Hydrolysis of lignocellulosic material of carob pulp in Batch, SSF and NSSF processes for ethanol production

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Abstract

The progress of technologies for fuel ethanol production is a priority, because this biofuel is one of the most important resources used as renewable energy sources [1]. Usually the main types of feedstocks for bioethanol production are raw materials containing fermentable sugars, polysaccharides that can be hydrolyzed for obtaining fermentable sugars and lignocellulosic biomass [1]. Carob (*Ceratonia siliqua L.*) is a perennial leguminous tree and an important component of the Mediterranean vegetation. It has an important role in the economy of the south of Portugal (Algarve) where 50,000 tons of carob fruit are produced each year, making the region the third largest producer in the world [2]. The global amounts of carob pod production are nearly 400,000 tons per year from about 200,000 ha. This raw material contains fermentable sugars and, to take all benefits of the fruit, in a first step all soluble sugars are extracted and also the fibers are treated. These lignocellulosic materials are composed of carbohydrate polymers (cellulose and hemicellulose), lignin and a remaining smaller part comprising extractives and minerals in a complex structure. The cellulose and hemicellulose typically involve up to two thirds of the lignocellulosic materials and are the substrates for second generation ethanol production [3]. By hydrolysis of cellulose and hemicelluloses fractions, bioethanol can be produced from lignocellulosic material, by fermentation of the obtained sugar monomers [4]. The so-called 'Simultaneous Saccharification and Fermentation' (SSF) process, has several advantages, performing hydrolysis and fermentation in a single step [4]. High concentration of cellobiose and glucose, sugars produced by hydrolytic enzymes, can induce inhibition effects to enzyme activity. In that way, the fermenting microorganisms present in the culture, consume these sugars, preventing this inhibition step [5]. The enzyme activity is forced to be far below its potential, because the enzymatic hydrolysis reaction in SSF process is operated at a temperature lower than the optimum level of enzymatic hydrolysis. A Nonisothermal Simultaneous Saccharification and Fermentation process (NSSF) was suggested, in order to overcome this problem [5]. In NSSF process, saccharification and fermentation occur simultaneously but in two separate shake flasks at different temperatures [5]. The lignocelluloses is retained inside a hydrolysis shake flask and hydrolyzed at the optimum temperature for the enzymatic reactions (i.e. 50ºC). The supernatant from the hydrolysis shake flask is recirculated through a new shake flask to ferment sugars from hydrolysis, which runs at its optimum temperature (i.e. 30ºC). When the hydrolysis temperature is raised from 30 to 50 ºC, the cellulose activity is increased 2-3 times [5]. In this work, tests were performed with different pretreatment solutions, to choose the best one. The best result was obtained with diluted acid at 100 ºC with a total sugar recovered of 25.54 g/l. This pretreatment will increase the sugar concentration in the medium as well as preparing the lignocellulose material for cellulase activity. After the hydrolysis reaction using Celluclast 1.5L and Novozyme 188 enzymes, an increase of 2.34 g/l of total sugar was obtained. In order to find out if there are compounds that will inhibit the growth of fermenting microorganisms, all the sugars obtained through acid and enzymatic hydrolysis were tested. Fermentation with pretreatment supernatant had a yield of 49 % (ethanol/fermentable sugars) and the mixture of both supernatants mentioned above had also approximately 50 % of ethanol production. Since no inhibition occurred, SSF and NSSF were tested in different specifications: i) SSF with pretreated pellet; ii) NSSF with enzymatic hydrolysis followed by fermentation; iii) Final pretreated mixture in a SSF system and iv) the final pretreated mixture in a NSSF system. The best product/substrate yield was achieved in

SSF mode (i) with 43 %, however, in this system there was an inhibition by initial substrate concentration, with a maximum ethanol concentration obtained of 46.5 g/l. The best production of ethanol (73.2 g/l) was achieved by the NSSF process (iv), with a product/substrate yield of 38% (see Figure 1). The low value of total sugar obtained by enzymatic hydrolysis is compensated by sugars from acid hydrolysis. It is reported that the existence of inhibitors after hydrolysis can interfere in ethanol production, however, in our case, only the initial high sugar concentration caused this inhibition. Therefore, we can reach values very close to the theoretical which is very promising for bioethanol production.

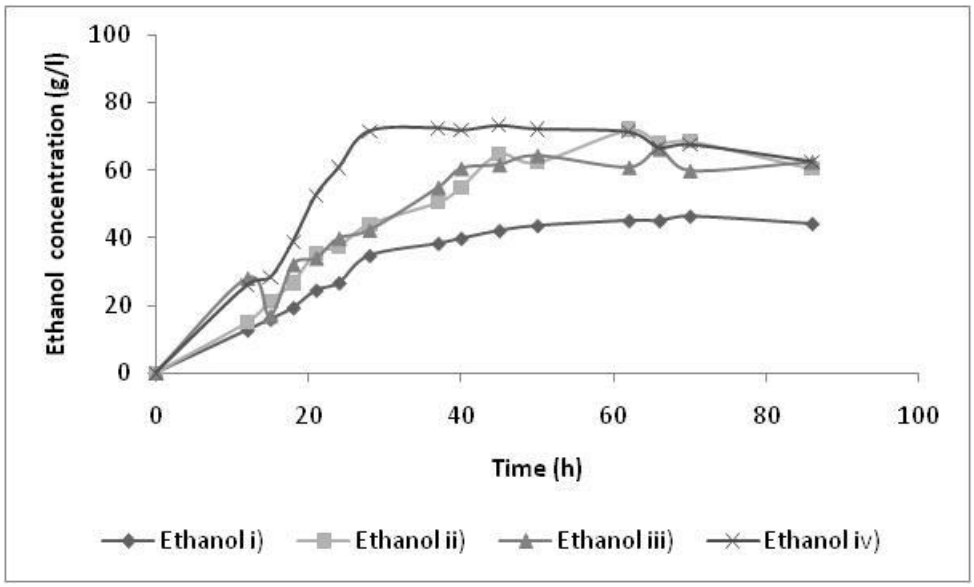

Figure 1. Ethanol production through time where i) SSF; ii) NSSF; iii) SSF with pretreated mixture; iv) NSSF with pretreated mixture.

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