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Application of Full Mesophilic Anaerobic Effluent Recycling to Very High Gravity Ethanol Fermentation from Cassava

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A zero-discharge system composed of ethanol fermentation and biogas fermentation (anaerobic digestion) for cassava-based ethanol production was developed by recycling mesophilic anaerobic effluent. Stillage originated from ethanol fermentation was first treated by the biogas fermentation system and, then, the anaerobic effluent was used as cooking water for the next ethanol fermentation batch. When recycling the anaerobic effluent, the average final ethanol concentration was 14.49 ± 0.29 %, (v/v), which was close to that of the control (14.6 ± 0.1 % (v/v), using tap water for cooking water). This coupled process was confirmed to have stable operation by 10 recycles. This clean technology could thoroughly eliminate the stillage pollution and save about 90 % fresh water used in ethanol fermentation.

Key Words: Anaerobic effluent, Cassava, Ethanol, Stillage, Zero discharge.

INTRODUCTION

Cassava-based fuel ethanol has rapidly developed recently in China because cassava is not a stable food crop for the Chinese people. However, the pollution caused by stillage is one of the most critical environmental issues and has become a limiting factor in the further development of the cassava-based ethanol production[1].

Generally, about 15-30 % of the stillage was recycled for the ethanol fermentation after treating it with appropriate separation processes[2,3] and the residual was successively treated by anaerobic and aerobic biological wastewater treatment. The anaerobic digestion step generally could remove more than 90 % COD in stillage alone with a large amount of methane formation and with lower operation cost[4-7]. In contrast, much energy was consumed for aeration during the aerobic biological wastewater treatment and a large mount of sludge generated, which is a potential secondary pollution[8]. Furthermore, sometimes the aerobic effluent still could not be discharged and must be treated further to accord with the national emission standards for wastewater[9].

To solve the stillage pollution and reduce wastewater treatment costs, an ethanol-methane coupled fermentation process was proposed for cassava-based ethanol production. In this process, the stillage originated from ethanol fermentation was first treated by a two stage thermophilic-mesophilic biogas fermentation system and, then, the mesophilic anaerobic effluent was totally recycled as cooking water for the next ethanol fermentation batch (Fig. 1). As a result, the wastewater pollution caused by stillage could be avoided and the aerobic biological treatment cost could be saved.

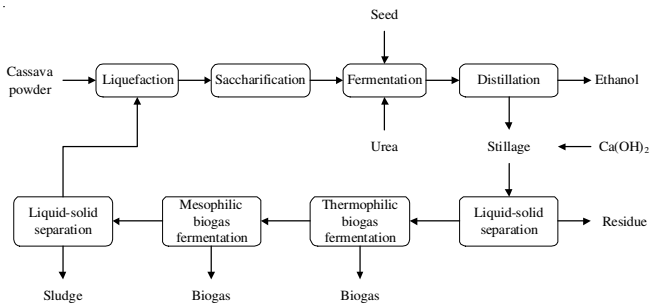

Fig. 1. Flowchart of ethanol-methane coupled fermentation process

In this study, we focused on investigating the feasibility of the ethanol-methane coupled fermentation process under very high gravity (VHG) ethanol fermentation conditions. Accumulations of organic acids, chemical oxygen demand (COD), conductivity and NH_4^+-N in cooking water (mesophilic anaerobic effluent) were also investigated.

EXPERIMENTAL

Organism and cultivation conditions: Angel alcohol active dry yeast (ADY, a commercial strain of *Saccharomyces cerevisiae* for ethanol production) was obtained from Hubei Angel Yeast Co. Ltd., China. 2 g ADY was dissolved and

activated in 100 mL of 20 g/L glucose solution at 35 ºC for 0.5 h prior to fermentation.

Ethanol fermentation condition: The mash preparation and ethanol fermentation were carried out in a 10-l jar fermentor (Baoxing Bio-engineering Co. Ltd., China) and the culture containing 2.80 kg cassava powder (starch content 65-68 %, particles size was 40 mesh) and 6.16 L water (culture volume is about 8 L). The culture pH was adjusted to 6.2-6.4 with 30 % (w/w) H_2SO_4 and 10 IU thermostable α-amylase (20,000 IU/mL, optimum pH 6.2-6.4, temperature 95-105 ºC, Genencor China Co. Ltd.) per gram of cassava powder was added. The temperature was raised to 95 ºC and held for 1 h at 200 rpm. Then temperature was cooled down to 30 ºC and pH was adjusted to 5.0-5.5 with 30% (w/w) H_2SO_4. 150 IU glucoamylase (130,000 IU/mL, Genencor China Co. Ltd.) per gram of cassava powder, 4 g urea and 100 mL ADY solution were added to start the fermentation. Ethanol fermentation temperature and time were 30 ºC and 60 h, respectively.

