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Alleviation of harmful effect in stillage reflux in food waste ethanol fermentation based on metabolic and side-product accumulation regulation

Hongzhi Ma [a,b,*], Jian Yang [a,b], Yan Jia [a,b], Qunhui Wang [a,b], Xiaoyu Ma [a,b], Kenji Sonomoto [c]

[a] Department of Environmental Engineering, University of Science and Technology Beijing, 100083, China
[b] Beijing Key Laboratory of Resource-oriented Treatment of Industrial Pollutants, Beijing, China
[c] Department of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

HIGHLIGHTS

- Alleviation harmful effect in stillage reflux in food waste ethanol fermentation.
- Controlling proper ORP value could reduce the harmful effect.
- Adding $CaCO_3$ adjust the accumulated lactic acid benefit for ethanol fermentation.
- Regulation by yeast metabolism approach slightly better than by-product accumulation.

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ABSTRACT

Stillage reflux fermentation in food waste ethanol fermentation could reduce sewage discharge but exert a harmful effect because of side-product accumulation. In this study, regulation methods based on metabolic regulation and side-product alleviation were conducted. Result demonstrated that controlling the proper oxidation–reduction potential value (−150 mV to −250 mV) could reduce the harmful effect, improve ethanol yield by 21%, and reduce fermentation time by 20%. The methods of adding calcium carbonate to adjust the accumulated lactic acid showed that ethanol yield increased by 17.3%, and fermentation time decreased by 20%. The accumulated glyceal also shows that these two methods can reduce the harmful effect. Fermentation time lasted for seven times without effect, and metabolic regulation had a better effect than side-product regulation.

1. Introduction

Ethanol fermentation from food waste is one of the simple resource technologies with the advantage of quick fermentation time (Ma et al., 2016). Its drawback is similar to that of other materials, that is, such process needs to consume large amounts of water. The stillage discharge volume is high in ethanol industry and a lot of wastewater with high BOD and COD needs to be dealt with (Pant and Adholeya, 2007). Many stillage treatments have been investigated to realize "zero discharge" of the process of the ethanol industry for further direction. These treatments include natural oxidation, single cell protein production, evaporation, and concentration.

Stillage recycling is one simple and economical method of solving stillage pollution. This process has the merit of reducing pollution and making full use of its rich protein, amino acids, and various metal ions. Ethanol yield would be improved, and water is simultaneously saved in the process (Tao et al., 2005). However, stillage contains a large number of by-products toxic to yeast with high boiling point. Stillage recycling inevitably leads to the steady accumulation of toxic substances, which thereby inhibit the normal growth and metabolism of yeast (Su et al., 2015).

Many relevant bodies of research focus on the effect of stillage reflux on ethanol fermentation. Chenguang Liu studied the stillage reflux of corn starch zymotic fluid using a self-flocculating yeast. He adopted manipulation measures of BERs, intermittent aeration, addition of potassium ferricyanide and other saline materials to

* Corresponding author at: Department of Environmental Engineering, University of Science and Technology Beijing, 100083, China.
E-mail addresses: mhz527@sina.com, 66243295@qq.com (H. Ma).

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control fermentation of liquor oxidation–reduction potential (ORP), and ease the side reactions for the stillage reflux. Such experience could provide a direction for stillage reflux in food waste ethanol fermentation (Liu et al., 2011b, 2012).

Previous studies have shown that high lactic acid content is the most serious by-product inhibiting fermentation in the stillage reflux for food waste ethanol fermentation (Ma et al., 2016). The stillage has unique properties of saline and oil contents. Zhu pointed out that the stillage of food waste ethanol fermentation is acidic with high suspended solids (1000 mg/L), COD could be up to 60,000 mg/L, high nitrogen and phosphorus contents, corresponding TN and TP could be 700 mg/L and 180 mg/L, respectively (Zhu et al., 2016). Accordingly, stillage reflux fermentation of food waste is more likely to form accumulation of high-boiling by-products than that of the starch material. Previous studies have shown that high lactic acid concentration becomes the most serious by-product inhibiting the fermentation in the stillage recycling process. The lactic acid concentration even reaches 120 g/L when the reflux fermentation moves to the seventh batch. The fermentation time is prolonged from less than 24 h in the first batch to 96 h in the seventh batch. The ethanol yield in the sixth and seventh batches has significantly decreased (Ma et al., 2016).

This research aims to solve the side-product accumulation during stillage reflux in food waste from the angle of yeast metabolism and by-product separation and relieve the harmful effect. Controlling the ORP and the lactic acid concentration is done to achieve more reflux time and less side effect during ethanol fermentation from food waste.

