA concentration in the reactor during stage 2 operation.

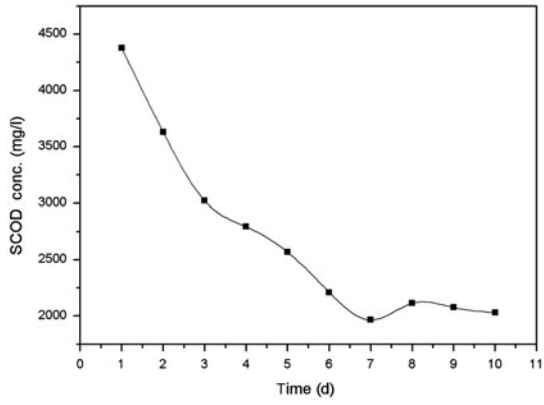

Fig. 5. SCOD concentration in the reactor during stage 2 operation.

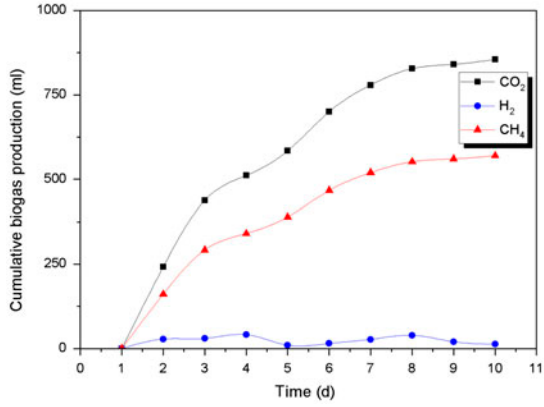

Fig. 6. Cumulative biogas volume during stage 2 operation.

quantity of the methanogens began to increased obviously in stage 2.

4. Conclusions

This study demonstrated that PMW could be effectively converted into VFA by using RM in batch cultures. Acetate and propionate were the major aqueous fermentation products, whereas butyrate and valerate were formed in small quantities. Biogas was composed of carbon dioxide, methane and hydrogen. In stage two, under current experimental conditions (pH = 7, $T = 39 \pm 1$ ℃), the highest value of degradation efficiency is about 53.6%, the lowest SCOD of the effluence is 1964 mg/L and the corresponding production of the carbon dioxide and methane, are, respectively 863 and 530 ml. These results suggest that anaerobic fermentation by RM could be a promising way for effective disposal of PMW.

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