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Application of low-cost algal nitrogen source feeding in fuel ethanol production using high gravity sweet potato medium

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ABSTRACT

Protein-rich bloom algae biomass was employed as nitrogen source in fuel ethanol fermentation using high gravity sweet potato medium containing 210.0 g l^{-1} glucose. In batch mode, the fermentation could not accomplish even in 120 h without any feeding of nitrogen source. While, the feeding of acid-hydrolyzed bloom algae powder (AHBAP) notably promoted fermentation process but untreated bloom algae powder (UBAP) was less effective than AHBAP. The fermentation times were reduced to 96, 72, and 72 h if 5.0, 10.0, and 20.0 g l^{-1} AHBAP were added into medium, respectively, and the ethanol yields and productivities increased with increasing amount of feeding AHBAP. The continuous fermentations were performed in a three-stage reactor system. Final concentrations of ethanol up to 103.2 and 104.3 g l^{-1} with 4.4 and 5.3 g l^{-1} residual glucose were obtained using the previously mentioned medium feeding with 20.0 and 30.0 g l^{-1} AHBAP, at dilution rate of 0.02 h^{-1}. Notably, only 78.5 g l^{-1} ethanol and 41.6 g l^{-1} residual glucose were obtained in the comparative test without any nitrogen source feeding. Amino acids analysis showed that approximately 67% of the protein in the algal biomass was hydrolyzed and released into the medium, serving as the available nitrogen nutrition for yeast growth and metabolism. Both batch and continuous fermentations showed similar fermentation parameters when 20.0 and 30.0 g l^{-1} AHBAP were fed, indicating that the level of available nitrogen in the medium should be limited, and an algal nitrogen source feeding amount higher than 20.0 g l^{-1} did not further improve the fermentation performance.

1. Introduction

Root crops such as sweet potato and cassava are widely planted in China and other South Asian countries as starchy crops. The high starch output as well as high climate and field adaptation of root crops reveal their higher potential in fuel ethanol feedstock than corn grains and cane sugar. Consequently, in fuel ethanol production, great attention has been paid on root crops in laboratorial or industrial scale in recent years (Nguyen et al., 2007; Jin et al., 2009; Strichuwong et al., 2009; Yuwa-Amornpitak, 2010; Sriroth et al., 2010; Akaracharanya et al., 2011; Papong and Malakul, 2011). As a starch-rich crop, sweet potato is a cheap and widely accessible feedstock substitute for grain and cane sugar in fuel ethanol production (Zhang et al., 2010, 2011). In this field, studies focusing on raw material pretreatment, medium optimization, novel fermentation technologies, and micro-organisms isolation have been taken (Jin et al., 2009; Zhang et al., 2011; Sree et al., 1999). However, more effective and economical process is still needed to be explored to enhance the competitive capacity of root crops in the production of fuel ethanol against traditional grain-based or cane sugar-based fuel ethanol.

Nitrogen is an indispensable nutrition for the growth and metabolism of yeast cells, and some studies prove that any kind of nitrogen source feeding (ammonium, free amino acid, urea, or yeast extract (YE)) can increase the fermentation rate and improve the growth of yeast cells under either aerobic or anaerobic conditions (Thomas and Ingledew, 1990; Albers et al., 1996; Bach et al., 2009; Pereira et al., 2010; Yue et al., in press). On the contrary, lack of nitrogen nutrition will lead to the reduction of ethanol formation rate and yield, and such negative effect cannot be omitted particularly when the ethanol concentration in the medium keeps at a high level (Watanabe et al., 2010; Sablayrolles et al., 1996). Metabolic balance analysis further reveals that free amino acids feeding can enhance ethanol yield and reduce glycerol formation by

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inhibiting the intracellular amino acids synthesis from glucose and ammonia. While, a limit of free amino acids providing will generate more intracellular amino acids synthesis, which would consume glucose and produce surplus NADH, and further lead to more glycerol formation, resulting in a reduction of ethanol yield (Broa et al., 2006).

In fuel ethanol fermentation, the lower protein content in sweet potato tubers requires higher amounts of an additional utilizable nitrogen source, which will increase the production costs of fuel ethanol. Ammonium sulfate and urea are the most popular nitrogen nutrition used in grain-based and sugar-based industrial scale fuel ethanol processes. While, YE and peptone are widely used in laboratory-scale yeast culture and fuel ethanol fermentation studies. In industrial processes, an additional nitrogen source feeding is necessary and costs considerable part of total fuel ethanol cost. Some replacements of ammonium and YE such as finger millet flour and corn steep liquor had been tried (Reddy and Reddy, 2006; Pradeep and Reddy, 2010). These substitutes show good application prospect but the seeking of cheap and accessible nitrogen source for fuel ethanol production is still an open issue.

