



Techno-economic evaluation of strategies based on two steps organosolv pretreatment and enzymatic hydrolysis of sugarcane bagasse for ethanol production

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ARTICLE INFO

Article history:

Received 17 December 2014

Received in revised form

14 July 2015

Accepted 31 July 2015

Available online xxx

Keywords:

Sugarcane bagasse

Organosolv

Enzymatic hydrolysis

Ethanol

Techno-economic analysis

ABSTRACT

Several strategies based on a two steps organosolv pretreatment followed by enzymatic hydrolysis of sugarcane bagasse (SCB) were evaluated with the objective of selecting operational conditions suitable to promote an efficient and low cost production of ethanol. Initially, the influence of six variables used for the organosolv pretreatment was studied. The variables included the time of the first organosolv pretreatment step, the use of 45% ethanol as pulping solution, solid-to-liquid ratio of the ethanol solution used during the first pretreatment step, time of second organosolv pretreatment, concentration of ethanol and concentration of NaOH solution used in the second pretreatment step. Further assays of enzymatic hydrolysis were carried out to promote additional reduction in the costs of the process and improve the results of cellulose conversion to glucose. Eliminating the milling step of the pretreated SCB, using a commercial tensoactive (composed of esters and several surfactants), and recycling 50% of the slurry obtained during the second step of organosolv pretreatment as reaction medium proved to be feasible for use during the enzymatic hydrolysis. Fermentation of the glucose medium produced under the selected pretreatment conditions to ethanol by *Saccharomyces cerevisiae* occurred with 81% efficiency and a cost of 102.88 \$/hL of ethanol.

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1. Introduction

Sugarcane bagasse is a low cost agro-industrial residue that contains significant amount of carbohydrates with potential to be used on ethanol production. In order to produce ethanol from lignocellulosic biomass such as sugarcane bagasse, a pretreatment stage is required to render the cellulose fibres more accessible to the subsequent step of enzymatic hydrolysis, which is then performed to break down the polysaccharides to simple sugars [1]. A number of technologies for biomass pretreatment, including biological, chemical and physical processes, have been investigated. However, to be of industrial interest, the pretreatment must be

economical; i.e. the energy demands and costs associated with feedstock size reduction, materials, treatment of residues, among others, must be minimized [2–4].

Among the existing pretreatment technologies, organosolv process (especially when using ethanol) is reported to improve the cellulose digestibility with additional advantages, since it also promotes an efficient extraction of sugars from the hemicellulose structure and produces hydrolysates with low concentration of compounds derived from the degradation of sugars and lignin, which are inhibitory for the microbial metabolism [5–8]. Furthermore, the use of ethanol in this pretreatment process also promotes: i) higher solid recovery, ii) protection of the cellulosic fraction, iii) increased porosity of the residual solid material, and iv) recovery of a higher-quality lignin fraction with potential for use in several industrial applications [9,10].

The use of organosolv process comes from the pulp and paper industry, and therefore, the existence of pilot reactors in this

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industry allows minimizing the uncertainty of handling and operational difficulties related to the implementation of this process on industrial scale. A combined use of organosolv process in two steps is very useful to produce a pulp enriched in cellulose, avoiding losses of sugars from hemicellulose. Additionally, this two steps process increases the cellulose digestibility and yields high-quality lignin, which is of great interest for subsequent applications in a biorefinery. However, treatments of the lignocellulosic materials previous enzymatic hydrolysis are usually done with particles of little size, and the energy requirements for particle size reduction can be significant. For example, the dilute acid pretreatment process used in the National Renewable Energy Laboratory (NREL) involves grinding to 1–3 mm, which accounts for one third of the power requirements of the entire process [2]. Moreover, industrial-scale processes differ from small-scale laboratory experiments. In the latter, process parameters can be carefully controlled and usually distilled water or buffer solutions are employed to optimize the conditions. On the other hand, the fresh water requirements must be minimized in industrial processes in order to decrease the amount of wastewater produced [11,12]. Then, the pretreatment process should minimize the energy demands and limit the costs involved with the reduction of the material particle size, materials of construction, and treatment of wastes [2,4].

Economic analysis is necessary to determine the best whole process options for a particular feedstock and product opportunity, once the experimental data are available [13]. This analysis helps to direct research and development efforts by identifying process parameters that have the greatest impact on overall economics. These parameters can be used to benchmark a conceptual process design. Process economic analysis also enables estimation of an absolute production cost for ethanol or other potential products necessary for comparing biorefinery-based costs to existing processes [3]. The combination of experimental data and economic modelling results in a matrix that matches feedstocks with different pretreatment options, as well as for comparing the impact of each stage of processes.

In the present study, the influence of six variables used for the two steps organosolv pretreatment of sugarcane bagasse was studied. The variables included the time of pretreatment, the use or not of ethanol and the solid-to-liquid ratio used during the first step, as well as the time, the ethanol and the NaOH concentration used in the second step. The glucose concentration and yield obtained during the subsequent enzymatic hydrolysis of the pretreated solids was taken as response of these experiments. An economic analysis of all the studied pretreatment conditions was also performed. Then, with the objective of promoting additional reduction in the costs of the process and improving the results of cellulose conversion to glucose, additional strategies of enzymatic hydrolysis were evaluated and economically assessed. Finally, the ethanol production from the glucose medium obtained under the selected conditions of organosolv and enzymatic pretreatments was evaluated. Fig. 1 shows the heuristic diagram that represents the research strategy by knowledge-based process development used in the present study.

2. Material and methods

2.1. Raw material

Sugarcane bagasse (SCB) was supplied by the “Sergio González” paper industry in Cienfuegos, Cuba. Chemical composition of SCB was (wt% on dry basis): glucan 44.46, xylan 20.68, lignin, 18.0, extractives 8.8, and ashes 1.3 (average values of three replicates, error lower than 1% for all the fractions). These values are in the range found for this kind of materials [14,15].

2.2. Two steps organosolv pretreatment

The organosolv pretreatment was carried out in a semi pilot plant equipped with a 50-L reaction vessel, located in a pulp and paper mill in Cienfuegos, Cuba. For the reactions, the reactor was loaded with 500 g (dry matter) of SCB and the catalyst solution (sulphuric acid 1.25% w/w on dry fibre) [6]. The system was then heated with direct steam from the boiler of the mill to 175 °C, being the temperature maintained constant during the desired time (15–40 min). In some cases, according to the conditions of the experimental design, aqueous ethanol (45% v/v) was used as pulping solution under different solid-to-liquid ratios (1:5–1:7 g/mL) (Table 1). After this first pretreatment step, the residual solid material was separated from the hydrolysate, washed with water until neutralization, and stored at 4 °C for use in the further organosolv experiments.

The second step of organosolv pretreatment was performed in the same 50-L reaction vessel and under the same temperature used during the first pretreatment (175 °C). In this second step, the residual solid material obtained from the previous stage was subjected to organosolv reaction for longer times (60–90 min), using aqueous ethanol solution (10–45% v/v) in a solid-to-liquid ratio of 1:5 g/mL, and a NaOH solution in concentration varying between 1.5 and 3% w/w on dry fibre (Table 1). After this second organosolv step, the residual solid material was separated from the hydrolysate, washed with water until neutralization and stored at 4 °C for use in the subsequent assays of enzymatic hydrolysis. The lignin solubilized in the basic hydrolysate medium was precipitated by adding H₂SO₄ until pH < 3. The lignin precipitate was collected by filtration in a filter paper Whatman 1.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis of the SCB obtained after one or two steps of organosolv pretreatment was carried out with a commercial cellulase concentrate (Celluclast 1.5 L – Novozymes A/S, Bagsvaerd, Denmark). For the reactions, an enzyme loading of 15 Filter Paper Units (FPU) was used per gram of substrate material. The reaction medium was supplemented with β-glucosidase (Novozym 188, Novozymes A/S, Bagsvaerd, Denmark) in a load of 15 International Units (IU)/g substrate. Prior to enzymatic hydrolysis, the pretreated material was ground to particle sizes of about 2 mm using a knife mill. Enzymatic hydrolysis experiments were carried out in 100 mL Erlenmeyer flasks containing 25 mL reaction mixture. The substrate consistency was adjusted to 5% (w/v) in citrate buffer (pH 4.8), and the flasks were incubated in a rotatory shaker at 150 rpm, 50 °C, for 24 h.

The subsequent assays of enzymatic hydrolysis of the best condition of two-step pretreatment were carried out under similar process conditions but varying the particle size (pretreated SCB milled to about 2 mm or not milled (1–3 cm)) and reaction medium (medium citrate buffer pH 4.8, or mixed with 50% (v/v) of the hydrolysate obtained during the second organosolv pretreatment step after separate the lignin) used in the experiments. After established the best conditions of these variables for use on the enzymatic hydrolysis process, additional experiments were carried out using 10% (w/v) pretreated solids, a cellulase loading of 15 FPU/g of pretreated solid, 15 International Units (IU) of β-glucosidase/g substrate, and with the reaction medium supplemented with Tween 20 or with QuimiFoam-Master (a commercial tensioactive commonly used in ethanol distilleries in Cuba, composed of a mixture of esters and several surfactants with predominance of polydimethylsiloxane) in a concentration of 2.5% (w/w) on a dry weight basis.

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