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ORIGINAL ARTICLE

Suitability of *Sorghum bicolor* L. stalks and grains for bioproduction of ethanol

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Abstract The goal of this proposed research is to utilize each of stalks juice, the acid hydrolysate of both lignocellulosic components of the juice extracted stalks and grains of non food sweet sorghum crop as a carbon source during bioproduction of ethanol using *Saccharomyces cerevisiae* and simultaneous saccharification fermentation (SSF) process. The obtained results revealed variation in proximate composition of carbohydrate compounds and minerals content between the sweet sorghum crop parts used in this study. The acid hydrolysis of the lignocellulosic components of stalks led to hydrolyze 11.18% of cellulose, 76.91% of hemicelluloses and 24.27% of lignin. It was also converted 67% of the starch in the grains into reducing sugars. After 72 h of fermentation, the medium containing starch grains hydrolysate gave the highest ethanol production (23.93 g/l) with yield of 0.50 g alcohol per g sugar and fermentation efficiency of 97.39% comparing with other two mediums having stalks juice and stalks hydrolysate (SH) as carbon sources. Also the yeasts in this medium consumed highest amount of sugars and gave the highest biomass yield followed by that containing stalks hydrolysate. Waste water which was remained after recovering of ethanol and removing the biomass from the fermentation medium had considerable levels of potassium, sodium, Mg, Ca, Fe, Cu and Mn and can be recommended for use in plant irrigation.

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Introduction

Production of bio-ethanol as an alternative energy source should be produced from non food crops, an agro-industrial wastes and lignocellulosic feedstock rich in carbohydrates (Oliveira et al., 2006; Balat, 2011).The available carbohydrates in such sources can be converted into ethanol either by simultaneous saccharification then fermentation (SSF) or by separate enzymatic hydrolysis and fermentation (SHF) processes (Endo et al., 2008). SSF process is more favored due to it's low potential costs (Hamelink et al., 2005).

Saccharomyces cerevisiae can be used for fermentation. It has been used in an industrial large-scale in fermentation of sugar- and starch-based materials into ethanol (Hahn-Hägerdal et al., 2007; Tian et al., 2009).

Sweet sorghum (*Sorghum bicolor* L. Moench) is a non food crop. It gives high green biomass yield (20–30 dry tons/ha), needs low water (1/3 of sugarcane and 1/2 of corn), and fertilizer requirements, short period (3–5 months) for growth, grows at diverse climate and soil conditions. It contains fermentable sugars (sucrose, glucose and fructose) in its stalks juice, starch in its grain, and lignocelluloses feed stock in its juice extracted stalks (Kresovich and Henderlong, 1984; Prasad et al., 2007; Ronghou et al., 2008; She et al., 2010). Therefore this plant considers one of the promising non food crop for fuel ethanol production (Wu et al., 2010).

The main objective of this study was to evaluate the suitability of using stalks juice, lignocellulosic components of the juice extracted stalks and grains of sweet sorghum crop in bioproduction of ethanol using *S. cerevisiae* and SSF process.

Materials and methods

Materials

The stalks free from leaves and husks as well as the grains of sweet sorghum (*S. bicolor* L. Moench) crop were obtained from Sabahia Agric. Research Station, Agric. Research Center, Alexandria, Egypt. The crop was planted in May, 2010 and harvested in late October of the same year. The following substrates were prepared to use as a carbon source in the broth used for the bioproduction of ethanol by *S. cerevisiae*.

Stalks juice (SJ)

The obtained stalks were washed with tap water then squeezed using sugar cane roller mill at Technological Lab. in Sugar Crops Res. Inst. of Sabahia, Agric. Res. Station. The collected extracted juice was filtered through fine plastic screen to remove suspended matters, packed in polyethylene pouches and stored at −18 °C in deep freezer until used.

Lignocellulosic hydrolysate of the juice extracted stalks (HS)

After juice extraction, the extracted stalks were collected, washed several times with hot water to remove their content of any residual sugars, then dried in an electric oven (E. Schulg & Co. Inh. Franz. KG), at 60 °C ± 2 °C for 5 h, ground and sifted before subjecting to acid hydrolysis using H_2SO_4 at ratio of 1 g sample: 10 mL of 3% acid solution 121 °C for 30 min in a labtech. autoclave (Labtech, USA). After cooling, the resulted hydrolysate was filtered under vacuum, neutralized with 2% NaOH, packed in glass jars and stored at −20 °C until used.

Starch hydrolysate of grains (HG)

The grains were dried at 50 °C ± 2 °C for 5 h using the above mentioned electric oven, then ground, screened through 30 mesh sieve and subjected for acid hydrolysis, filtration, neutralization packing and storage as mentioned above.

Methods

Bio-ethanol production

Preparation of yeast inoculums

Dry *S. cerevisiae* was activated by adding 10 g of dry yeast to 50 mL of pre-culture broth containing 1 g glucose, 0.4 g peptone, 0.15 g yeast extracts, 0.05 g KH_2PO_4, and 0.025 g $MgSO_4{\cdot}7H_2O$, and incubated in an a rotary incubator shaker, at 38 °C and 200 rpm for 60 min. before using it as an inoculum for ethanol production.

Fermentation method

One hundred milliliter of each of SJ, acid hydrolysate of HS and/or HG was added as a carbon source to flask containing 5 g of yeast extract, 5 g of peptone, 1.2 g $(NH_4)_2SO_4$, 1 g KH_2PO_4, and 0.5 g $MgSO_4{\cdot}7H_2O$, to prepare one liter of the required fermentation medium for ethanol production .The pH value of the medium was adjusted to 6 ± 0.3 before autoclaving at 121 °C for 20 min .The sterilized fermentation medium was incubated with 10 mL (~10% inoculums size) of activated yeast, then, incubated in a shaking (rotatory incubator Innova 4230, Edison, NJ., USA) at 30 °C and 200 rpm for 72 h (Wu et al., 2006). Through this period both ethanol production and sugars consumption in medium were periodically determined. At the end of the fermentation period, the temperature of broth was raised to above the boiling point of ethanol to recover alcohol by distillation. The biomass in the fermentation broth, was separated by centrifugation, at 5000 rpm for 20 min, dried at 80 °C and weighed (Norris and Ribbons, 1970). Meanwhile some minerals (Mg, Na, Fe, K Mn, Cu and Ca) were estimated in the waste water of the fermentation broth.

Analytical methods

Total solid, ash, crude, protein, crude fat and crude fiber of the used sweet sorghum raw materials were analyzed by AOAC approved methods (1998). Total carbohydrate (%) was calculated by difference. Total sugars were determined by phenol–sulfuric method (Dubois et al., 1956). The concentration of reducing sugars was determined by dinitrosalicylic colorimetric assay using glucose as sugar standard (Miller, 1959). The hemicellulose, cellulose and lignin were analyzed using the method of Goering and Van Soest (1970). Minerals (Na, K, Mg, Ca, Fe, Mn and Cu) were estimated using Perklin Elmer atomic absorption spectrophotometer (Model 2380), England, as described in the AOAC (1998). The produced ethanol was measured by gas chromatography (Shimadzu, GC-17A, Japan) using a RTX-1 column (20 m by 0.25 mm) packed with 100% dimethyl polysiloxane and a flame ionization detector (Palo Alto, CA), N_2 as carrier gas at a flow rate of 30 mL/min, and maintaining the injector and detector temperature at 150 °C (Laopaiboon et al., 2007). All the experiments were carried out in triplicate.

Calculation

Ethanol concentration (P, g L^{-1}), ethanol yield (Y), volumetric productivity of ethanol; fermentation efficiency (FE) were