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Research paper

Enzymatic hydrolysis of biomass at high-solids loadings through fed-batch operation

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ABSTRACT

A fed-batch approach in stirred tank bioreactors for achieving enzymatic hydrolysis with a high load of lignocellulosic mass is described in this paper. This approach allows ease of mixing, increasing the load of a higher concentration of biomass without performance decrease, in comparison with the typical batch process, and consequently it provides a higher concentration of reducing sugars for a down-stream fermentation process; additionally, the fed-batch process times are shorter than the ones that have been reported so far. The work basically consisted of experiments in fed-batch operation with final sorghum straw loadings of 5, 10, 15 and 20% w v^{-1}, using a commercial enzyme complex, and conventional batch experiments for comparison purposes. Without typical operation problems of batch processes such as the lack of effective initial mixing and difficult adjustment of pH and temperature, hydrolysate broths up to a reducing sugars concentration of 130 g L^{-1} were obtained. Finally, hydrolysates were fermented to verify effective bioethanol production, reaching concentrations of bioethanol of 48 g L^{-1}.

1. Introduction

The latent possibility of substituting gasoline by bioethanol has motivated the R&D on bioprocesses and bioreactants to render feasible the large-scale production of fuel bioethanol. The diverse ecological, social, and economic matters that drive it may be found elsewhere, e.g. Ref. [1].

According to the raw material, bioethanol is classified in "generation" terms, in which diverse bioprocesses can be followed, diverse bioreactants can be applied, and different product concentrations can be obtained, e.g. Ref. [2]. For any bioethanol generation, a challenge to make large-scale production economically feasible lies in obtaining fermentation broths of at least 4% w v^{-1} of ethanol [3,4]; this is because bioethanol purification costs heavily increase with lower ethanol concentrations [5]. In this way, for example, in the case of using any high-performance yeast, the fermentation process must be loaded with a solution of at least 80 g L^{-1} of six-carbon reducing sugar [6]. Then, the higher the concentration of reducing sugars, the higher the ethanol concentration and, thus, the lower the bioethanol purification cost.

Second-generation bioethanol (BioEtOH-2G), whose raw material is lignocellulosic biomass coming from agro-industrial wastes (e.g., sorghum straw, wheat straw, sugarcane bagasse), is produced via four main steps: (1) pretreatment of raw material to break the lignin seal and make available the holocellulose (cellulose and hemicellulose) to the enzymes, (2) enzymatic hydrolysis of pretreated raw material to depolymerize its holocellulose into reducing sugars, (3) fermentation of reducing sugars to convert them in bioethanol, and (4) purification of bioethanol from the fermentation broth, which has a high content of water [7].

The enzyme-based application is advantageous over chemical treatments due to its use of moderate and noncorrosive operating conditions. In addition, very mild process conditions give potentially high yields, and maintenance costs are low compared with acid or alkaline hydrolysis. Besides, the process is compatible with many pretreatment options [8].

In this framework, to obtain a reducing sugars solution with a concentration greater than 80 g L^{-1}, enzymatic hydrolysis must be carried out with a load of pretreated material greater than 15% w v^{-1} [9,10]. This considers that pretreated material gets a composition around 70% of cellulose after pretreatment and that the enzymatic process performs with a yield of 90%. Hence, enzymatic hydrolysis at moderate/low solids load is not attractive from an economical and environmental point of view [11].

Lignocellulosic mass is low density, meaning that a small amount fills up a big space in a stirred tank bioreactor. In this light, it is convenient to reduce the size of the raw material as much as possible (by

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milling), to get a higher biomass load into the bioreactor [12]. However, the higher the amount of biomass, the less the effect of the stirrer in mixing, even with a high-speed stirring. The poor mixing by high biomass loading causes heat and mass transfer problems; e.g., the pH adjustment becomes difficult, gradients of temperature and reactants concentration in the reactor appear along a considerable initial period of the process, and the enzyme work is delayed [13]. In this way, the performance deteriorates: yield likely decreases, and process time increases.

Reaction time is an important trait of process performance, since it affects the transformation cost in an industrial framework. It is worth noting that studies of enzymatic hydrolysis at high biomass load have implied long-time experiments; for example, while Xue et al. [14] performed their experiments at a time of 48 h, Hodge et al. [15] and Knutsen and Liberatore [16] have preferred to hold the experiments until 168 h, to achieve the highest possible holocellulose-to-reducing sugars conversion. Moreover, Modenbach and Nokes [17], and more recently Fockink et al. [18], in their reviews on enzymatic hydrolysis at high-solids loads, report reaction times between 72 and 168 h.

