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Production of biofuels from sweet sorghum juice via ethanol–methane two-stage fermentation

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ABSTRACT

Sweet sorghum juice is rich in fermentable sugar. Combining ethanol fermentation with methane fermentation to convert sweet sorghum juice to biofuels not only maximizes the energy recovery, but also reduces the environmental load. A two-stage fermentation, consisting of continuous ethanol fermentation and thermophilic methane fermentation, was developed to convert sweet sorghum juice to productions of ethanol and methane. The results of batch ethanol fermentation indicated that it was essential to supplement the feedstock with nutrients in order to improve the ethanol yield. Continuous ethanol fermentation could be performed at 35 °C without decreasing the ethanol yield at a dilution rate of 0.3 h^{-1}, and ethanol yield and productivity of 88.5% and 20.3 g/L/h were obtained, respectively. In contrast, the productivity was improved to 27.4 g/L/h by increasing the dilution rate to 0.4 h^{-1} at a fermentation temperature of 33 °C. The stillage eluted from the ethanol production process was subjected to thermophilic methane fermentation. After adjusting the C/N ratio of the stillage to 40, a total organic carbon (TOC) removal efficiency of 87.0% and gas evolution rate of 1200 mL/g-TOC were achieved, even at a high TOC loading rate of 10 g/L/d by adding $(NH_4)_2SO_4$ of 0.1 g/L-stillage.

1. Introduction

Bioethanol is a sustainable alternative fuel that could reduce CO_2 emissions by 90% if substituted for gasoline (Ward and Singh, 2002). When used as feedstock, sweet sorghum has the following advantages for the production of bioethanol: a high biomass yield, high carbohydrate content (e.g., fermentable sugars in the juice of the stem and structural sugars in the bagasse) and non-competition with food and arable land (Chohnan et al., 2011; Rohowsky et al., 2013).

Juice from the sweet sorghum stem contains glucose, fructose and sucrose, and these sugars can be directly converted to ethanol by *Saccharomyces cerevisiae*. Studies on the production of bioethanol from sweet sorghum juice by batch fermentation have focused on screening strains (Bulawayo et al., 1996), optimizing fermentation conditions (Kundiyana et al., 2010) and supplementing the feedstock with nutrients (Cao and Liu, 2013). However, most of these studies were performed in batch (Wu et al., 2010) or repeated-batch fermentation (Chohnan et al., 2011), and the continuous fermentation process has been rarely studied (Liu et al., 2008). In our previous studies, we successfully performed continuous ethanol fermentation to convert molasses (Kida et al., 1990), the acid hydrolysate of wood biomass (Tang et al., 2006) and kitchen garbage (Tang et al., 2008) into ethanol using the flocculating yeast *S. cerevisiae* strain KF-7.

Organic compounds such as proteins and starch contained in sweet sorghum juice cannot be utilized during ethanol fermentation (Andrzejewski et al., 2013a). However, little attention has been paid on the surplus organic compounds, although Antonopoulou et al. (2008) performed anaerobic digestion of the remaining biomass after hydrogen fermentation of sweet sorghum juice. Thus, it is important to add an additional process, such as methane fermentation, following ethanol fermentation to recover the most energy from sweet sorghum juice and reduce the content of organic compounds released into the environment.

Abbreviations: TOC, total organic carbon; VFAs, volatile fatty acids; GC, gas chromatography; HPLC, high-performance liquid chromatography; k_La, volumetric oxygen transfer coefficient; SRB, sulfate-reducing bacteria.

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Table 1
Effects of supplementing the feedstock with nutrients on the batch ethanol fermentation of the raw sweet sorghum juice.

Case	Nutrients (g/L)			Ethanol yield (%)
	KH_2PO_4	$MgSO_4{\cdot}7H_2O$	$(NH_4)_2SO_4$	
a	0	0	0	70.0
b	0	0	0.5	80.5
c	0.1	0	0.5	86.8
d	0.1	0.5	0.5	89.6

In the present study, the production of ethanol and methane from sweet sorghum juice was studied using an ethanol–methane two-stage fermentation process. Flocculating yeast *S. cerevisiae* strain KF-7 was used in ethanol fermentation. Ca^{2+} was supplemented to enhance the flocculability of yeast cells. First, batch ethanol fermentation was performed to evaluate the effects of supplementing the feedstock with mineral nutrients on the growth of KF-7 and ethanol yield referred to fermentation efficiency. In continuous ethanol fermentation, balance of mineral ions in the inlet and outlet of the reactor were conducted based on the results of batch fermentation, and the effects of the dilution rate, temperature and aeration rate were evaluated, with the aim of achieving significantly high ethanol yield and productivity by using the flocculating yeast with high cell activity. Thermophilic methane fermentation of the stillage eluted from the ethanol production process (hereafter called stillage) was subsequently studied to achieve a high total organic carbon (TOC) loading rate and treatment efficiency.

2. Materials and methods

2.1. Sweet sorghum and yeast strain

Sweet sorghum was kindly provided by the Kyusyu Okinawa Agricultural Research Center. The stems were squeezed after the leaves and husks were stripped, and the fresh juice was stored at −20 °C. The pH of the juice was 5.18 ± 0.15, and the juice consisted of the following sugar content: glucose, 55.7 ± 2.8 g/L; fructose, 40.1 ± 5.5 g/L; and sucrose, 19.9 ± 3.6 g/L.

