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**SHORT COMMUNICATION**

# Application of full permeate recycling to very high gravity ethanol fermentation from corn

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**Abstract**−A ceramic membrane with pore size of 0.2 μm was used to percolate grain stillage of very high gravity (VHG) ethanol fermentation from corn, and the micro-filtration permeate was completely recycled for the cooking step in the next fermentation process. The concentrations of solids, sugars, total nitrogen and $Na^+$ in the grain stillage and permeate reached a relative steady state after two or three batches of filtration and recycling process. There are no negative effects of by-products on VHG ethanol fermentation, and the final ethanol yield was above 15% (v/v). The conditions of filtration were examined to determine the optimum conditions for the process and included an initial flux of clean water above 550 $L \cdot m^{-2} \cdot h^{-1}$ (0.1 MPa), an operating differential pressure of 0.15 MPa, an operating temperature above 70 °C, and a permeation flux greater than 136 $L \cdot m^{-2} \cdot h^{-1}$. It could be concluded that full permeate recycling during ethanol production was an efficient process that resulted in less pollution and less energy consumption.

Key words: Very High Gravity (VHG), Ethanol Fermentation, Ceramic Membrane, Full Permeate Recycling

## INTRODUCTION

Ethanol production has been a subject of tremendous interest as an energy resource and alternative to fossil fuels since the oil crisis of the 1970s. Therefore, high efficiency ethanol productivity with low cost in raw materials and energy consumption is an important goal in ethanol fermentation studies [1,2]. To achieve these objectives, several techniques have been employed, such as using strains with efficient ethanol production [3-5], immobilizing cells with or without carriers [6-9] and cell recycling through sedimentation or membrane retention [10-12].

Most ethanol is produced by using a batch fermentation system followed by a distillation process. The fermented broth usually contains less than 15% (v/v) ethanol, as well as more than 10 L of grain stillage, which can be converted to 1 L of spirits [13]. While grain stillage is an organic contaminant, it is rich in nutrients. Direct drainage of distillery waste water into rivers causes environmental pollution and grievous waste. Distillery waste water, if properly treated, can be reused in the next fermentation process [14].

Grain stillage recycling is one of the best ways to reduce environmental pollution in the ethanol fermentation industry. For conventional fermentation processes, stillage recycling minimizes the amount of stillage generated [15]. However, during very high gravity (VHG) ethanol fermentation, the concentration of solid phase materials in the stillage increases [16], and subsequent recycling results in the buildup of non-metabolized feed components and fermentation by-products in the fermentor, which leads to an inhibition of alcohol fermentation.

Therefore, improving the efficiency of solid phase separation is critical during VHG ethanol fermentation. A ceramic ultrafiltration membrane has been applied to separate suspended solids and high molecular weight organics during stillage recycling [17,18]. The ceramic membranes may be the ideal solution as they are highly selective, resistant to high temperatures and solvents and very stable, having a long life span [19]. Nevertheless, there are some limitations to the use of these membranes: modest fluxes, a high investment necessary to obtain a ceramic membrane unit, and fouling (the blockage of the pores), which can be considered the main concern of this technique [20]. In this study, we applied full permeate recycling using ceramic membrane micro-filtration in a single step to increase the efficiency of solid phase separation during VHG ethanol fermentation.

## MATERIALS AND METHODS

### 1. Microorganisms and Cultivation Conditions

Angel alcohol instant active dry yeast (ADY, a commercial strain of *Saccharomyces cerevisiae* for ethanol production) was obtained from Hubei Angel Yeast Co. Ltd., China. 14 gram ADY was dissolved and activated in 50 milliliters of hot water at 35-40 °C for 30 min prior to fermentation.

### 2. Reagents

α-Amylase (20,000 IU/mL, optimum pH 6-7, temperature 85 °C), glucoamylase (100,000 IU/mL, optimum pH 4.0-5.0, temperature 60 °C) and raw ground corn (starch: 68%, moisture: 12%, protein: 3.5%, fatty acid: 1.1%, the corn particles size: approximately 20 mesh) were supplied from Jiangyin Xinda Biochemistry Engineering Co. Ltd.