Bloom algae biomass, which is usually discarded or used as a fertilizer after being collected from eutrophic waters, contains extremely high level of protein (over 20% in dry weight) (Benemann, 1979). In this study, bloom algae biomass was collected and used as a low-cost nitrogen source fed in sweet potato medium for fuel ethanol fermentation. The effects of algal nitrogen source on the ethanol yield and the system performance in batch and continuous modes were investigated.

2. Materials and methods

2.1. Raw material treatment

Fresh sweet potato tubers were washed with tap water, sliced, dried at 85 °C in a vacuum oven for two days, and stored in plastic bags at 4 °C. Bloom algae biomass was collected from a local lake and washed with tap water separating via centrifugation (4200 × *g*, 10 min), dried at 85 °C in vacuum oven for two days, then milled and passed through a 40-mesh screen. The untreated bloom algae powder (UBAP) was stored in plastic bags at 4 °C. The same batch of raw materials was used for all experiments. The procedure outline for the experiments was summarized in Fig. 1.

2.2. Algal nitrogen source preparation

Approximately 100 g algae biomass powder was added into a 500 ml glass beaker and mixed with 200 ml 0.1 mol l^{-1} HCl. Then, the beaker was placed in boiling water for 2 h, with the temperature in the beaker kept at approximately 96 °C. After hydrolysis, the algal slurry was neutralized using 2.0 mol l^{-1} NaOH solution and dried at 85 °C in vacuum oven until the water evaporated completely. Ten batches of dried hydrolyzed algal biomass lumps were collected, milled, and passed through a 40-mesh screen. The acid-hydrolyzed bloom algae powder (AHBAP) was stored at 4 °C for the subsequent experiments.

2.3. Sweet potato medium preparation

Dried sweet potato chips were milled and passed through a 20-mesh screen. Twice of tap water (in weight) was added and mixed with the sweet potato powder in a steel tank. The pH of the slurry was adjusted to 6.0 using 0.5 mol l^{-1} HCl solution, then the slurry was heated to 85 °C under agitation. α-Amylase (4000 U per gram α-amylase powder; Donghua Co., China) was added at a dosage of 2.0 g α-amylase per kilogram sweet potato powder and the temperature of slurry was kept at 85 °C for 2 h for liquefaction. The temperature of slurry was then cooled to 55 °C, and 4.0 g glucoamylase (50,000 U per g glucoamylase powder; Donghua Co., China) per kilogram sweet potato powder was added. The temperature of slurry was then kept at 55 °C for 4 h under agitation for thorough hydrolysis of liquefied starch. The liquor was separated and collected via filtration and stored at −20 °C for the subsequent experiments.

2.4. Yeast strain and pre-culture

An ethanol-tolerant industrial yeast strain, *Saccharomyces cerevisiae* ATCC 6508, was bought from American Type Culture Collection (ATCC) and was used for ethanol fermentation in this study. The initial seed was cultured in a yeast extract peptone

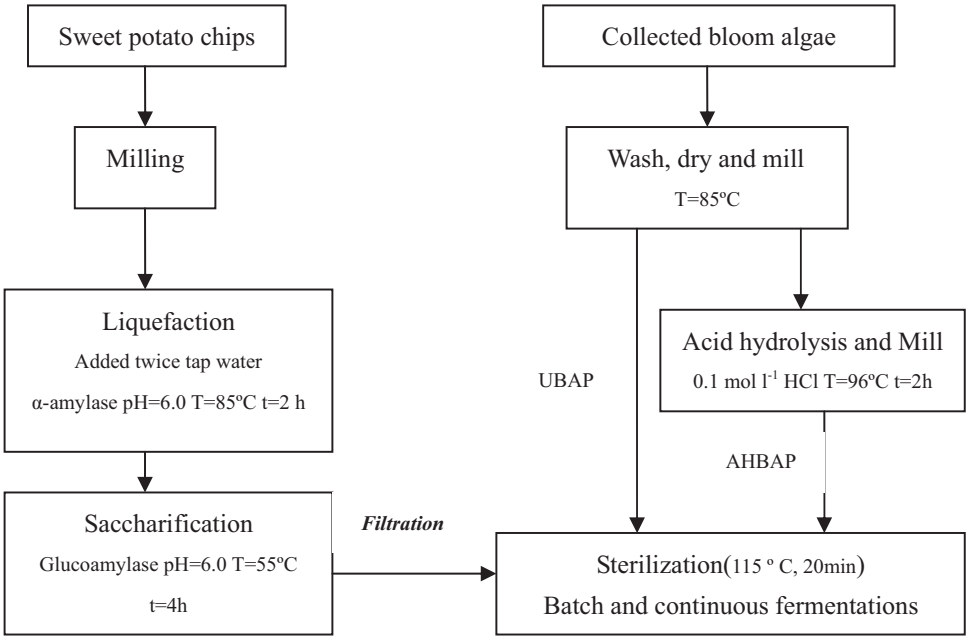

Fig. 1. Procedure outline of raw material treatment and fuel ethanol fermentation from sweet potato using bloom algae biomass as a nitrogen source.