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Ethanol production by solid state fermentation of sweet sorghum using thermotolerant yeast strain

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ABSTRACT

Solid state fermentation of chopped sweet sorghum particles to produce ethanol was studied statically using thermotolerant yeast. The influence of various process parameters, such as yeast cell concentration, particle size and moisture content, on the ethanol yield was investigated. Optimal values of these parameters were 4×10^6 cells/g raw sorghum, Dp = 1.5 mm and 75%, respectively. Addition of reducing agent H_2SO_3 into the fermentation medium provided anaerobic condition, and obtained the maximum ethanol yield of 7.9 g ethanol per 100 g fresh stalks or 0.46 g ethanol/g total sugar, which was 91% of the theoretic yield.

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1. Introduction

Due to the diminishing fossil fuel reserves, alternative energy sources need to be renewable, sustainable, efficient, cost-effective, and safe [1]. In the past decades, microbial ethanol production has been focused and considered as an alternative fuel in the future.

Ethanol has excellent fuel properties for spark ignition internal combustion engines. For example, its high octane and high heat of vaporization make alcohol more efficient as a pure fuel than gasoline [2]. Industrial alcohol is produced from various substrates like molasses, maize starch, sugarcane, sugar beet, tapioca etc. But most of the immediate expansion in ethanol production is expected to rely on traditional technologies for use of grains (e.g., from corn and wheat) and some sugar (e.g., cane and beet sugar). In fact, large scale use of grains puts upward pressure on grain prices [3].

Of the many crops currently being investigated for energy and industry in China, sweet sorghum is one of the most promising, particularly for ethanol production [4]. Sweet sorghum is a C4 crop in the grass family belonging to the genus *Sorghum bicolor* L. Moench which also includes grain and fiber sorghum and is characterized by a high photosynthetic efficiency. Sweet sorghum is often considered to be one of the most drought resistant agricultural crops as it has the capability of remaining dormant during the driest periods [5]. The plant grows to a height of from about 120 to above 400 cm, depending on the varieties and growing conditions and can be an annual or short perennial crop. The development of sweet sorghum in China is now an agriculture policy option of the government and international agencies that aim at improving agricultural land use by promoting sustainable crops and valuing semi arid and other undeveloped lands.

There are some reports of ethanol production using submerged fermentation or immobilized cell fermentation from sweet sorghum juice [6,7], but few reports are available using solid state fermentation (SSF). The technique of SSF involves the growth and metabolism of microorganisms on moist solids without any free-flowing water. It avoids the need to isolate the sugars into a separate liquid phase before fermentation. Meanwhile, it has many potential advantages especially for regions in defect of water [8–10]: (a) less

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requirement of water (especially attractive in summer months when water is scarce), (b) less physical energy requirement, (c) less capital investment, (d) less operating costs, (e) less liquid waste to be disposed of and hence less pollution problems. However, SSF has some limitations such as limited choice of microorganisms capable of growth under reduced moisture conditions, controlling and monitoring parameters such as temperature, pH, humidity and air flow [11,12].

In the present work, we investigated the solid state fermentation of fresh sweet sorghum stalks for ethanol production. The influence of various process variables on ethanol yield was studied.

2. Materials and methods

2.1. Sorghum

Sweet sorghum Rio harvested in October 2006 was kindly provided by Prof. M. J. Wang (Chinese Academy of Agriculture Engineering, China). Leaves and husks were stripped from the fresh stalks by hand and stored in the freezer at −20 °C. Following thawing at room temperature, the stalks were cut into small particles. To adjust the moisture content of the sorghum, the chopped particles were dried in an oven at 80 °C until the desired moisture level was achieved. Sugar contents of the fresh stalks were (g/100 g sweet sorghum stalks): glucose, 0.5; fructose, 1.1; sucrose, 15.5, and the initial moisture content was 71%.

2.2. Microorganism and media

The laboratory mutant strain of baker yeast AF37X was used throughout the experiments. The yeast strain was maintained in MY medium whose composition (in g l^{-1}) was glucose, 20; yeast extract, 3; polypeptone, 5; malt extract, 3; agar, 20. In all cases, cultures were maintained at 37 °C for 24 h and then stored at 4 °C. Subculturing was done every two months. The composition of the pre-culture medium for yeast (in g l^{-1}) was: glucose, 10; sucrose, 10; yeast extract, 3; polypeptone, 5; malt extract, 3. All the media were adjusted to pH 6.5 and autoclaved at 116 °C for 20 min before use.

