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Bioethanol production from hydrothermal pretreated wheat straw by a flocculating *Saccharomyces cerevisiae* strain – Effect of process conditions

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ABSTRACT

Wheat straw is nowadays being considered a potential lignocellulose raw material for fuel ethanol production of second generation and as an alternative to conventional fuel ethanol production from cereal crops. In the present study, hydrothermal pretreated wheat straw with high cellulose content (>60%) at 180 °C for 30 min was used as substrate in simultaneous saccharification and fermentation (SSF) process for bioethanol production using a thermotolerant flocculating strain of *Saccharomyces cerevisiae* CA11. In order to evaluate the effects of temperature, substrate concentration (as effective cellulose) and enzyme loading on: (1) ethanol conversion yield, (2) ethanol concentration, and (3) CO_2 concentration a central composite design (CCD) was used. Results showed that the ethanol conversion yield was mainly affected by enzyme loading, whereas for ethanol and CO_2 concentration, enzyme loading and substrate concentration were found to be the most significant parameters. The highest ethanol conversion yield of 85.71% was obtained at 30 °C, 2% substrate and 30 FPU of enzyme loading, whereas the maximum ethanol and CO_2 concentrations (14.84 and 14.27 g/L, respectively) were obtained at 45 °C, 3% substrate and 30 FPU of enzyme loading, corresponding to an ethanol yield of 82.4%, demonstrating a low enzyme inhibition and a good yeast performance during SSF process. The high cellulose content obtained in hydrothermal pretreatment and the use of a thermotolerant flocculating strain of *S. cerevisiae* in SSF suggest as a very promising process for bioethanol production.

1. Introduction

Bioethanol is an increasingly important alternative fuel for the replacement of gasoline, with a world production in 2009 of 19,535 millions of gallons and an estimate, only for USA in 2022, of 36,000 millions of gallons. It is thus expected that the production of bioethanol will keep on increasing in the next 10 years (Fig. 1) [1]. Second-generation bioethanol obtained from lignocellulosic materials (LCM) has received major attention due to their abundance and immense potential for conversion into sugars and fuels. However, there are relevant obstacles such as production costs, technology and environmental problems that need to be overcome in the production of second-generation bioethanol [2–4]. Wheat straw is one of the most abundant agricultural by-products, presents a low commercial value and most of it is being used as cattle feed and waste. In terms of total production, wheat is the second most important grain crop in the world. FAO statistics reported a world annual wheat production in 2009 of 682 million tons and, in average, the harvesting of 1.3 kg of grain is accompanied by the production of 1 kg of straw; this gives an estimation of about 524 million tons of wheat straw in 2009, an amount that clearly justifies the need to consider wheat straw as a complementary source of raw material for the production of bioethanol [5,6].

The process for the production of second-generation bioethanol includes three main steps: pretreatment, enzymatic hydrolysis and fermentation. Hydrothermal processing (autohydrolysis), a pretreatment based in the use hot compressed water, presents several advantages; no chemicals other than water are needed, no problems derived from equipment corrosion occur, toxic compounds formation is minimized, a high recovery of valuable hemicellulose derived products is obtained and cellulose structure is made susceptible to enzymatic hydrolysis [7–9]. Simultaneous saccharification and fermentation (SSF) processes, firstly described by Takagi et al. [10], combine enzymatic hydrolysis of cellulose with simultaneous fermentation of the obtained sugars to ethanol and are one the most promising process option for bioethanol production from LCM [11]. SSF process has shown to be superior to separate hydrolysis and fermentation (SHF) in terms of overall ethanol yield. Furthermore, SSF reduces the processing time, which in turn leads to increases in ethanol productivity this is a consequence of the fast conversion of glucose to ethanol by the fermenting microorganisms, that reduce the enzymes inhibition due the presence of sugars. Reduction in equipment costs is also obtained by performing the

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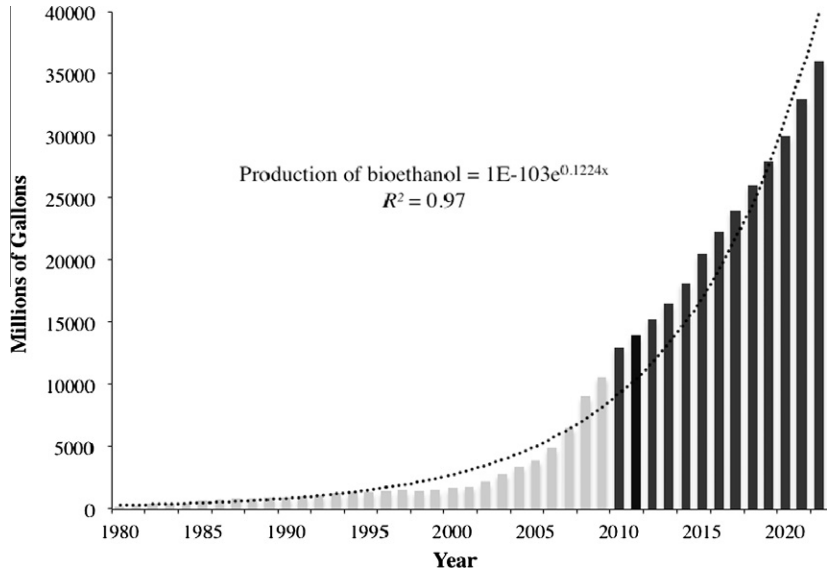