Analysis methods: Concentrations of ethanol, acetic acid, propanoic acid, butyric acid, valeric acid and glycerol were determined by high performance liquid chromatography (Dionex UltiMate 3000 HPLC, USA). Samples were pretreated as described by Graves *et al.*[10]. A 20-μL aliquot from a suitably diluted sample was analyzed using a Bio-Rad HPX-87H Aminex ion exclusion column coupled to a refractive index detector (Shodex RI-101, Japan). The column was operated at 65 ºC, 0.005 M sulfuric acid was the mobile phase at 0.6 mL/min and the data was processed using the Chromeleon software (Dionex, USA). Conductivity was measured by a conductivity meter (DDS-11C, Shanghai Leichi Instrument Co. Ltd., China), COD and NH_4^+-N was determined according to the standard APHA methods[11].

RESULTS AND DISCUSSION

Ethanol fermentation results in ethanol-methane coupled fermentation process: Ten recycles were carried out according to the procedure in Fig. 1. When recycling anaerobic effluents, the average of final ethanol concentration, glycerol concentration and starch utilization ratio were 14.49 ± 0.29 % (v/v), 11.23 ± 0.31 g/L and 87.2 ± 2.2 %, respectively, which were similar to that of the control (14.6 ± 0.1 % (v/v), 11.2 ± 0.2 g/L and 88.0 ± 2.8 %, respectively, using tap water for medium preparation). The results (Fig. 2) suggested that, the ethanol fermentation was not effected when using anaerobic effluents as cooking water. Zhang *et al*. has reported that, high concentrations of organic acids contained in the anaerobic effluents could inhibit the yeast growth and extend the ethanol fermentation time[12]. As a result, organic acids in the anaerobic effluents were detected (Fig. 3).

Biogas fermentation process involves three major groups of bacteria and each group performs hydrolysis and acidification, smaller organic acids formation and methane conversion, correspondingly[13]. Macromolecular substrate was first decomposed and converted into small molecular organic acids and then the small molecule organic acids were converted to methane by the methanogenic bacteria. Normally, organic acids contained in the anaerobic effluent maintained at a very low concentration[8,9]. However, organic acids in the anaerobic effluent would accumulate when the organic load rate (OLR) was too high[12].

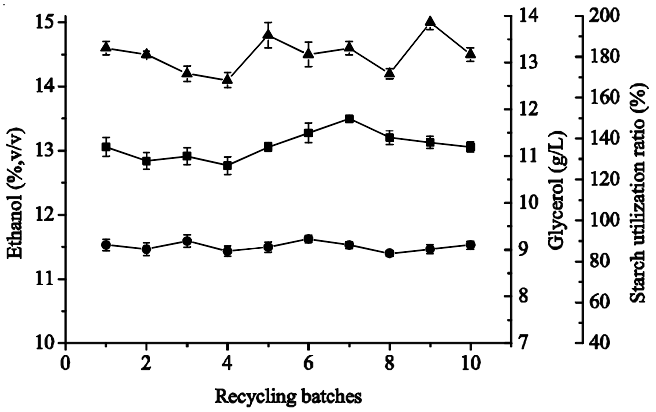

Fig. 2. Variations of ethanol (▲), glycerol (■) and starch utilization ratio (●) when consecutively reutilizing anaerobic effluents as cooking water for ethanol fermentation

During operation of ethanol-methane coupled fermentation process, total organic acids concentration in anaerobic effluent was below 0.15 g/L (Fig. 3), which resulted from the lower organic load rate. For example, the organic load rate for thermophilic and mesophilic biogas fermentation was 7.8 ± 1.3 and 1.25 ± 0.25 kg COD/(m^3·day). Acetic acid and propionic acid were the main acids in the anaerobic effluent and butyric acid, valeric acid was not detected, which was similar to the results reported by Zhang *et al.*[12]. Zhang *et al*. reported that, to avoid the ethanol fermentation inhibition caused by organic acids, acetic and propionic acid in the medium should be < 4.8 and < 2.22 g/L when they individually existed[14]. Obviously, organic acids contained in the anaerobic effluent were too low to inhibit the ethanol fermentation in this study.

