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Ultrasound improved ethanol fermentation from cassava chips in cassava-based ethanol plants

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ABSTRACT

The effects of ultrasound and heat pretreatments on ethanol yields from cassava chips were investigated. Cassava slurries were sonicated for 10 and 30 s at the amplitudes of 80, 160, and 320 μm_{pp} (peak to peak amplitude in μm) corresponding to low, medium, and high power levels, respectively. The sonicated and non-sonicated (control) samples were then subjected to simultaneous liquefaction-saccharification and ethanol fermentation. Cassava starch-to-ethanol conversion efficiencies showed that higher ethanol yields were directly related to sonication times, but not to power levels. Significantly higher ethanol yields were observed only for sonicated samples at the high power level. The ethanol yield from the sonicated sample was 2.7-fold higher than yield from the control sample. Starch-to-ethanol conversion rates from sonicated cassava chips were also significantly higher; the fermentation time could be reduced by nearly 24 h for sonicated samples to achieve the same ethanol yield as control samples. Thus, ultrasound pretreatment enhanced both the overall ethanol yield and fermentation rate. When compared to heat-treated samples, the sonicated samples produced nearly 29% more ethanol yield. Combined heat and ultrasound treatment had no significant effect on overall ethanol yields from cassava chips. Ultrasound is also preferable to heat pretreatment because of lower energy requirements, as indicated by energy balances. Integration of ultrasound application in cassava-based ethanol plants can significantly improve ethanol yields and reduce the overall production costs.

1. Introduction

Prospering modern society heavily relies on energy. Our energy needs range from home appliances, lighting, transportation, heating/cooling, medical, to industrial processes to supply commodities. Many developing countries have just begun to enjoy the comfort of modern gadgets, and the demand for energy will continue to grow. Increasing energy demand especially in developing countries will consequently increase global energy demand over 50% by 2025 (Ragauskas et al., 2006). Fossil-derived fuels currently provide more than 90% of the world's total commercial energy needs (OPEC, 2007). Dwindling reserves coupled with rapidly increasing consumption rates from emerging Asian nations and the environmental devastation resulting from global warming demand for the development of sustainable, affordable, and environmentally friendly energy sources. Biofuels, particularly ethanol, are renewable and environmentally clean fuels.

Bioethanol is currently produced mainly from corn (United States) and sugarcane (Brazil). Tropical countries like Thailand are developing cassava-based ethanol plants. Cassava is one of the most important cash crops in Thailand (KAPI, 2003). Annual production of cassava tubers in Thailand is expected to rise to 20 million tons by 2012 (Nguyen et al., 2007). With the production capacity improvement, cassava supply is expected to exceed the demand. Thus the utilization of cassava root as a raw material for ethanol production will stabilize the price of cassava tubers and enhance the rural economy. Raw cassava tubers can be converted into dried chips, enabling year-round operation of ethanol plants. Therefore, cassava is recognized as one of potential crops for ethanol production in Thailand (Sriroth et al., 2000).

In view of these merits, in 2005, the Royal Thai Government issued permit for building 12 cassava-based ethanol plants with total daily production capacity of 3.4 million liters (~0.9 million gallons) (Sukphisal, 2005).

Utilization of cassava in cassava-based ethanol plants involves hammer milling, mesh screening, mashing, cooking, and enzymatic hydrolysis of starch to fermentable sugar. The released sugar is

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fermented to ethanol, which is then recovered by distillation and dehydration using molecular sieves (Sriroth and Piyachomkwan, 2005). The conventional process, however, requires high enzyme loadings and long fermentation times, and yet results in low ethanol yields. Additional forms of pretreatment may improve ethanol yields and reduce production costs, by shortening fermentation times, lowering enzyme dosages, improving enzyme hydrolysis, and eliminating some unit processes/operations. Cassava starch molecules are tightly bound within the fibrous structure. Reducing particle sizes and opening up the fibrous structure could essentially reduce the enzyme loading, shorten the processing time, improve the starch hydrolysis, and enhance the overall sugar yield for ethanol fermentation.

The application of ultrasound in the field of biorenewables is a relatively new concept, and has demonstrated potential as a pretreatment method to enhance enzymatic hydrolysis and subsequent ethanol fermentation. Ultrasound produces a hydrodynamic shear force in aqueous phase due to the rapid collapse of microbubbles formed during cavitation (Kuttruff, 1991). The hydrodynamic shear force facilitates the disintegration of coarse particles in slurry into finer particles thereby significantly increasing the surface area for enzyme activity. The study from Khanal et al. (2007) found 10–20-fold reduction of corn particles derived from dry-grind ethanol plant following ultrasound pretreatment. The considerable corn particle size reduction resulted in significant improvement in sugar release during enzymatic hydrolysis. In another study by our group, ultrasound pretreatment of cassava chip slurries enhanced the reducing sugar yield by 180% (Nitayavardhana et al., 2008).

