Comparison of ethanol production performance in 10 varieties of sweet potato at different growth stages

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**Abstract**

The performance in the ethanol production of 10 varieties of sweet potato was evaluated, and the consumption in raw materials, land occupation and fermentation waste residue in producing 1 ton of anhydrous ethanol were investigated. The comparative results between 10 varieties of sweet potato at 3 growth stages indicated that NS 007 and SS 19 were better feedstocks for ethanol production, exhibiting less feedstock consumption (6.19 and 7.59 tons/ton ethanol, respectively), the least land occupation (0.24 and 0.24 ha/ton ethanol, respectively), less fermentation waste residue (0.56 and 0.55 tons/ton ethanol, respectively), the highest level of ethanol output per unit area (4.17 and 4.17 ton/ha, respectively), and a lower viscosity of the fermentation culture (591 and 612 mPa S, respectively). The data above are average data. In most varieties, the ethanol output speed at day 130 was the highest. Therefore, NS 007 and SS 19 could be used for ethanol production and harvested after 130 days of growth from an economic point of view. In addition, the high content of fermentable sugars and low content of fiber in sweet potatoes are criteria for achieving low viscosity in ethanol fermentation cultures.

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1. Introduction

In recent years, due to rising environmental concerns and periodic crises in some large oil exporting countries, fuel ethanol has become a viable substitute for gasoline. The fuel ethanol used today is mainly produced from sugarcane (Brazil) and corn (USA), but it is not sustainable to use grain for ethanol production in China, which has the largest population in the world to feed. Therefore, ethanol should be produced from non-grain materials, especially in China. Sweet potato, a non-grain crop that is rich in starch, is a promising feedstock for fuel ethanol production. Recent studies have shown that sweet potatoes can yield 2–3 times more ethanol than corn, approaching the amount that sugarcane can produce (Comis, 2008). China is the biggest producer of sweet potatoes in Asia and throughout the globe. With a stable annual production of more than 100 million tons, China produces greater than 80% of the world’s sweet potato crop (Scott, 1992). Furthermore, Sichuan province in southwest China alone produces 20 million tons, which exceeds that of all non-Chinese developing countries (Christopher and Song, 2002). China’s largest oil and gas producer, China National Petroleum Corporation (CNPC), has signed an agreement with the government of Sichuan Province to develop facilities to produce 600,000 tons of ethanol from sweet potatoes each year.

The varieties of sweet potato in China are abundant, and each variety has its own characteristics. With respect to using sweet potatoes for ethanol production, economic efficiency is the most important characteristic. Determining the variety of sweet potato appropriate for ethanol production is essential for the ethanol production industry.

Sweet potatoes in China are traditionally planted at the end of May and harvested at the beginning of November, requiring approximately 160 days of growth. After being harvested, the sweet potatoes can be stored in good condition until February of the following year because of climatic conditions. To meet the requirements of fuel ethanol manufacture, the shelf life of the sweet potato must be prolonged. In addition to improving storage methods, harvesting ahead of schedule may be another method for prolonging shelf life.

To our knowledge, no studies characterizing the varieties of sweet potato crops in terms of ethanol production performance have been conducted, and an optimal variety for ethanol production remains unidentified. The aim of this study is to investigate ethanol production performance and to select the appropriate variety and growth period for ethanol production among the 10
varieties tested. This comparative study was realized using some parameters, such as feedstock consumption, ethanol output, ethanol output speed and fermentation residue of 10 varieties of sweet potato at 3 growth stages.

2. Materials and methods

2.1. Microorganism

Saccharomyces cerevisiae strain CCTCC M206111 was separated from wine lees in our laboratory. The yeast strain was maintained on malt wort agar medium at 4 °C.

2.2. Inoculum culture

The yeast strain was added to 250 mL flasks containing 100 mL of sterilized medium. The medium composition was as follows: 100 g/L glucose, 1.3 g/L (NH4)2SO4, 0.1 g/L MgSO4 and 8.5 g/L yeast extract. The culture was incubated at 30 °C for approximately 14 h. The liquid cultures had an initial cell dry weight of 3.0–3.5 g/L.