2.3. Pre-cultivation

Two loops from yeast slants were used to inoculate 100 ml of the pre-culture medium in 250 ml Erlenmeyer flasks and cultivated on a rotary shaker (180 rpm) at 37 °C for 20 h.

2.4. Solid state fermentation

Experiments were carried out in 250 ml conical flasks, each of which contained 100 g fresh sorghum stalks. Details of the cell concentration, particle size and moisture content were described in the following experiments. After inoculation, the contents were mixed thoroughly and incubated at 37 °C. The solid samples withdrawn from the fermentation medium were pressed using a syringe to remove the liquid from the solids. This liquid was centrifuged and then filtered to remove cells and other suspended solids. The sugars and ethanol concentrations were determined in this clear liquid phase [8,13].

2.5. Analytical methods

Sweet sorghum stalks contained sugars primarily in the form of sucrose, glucose and fructose. Glucose was determined enzymatically with a glucose oxidase-chromogen reagent (Shandong University). Sorghum sucrose was hydrolysed in 1.2 N HCl for 7 min at 60 °C and neutralized with 1 N NaOH prior to its determination by the method of reducing sugars. Reducing sugar was determined using the 3,5-dinitrosalicylic acid (DNS) method [14,15]. The moisture content of sweet sorghum stalks was determined after drying and measuring the weight before and after the drying procedure. The ethanol content was measured by using Shimadzu GC-2050 gas chromatography with cbp-20 capillary column and a flame ionization detector. The chromatogram was run at 180 °C oven temperature and 90 °C injection temperature using N_2 as a carrier gas and H_2 as a flaming gas.

3. Results and discussion

3.1. Influence of yeast cell concentration

A series of experiments with different initial cell concentrations were performed. The particle size and moisture content were 0.5–1.5 mm and 75%, respectively. No reducing agent was added. The inoculum culture was concentrated in 0.9% NaCl aseptically. Serial dilutions of this concentrated cell suspension were used to inoculate the flasks to yield initial cell concentrations of 2, 4, 10×10^6 cells/g raw sorghum. Samples were withdrawn from each flask every 6 h.

Fig. 1 depicts the ethanol formation profile with different initial cell concentrations. The maximum ethanol yield was 6.81 g ethanol/100 g sweet sorghum with initial cell concentration of 4.0×10^6 cells/g raw sorghum stalks. Flask with initial cell concentration of 2.0×10^6 cells/g raw sorghum stalks could reach almost the same value. Lower ethanol yield was obtained with higher initial cell concentration. This may result from overuse of sugars for growth and maintenance at high cell concentrations. However, it's obvious that higher initial cell concentrations resulted in shorter fermentation period and reduced the chance of contamination. Therefore, cell concentration of 4.0×10^6 cells/g raw sorghum stalks was used in the following experiments.

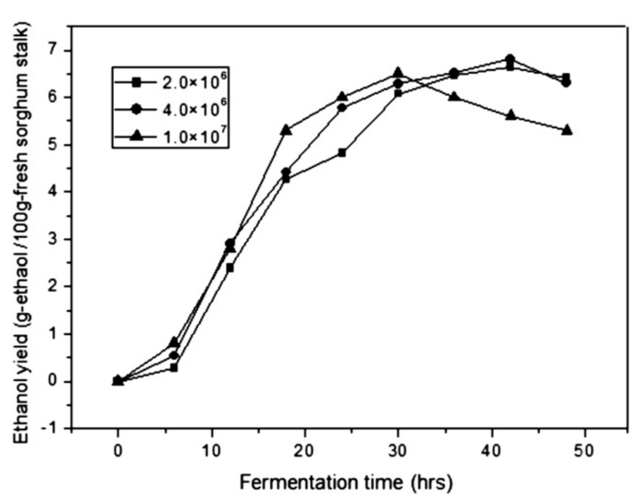

Fig. 1 – The ethanol formation profiles in SSF of sweet sorghum stalks at different initial cell concentrations (cells/g raw sorghum).

