Industrial Crops and Products 56 (2014) 160–165

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Pilot scale simultaneous saccharification and fermentation at very high gravity of cassava flour for ethanol production

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ARTICLE INFO

Article history:
Received 30 August 2013
Received in revised form 5 February 2014
Accepted 8 February 2014
Available online 28 March 2014

Keywords:
Simultaneous saccharification and fermentation (SSF)
Very high gravity (VHG)
Ethanol
Cassava flour

ABSTRACT

We developed a simultaneous saccharification and fermentation (SSF) process of cassava flour at very high gravity (VHG). Cassava flour (CF) was dissolved in water to reach 315.4 g/l dry matter, and then the mixture was liquefied at 80 °C for 90 min by using alpha-amylase (3532 AAU/kg CF) and beta-glucanase (2812 U/kg CF). SSF of liquefied mash of cassava was performed at 30 °C with the simultaneous addition of two glucoamylases (Distillase ASP at 540 GAU/kg CF and Amigase Mega L at 0.035% w/w), active dry yeast (1.5×10^7 cells/l), urea (12 mM) and KH_2PO_4 (4 mM). Under these conditions, the SSF process finished after 72 h. The ethanol content achieved 17.2% v/v corresponding to 86.1% of the theoretical ethanol yield at lab scale and decreased to 16.5% v/v corresponding to 83.6% of the theoretical ethanol yield at pilot scale. Therefore, the SSF of cassava flour under VHG condition could have a great potential for the ethanol industry in Vietnam and South East Asia.

1. Introduction

According to the increasing price of oil, bio-ethanol is known as an ideal candidate to replace the role of fossil fuel. Thus, the research on this renewable source becomes growingly important for humans, especially in terms of improving the productivity, the efficiency and decreasing production cost. In Vietnam and in South East Asia, cassava is considered an attractive raw material for bio-ethanol production thanks to the following advantages: (i) the ease of plantation in various soil types and climate conditions; (ii) a very low input and investment for planting; (iii) "all year round" availability of feedstock in the form of fresh roots and dry chips; (iv) a high starch-containing raw materials and a lower proportion of fibers (Sriroth et al., 2007). Indeed, the Vietnamese Ministry of Industry and Trade declared that bio-fuel production will achieve 1.8 million tons in 2025, which accounts for 5% of country's demand (Ministry of Industry and Trade, 2007b). Moreover, the government also adapted the policy to improve the beverage ethanol industry in Vietnam. By the development strategy of beverage ethanol production in Vietnam (Ministry of Industry and Trade, 2007a), ethanol industry will produce 188 million liters ethanol for food industry in 2025. Overall, the beverage and bio-ethanol industry has a great potential in Vietnam in the future.

Besides the conventional process of ethanol production, simultaneous saccharification and fermentation (SSF) process has been widely used in the world, but only recently introduced to Vietnam in order to augment ethanol yield and shorten time production. Indeed, after liquefaction by alpha-amylase, glucoamylase is added to the slurry, concomitantly with yeasts, and the SSF is conducted in a single reactor. The presence of yeast along with enzymes minimizes the sugar accumulation in the bioreactor. Moreover, since the sugar produced during starch or cellulosic breakdown slows down alpha-amylase action, higher yields and concentrations of ethanol are possible using SSF (Das Neves, 2006; Klasson et al., 2013; Molaverdi et al., 2013; Scordia et al., 2013; Wang et al., 2013; Yingling et al., 2011a,b). The SSF process has been successfully carried out on different substrates such as flax shive (Klasson et al., 2013), sweet sorghum stalk (Molaverdi et al., 2013), giant reed (Scordia et al., 2013), sweet sorghum bagasse (Wang et al., 2013), potato tubers (Srichuwong et al., 2009) and cassava (Chu-Ky et al., 2009; Yingling et al., 2011b). Therefore, it is of interest to improve the efficiency of the SSF process in the ethanol industry in Vietnam.

Very high gravity (VHG) technology has been introduced to increase the volumetric productivity and the cost effectiveness of the SSF process. In VHG technology, mash preparation contains at minimum of 270 g/l dry matter (Bayrock and Ingledew, 2001). This technology has a great deal of advantages in ethanol production: (i) increasing plant capacity and reduction in capital costs; (ii) increasing plant efficiency; (iii) reducing risk of contaminating bacteria (Thomas et al., 1996; Yingling et al., 2011a,b).

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http://dx.doi.org/10.1016/j.indcrop.2014.02.004
0926-6690/

Table 1
Characterization of the enzyme products used in this work.

No.	Enzymes products	Nature	Optimal pH	Optimal temperature (°C)	Activity
1	Spezyme Alpha	Alpha-amylase	5.7–5.8	83–85	13,775 AAU/g[a]
2	Optimash TBG	Beta-glucanase	4.5–6.0	75–85	5,625 U/g[b]
3	Distillase ASP	Glucoamylase	4.0–4.5	58–65	580 GAU/g[c]
4	Amigase Mega L	Glucoamylase	4.0–4.5	55–60	–

[a] AAU: Alpha Amylase Unit defined by Dupont (One AAU unit of bacterial alpha-amylase activity is the amount of enzyme required to hydrolyze 10 mg of starch per minute under specified conditions).
[b] U: Unit defined by Dupont (one unit of beta-glucanase activity is defined as the quantity of enzyme which produces reducing sugars equivalent to 1 μmol of dextrose per minute from barley beta-glucan under standard assay conditions).
[c] GAU: GlucoAmylase Unit defined by Dupont (One Glucoamylase Unit (GAU) is the amount of enzyme that liberates 1 g of reducing sugars calculated as glucose per hour from soluble starch substrate under the conditions of the assay).