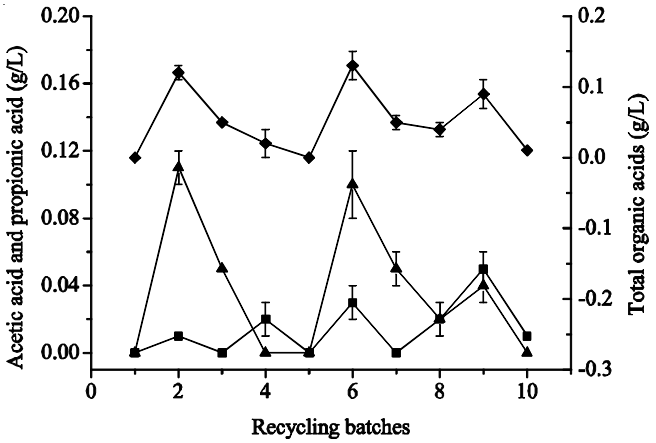

Fig. 3. Organic acids contained in the cooking water in different recycle batches symbols: acetic acid (▲), propionic acid (■), total acids (◆)

Biogas fermentation operation status in ethanol-methane coupled fermentation process: About 7.8 L stillage generated from ethanol fermentation in each recycle. $Ca(OH)_2$ was added to precipitate the sulfate in the stillage, to avoid the impact of sulfate on the biogas fermentation. 6 L thin stillage was obtained after liquid-solid separation and was treated by the biogas fermentation system. The COD removal rate was 92 ± 2 % (thermophilic biogas fermentation) and 75 ± 2 % (mesophilic biogas fermentation) and the COD of mesophilic anaerobic effluent was only 2,500 ± 700 mg/L (Table-1). Inhibitory

substances for ethanol fermentation in stillage, such as acetic acid and lactic acid[10,15,16], could be degraded and converted to biogas[12], which was benefit to reusing the anaerobic effluent as the cooking water for the ethanol fermentation.

TABLE-1
PARAMETERS AND OPERATION CONDITIONS OF THE METHANE FERMENTATION

Parameters and operation conditions	Thermophilic biogas fermentation	Mesophilic biogas fermentation
Working volume (L)	16.0	8.0
Temperature (ºC)	55 ± 1	35 ± 1
Initial anaerobic sludge size (L)	4	4
Organic load rate (kg COD/(m^3·day))	7.8 ± 1.3	1.25 ± 0.25
Hydraulic retention time (days)	16	8
Influent COD (mg/L)	125,000 ± 22,000	10,000 ± 2,000
Effluent COD (mg/L)	10,000 ± 2,000	2,500 ± 700
COD removal rate (%)	92 ± 2	75 ± 2

Accumulative effects in ethanol-methane coupled fermentation process: For any closed loop system, substance accumulation was inevitable. COD, ammonia and conductivity of the cooking water (mesophilic anaerobic effluent) in each recycle were detected to reflect the substance accumulation of the ethanol-methane coupled fermentation process. Low molecular weight fermentation by-products, unused raw materials and yeast cells can be removed by distillation and solid-liquid separation and most of the soluble organic matter could be decomposed and converted to biogas during the biogas fermentation system. However, ammonia nitrogen and inorganic salts could not be effectively removed by the above process[17,18]. Consequently, COD of the cooking water almost did not accumulate, but the ammonia and conductivity accumulated and reached their balance concentrations after the 5th recycle. Removal of the inorganic salts mainly by the solid-liquid separation and coagulation and/or coprecipitation might exert a major role as their concentrations exceed solubility products[18] (Fig. 4).

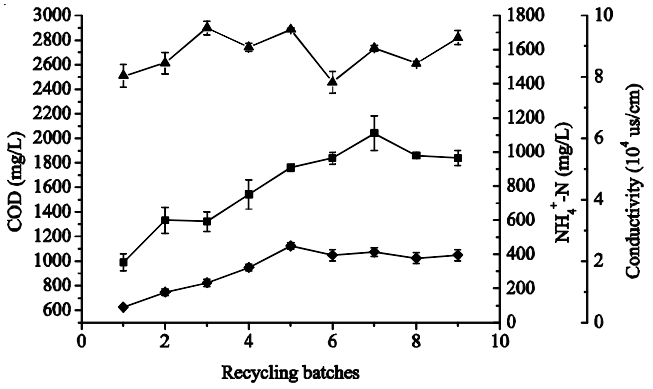

Fig. 4. Substance accumulation in ethanol-methane coupled fermentation process; Symbols: COD (▲), NH_4^+-N (■), conductivity (◆)

Conclusion

A clean technology was applied in the ethanol production to resolve the stillage pollution. After treating by a thermophilic-mesophilic biogas fermentation system, the stillage could be totally recycled to the next ethanol fermentation batch and a zero-discharge system could be established. Using anaerobic effluent as cooking water, the average ethanol production (14.49 %, v/v) obtained was close to that of the control (14.60 %, v/v) and the fermentation time kept the same as the control. The coupled process was also confirmed to have stable operation over ten recycles.

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