An approach that has been explored is starting the enzymatic hydrolysis with a moderate load of lignocellulosic mass, and after a considerable experiment time, a bolus dose of biomass of definite weight is fed time-to-time; in this way, an effective load equivalent to a high-solids process is achieved. For example, Zhang et al. [19] started a process of 9% w v^{-1} of sugarcane bagasse, and later added corresponding amounts of biomass to 8, 7, and 6% w v^{-1} in reaction times of 8, 24, and 48 h, respectively. In this way, an effective load of 30% w v^{-1} was achieved; reaching a glucan conversion of 51%. In turn, Wang et al. [20] started with an initial load of 10% w v^{-1} of sweet sorghum straw, and later, at the times of 24 and 48 h, they added corresponding biomass to obtain 5% w v^{-1} in both instances. The effective biomass load achieved was 20% w v^{-1}, and the glucan conversion was 60%. Then, feeding biomass from time-to-time resulted in enzymatic hydrolysis processes of an effective high-solids load, where troubles by poor mixing have been dodged.

In this light, this work explored systematically a fed-batch operation strategy in the enzymatic hydrolysis carried out in a stirred tank bioreactor. Such strategy pretends to avoid operative problems that lack the loading of high amounts of biomass, and to reduce the process time. It is considered a dynamical framework in which, in addition to frequent and periodic loadings of a small amount of alkaline-oxidative sorghum straw, the process evolution is monitored through samples taken and analyzed periodically. Finally, comparisons with conventional batch processes are discussed to show the advantages.

2. Material and methods

2.1. Lignocellulosic material

The lignocellulosic material was sorghum straw (RSS), collected from region Bajío in Guanajuato, México. It was milled up to a particle size of mesh 20–40 and pretreated with an alkaline-oxidative medium.

The alkaline-oxidative medium consisted of 2% v v^{-1} sodium hydroxide and 1.5% v v^{-1} hydrogen peroxide; the pretreatment reaction was carried out at 60 °C and pH 11.5 during 5 h. At the end, the alkaline-oxidative sorghum straw (ASS) was filtered and washed with 1 L of distilled water.

The dry-basis composition of the raw and pretreated material with respect to cellulose, hemicellulose, and lignin were determined following [21,22]. The moisture of ASS was measured with a moisture analyzer.

2.2. Enzyme complex

A commercial enzyme complex was used: Cellic® CTec2 kindly provided by Novozymes® Latin America Ltd (Paraná, Brazil). CTec2 is a blend of aggressive cellulases for degradation of cellulose to fermentable sugars; it also contains a high level of β-glucosidases plus hemicellulase. This enzyme complex is a brown liquid with a density of 1.15 g mL^{-1} and it is composed of a weight percent by cellulases and xylanases of 1–5% and 10–20%, respectively. The optimal temperature and pH are 45–50 °C and pH 5.0–5.5.

The enzyme complex activity was 204 FPU mL^{-1}, which was determined following the NREL technique [23].

2.3. Reaction system

The experiments were carried out in a stirred tank reactor system composed by a flask of 0.5 L (Proculture® glass spinner flask by Corning®) warmed by a hot plate with a temperature controller device (stirring hot plate Corning® PC-420D) and mechanically stirred (overhead stirrer IKA® RW-20 Digital) with three marine propellers of four blades.

All experiments were performed under the same conditions of temperature (*T*), pH, and ratio enzyme/biomass (*EL*), whose setting up is described below. During every experiment, samples were frequently taken out to monitor concentrations of reducing sugar, with which evolution trajectories of experiments were constructed and characterized.

2.4. The fed-batch strategy

The procedure to perform the enzymatic hydrolysis at high-solids, through a fed-batch operation, was as follows: first, the corresponding distilled water was charged into the reactor (the water contained in the moist ASS was considered as well), and the stirrer was turned on at a speed that guarantees good mixing (e.g., 150 rpm). Next, temperature and pH were adjusted, and the corresponding amount of enzyme complex was loaded; a readjustment of pH and temperature was necessary afterward. Once homogeneity was reached, a first sample was taken out because enzyme complex contains reducing sugars; next, the addition of amounts of ASS was started. Increments were of 2.5 g (dry basis), and were added periodically up to completing a specified total load of biomass. Four scenarios of total ASS load ({5, 10, 15, 20}% w v^{-1}) and two intervals between biomass addition instants ({5, 10} min) were explored. After biomass total load, the enzymatic hydrolysis was continued up to reaching a total experiment time of 10 h.

2.5. Conventional enzymatic hydrolysis

For comparison purposes, enzymatic hydrolysis in a typical batch operation was carried out as follows: a mixture of distilled water and moist ASS was prepared in the reactor (considering the water contained in the moist ASS). pH and temperature were adjusted; next, the corresponding amount of enzyme complex was added. The experiments were performed at ASS concentrations of 5 and 10% w v^{-1} (dry basis), and the reaction time was 10 h.

2.6. Optimal process conditions in the enzymatic hydrolysis

The enzymatic hydrolysis experiments were carried out at the same conditions of temperature (*T*), pH, and ratio of enzyme/biomass (*EL*). The values of these process conditions were optimally set up by following a three-factorial experimental design approach based on microreaction [24], with three levels for *T*, three for pH, and four for *EL*; in this way, the set of scanned process conditions was formed by the complete combination of the following conditions:

T = {45, 50, 55} °C, pH = {4.0, 4.5, 5.0}, EL = {78.95, 184.21, 289.47, 394.74} μL g^{-1}-*ASS*.

This experimentation was carried out in a shaking thermoblock of