Nevertheless, VHG technology causes also some inconvenience, including the high viscosity of starch paste after liquefaction, which leads to the resistance to solid–liquid separation, difficulties in handling process, incomplete hydrolysis of starch to fermentable sugars and lower fermentation efficiency (Ingledew et al., 1999; Srikanta et al., 1992). Therefore, the success of its application depends on the preparation of mash with low viscosity. For instance, in order to reduce starch paste's viscosity, sweet potato was pretreated in a VHG process by using cell-wall degrading enzymes such as cellulases, pectinase, hemi-cellulases and viscosity reduction enzyme (xylanase). As a result, the ethanol yield was achieved approximately 90% of the theoretical ethanol yield (Srichuwong et al., 2009; Zhang et al., 2010, 2011). Thomas et al. (1993) reported that in VHG (dissolved solids 300 g/l) of wheat mash fermentation at 20 °C for 200 h, maximal final ethanol concentration of 23.8% v/v was obtained.

In another approach to VHG technology with cassava, optimization has been applied to study the effects of some key factors that influence ethanol production such as gravity, particle size, initial pH, liquefaction and fermentation temperature, liquefaction time and enzyme concentration. Under optimized conditions, high ethanol concentration (greater than 15%) and high starch utilization ratio (c.a. 90%) were obtained (Yingling et al., 2011b). However, the investigation on VHG technology with cassava at a larger scale than that of laboratory has still been limited.

In this work, our approach is to develop cost-effective ethanol processes which are based on: (i) decreasing energy consumed by utilizing enzymes which are capable of hydrolyzing raw starch at lower temperatures; (ii) saving equipment investment and increasing ethanol yield by using SSF process of cassava flour under VHG condition. This work aimed to develop SSF processes under VHG condition of cassava flour at lab and pilot scales for ethanol production.

2. Materials and methods

2.1. Microorganism

Commercial active dry yeast *Saccharomyces cerevisiae* (Ethanol Red), kindly provided by Fermentis (France), was used in this study. Dry yeast was hydrated in tap water at 38 °C for 20 min prior to addition to the liquefied mash of cassava flour.

2.2. Materials

Cassava flour was obtained in Tuyen Quang province (North Vietnam). After thoroughly dried, cassava chips were ground into cassava flour to the size minor than 0.3 mm, and stored at dry and cool place in the lab. Starch content of the cassava flour used in this work was 77 ± 1% and its humidity was 11 ± 1%.

Different kinds of commercial enzyme products kindly provided by Dupont (previously known as Genencor—A Danisco Division) were used in this work including Spezyme Alpha (containing alpha-amylase from *Bacillus licheniformis*), Optimash TBG (containing beta-glucanase from *Talaromyces emersonii*) and Distillase ASP (containing glucoamylase from *Bacillus licheniformis* and *Trichoderma reesei*). Amigase Mega L (containing glucoamylase from *Aspergillus niger*) was provided by DSM – Food Specialties – Beverage Ingredients. Properties of these enzyme products are presented in Table 1.

2.3. Simultaneous saccharification and fermentation (SSF) at lab scale

Three SSF processes at VHG were developed in this work (Fig. 1). Cassava flour (CF) was mixed with tap water in 2-l fermentor to achieve a concentration of 315.4 g/l dry solid in a final volume of 1 l. For all three investigated processes, the liquefaction step was conducted at 80 °C and stirred at 200 rpm for 90 min at pH 5.5. After liquefaction, the mash was cooled to room temperature (30 °C) before subsequent SSF. The SSF of liquefied cassava mash was performed at 30 °C in a 2-l fermentor, with the simultaneous addition of glucoamylase, active dry yeast (Ethanol Red at 1.5×10^7 cells/ml), urea (12 mM) and KH_2PO_4 (4 mM). During the first 8 h of SSF, the fermentation broth was agitated every hour for 5 min at 120 rpm to ensure homogenization. After this period, the SSF was conducted under static condition and finished after 72 h. In our work, three SSF processes were performed and differentiated as follows:

- SSF1 process: alpha-amylase (Spezyme Alpha) at the dosage of 3,532 AAU/kg CF was added to the cassava slurry under VHG condition, and glucoamylase (Distillase ASP) at the dosage of 540 GAU/kg CF was added to conduct SSF.
- SSF2 process was similar to SSF1 process with only one modification as follows: additional beta-glucanase (Optimash TBG) at the dosage of 2,812 U/kg CF was added to the cassava slurry under VHG condition during liquefaction to reduce viscosity of liquefied mash of cassava.
- For SSF3 process, both alpha-amylase (Spezyme Alpha) at 3,532 AAU/kg CF and beta-glucanase (Optimash TBG) at 2,812 U/kg CF were added for liquefaction. For SSF, besides using glucoamylase (Distillase ASP) at 540 GAU/kg CF, additional glucoamylase (Amigase Mega L) at 0.035% w/w was added to improve the efficacy of hydrolyzing residual starch in the slurry.

2.4. Simultaneous saccharification and fermentation (SSF) at pilot scale

The SSF under VHG condition was upgraded to the pilot scale based on the results obtained with SSF3 process which was conducted at the lab scale as described in Section 2.3. The pilot scale experiment was carried out in a total volume of 100 l using a double jacket reactor (200 l) for liquefaction and a fermentor (200 l) for SSF, respectively. As the same for SSF3 process, both alpha-amylase