### 3. Mash Preparation and Ethanol Fermentation

The mash preparation and ethanol fermentation were carried out in a 30 liter fermentor (Xinchang Deli Petrochemical Equipment Co. Ltd., Zhejiang, China) and the culture containing 7 kilogram

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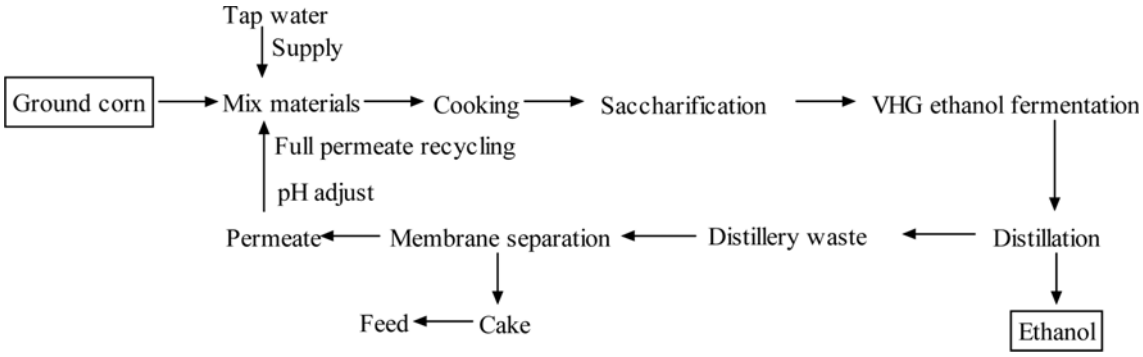


**Fig. 1. Schematic diagram of the laboratory-scale, full permeate recycling process.**

ground corn and 14 liter water was mixed and agitated in it. The culture pH was adjusted to 6.5-6.7 with 20% (w/v) NaOH, and 5 international units (IU) of $\alpha$-amylase per gram of dry corn was added. The temperature was raised to 85 ºC and held for 60 min with continuous stirring at 100 rpm. Then the temperature was cooled to 60 ºC by tap water as the cooling agent. The pH of the mash was adjusted to 4.3 with 6 M HCl, and then 150 IU glucoamylase per gram of corn were added. After the temperature of mash was cooled to 30 ºC, 100 grams $(NH_4)_2SO_4$ and 50 mL ADY solution were added and cultivated at 30 ºC.

**4. Recycling Process**

The bench-scale ethanol fermentation process and recycling scheme are illustrated in Fig. 1. The stillage was filtered through a ceramic membrane with pore size of 0.2 μm (Hefei Shijie Membrane Filter Co. Ltd., Anhui, China). The micro-filtration permeate was completely recycled and reused in the cooking step for the next fermentation. Tap water was added to make up for the water loss during distillation. The permeate had a low pH of 3.5-4.5, which was adjusted to neutral (pH 6.5-7.0) before the next cooking step.

**5. Analysis**

The reducing sugars were measured by using the dinitrosalicylic acid (DNS) method [21]. The standard curve for this volumetric assay was calculated by linear regression. Total sugars were also measured by DNS method after the samples were acid hydrolyzed (0.54 N HCl, 60 min, 100 ºC) and neutralized with 20% (w/v) NaOH. The ethanol content was determined by gas chromatography with a flame ionization detector. For GC analysis, the carrier gas was $N_2$, and butanol was used as the internal standard. The concentrations of sodium ions, solids and total nitrogen were determined according to previously described methods [22]. The viscosity was measured with an NDJ-1 rotational viscometer (Shanghai Balances Instrument Factory, China). All the measurement experiments were run in triplicate and the relative standard deviation for all analysis and experimental results was less than 3.0%.

## RESULTS

**1. Effect of Operating Differential Pressure on Permeation Flux of Ceramic Membrane Filtration**

In this study, separation was a micro-filtration process using differential pressure. Operating differential pressure can affect the permeation flux and energy consumption of the filtration process. Therefore, optimization of operating differential pressure in the filtration process can reduce the energy cost and increase the permeation flux.

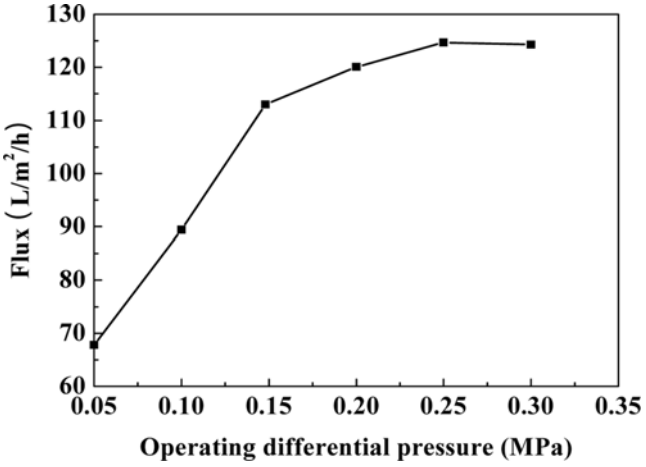


**Fig. 2. Effect of operating differential pressure on permeation flux.**

Fig. 2 depicts the relationship between operating differential pressure and flux at steady state for VHG ethanol fermentation with filtered grain stillage. During the low pressure stage, there was a linear dependence between flux and operating differential pressure. That is, an increase in operating differential pressure resulted in a large increase in permeation flux. However, when the operating differential pressure reached 0.15-0.20 MPa, there was no longer a linear relationship between permeation flux and operating differential pressure. Thus, the critical pressure for filtration was 0.2 MPa. Pressures ≥0.2 MPa resulted in a relatively stable flux that was unaffected by pressure increase.

The cross flow rate was another important factor that influenced permeation flux. Cross flow rate depends on both the raw material liquid (viscosity and solid content) and the mechanical strength of the membrane materials. A high cross flow rate would increase flux by removing the grain sediment and pollution on the membrane surface. The rate used in this study depended on the pump characteristics and energy consumption. It was found that a rate of 3-5 m/s allowed for successful filtration, and an operating differential pressure was optimized at 0.15 MPa in terms of energy consumption.

**2. Effect of Operating Temperature on Permeation Flux**

Permeation flux was affected by viscosity and the operating temperature. On the other hand, grain stillage from VHG ethanol fermentation is highly viscous organic waste water. Therefore, in order

to increase the permeation flux of highly viscous liquids, either operating temperature or proper pretreatment should be used.

Following ethanol distillation, the temperature of grain stillage was 100 °C. Filtration at this high temperature did not require any extra equipment or energy consumption. It was observed that stillage viscosity decreased with increasing temperature and the permeation flux increased slightly (Fig. 3). When the temperature was increased from 40 to 70 °C, the permeation flux increased from 85 to 136 L·