Although, ultrasound pretreatment was found to enhance liquefaction and saccharification of starch-based feedstocks (corn and cassava), the subsequent effect of ultrasound on ethanol yield has not been examined. Therefore, the objectives of this study were to evaluate: (i) the effect of ultrasound pretreatment on ethanol fermentation efficiencies and yields from cassava chip slurries, (ii) the effect of different sonication conditions, e.g. power inputs and sonication times on reducing sugar release from cassava chip slurries and subsequent ethanol fermentation, and (iii) the effect of starch gelatinization on the ethanol fermentation.

2. Methods

2.1. Cassava chips and enzyme

Cassava chips provided by Sui Heng Lee Co. Ltd. (Bangkok, Thailand) were ground and passed through a 10-mesh screen. The total starch content of the chips was 79.20% (dry basis) as determined by GOPOD assay (Megazyme International Ireland Co. Ltd., Wicklow, Ireland). The moisture content of cassava chip was 13.60% (dry basis) as measured by using a forced-air oven at 135 °C for 2 h. The enzyme *STARGEN™ 001* was obtained from Siam Victory Chemical Co. Ltd. (Bangkok, Thailand). *STARGEN™ 001* (456 granular starch hydrolyzing unit (GSHU)/g) is a cocktail of α-amylase (gene from *Aspergillus kawachi* was expressed in *Trichoderma reesei*) and glucoamylase (from *Aspergillus niger*). The enzyme mixture works synergistically in hydrolyzing starch into glucose.

2.2. Ultrasonic equipment

The Branson 2000 Series bench-scale ultrasonic unit (Branson Ultrasonics Corporation, Danbury, CT, USA) was used for sonication. The ultrasound unit has a maximum power output of 2.2 kW and operates at a constant frequency of 20 kHz. The components of the ultrasound system include the booster (gain 1:2) and the catenoidal titanium horn (gain 1:8), with a flat 13-mm diameter face.

2.3. Ultrasonic pretreatment

Cassava chip slurries at 5% total solids (TS) were prepared in 0.05 M acetate buffer at pH 4.3. Sonication of 35 ml slurries, in 50-ml polypropylene (PP) centrifuge tubes, was performed in batch mode at three different peak to peak amplitudes (measured as μm_{pp}) levels: low (80 μm_{pp}), medium (160 μm_{pp}), and high (320 μm_{pp}). The respective ultrasonic power densities were 2.00 ± 0.10, 4.00 ± 0.10, and 8.50 ± 0.20 W/ml for low, medium, and high amplitude levels. The slurries were sonicated for 10 and 30 s for each power level. The procedure is summarized in Fig. 1.

Uncooked and cooked cassava slurries were used to determine the effect of starch gelatinization. In order to cook the samples, the slurries with 5% TS were placed in a steamer cabinet at 95 °C for 15 min.

2.4. Simultaneous liquefaction-saccharification and fermentation

Simultaneous liquefaction-saccharification and fermentation was performed in sterile 250-ml PP bottles containing 70 ml of sonicated slurry, enzyme: *STARGEN™ 001*, added at ratio of 0.5% (v/w of starch content in cassava chips) and 25 ml yeast extract medium (2.4% yeast extract, 0.092% $CaCl_2{\cdot}2H_2O$, 0.4% $MgSO_4{\cdot}7H_2O$, 0.6% KH_2PO_4, and 1.6% $(NH_4)_2SO_4$) (Veale et al., 2003). The enzyme–cassava chip slurry mixture was inoculated with 5 ml of yeast cells – *Saccharomyces cerevisiae* (ATCC 24859; 9.5×10^7 cells/ml) – for a total volume of 100 ml. The bottles were capped loosely to create an anaerobic environment and to allow the release of CO_2 from the bottles. The bottles were incubated in a shaker for up to 72 h at 32 °C and 180 rpm. Samples were taken at 12-h intervals and analyzed for reducing sugar and ethanol concentrations. Each experiment was conducted in triplicate.

2.5. Reducing sugar and ethanol determination

The samples were centrifuged at 3400*g* for 20 min and filtered through Whatman PP 0.45 μm syringe filters. The filtrate was analyzed for reducing sugar and ethanol concentrations. Reducing sugar content was determined using a modified dinitrosalicylic acid (DNS) colorimetric method (Miller, 1959). Ethanol concentration was determined by using a waters high pressure liquid chromatograph (HPLC) (Millipore Corporation, Milford, MA, USA). The HPLC was equipped with a water model 401 refractive index detector, column heater, auto-sampler and computer controller. The Bio-Rad Aminex HPX-87H column (7.8 × 300 mm; Bio-Rad Chemical Division, Richmond, CA, USA) was used with 0.012 N sulfuric acid as a mobile phase at 0.8 ml/min, an injection volume of 20 μl, and a column temperature of 65 °C (Shrestha et al., 2009). All analyses were carried out in triplicate with the same batch of cassava slurry samples and the mean values are reported. The yields of reducing sugar and ethanol were determined as percentages of initial sample dry weight.

2.6. Starch-to-ethanol conversion efficiency

The conversion efficiency was calculated from the theoretical yield of 56.79 g of ethanol from 100 g starch (e.g. 1 g of starch is hydrolyzed into 1.11 g of glucose, and 1 mol of glucose is converted into 2 mol of ethanol). The ethanol conversion efficiency was calculated as illustrated below: