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Enzymatic saccharification and fermentation of cellulosic date palm wastes to glucose and lactic acid

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ABSTRACT

The bioconversion of cellulosic wastes into high-value bio-products by saccharification and fermentation processes is an important step that can reduce the environmental pollution caused by agricultural wastes. In this study, enzymatic saccharification of treated and untreated date palm cellulosic wastes by the cellulases from *Geobacillus stearothermophilus* was optimized. The alkaline pre-treatment of the date palm wastes was found to be effective in increasing the saccharification percentage. The maximum rate of saccharification was found at a substrate concentration of 4% and enzyme concentration of 30 FPU/g of substrate. The optimum pH and temperature for the bioconversions were 5.0 and 50 °C, respectively, after 24 h of incubation, with a yield of 31.56 mg/mL of glucose at a saccharification degree of 71.03%. The saccharification was increased to 94.88% by removal of the hydrolysate after 24 h by using a two-step hydrolysis. Significant lactic acid production (27.8 mg/mL) was obtained by separate saccharification and fermentation after 72 h of incubation. The results indicate that production of fermentable sugar and lactic acid is feasible and may reduce environmental pollution by using date palm wastes as a cheap substrate.

Introduction

At present, the conversion of low-value agriculture wastes into valuable commodities, energy, chemicals and microbial protein by saccharification and fermentation processes is not economically feasible, largely due to the costs of cellulosic materials and cellulolytic enzymes, as well as technical problems associated with cellulose saccharification.[1–5] There is an increased interest in using thermophilic bacteria *Geobacillus stearothermophilus* for the production of cellulases and separate saccharification and fermentation of lignocellulosic biomass due to their higher operating temperatures and broad substrate range.[6,7] The complete cellulose hydrolysis can be achieved by a combination of three types of cellulases: endoglucanases, which cleave internal glucosidic bonds;

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exoglucanases, which cleave cellobiosyl units from the ends of cellulose; and glucosidase, which cleaves glucose units from cello-oligosaccharides. Endoglucanases are characterized by their activity toward substituted cellulose derivatives such as carboxymethylcellulose, while exocellulases have been operationally defined by their ability to degrade microcrystalline cellulose.[8]

Out of the total global production of 7.4 million tons of dates, 5.4 million come from the Arab world.[9] The Kingdom of Saudi Arabia (KSA) is a major date-producing country and is ranked second in the world. The total area planted with date palm trees is about 162,000 hectares, while the number of palm trees has reached nearly 23 million.[10] Besides fruits, date palm also provides a large number of other products that have a wide range of applications. It can be used as a raw material for certain industrial purposes. Practically all parts of date palm are usable such as trunk, leaves (whole leaves, midribs, leaflets and spines, and the sheath at the leaf base), reproductive organs (spathes, fruit stalks, spikelets and pollens) and many of their extracts.[11] Date palm (*Phoenix dactylifera*) has high values of cellulose (45.3%), hemicellulose, (29.13%), and lignin (25.82%).[12] Lignocellulosics cannot be saccharified by cellulases to yield sugar unless they are processed through mechanical, physical, and chemical pre-treatments to remove the inhibitory lignin complex, to reduce the crystallinity and degree of polymerization of cellulose, to increase the surface area available for the enzymes, and to enhance the susceptibility of the substrates to enzymes.[13–16] Lactic acid is a valuable organic acid due to its broad applications in pharmaceutical, leather, and food industries, and its potential for the production of biodegradable poly-lactic acid—an environmentally friendly alternative to plastic.[17–19] In this study, an optimized production of glucose syrup by enzymatic saccharification of treated and untreated date palm wastes was investigated. Besides this, an attempt was also made to produce lactic acid by separate saccharification and fermentation.