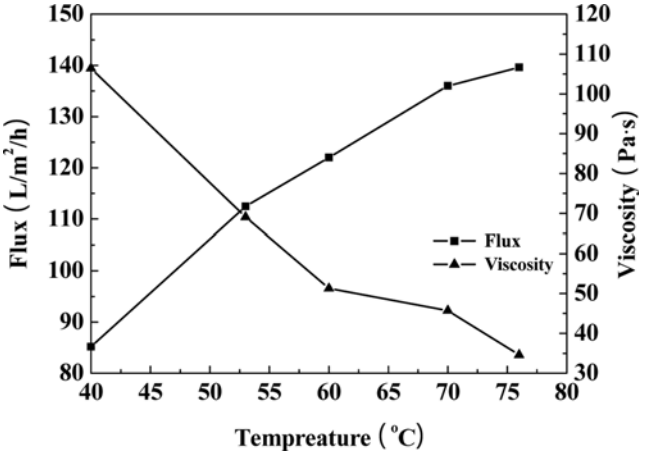


**Fig. 3. Effect of operating temperature on permeation flux and viscosity.**

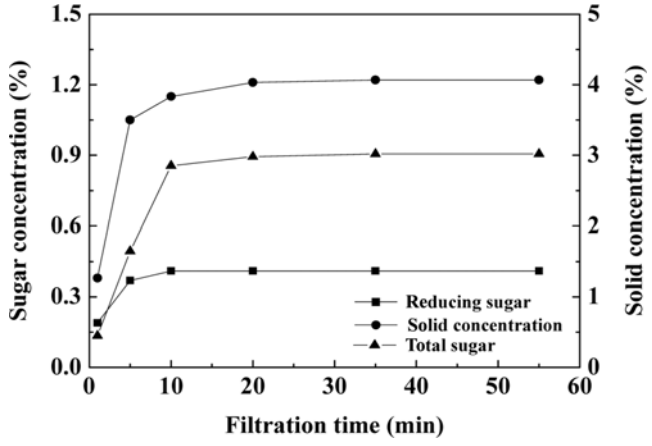


**Fig. 4. Variations in total and reducing sugars and solid concentrations in permeate during batch filtration.**

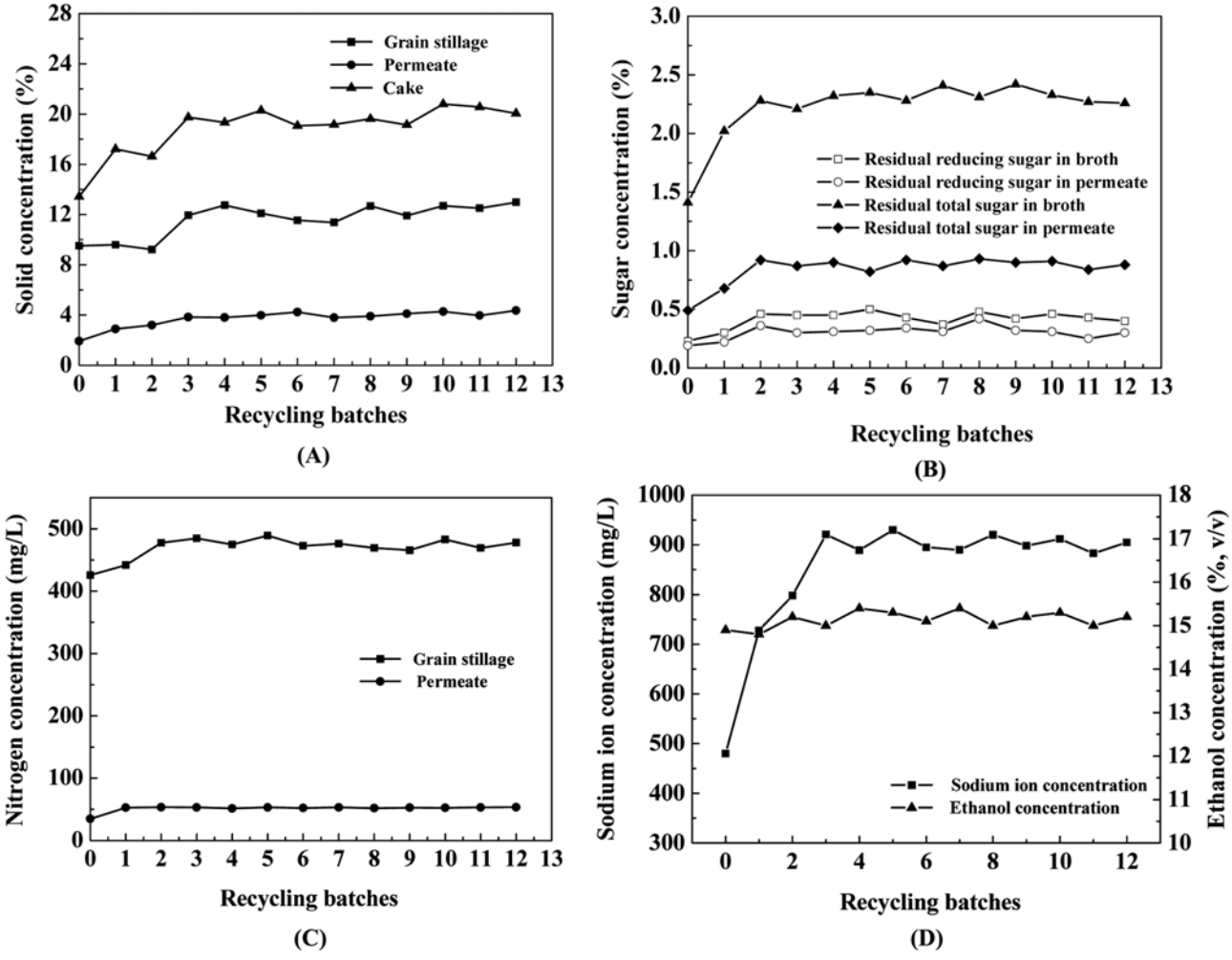


**Fig. 5. Variations of main parameters during 12 cycles of full permeate recycling: (A) solid concentration, (B) total and reducing sugar concentrations, (C) nitrogen concentration, (D) sodium ion and ethanol concentrations.**

$m^{-2}\cdot h^{-1}$ with a growth rate of approximately 60%. After the temperature exceeded 70 °C, there was no obvious effect of temperature on flux, and the permeation flux was 142 $L\cdot m^{-2}\cdot h^{-1}$ at 90 °C. Thus, the operating temperature used in our study was ≥70 °C.

**3. Character Stability of Filtrate in Ceramic Membrane Filtration Process**

The concentrations of the three principal filtrate components (solids, reducing sugars and total sugars) changed with filtration time as illustrated in Fig. 4. The concentrations of solids, total sugars and reducing sugars in the feed grain stillage were 9.908%, 2.43%, 0.664% (w/v), respectively. After 10 min of ceramic membrane filtration, the filtrate concentrations were relatively stable.

**4. Effect of Full Filtrate Recycling on VHG Ethanol Fermentation**

Thirteen batches of VHG ethanol fermentation and stillage treatment (12 batches of recycling) were carried out according to the procedure in Fig. 1. The concentrations of solids, sugars, nitrogen, $Na^+$ and ethanol in different phases are shown in Fig. 5. The concentrations of solids, total sugars, reducing sugars, total nitrogen and $Na^+$ in the grain stillage and filtrate showed minor undulations and reached a relative steady state after two or three recycling batches.