2.3. Enzymes

The commercial enzymes for liquefaction (Liquozyme Supra, alpha-amylase) and for saccharification (Suhong GA II, glucoamylase) were purchased from Novozymes. The amount of enzyme that can hydrolyze 5.26 g soluble starch per hour at pH 5.6 and 37 °C is defined as 1 KNU (Kilo Novo alpha-amylase Unit). The amount of enzyme that cleaves 1 mmol of maltose per min at pH 4.3 and 25 °C is defined as 1 AGU (Amyloglucosidase Novo Unit) (Novo, 1981). Liquozyme Supra is a heat-stable α-amylase (EC 3.2.1.1) from Bacillus licheniformis with a declared activity of 90 KNU/g. Suhong GA II is a glucoamylase (EC 3.2.1.1) from Aspergillus niger with a declared activity of 500 AGU/g.

2.4. Raw materials and fermentation culture

Fresh sweet potatoes were collected from Nanchong Agricultural Research Institute, Sichuan, China. The sweet potato crops were planted on May 29th, 2008 and harvested after growing for 100, 130 or 160 d.

The fresh sweet potatoes were cleaned and crushed into small pieces (<3 mm). Then, water was impregnated in the various hydromoduli of different varieties of sweet potato, which corresponded to the initial total fermentable sugar concentration of 180 g/kg in all fermenters. After low-temperature cooking at 85 °C for 10 min, the mixture was liquefied using Liquozyme Supra (10 min, 85 °C, pH 5.2, 120 KNU/kg starch). All fermentations were carried out using 250 mL Erlenmeyer flasks containing 100 g fresh sweet potato hydrolysis. The initial pH of all media was adjusted to 5 with 1 M NaOH and 1 M HCl. The flasks with media were sterilized by autoclaving at 115 °C for 20 min prior to inoculation. Glucoamylase was added at a dosage of 750 AGU/kg starch at the time of inoculation. The ethanol fermentations were carried out at 30 °C under anaerobic conditions with agitation at 150 rpm.

2.5. Analytical methods

The total fermentable sugar content of the fresh sweet potatoes was determined by acid hydrolysis (Wang et al., 2007) in which the samples were treated with HCl at 100 °C for 2 h and the amount of reducing sugar was measured by the DNS method (3.5-dinitro salicylic acid) using glucose as the standard (Maldonado and Strasser de Saad, 1998). The crude fiber in the fresh sweet potato is the acid and alkali-insoluble residue analyzed according to the Chinese Standard GB/T 5009.10-2003. The pectic substances in fresh sweet potato were extracted using the ethanol and water method and determined via galacturonic acid (Walter et al., 1997).

Fermentation samples were taken from the fermenter and centrifuged at 8000 rpm to remove any solids from the media. The ethanol concentration was determined by gas chromatography, in which 0.8 mL of a 10% sample supernatant filtered through a 0.45 μm membrane filter (Millipore, USA) was mixed with 0.2 mL of 10% n-propanol. All determinations were performed using standard curves. A gas chromatograph (model FULI 9790; FULI Corp., China) fitted with a flame ionization detector was operated under the following conditions: gas column stainless steel column (3.2 mm × 2 m) packed with Chromosorb (FULI Corp., China); temperature of injector and detector, 200 °C; nitrogen carrier gas flow rate, 30 mL/min; and temperature of column oven, 160 °C (Liu et al., 2007). The fermentation residue was measured by the gravimetric method at 105 °C until a constant weight was achieved. The viscosity was measured using a rotational viscometer (DV-II+ PRO, Brookfield, USA) equipped with a recirculating water-bath (TC 200, Brookfield, USA) for control of the sample-container temperature. The viscosity changes were determined at 30 °C with a paddle speed of 100 rpm. Every experiment was conducted in duplicate, and the data presented represent mean values.