Materials and methods

Enzyme source

The bacterial species *Geobacillus stearothermophilus* Y-1, as a source of cellulases, was isolated from the Najran region, KSA. Culture from agar plate was inoculated into a 50 mL tube containing 5 mL of nutrient broth and incubated at 50 °C in an orbital shaker at 200 rpm. This culture was used to inoculate a 250 mL Erlenmeyer flask containing 50 mL Bushnell Haas medium (BHM).[20] The production medium (BHM) consisted of: $MgSO_4{\cdot}7H_2O$ (0.2 g/L), K_2HPO_4 (1 g/L), KH_2PO_4 (1 g/L), yeast extract (1.0 g/L), $FeCl_3{\cdot}6H_2O$ (0.05 g/L), $CaCl_2$ (0.02 g/L), and Tween 80 (0.2%). It was supplemented with 2.0% alkaline-treated date palm leaves as a carbon source. The optimum conditions for cellulase production, when the fermentation period was extended up to 48 h, were as follows: cultivation temperature 45 °C, pH 7.0, and agitation rate 200 rpm (data not shown). The cells and insoluble materials were removed by centrifugation at 10,000 rpm for 10 min and the cell-free supernatant was used as the enzyme source. The cellulases system contained: FPase 8.105 U/mL, CMCase, 12.84 U/mL and β-glucosidase 3.74 U/mL.

Pre-treatments of date palm wastes

Date palm cellulosic wastes (leaves, leaf bases, and fibers) were collected from a date palm plantation in Abha city, KSA and used as the cellulosic substrate. The wastes were ground and pre-treated by two methods: (1) Alkaline pre-treatment: 2 N NaOH at 30 °C for 48 h[21] and (2) acid-steam pre-treatment: 1% H_2SO_4, 120 °C for 100 min.[22] After the treatment, the wastes were washed thoroughly with tap water until neutralized and oven dried at 70 °C. Dried materials were ground through a Wiley Mill (Model 2 Thomas Co., USA) to obtain a particle size ≤1 mm. Determination of cellulose, hemicellulose and lignin contents in the treated and untreated wastes was performed according to Saura-Calixto et al.[23]

Enzymatic saccharification of date palm cellulosic wastes

Enzymatic hydrolysis of date palm cellulosic wastes was carried out following the methods of Holtzapple et al.[24] Briefly, 2% cellulosic waste was mixed with an appropriate amount of enzyme (20 FPU/g of substrate) in a 100-mL Erlenmeyer flask containing 20 mL acetate buffer (pH 5.0), and sodium azide (0.3 g/L) was added to inhibit microbial contamination. The enzymatic hydrolysis was carried out for 24 h at 50 °C using a shaking incubator (100 rpm). After the saccharification period, the reaction mixture was centrifuged at 4000 rpm for 30 min to remove unhydrolyzed substrate and the supernatant was subjected to glucose determination. The effects of incubation temperature, pH, substrate concentration, enzyme concentration, and incubation time on saccharification and glucose production were investigated.

Reducing sugars assay

The amount of reducing sugars released by the enzymatic hydrolysis was estimated by dinitrosalicylic acid (DNS) method.[25] The sample (1.0 mL) was mixed with 2 mL of DNS reagent. The tubes were then heated in a boiling water bath for 5 min, after cooling at room temperature; the absorbance was measured at 540 nm. The amount of the released reducing sugar was calculated by using a standard curve of glucose, and expressed as mg/mL. The percentage saccharification was calculated using the equation of Mandels and Sternberg[26] as follows:

$$\%\text{Saccharification} = \frac{\text{Reducing sugars (mg/mL)} \times 0.9 \times 100\%}{\text{initial substrate concentration (mg/mL)}}.$$

The factor 0.90 was used to convert polysaccharide to monosaccharide accounting for water uptake during hydrolysis. All experiments were carried out in triplicates.

Lactic acid fermentation

Strain and growth medium

The culture of *Lactobacillus delbrueckii* subsp. Lactis (B. 01357), a homo fermentative lactic acid producer, was utilized in