2. Materials and methods

2.1. Raw materials

Food waste was collected from a dining room in the University of Science and Technology, Beijing, China. The corresponding parameters were shown in our previous publications (Zhang et al., 2012). Dry yeast (Anqi Company, China) was used for fermentation and activated by dissolving into glucose broth at 2% concentration for 2–3 h in a thermostatic shaker culture at 35 °C.

2.2. Ethanol fermentation

After pretreatment, food waste was mixed with water at a ratio of 2:1. Accordingly, 1000 g of food waste was mixed with 500 g of water, with the addition of 100 U/g glucoamylase (Ao Bo Xing Company, China). The solution was saccharified at 60 °C for 6 h. The liquid was then centrifuged for 10 min with a rotation rate of 4000 rpm. The supernatant was stored at 0–4 °C for the following fermentation. During fermentation, the supernatant was inoculated with yeast at a size of 10%. Broth was sampled every 12 h. The corresponding fermentation parameters and by-products were subsequently determined (Ma et al., 2014).

2.3. Stillage reflux fermentation method

The fermentation broth discharged from the fermenter was collected and distilled to remove ethanol and volatile by-products. The remaining stillage with non-volatile by-products was recycled for saccharification and broth preparation. The broth was then used for ethanol fermentation as mentioned in Section 2.2. The stillage reflux was utilized for a maximum of seven times.

2.4. Regulation methods

Metabolic regulation for yeast: The ORP value in the fermentation process was continuously observed. Accordingly, 25 ml of 4.2 g/L $K_3[Fe(CN)_6]$ (Chinese Medicine Group Chemical Reagent Co., Ltd.) solution was added to the broth when the ORP value was below −500 mV.

By-product regulation: The lactic acid concentration was detected at the end of each fermentation test. About 67 g solid calcium carbonate was added to 1 L fermentation broth when the lactic acid concentration was higher than 80 g/L. (Chinese Medicine Group Chemical Reagent Co., Ltd.) Corresponding technological roadmap was showed in Fig. 1.

2.5. Analytical method

Fermentation was performed in a fermentation tank (BLBIO-mini-1GC), which was sampled at a certain time, and 5 ml of fermentation broth was obtained at each time. After centrifugation at 10,000 r/min for 5 min, supernatant was subjected to determinations of ethanol contents, ethanol concentrations were analyzed by gas chromatography (Shimadzu GC-2010) with a flame ionization detector. Lactic acid was measured by biosensor SBA-40C. VFAs were analyzed by gas chromatography (Shimadzu GC-2010) with flame ionization detector. ORP was monitored using an ORP electrode (G301ORP-120S/N:121965), and pH was monitored by a pH electrode (G301k-120). ORP and pH were recorded in the fermentation tank (BLBIO-mini-1GC), and glycerol was used for titration (Cardoso et al., 2015). All experiments were repeated thrice, and the average results were used for analysis.

3. Results and discussion

Seven batches of ethanol fermentation experiments from food waste were performed with stillage reflux. The A–E groups were used to indicate the regular batches of fermentation cycles. From the sixth time, the metabolic regulation and side-production regulation methods were adopted to control the fermentation process. The corresponding parameters were analyzed and discussed.

3.1. Fermentation parameters for stillage reflux ethanol fermentation from food waste without regulation

Some studies were conducted on the effect of stillage reflux on ethanol fermentation. Kishimot et al. reported the whole circulation process of distillation liquid waste in the continuous ethanol fermentation. Non-fermentation polysaccharide and inorganic ions finally reached the inhibiting concentration in the fermentation broth (Kishimoto et al., 1997). Kechang Zhang et al. designed two

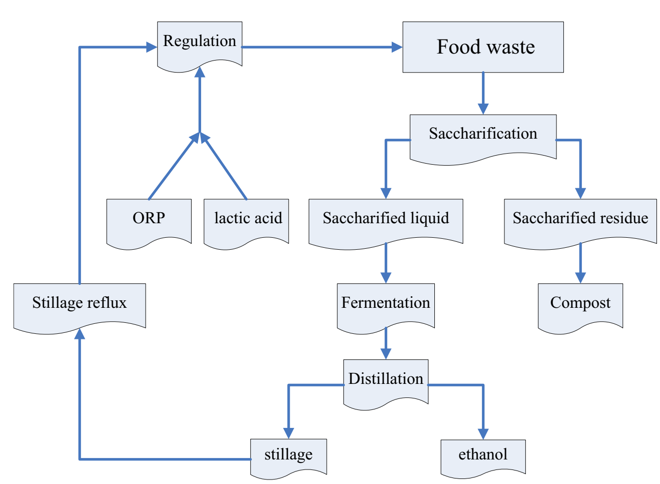

Fig. 1. Technology roadmap in this study.