2.6. Statistical methods and calculations

The limitation of using root and tuber mashes for ethanol production is attributed to their highly viscous nature. High viscosity caused resistance to solid–liquid separation and lower fermentation efficiency (Srikanta et al., 1992), which will increase the cost of ethanol production. Therefore, choosing a sweet potato with low viscosity as the feedstock may be beneficial for reducing the cost of ethanol production. According to several studies, the viscosity of a plant is mainly related to fiber and pectin (Dikeman and Falhey, 2006; Sreenath et al., 1987). The addition of water may also affect the viscosity of the fermentation mash. However, too much water will result in a low concentration of ethanol, especially when the fermentable sugar content in the feedstock is low. To clarify the factors affecting the viscosity of the fermentation mash from sweet potatoes, correlation analyses between the viscosity and the fiber, pectin and fermentable sugar contents were carried out using SPSS 13.0 for Windows.

Fermentation efficiency is an important parameter for evaluating the efficiency of the conversion of feedstock to product. It can be calculated by the following equation: fermentation efficiency = ethanol produced during fermentation/theoretical yield of ethanol produced × 100% (Zhang et al., 2010).

3. Results and discussion

3.1. Fermentable sugar, fiber and pectin contents of fresh sweet potatoes

The main composition of fresh sweet potatoes was expressed using wet weight (g/100 g fresh sweet potato; Table 1). The fermentable sugar content varied from 15.89 to 36.04%. Thus, sweet potatoes may be considered a substrate-rich source of feedstock for ethanol fermentation. NS 007 had the highest content of fermentable sugars among the fresh sweet potato varieties analyzed. The fiber and pectin contents of the sweet potatoes varied from 0.68 to 1.04% and from 0.12 to 1.83%, respectively. Variation not only existed among the different varieties but also at different growth periods for each variety. In most varieties, the content of fermentable sugars was highest at day 130, while the
content of fiber was lowest at day 130. The pectin content of all varieties increased as the growth period increased.

3.2. Ethanol production characteristics of fresh sweet potatoes

3.2.1. Feedstock consumption of fresh sweet potato

Based on the fermentable sugar content (Table 1) and ethanol fermentation parameters at the bench scale (Table 2), the feedstock consumptions required to produce 1 ton of anhydrous ethanol from the 10 varieties of sweet potatoes were calculated (Table 3). The consumptions varied from 5.99 to 13.86 t, which was mainly due to the different contents of fermentable sugar in the fresh sweet potatoes. The feedstock accounts for half of the ethanol production cost. Cases in which less feedstock is consumed will lower the cost of ethanol production. Thus, if 600,000 tons of ethanol were produced yearly (according to the agreement between CNPC and the government of Sichuan Province), the yearly feedstock cost of NS 007, excluding the costs of feedstock collection, transportation, preservation, processing and pretreatment, would be half that of 200730.

3.2.2. Land occupation

According to the feedstock consumption (Table 3) and yield of sweet potatoes per unit area (Table 4), the respective land occupations of the 10 varieties of sweet potato to produce 1 ton of anhydrous ethanol were calculated (Table 5). To produce 1 ton of ethanol, the land occupation requirements varied from 0.19 to 0.57 ha. The average land occupation was lowest for strains NS 007 and SS 19. The ecological footprint of a biofuel, in terms of the land area needed to grow sufficient quantities of the feedstock, should be minimized (Groom et al., 2008). Less land occupation not only results in lower rent of the land but also a reduced cost of cultivation. These factors all contribute to a lower cost of feedstock for ethanol production.

3.2.3. Waste residue

To produce 1 ton of anhydrous ethanol, the quantity of fermentation waste residue varied from 0.43 to 0.96 t (Table 6). Because of pollution problems, the treatment of the ethanol fermentation residue is 1 of the most significant and challenging issues in the industrial production of ethanol (Fitzgibbon et al.,
In addition to its disposal, the issue of decreasing the output of the fermentation residue also should be addressed. Choosing the right variety of sweet potato may be beneficial in this sense.

