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Simultaneous liquefaction, saccharification and fermentation at very high gravity of rice at pilot scale for potable ethanol production and distillers dried grains composition

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ABSTRACT

In this work, a simultaneous liquefaction, saccharification, and fermentation (SLSF) process at very high gravity (VHG) of broken rice for potable ethanol production was developed at pilot scale. The SLSF–VHG process was performed in a unique fermenter, at 30 °C. Rice flour (RF) was dissolved in tap water to reach 311.5 g/l dry matter and then the mixture was simultaneously liquefied, saccharified, and fermented. Thanks to the addition of a raw starch hydrolyzing enzyme containing a mixture of alpha-amylase and gluco-amylase (Stargen 002 at 991.8 GAU/kg RF), gluco-amylase (Amigase Mega L at 0.035% w/w), protease (Fermgen at 600 SAPU/kg RF), active dry yeast *Saccharomyces cerevisiae* (Red Ethanol at 3.5×10^7 cells/ml), KH_2PO_4 (4.8 mM), and urea (16.0 mM). Under these conditions, the SLSF–VHG process finished after 120 h and achieved an ethanol content of 17.6% v/v corresponding to 86.3% of the theoretical ethanol yield. We scaled up this SLSF process at very high gravity at pilot scale (25 l) and achieved an ethanol content of 17.0% v/v corresponding to a yield of 83.2% of the theoretical ethanol yield. Rice-based distillers dried grains (DDG) was produced from the whole stillage of SLSF process at very high gravity by being plate-filtered and dried. The obtained DDG had high contents of crude protein (47.5%) and fibers (15.8%). Our results suggest, the SLSF under VHG condition of broken rice as well as the recovery of protein-rich DDG could have a great potential for the ethanol and animal feeding industry in Vietnam.

1. Introduction

In Vietnam, potable ethanol is widely produced from cane sugar molasses, rice and cassava. Among these raw materials, rice is particularly considered a suitable one for potable ethanol production as it possesses many benefits. The major rice consumers are food and drink industries, such as pasta, and bread industries, beer and other liquor distilleries, as well as pharmaceutical industry (Gang et al., 2007). Over 20 years, the total production of rice in Vietnam increased twofold and

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reached 43.4 million tons in 2013. Vietnam exported about 8 million tons of rice annually (General-Statistic-Office-of-Vietnam, 2013). Besides being consumed as food, rice is often transformed into different products. One of these is ethanol whose productivity will be 800 million liters in 2015.

Most ethanol distilleries in Vietnam use conventional processes for the production of potable ethanol which basically involves an energy-consuming liquefaction (95–105 °C), separate saccharification (60–62 °C), and fermentation (30–32 °C) of starch slurry. Liquefaction and saccharification require the starch granules to be extensively gelatinized at high temperature. This is an energy-intensive process requiring the addition of heat energy to starch granule slurries, until the gelatinization temperature of the starch is exceeded. The whole process requires a high-energy input, important investment thus increases the production cost.

With biotechnological advances in recent years, a new generation of liquefying enzymes such as thermostable starch hydrolyzing α-amylase is produced by a genetically modified strain of *Bacillus licheniformis*. These enzymes can reach liquefaction step efficiently at lower temperature (Gang et al., 2007; Wang et al., 2007). New blends of enzymes produced by controlled fermentation of genetically modified strains of *B. licheniformis* and *Trichoderma reesei* are also available to perform saccharification more effectively (Gang et al., 2009). Stargen, capable of hydrolyzing raw starch, contains alpha-amylase and gluco-amylase synthetized by *Aspergillus niger* and *Aspergillus kawachi*. These enzymes are adsorbed on the surface of starch grain and induce holes on this surface where glucose is released. The capability of hydrolyzing raw starch depends on the nature of starch. When the starch contains a high quantity of amylopectin, the enzymatic hydrolysis is performed easily (Wang et al., 2007).

Simultaneous Liquefaction, Saccharification, and Fermentation process (SLSF) or no-cook process has been recently introduced to increase ethanol yield and to save energy and investment cost (Gohel and Duan, 2012; Kelsall and Piggot, 2009). Alpha-amylase, gluco-amylase are added to the slurry, concomitantly with yeasts. The SLSF is conducted in a unique bioreactor, at a unique pH and at ambient temperature. The presence of yeast along with enzymes minimizes the sugar accumulation in the vessel. Since the sugar is produced slowly during starch breakdown, higher rates, yields and concentrations of ethanol are possible with the use of SLSF (Robertson et al., 2006; Xu and Duan, 2010).

A recent work conducted by Xu and Duan (2010) showed that the use of new enzymes for ethanol production without heating at very high gravity was achievable with sorghum. Indeed, the temperature control in combination with enzymatic hydrolysis using raw starch hydrolyzing enzyme (Stargen) could significantly improve the efficiency of fermentation process. Ethanol reached up to 20% v/v after 90 h of fermentation, with the use of commercial dry yeast and a sorghum concentration up to 35% dry matter.

Nguyen et al. (2014) developed a simultaneous saccharification and fermentation (SSF) process of cassava flour at very high gravity (VHG). Indeed, cassava flour was resuspended in water to reach 311.5 g/l dry matter, and then the mixture was liquefied at 80 °C for 90 min by using a mixture of alpha-amylase and beta-glucanase. SSF of liquefied mash of cassava was performed at 30 °C with the simultaneous addition of gluco-amylases, active dry yeast, urea, and KH_2PO_4. Under these conditions, the SSF process finished after 72 h. The ethanol content achieved 17.2% and 16.5% v/v corresponding to 86.1% and 83.6% of the theoretical ethanol yield at lab and pilot scales, respectively.

In another work, Poonsrisawat et al. (2014) investigated the viscosity reduction of cassava for ethanol fermentation at very high gravity by using cell wall degrading enzymes from *Aspergillus aculeatus*. Cassava root mash was adjusted to 32% (w/w) dry matter and was pretreated with 0.5% (w/w) viscosity reducing enzyme preparation and incubated at 45 °C, pH 5.0 for 2 h. The pretreated cassava mash was liquefied by 0.03% (w/w) thermostable α-amylase (Liquozyme SCDS) in a bioreactor at pH 5.5–6.0, 85 °C for 2 h. The liquefied mash was then simultaneously saccharified by 0.06% (w/w) gluco-amylase (Spirizyme Fuel) or low-temp amylase (in-house enzyme prepared from *Aspergillus* strain) was added at 0.35 IU/g of raw starch and fermented by *Saccharomyces cerevisiae* at pH 4.5, 32 °C for 96 h. The ethanol content reached 19.65% v/v corresponding to 87.55% yield with thermal process and only 17.54% v/v corresponding to 75.33% with non-thermal process, respectively.

In another research related to VHG technology with rice flour, the optimized process was applied to study the effects of some key factors that influence ethanol production such as gravity, particle size, initial pH, fermentation temperature, 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., 2011). However, the investigation on VHG technology with broken rice at a larger scale than that of laboratory has still been limited.

In ethanol industry, fermentation produces a co-product, a so-called dried distiller's grain with solubles (DDGS). Since only starch and sugars are converted into ethanol, non-fermentable components in cereal grains are concentrated by a factor of more than two in DDGS (Monceaux and Kuehner, 2009). Currently, the majority of the DDGS has been used as an ingredient for livestock feed. In dry-grind processes, the fermentation beer is distilled and therefore ethanol is recovered. The non-volatile components then leave this step as a product called whole stillage. The whole stillage contains the fiber, fats, protein, other unfermented components of the grain, and yeast cells. Whole stillage is usually centrifuged to produce a liquid fraction (thin stillage) and a solids fraction (wet distillers' grains). The remaining thin stillage is concentrated through multiple effect evaporators to produce syrup called condensed distillers' solubles (CDS) (Monceaux and Kuehner, 2009). While wet distillers' grains, syrup, or the combination of both (wet distillers' grains with solubles, WDGS) can be sold as an animal feed, the combination of wet distillers' grains and syrup is often dried to produce dried distillers' grains with solubles (DDGS) in order to greatly lengthen its shelf-life. Indeed, the composition of DDGS has been of great interest to researchers in the area of animal science, ethanol producers, and especially to the animal feeding industry as the majority of this has been sold as feed ingredients for livestock (Liu, 2011).

In this work, we aimed at developing SLSF processes of broken rice at very high gravity (>300 g/l dry solid) either at laboratory or pilot scales by utilizing enzymes capable of hydrolyzing raw starch at ambient temperature. Other objectives were to recover rice-based distillers dried grains (DDG) obtained from ethanol by-products of SLSF-VHG process and to determine the main composition of rice-based DDG for animal feeding usage.