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Simultaneous cold hydrolysis and fermentation of fresh sweet potato

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ABSTRACT

In recent decades, environmental and economic issues have pushed the production of biofuels worldwide. In this scenario, ethanol is the most produced biofuel. Starch is a potential substrate for this purpose, but the extra cost needed to hydrolyze it into glucose is still a drawback. As an alternative for the expensive and energy demanding conventional hydrolysis process, the cold hydrolysis is being studied. In this process, granular starch degrading enzymes act directly on raw starch granules; therefore, this hydrolysis is carried out below gelatinization temperature. As a consequence, the energy requirement can be significantly reduced. In this work, the cold hydrolysis and fermentation of fresh sweet potato were experimentally studied. For that, it was employed the sweet potato strain BRS Cuia, whose carbohydrate level reaches 28.7%. It can be translated into a potential to produce 185 L t^{-1} ethanol, or equivalently 7400 L ha^{-1}. The enzymes blend adopted for the hydrolysis stage was Stargen™ 002. The surface response method indicated 200 g L^{-1} of sweet potato and 45 GAU g^{-1} of sweet potato as the best balance between high glucose formation rate and low enzyme consume. The 1 h pretreatment that achieved the highest glucose concentration was at 52 °C in the presence of the enzymes blend. Finally, the study of the simultaneous hydrolysis and fermentation showed that the medium supplementation has no significant effect over the fermentation performance, while the pH control is beneficial, improving the ethanol production in 54%.

1. Introduction

In recent decades, environmental and economic issues have boosted the world production of biofuels. They can provide a more balanced carbon cycle, reducing the greenhouse effect, and collaborate to energetic independency, shorting oil consume and importation [1,2]. In this regard, ethanol is the most important biofuel, contributing with more than 90% of the total usage of renewable fuels [3]. Brazil, which is the pioneer in ethanol production and usage, employs sugar-based raw materials as sugarcane juice and molasses. The

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United States of America, which is currently the biggest producer, utilizes mainly starch-based substrate as corn [4–6].

After cellulose, starch is the most abundant carbohydrate found in plants. Once its hydrolysis liberates a great amount of reducing sugars (RS), it is a potential substrate for biofuels production when not enough or economic sugar-based feedstock is available. At the same time, there is still a drawback in using starch for this purpose, which is the extra cost needed to the hydrolysis step [7,8]. The conventional conversion of starch to glucose requires a two-step process: liquefaction and saccharification. During liquefaction, gelatinization of starch is promoted by applying high temperature (around 90 °C) in excess of water, converting semi-crystalline starch granules to amorphous conformation, which is more susceptible to enzymatic hydrolysis [9,10]. This process is energy-intensive, increasing significantly the production cost of starch-based ethanol. It has been estimated that the energy input of those two steps is about 30%–40% of the total energy demanded during ethanol production from starch [11].

As an alternative for the conventional process, the cold hydrolysis, also called unconventional, granular or non-cooked starch hydrolysis, is being studied. Actually, the study of this process is not recent [12–14], but researches in this theme have become more intense in the last decade [15–17]. Granular starch degrading enzymes (GSDE) can act directly on raw starch granules; therefore, cold hydrolysis is carried out below gelatinization temperature. As a consequence, GSDE significantly reduce the energy requirement for hydrolysis step. Robertson et al. [18] estimated that this reduction in energy usage achieved 10%–20% in ethanol production. The use of GSDE presents other advantages than lower energy demand. Once the hydrolysis temperature is similar to the fermentation one, the two steps can run concomitantly in a single step. The process is then intensified, allowing more compact units of production. The release of RS is gradual, reducing osmotic stress and avoiding catabolic repression. In addition, the moderate temperatures mitigate by-products reactions, such as Maillard, leading to an increase in the yield of RS [8,19].

On the other hand, there are still challenges to be overcome in order to make cold hydrolysis economically competitive when compared to the conventional process. Firstly, the lower temperatures employed usually generate contamination problems. In addition, differently from gelatinized starch, granular starch remains solid and then, the degradation reaction takes place in a solid–liquid system, where the associated mass transfer is a well-known limitation. As a consequence both greater amount of enzymes, which influences significantly the cost, and longer times are needed to complete the conversion to glucose [20]. Zhang et al. [21], for example, reached 112 g L^{-1} of ethanol in 24 h applying simultaneous saccharification and fermentation (SSF) of liquefied sweet potato, while Białas et al. [22] reported ethanol concentrations around 100 g L^{-1} after 60 h applying simultaneous hydrolysis and fermentation (SHF) of granular starch. For minimizing the cited problems, a preheating step, at sub-gelatinization temperature, is sometimes suggested by the producer of enzymes [23]. This pretreatment aims to make the granular starch more susceptible to enzyme action and its influence has already been discussed in the literature [24,25]. Authors have also employed auxiliary enzymes, such as cellulases and pectinases, to improve the hydrolysis performance [26–28].

Among starchy feedstock, tubers present an advantage over others food crops: high carbohydrate content. This characteristic leads to a high potential for ethanol production per hectare. Secondly, these crops can be cultivated on marginal lands where other crops cannot be grown well [29,30]. In the present work, it was studied the influence of sweet potato concentration (C_{sub}) and enzymes ratio (E) on the cold hydrolyzes of fresh sweet potato by response surface methodology, being also evaluated different conductions of preheating treatments. Afterwards, the simultaneous hydrolysis and fermentations (SHF) were performed to study the effect of pH control and medium supplementation. Differently from most studies of cold hydrolysis found in the literature [10,25,31,32], it was not employed isolated starch, but directly the fresh tubers.

2. Materials and methods

2.1. Raw materials, enzymes and microorganism

The fresh sweet potato (*Ipomoea batatas* (L.) Lam.) roots of the variety BRS Cuia, whose average yield is 40 t ha^{-1} [35], were harvested from an experimental field of EMBRAPA Clima Temperado in Pelotas-RS, Brazil, after 120–140 days of cultivation during January–May. They were then transported to the laboratory, where they were peeled, sliced, weighted and stored in a freezer at −18 °C.

The enzymes used were granular starch hydrolyzing enzymes, Stargen™ 002, which is a mixture of á-amylase and glucoamylase produced by Genencor (Palo Alto, USA). The activity and optimal pH range declared by the producer are 570 GAU g^{-1} and 4.0–4.5, respectively. One Glucoamylase Unit (GAU) is the amount of enzyme that will release 1 g of RS calculated as glucose per hour from soluble starch substrate under the conditions of the assay [33].

The strain CAT-1 of the yeast *Saccharomyces cerevisiae* used in this study was supplied by LNF Latino Americana Ltda. in its lyophilized form. This strain was isolated from industrial processes for ethanol production by Fermentec and Escola Superior de Agricultura Luiz de Queiroz [34]. The lyophilized yeast was stored at a temperature between 1 °C and 5 °C. For the fermentations, it was weighed and inoculated directly without pre-cultivation.

2.2. Sweet potato characterization

The content of free sugars and total starch were determined by the methods 039/IV and 043/IV and the moisture content by gravimetric analysis after drying at 105 °C to constant weight according to method 012/IV of the Adolfo Lutz Institute [36]. Lipids, proteins, fibers and ashes were quantified by Association of Official Agricultural Chemists (AOAC) methods.

The gelatinization temperature was determined in duplicate by using a Differential Scanning Calorimeter (DSC 6000, Perkin Elmer, USA), equipped with a refrigerated cooling system. For the determination, around 15 mg of a suspension of