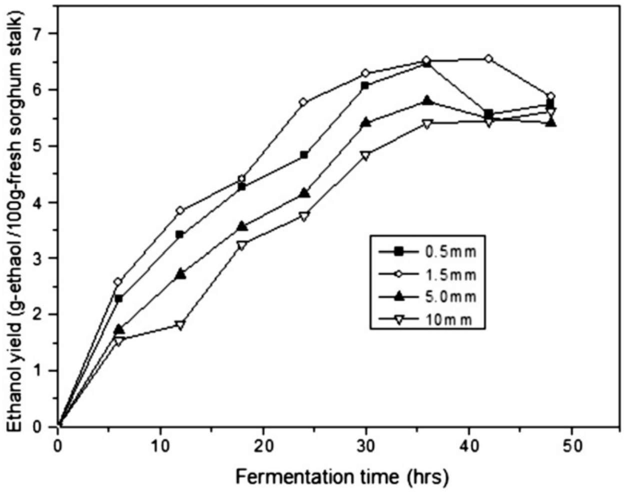

Fig. 2 – Influence of particle size on ethanol production from sweet sorghum by SSF.

3.2. *Influence of the particle size*

Particle size of the substrate is important. It affects the surface area to volume ratio of the particle, which is initially accessible to the microorganism [16,17]. Since ethanol fermentation is anaerobic, the particle size of the substrate affects the process significantly. The cell concentration and moisture content were 4.0×10^6 cells/g raw sorghum and 75%, respectively. No reducing agent was added. Due to higher packing density and reduction of void space between particles, more ethanol was therefore produced with smaller particle size (0.5–1.5 mm). Meanwhile, it tended to decrease the area for heat transfer and gas exchange with the surrounding atmosphere, as a result of which anaerobic conditions generated quickly for ethanol fermentation. However, too small particle size is also disadvantageous for heat transfer and exchange of carbon dioxide between the air and the solid surface. Increase in the particle size resulted in a lower ethanol yield (Fig. 2). In relation to particle size, it must be remembered that SSF process does not remain constant but tends to diminish, so particle size 0.5–1.5 mm was chosen during the experiments below.

3.3. *Influence of moisture*

An optimum moisture level has to be maintained, as lower moisture level tends to reduce microbial growth, enzyme stability and substrate swelling [18,19]. Higher moisture level leads to particle agglomeration, gas transfer limitation and competition from bacteria [20]. In general, the moisture levels in SSF processes vary between 30 and 85%. For bacteria, the moisture of the solid matrix must be higher than 70%, and in the case of filamentous fungi it could be as wide as 20–70% [21].

The cell concentration and particle size were 4.0×10^6 cells/g raw sorghum and 0.5–1.5 mm, respectively. No reducing agent was added. Chopped sorghum was dried to the moisture levels of 60, 65, 70, 75 and 80, respectively. Another 100 g chopped sorghum stalks were adjusted to reach the moisture content of 85% by adding distilled water. After sterilization, each flask was inoculated with concentrated yeast cells. The flasks were incubated in a constant environment shaker at 37 °C. Samples were withdrawn every six or 4 h.

The moisture level of 75–80% was optimal. It resulted in the maximum ethanol yield of 7.5 g ethanol per 100 g fresh stalks (Fig. 3). Low moisture content of chopped sorghum is advantageous since the chance of contamination of fermentation medium is reduced. However, there is a limit of moisture content below which yeast cells may not be functional to produce ethanol. As the moisture level decreased from 75% to 60%, the ethanol yield declined sharply from 7.5 to 2.2 g ethanol per 100 g fresh stalks. High moisture level made the stalks stick together during the SSF, which can hinder the heat transfer and exchange of carbon dioxide between the air and the solid surface, too. At 75–80% moisture level, the highest ethanol yield of 7.5 g ethanol per 100 g fresh stalks was obtained. Lower moisture content it is, less energy is needed for product recovery. So, moisture level of 75% was applied below.