### 3.2.4. Viscosity

High viscosity causes handling difficulties during processing and may lead to incomplete hydrolysis of the starch to fermentable sugar, thus lowering the fermentation efficiency (Ingledew et al., 1999; Wang et al., 2008). The excessive addition of water may reduce viscosity; however, the concentration of fermentable sugars in the fermenter is also decreased by the dilution, and more energy is required for water evaporation. In this experiment, water was impregnated in various hydromoduli of different varieties of sweet potato, which corresponded to an initial total fermentable sugar concentration of 180 g/kg. Based on these figures, the fiber and pectin contents and the hydromoduli of the 10 varieties of sweet potato as well as the fiber and pectin contents in the fermentation cultures were calculated. SPSS 13.0 software was employed to analyze the correlations of these variables with the viscosity of the fermentation cultures.

The viscosity of the fermentation cultures varied from 322 to 42,820 mPa S (Table 7). Viscosity had a negative correlation with fermentable sugar content in the fresh sweet potatoes, and this correlation was significant at the 0.01 level. Because the initial total fermentable sugar in the fermentation cultures was the same in all varieties of sweet potato, a higher fermentable sugar content in a sweet potato strain resulted in more water that could be added to lower the viscosity. Meanwhile, viscosity had a positive correlation with fiber content in the fermentation culture, and this correlation was significant at the 0.01 level. Therefore, a high content of fermentable sugar and low content of fiber can be used as criteria to achieve low viscosity for ethanol production using sweet potatoes. Although pectin was responsible for adhesion among cells (Willats et al., 2001), it did not bear a statistically significant correlation with viscosity in our study. The explanation for this observation is still unknown.

### 3.3. Effect of growth period on ethanol production characteristics

In a general theoretical sense, sweet potatoes can be harvested whenever they reach marketable size, and in the tropics or in a greenhouse, sweet potatoes may grow without interruption for years. However, under the climatic conditions of China, sweet potatoes may cease growing at a certain growth stage due to a lack of moisture or nutrients, low temperatures or other unfavorable growing conditions.

The ethanol output per unit area of most varieties of sweet potato was increased at day 130 when compared with day 100. However, with a prolonged growth period, the ethanol output per unit area was not notably increased after day 130 (Fig. 1). Among the 10 varieties, NS 007 and SS 19 had the highest level of ethanol output per unit area. In most varieties of sweet potato, the ethanol output speed at day 130 was the highest (Fig. 2). Among the 10 varieties, NS 007 and SS 19 had the highest speed of ethanol output per unit area. Thus, sweet potatoes can be harvested and utilized 1 month earlier than the typical harvest. Early harvest could prolong a sweet potato’s shelf life as feedstock for ethanol production, enhance the multi-cropping index, and increase the level of energy output in a unit area, thus decreasing feedstock costs.

![Fig. 1. The level of ethanol output per unit area of 10 varieties of sweet potato at day 100, day 130 and day 160.](image-url)
4. Conclusions

Sweet potato, a crop that is rich in starch, is a promising material for ethanol production. In ethanol manufacture, energy efficiency, fermentation residue quantity and production costs are different for different varieties of sweet potato as the raw material. Ensuring the appropriateness of a variety of sweet potato for ethanol production is essential for the ethanol production industry. An exclusive evaluation system to assess the potential of the sweet potato for ethanol production should be established as soon as possible. In this study, we found that the high content of fermentable sugar and low content of fiber in sweet potato makes it an ideal crop to achieve low viscosity in ethanol production.

In this work, among the 10 strains tested, the sweet potato strains NS 007 and SS 19 had less feedstock consumption, the least land occupation, and the highest level and speed of ethanol output per unit area as well as a lower viscosity of fermentation culture and reduced fermentation waste residue. Although they did not have the lowest viscosity of fermentation cultures or the least fermentation waste residue, these strains could be used for ethanol production and harvested after growing for 130 days or act as parent crops to obtain hybrids that have ideal characteristics for ethanol production.

Acknowledgments

This study was supported by State 863 projects of China (No.2010AA101603), the China Agriculture Research System (No.CARS-11-A-04), the National Key Technology R&D Program of China (No.2011BAD22B03) and the Knowledge Innovative Program of the Chinese Academy of Sciences(NO.KSCX2-EW-G-1-1).

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