Fig. 1. Trends in US fuel ethanol production; (▒) real production; (■) estimate production. The points represent the trend model.

hydrolysis and fermentation in a single reactor. However, differences between the optimal temperature for cellulose activity and yeast growth is an issue that needs to be solved for an efficient SSF. The optimal temperature for cellulase enzymes (about of 50 °C) is higher than the tolerance range reported by the most used yeast for industrial ethanol production (about 30–37 °C) [12–16]. This requires the matching of the temperature conditions required for the optimum performance of the enzymatic and the fermenting microorganism.

On the other hand, yeasts isolated from extreme environments, exhibit the capability of growth at high temperatures while producing ethanol; a proof of this is the industrial ethanol fermentation in some tropical countries as Brazil where fermentation takes place at ambient temperatures and during the process the temperature can reach 41 °C as, due to its expensive costs [17], no cooling systems are used. This requires the use of yeast strains able to produce high ethanol yields at such high temperatures. Abdel-Banat et al. [18] demonstrated that if the fermentation step could be performed at higher temperatures, for instance within a 40–50 °C range, significant cost reductions in fuel ethanol production could be obtained. Advantages of processing at higher temperatures include a more-efficient simultaneous saccharification and fermentation, significant reduction of contamination and a continuous shift from fermentation to distillation [19,20].

Additionally, the use of flocculating yeasts is one of the most interesting ways to provide an increase of the efficiency of bioethanol production processes as a significant reduction of capital costs is achieved with the elimination of centrifugation (or at least a substantial reduction of the demand for such an expensive operation), making the process more competitive. The flocculation of yeast cells is a reversible, asexual and calcium-dependent process in which cells adhere to form flocs consisting of thousands of cells, the use of high cell density systems being investigated and used for separating yeast cells from beer in the brewing industry. In fact, these systems present several advantages as reduced downstream processing costs, reuse of the biomass for extended periods of time, higher productivity, protection against ethanol stress and resistance to contamination by other microorganism [21–25]. Overall, improved efficiency of the SSF will be obtained by using a yeast strain that can work at higher temperatures and has flocculant properties.

The aim of the present work was to evaluate the effect of SSF operating conditions (temperature, substrate and enzyme loading) of hydrothermal pretreated wheat straw as substrate on ethanol conversion yield, ethanol and CO_2 production with a flocculating strain *Saccharomyces cerevisiae* CA11.

2. Materials and methods

2.1. Wheat straw pretreatment by hydrothermal processing

Wheat straw used as raw material in this study and was kindly provided by a local farmer (Elvas, Portugal). Wheat straw was cut into small pieces (1–3 cm) and milled using a laboratory knife mill (Cutting Mill SM 2000, Retsch, Germany). The material composition was previously analyzed by Ruiz et al. [9], containing cellulose (glucan) as the most abundant fraction (37.4%) followed by xylan (29.4%), lignin (26.8%), arabinan (1.9%), and ash (1.6%). This chemical composition is in good agreement with other values found in the literature for this material [26,27]. The particle size distribution (w/w%) used in this work was as follows: 10% > 1 mm; 40% between 1–0.5 mm; 40% between 0.5–0.3 mm; and 10% to <0.3 mm. The same batch of raw material was used for all experiments.

Milled wheat straw samples were mixed with water in order to obtain a 10:1 liquid/solid mass ratio and treated in a 3.75 L total volume stainless steel reactor (Parr Instruments Company, Moline, Illinois, USA) with PID temperature control. The moisture content of wheat straw was considered as water in the material balances. The reactor was filled and heated to 180 °C at a heating rate of 3 °C/min until reaching the desired temperature, the reaction time was 30 min, these conditions having been previously evaluated by Ruiz et al. [9]. After completing the reaction time, the reactor was cooled down at a rate about of 3.2 °C/min and the agitation speed was set at 135 rpm. Fig. 2 shows a typical and excellent reproducibility of the heating and cooling temperature profiles obtained for a triplicate experiment at 180 °C. At the end of the treatment, the liquid and solid phases were separated by centrifugation and the solid residues were washed with distilled water. Quantification of structural carbohydrates, sugars and degradation products in both solid and liquid phases has been previously reported by Ruiz et al. [28]. The solid residue was used as substrate for simultaneous saccharification and fermentation.

2.2. Yeast strain cultivation

The flocculating *S. cerevisiae* CA11 was obtained from the microbial collection at the Microbial Physiology Laboratory/Department