3.4. *Influence of reducing agent of H_2SO_3*

In order to provide anaerobic condition to enhance the ethanol fermentation [8], different quantities of 30% sulfurous acid were added into the fermentation media. The cell concentration and moisture content were 4.0×10^6 cells/g raw sorghum and 75%, respectively. After sterilization, 30% sulfurous acid was added into the flasks to form the concentrations of 0, 15, 30, 40, 50 mg/100 g sorghum stalks. Each flask was inoculated with concentrated yeasts which resulted in 4.0×10^6 cells/g raw sorghum in the medium. The flasks were incubated in a constant environment shaker at 37 °C. Samples were withdrawn every six or 4 h.

The results are presented in Fig. 4, which compares the performance of flasks containing different concentrations of sulfurous acid. The highest ethanol yield was 7.9 g ethanol per 100 g fresh stalks obtained with 30 mg sulfurous acid per 100 g sorghum stalks. It is 91% of the theoretic yield, which is 5.3% higher than the one without reducing agent of H_2SO_3.

However, higher sulfurous acid concentration resulted in a lag period of nearly 50 h (not presented). This may be because of the inhibitory effects of sulfurous acid on yeast cells. At the

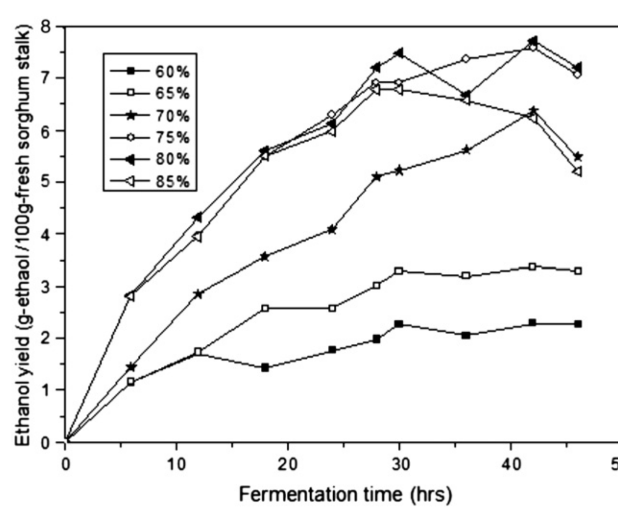

Fig. 3 – Solid state fermentation of sweet sorghum at various initial moisture levels (g water/100 g wet sorghum).

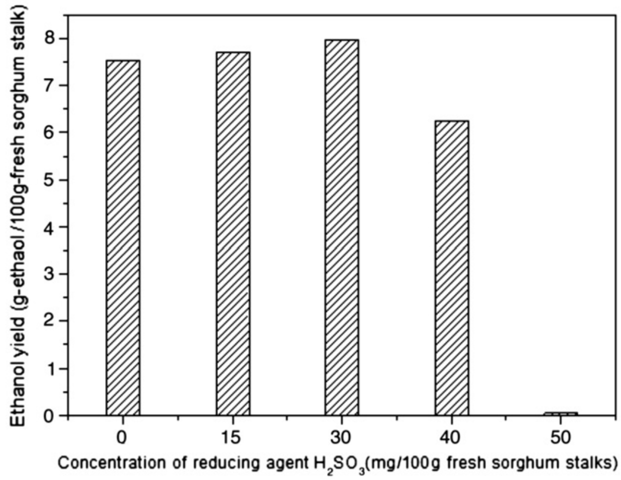

Fig. 4 – The influence of reducing agent H_2SO_3 on ethanol yield.

same time, little ethanol was produced in the flask with sulfurous acid concentration of 50 mg/100 g sorghum.

4. Conclusions

Solid state fermentation of chopped sweet sorghum particles to produce ethanol was studied statically using thermotolerant yeast. The influence of various process parameters, such as yeast cell concentration, particle size, moisture content, on the ethanol yield was investigated.

Optimal values of these parameters were found to be 4×10^6 cells/g raw sorghum stalks, 0.5–1.5 mm and 75% moisture level, respectively. With the reducing agent of 30 mg H_2SO_3/100 g fresh sorghum stalks, the maximum ethanol yield was 7.9 g ethanol per 100 g fresh stalks or 0.46 g ethanol/g total sugar after 40 ± 2 h, which is 91% of the theoretic yield.

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