Bioethanol production from date palm fibers: Effect of alkaline hydrogen peroxide pretreatment and fermentation conditions

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Abstract
The present study focuses on the exploration of the potential use of date palm fibers (DPF) as feedstock for bioethanol production. Pretreatment was performed with dilute solutions of sodium hydroxide and hydrogen peroxide with 5 % solids loading of DPF, at 80 ˚C for 24 h. Enzymatic saccharification was performed using a commercial cellulase blend (CE) (30 FPU/ gTS). Batch experiments with pretreated DPF were conducted, under submerged fermentation either through simultaneous saccharification and fermentation (SSF) or a separate hydrolysis and fermentation (SHF) concept using the nonconventional yeast W*ikerhamomyces anomalus*, strain X19 and the yeast *Pichia stipitis* CECT 1922 either separately or in co-cultures.

Introduction
Bioethanol is considered to be one of the most promising renewable fuel candidates. In order to be sustainable, research efforts have focused on the search of new renewable carbon sources. In this context, lignocellulosic biomass represents viable substrates for bioethanol production due to their great availability and low cost. Several pretreatment methods have been developed in the effort of removing the structural and compositional barriers and for improving the yield of the enzymatic hydrolysis of lignocelluloses. Among them, alkaline hydrogen peroxide pretreatment has been shown to be a good choice for the pretreatment of lignocellulosic biomass as it leads to high glucose yields and can be carried out in conditions for moderate temperature and pressure without acids which leads to inhibitors formation. Furthermore, pretreatment with peroxides improves the enzymatic efficiency through oxidative delignification and decrease the crystallinity of the cellulose.
The date palm (*Phoenix dactylifera*) is one of the most cultivated palms in the arid and semi-arid regions of the world. The removal of dry leaves and trunk fibers after harvesting generates an important quantity of date palm residues amounting to more than 198,000 tons accumulated every year in Tunisian agricultural lands, resulting in an estimated worldwide annual volume of 1.5-2.8 million tons. A rational way of valorizing this abundant renewable resource could be its use as substrate for energy generation. Thus, the aim of the present work was the valorization of pretreated date palm fibers towards bioethanol production.

Materials and Methods
Feedstock used
The date palm leaves and trunk used in this study were collected from the waste of date harvesting in the region of Kébeli, located in southern Tunisia. A first sieve was done in order to remove big size wastes to make the grinding process easier. Then, fibers were grinded in a hammer mill and finally sieved using a laboratory test sieve (MATEST) with a mesh of 80 μm.

Pretreatment conditions
For the pretreatment method used, the mass/volume ratio of solid (g TS) to aquatic solution (mL) was 5:100 (solids load 5 % w/v). Alkaline hydrogen peroxide pretreatment was performed through the addition of dilute solution of sodium hydroxide (0.5 %, w/v) at 80 °C for 24 h and dilute oxygen peroxide (30 %). The mixture was then incubated at 80 °C for 24 h. The pretreatment experiment was performed in duplicate. Either the whole pretreatment slurry or the two fractions obtained after separation through filtering with 0.7 μm (liquid and solid fractions (solid DPF or SDPF) obtained after pretreatment), were used for bioethanol production.

Bioethanol experiments
Bioethanol production experiments using pretreated DPF and SDPF at a solid loading of 5 % w/v were performed under simultaneous saccharification and fermentation (SSF) or separate hydrolysis and fermentation (SHF), using a commercial cellulase blend (CE) (Cellic CTec2-CEL, Sigma-Aldrich) at 30 FPU/g TS and

pH=4.8, either at 30 °C (SSF) or at 50 °C for 24 h (SHF). The whole slurry as well as the liquid fractions obtained after alkaline hydrogen peroxide pre-treatment of DPF were also used for ethanol production. Fermentation experiments were carried out in duplicate in 160 mL serum vials with a working volume of 25 mL and incubated at 150 rpm and 30 °C, in batch mode. The vials were sealed with rubber stoppers and equipped with 0.22 μm filters for CO_2 venting and sterilization. In all experiments, cells were harvested from pre-cultures of the *W. anomalus* X19 and *P. stipitis* CECT 1922 (5 %, v/v) by centrifugation at 6000 rpm for 10 min was suspended in mineral solution containing KH_2PO_4, $MgCl_2$. $6H_2O$ and $(NH4)_2SO_4$ each at concentrations of 1 g/L. The initial pH was set to 5.0, by using NaOH or HCl solution (6 N).

Analytical methods

Total solids (TS) and volatile solids (VS) were determined according to Standard Methods (APHA, 1995). The chemical composition of the raw and pretreated DPF (cellulose, hemicellulose and lignin) was determined by the analytical methods of the National Renewable Energy Laboratory. Detection and quantification of furfural, and hydroxyl-methyl-furfural (HMF) were performed with an HPLC-RI equipped with an Aminex HPX-87H column (Biorad), while ethanol concentration was measured using the ethanol assay FS kit (Diagnostic System International, Germany). Soluble carbohydrates content determination was performed through the method described by DuBois et al. (1956), while reducing sugars concentration was estimated by the DNS (3,5-dinitrosalicylic acid) method and was expressed as glucose equivalents (Miller, 1959).

Results

Characterization of raw DPF

The composition of the DPF used in the present study was: TS (%) = 91.14 ± 1.37, VS (%)= 83.62 ± 0.14, cellulose (g/100 gTS)= 38.22 ± 0.20, hemicellulose (g/100 gTS)= 28.35 ± 1.34, lignin (g/100 gTS)= 34.95 ± 0.56, ash (g/100 gTS) = 14.92 ± 0.20.

Effect of NaOH-H_2O_2 pretreatment

To improve the efficiency of enzymatic hydrolysis of DPF, an alkaline hydrogen peroxide pretreatment was carried out. The compositions of pre-treated DPF were 47.08 ± 1.40 % cellulose, 18.58 ± 1.49 % hemicellulose, and 13.48 ± 0.08% lignin. Compared with fresh palm fibre, the cellulose proportion increased while the proportion of hemicelluloses and lignin proportion decreased. Partial lignin and hemicelluloses were removed in the process of pretreatment which can make the cellulose more accessible to enzyme. As anticipated, NaOH-H_2O_2 pre-treatment is an effective pathway for delignification and hemicellulose degradation. Pretreatment with NaOH-H_2O_2 solution can break the complex structure of lignocellulosic biomass and make the cellulose and hemicelluloses accessible to enzyme

Ethanol production from pretreated DPF

In the present study, either the whole slurry obtained after NaOH-H_2O_2 pretreatment or the separate fractions after the pretreatment of DPF, were used for ethanol production using *W. anomalus* X19 and the yeast *Pichia stipitis* CECT 1922 either separately or in co-cultures. The production of bioethanol from pretreated DPF was assessed using the commercial cellulase blend (CE) (30 FPU/g TS) at SSF and SHF mode. Results showed that the maximum ethanol concentration obtained was 11.04 g/L after 96 h of fermentation at SHF concept using co-cultures of *W. anomalus* X19 and *P. stipitis* CECT 1922 under anaerobic conditions. In both SSF and SHF mode, high ethanol yields were achieved, indicating that DPF is a promising substrate for ethanol production.

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