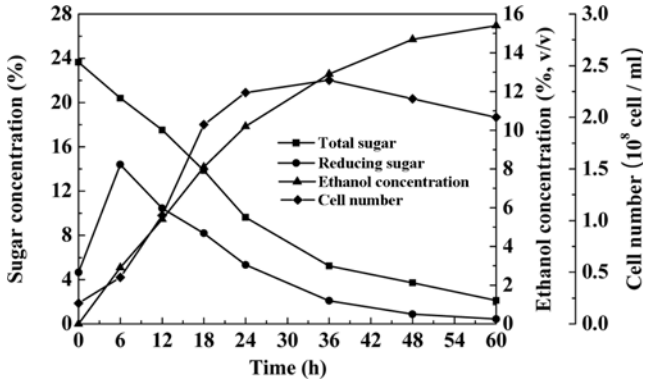


**Fig. 6. Time course curves of VHG ethanol fermentation using full filtrate recycling after five cycles.**

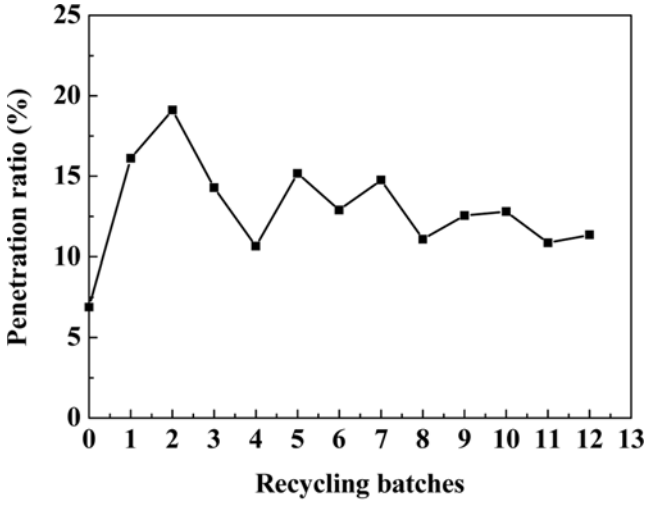


**Fig. 7. Variations in penetration ratio during 12 cycles of full permeate recycling.**

Therefore, VHG ethanol fermentation using the filtrate as process water had no adverse consequences. The concentration of by-products (solids, total nitrogen and $Na^+$) in the steady state did not affect VHG ethanol fermentation, and the final ethanol yield was above 15% (v/v). Representative fermentation curves for the ethanol and sugar concentrations are shown in Fig. 6. The filtration penetration ratio was selected as described by Kim et al. [23]. As shown in Fig. 7, the penetration ratio was also relatively stable after the second recycling batch and the steady state maintained during the subsequent batches.

## DISCUSSION

High efficiency ethanol productivity with a low cost, low energy consumption and minimum pollution was an ideal ethanol fermentation [1,24,25]. Hence, we introduced a ceramic micro-filtration membrane to recycle distillery wastes in a batch ethanol fermentation process to conserve energy, protect the environment and reduce costs. The optimum conditions for the filtration process included an initial flux of clean water above 550 $L\cdot m^{-2}\cdot h^{-1}$ (0.1 MPa), an operating differential pressure of 0.15 MPa, an operating temperature above 70 °C, and a permeation flux greater than 136 $L\cdot m^{-2}\cdot h^{-1}$.

It was proved that the critical pressure for filtration was 0.2 MPa, and pressures ≥0.2 MPa resulted in a relatively stable flux unaffected by increasing pressure. This could be due to the greatly compressed solids in the grain stillage. As the membrane was covered with compacted solids, the filtration resistance increased, which counteracted the effect of pressure on flux. An operating differential pressure of 0.15 MPa was chosen, which was lower than the critical pressure in order to obtain a high permeation flux and minimize membrane pollution.

By reusing the membrane permeate in the next cooking cycle, we obtained a similar ethanol yield in up to thirteen recycling steps with only a slight increase in fermentation by-products. The sugar content in the fermented broth and the membrane permeate accumulated up to the second cycle and then leveled off. And also, the solid mass content in the membrane permeate leveled off after the third recycling step. Although some component concentrations increased after recycling the membrane permeate, there were no cumulative effects on fermentation.

Our data indicated that full filtrate recycling during ethanol production was an efficient process that resulted in less pollution with waste water and less energy consumption. Application of this process would improve the efficiency of solid phase separation during VHG ethanol fermentation. Therefore, this procedure was a step towards the goal of high and efficient ethanol production with a low cost in raw materials and energy consumption.

## CONCLUSION

The concentrations of solids, sugar, total nitrogen and $Na^+$ in the grain stillage and permeate reached a relative steady state after two or three batches of filtrations and recycling process. There were no negative effects of by-products on VHG ethanol fermentation during full permeate recycling by ceramic membrane filtration, and the final ethanol yield was above 15% (v/v). The optimum conditions during filtration were determined: an initial flux of clean water above

550 $L \cdot m^{-2} \cdot h^{-1}$ (0.1 MPa), an operating differential pressure of 0.15 MPa, an operating temperature above 70 °C, and a permeation flux greater than 136 $L \cdot m^{-2} \cdot h^{-1}$.

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