The flocculating yeast *S. cerevisiae* strain KF-7 was used in the present study. This yeast strain was constructed by protoplast fusion of the flocculating yeast strain IR-2 and the thermotolerant yeast strain EP-1 (Kida et al., 1992).

2.2. Preparation of inoculum

Yeast strain KF-7 stored on a 2% (w/v) YPD (1% yeast extract, 2% dextrose, 2% peptone and 2% agar) slant was inoculated into 100 mL of sterilized 5% (w/v) YPD medium (1% yeast extract, 2% peptone and 5% dextrose) in a 500-mL conical flask. It was used as the yeast inoculum after it was cultivated at 30 °C for 16 h with shaking at 160 rpm in a rotary shaker (TB-9R-2F; Takasaki Kagaku, Saitama, Japan). After cultivation, ethanol concentration was 21.1 g/L, and no sugar was detected in the broth.

2.3. Batch ethanol fermentation

Mineral nutrients, including KH_2PO_4, $MgSO_4{\cdot}7H_2O$ and either $(NH_4)_2SO_4$ or $(NH_2)_2CO$ as nitrogen source, were added to 90 mL of sweet sorghum juice at the designated level (Table 1). After sterilization, 10 mL of inoculum was added into the juice to start fermentation. Batch ethanol fermentation was performed in a 300-mL conical flask. The flask was immersed into a water bath (30 °C), and the cultivation medium was stirred using a magnetic stirrer for 24 h. When evaluating $(NH_4)_2SO_4$ or urea as nitrogen source, the batch ethanol fermentation was carried out for 32 h without addition of other mineral nutrients. Ethanol yield was calculated as follow:

$$Y = \frac{C_{ethanol} - C_{inoculum}}{[(C_G + C_F) \times 92/180 + C_s \times 184/342] \times 0.9} \times 100\%,$$

where Y is the ethanol yield; $C_{ethanol}$ is the concentration of ethanol in the fermented broth; $C_{inoculum}$ is the concentration of ethanol introduced to batch fermentation when inoculating, which is 2.1 g/L; 0.9 is the volumetric ratio of sweet sorghum juice to total fermentation broth; and C_G, C_F and C_S are the concentrations of glucose, fructose and sucrose in sweet sorghum juice, respectively.

2.4. Continuous ethanol fermentation

Continuous ethanol fermentation was performed in a tower-shaped reactor with a working volume of 450 mL, as previously described (Tang et al., 2006). Before fermentation, the reactor was sterilized by circulating 2 mg/L of NaClO solution overnight, and then sterilized tap water was supplied continuously for 1 day. Approximately 450 mL of pre-cultivated broth (containing 1.5×10^8 cells/mL) was inoculated into the reactor, and then sweet sorghum juice was fed into the reactor at a dilution rate of 0.15 h^{-1}. Before feeding sweet sorghum juice into the reactor, the total sugar content (the sum of the concentrations of glucose, fructose and sucrose) was adjusted to 150 g/L by adding sucrose (hereafter called the adjusted sweet sorghum juice, while the unadjusted material is called the raw sweet sorghum juice), based on the total sugar concentration of 120–142 g/L reported by Andrzejewski et al. (2013a). In order to prevent the loss of sugar by bacterial contamination, the adjusted sweet sorghum juice was placed at 70 °C, based on the results of a preliminary study. The effects of supplementing the feedstock with mineral nutrients, dilution rate, temperature and aeration rate on continuous ethanol fermentation were investigated. Ethanol yield was calculated as follow:

$$Y = \frac{Cethanol}{(C_G + C_F) \times 92/180 + C_S \times 184/342} \times 100\%,$$

where Y is the ethanol yield, $C_{ethanol}$ is the concentration of ethanol in the fermented broth, and C_G, C_F and C_S are the concentrations of glucose, fructose and sucrose in sweet sorghum juice, respectively.

2.5. Distillation of fermented broth

The fermented broth eluted from the continuous fermentation was collected and distilled using a rotary vacuum evaporator (N-11; Tokyo Rikakikai, Tokyo). Distillation was stopped when 40% of the total weight of the fermented broth was collected as distillate. Tap water (the same amount of distillate) was added into the stillage before component analysis and methane fermentation. The stillage had a pH of 3.48 ± 0.04, and it was determined to have the following composition: TOC, 9958.0 ± 707.3 mg/L; NH_4^+, 11.3 ± 0.6 mg/L; and PO_4^{3-}, 137.0 ± 5.6 mg/L. The C/N (TOC/NH_4^+-N) ratio of the stillage was approximately 1100.

2.6. Thermophilic methane fermentation

Methane fermentation of the stillage was performed in an upflow anaerobic filter reactor with a working volume of 800 mL at 53 °C, as previously described (Sun et al., 2013). An 800-mL portion of thermophilic sludge was acclimatized by feeding synthetic wastewater [glucose, 35 g/L; corn steep liquor, 17.5 g/L; $(NH_4)_2CO_3$, 1.0 g/L; Na_2CO_3, 0.3 g/L; K_2HPO_4, 0.2 g/L; and $FeCl_3{\cdot}6H_2O$, 0.1 g/L] at a TOC loading rate of 1 or 2 g/L/d. After the reactor ran stable for approximately two weeks, the stillage was fed continuously at the same TOC loading rate. In order to enhance the methane fermentation, the stillage was supplemented with Ni^{2+} and Co^{2+}