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Review article

A brief review on the emerging technology of ethanol production by cold hydrolysis of raw starch

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HIGHLIGHTS

- This review briefly states the main concepts of cold hydrolysis for raw starch.
- It discuss the application of amylases and accessory enzymes in this field.
- It presents some data on ethanol production through the raw starch hydrolysis.
- This review presenting future trends in this field.

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ABSTRACT

Ethanol is one of the main biobased molecule produced worldwide, mainly from corn and other starchy crops. In the past few years, one promising technology that has been claimed to reduce capital and operational costs of industrial plants and increase overall yields for ethanol is named 'cold starch hydrolysis'. The saccharification is carried out at low temperatures (under starch gelatinization point) but require the use of accessory enzymes to achieve high conversions. The best result achieved so far is 98.6% of starch conversion into glucose. This review briefly states the main concepts of this technology, discussing recent literature data for ethanol production and finally presenting future trends in the field of ethanol production from starchy raw materials.

Contents

1. Introduction

Throughout the 20th century, oil and products derived thereof became the main energy source and the main basis for the chemical industry, thus leading to a global dependence on oil and its derivatives, with many nations today being extremely susceptible

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to variations in international oil prices. Besides the political and economic issues, fossil fuels are responsible for the emission of one of the main greenhouse gases, contributing to global climate changes, and thus their environmental impact has become a major concern in modern society.

Nowadays, it is widely accepted that the gradual replacement of oil with renewable biomass resources can effectively contribute to solve environmental problems and to develop a sustainable society [1]. We are currently experiencing a change of paradigm, moving society away from an oil-based economy to one based on renewable energy resources [2,3].

The use of ethanol as liquid biofuel is one of the most important alternatives to fossil fuel resources. However, the conventional starch-based ethanol production technology presents a high demand for energy from fossil sources. Studies indicate that current corn ethanol technologies, in spite of being much less oil-intensive than gasoline, have greenhouse gas emissions similar to those of gasoline, so that as a whole only 5–26% of the energy content is renewable, and the rest is primarily derived from natural gas and coal [4].

The costs due to the high energy demand of starch-based ethanol production can be reduced if starch hydrolysis is performed at temperatures below its gelatinization temperature, i.e., if hydrolysis of starch in raw, granular form takes place [5]. This is an emerging technology, which is enabled by several enzymes that act on starch in its granular form and thus do not need the liquefaction and cooking steps of the conventional process, allowing a reduction in energy consumption [6]. Robertson et al. [5] introduced the term "fermentation-excess enthalpy", defined as the energy required to heat the liquid suspension containing the starch granules from the fermentation temperature (30 °C) to the cooking temperature. According to the authors, the energy demand of the conventional process is about 10–20% of the fuel value of ethanol produced.

Among the enzymes needed for granular starch hydrolysis, the main group comprises amylolytic enzymes that act synergistically to breakdown starch polysaccharides (linear-chain amylose and branched-chain amylopectin) to glucose. The amylolytic complex is composed of endoamylases, exoamylases, and debranching enzymes. The understanding of the action of amylases for granular starch hydrolysis has been the focus in some other recent reviews [7–9]. According to Castro et al. [10], it is important to mention that other hydrolases also play an important role in the process. These so called "accessory" hydrolases include cellulases, xylanases and proteases, and their action contributes to expose the raw starch to amylases. Therefore, the production of multienzyme complexes containing amylases and accessory enzymes can significantly contribute to improve the conversion and the feasibility of granular starch hydrolysis.

In this context, this brief review aims to discuss the main recent findings on the application of amylases and accessory enzymes for granular starch hydrolysis, either sequentially or simultaneously to ethanol fermentation.

2. Granular starch hydrolysis: "cold hydrolysis"

The non-cook concept for starch processing, although firstly reported in the 1940's [11], has only been considered at large scale during the last decade [12,13]. The process comprises the use of raw starch degrading amylases, which are used after feedstock milling, without previous high-temperature cooking and liquefaction [14,15]. As a consequence, capital and operational costs are approximately 41% and 51% lower, respectively, and overall yields are higher due to the absence or almost absence of cross reactions (e.g. Maillard) [15,6]. This process concept is being considered a major breakthrough in the starch-to-ethanol industry, and is known as granular starch hydrolysis, raw starch hydrolysis, cold hydrolysis, native starch hydrolysis or sub-gelatinization temperature starch hydrolysis.

Because granular starch is insoluble in aqueous media at temperatures below gelatinization, the enzymes attack the granules in the solid phase. Granular starch degrading-enzymes are ubiquitous and can be produced by plants, animals and microorganisms, with microbial sources being preferred for industrial applications, due to the larger productivity and easy scale-up [9]. Studies have shown that the enzymes first adsorb onto the granule surface to then initiate starch degradation [16–18], and that the adsorption is higher in more exposed/available amorphous α-glucan chains of the starch polysaccharides [19]. Fig. 1 illustrates how a starchy biomass surface becomes degraded after cold enzymatic hydrolysis.

The enzymatic action over insoluble substrates, such as starch granules, occurs in several stages involving solid surface diffusion, adsorption and finally catalysis. The adsorption to the granules enables the enzyme to move from the aqueous to the solid phase and is considered to be a prerequisite for the subsequent catalytic activity [20]. The heterogeneous catalytic reaction, however, suffers from mass transfer limitations, and thus presents hydrolysis rates that are lower than those observed for gelatinized starch.

Naguleswaran et al. [21] compared hydrolysis of small and large starch granules from barley and corn and observed higher initial rates for the small granules and higher hydrolysis duration for large granules. The authors concluded that the molecular architecture and granule porosity influence amylolysis.

According to Vidal et al. [16], the mechanism of adsorption equilibrium in the enzymatic hydrolysis to form glucose applies only at the beginning of the reaction, since, as the enzymatic hydrolysis progresses, small cavities are formed and the diffusion of enzymes in the pores and channels becomes a new limiting step of the process. The structure of starch in granules directly affects the action of the enzymes. The ability of amylases to unfold the double helix structure is influenced by the distortion of neighboring chains, and the lower the segmental mobility (i.e. higher crystallinity), the more difficult the hydrolysis will be [20]. Also, the higher the surface area/volume ratio of granules, the higher its potential to be adsorbed and thus to be enzymatically hydrolyzed will be [22].

Helbert et al. [23] were able to visualize enzymes on the surface of granules, inside channels and inside granules with degraded core, and observed that enzymatic action occurs primarily from the surface to the center (centripetal-type hydrolysis). Then the core is completely degraded by erosion within granules, acting peripherally (centrifugal-type hydrolysis). In the first case, enzymes act progressing along the polysaccharide chains, while the centrifugal hydrolysis leads to erosion by a more diffusive movement of enzymes. Thus, the mechanism of the enzymatic degradation of granular starch by α-amylase can be described by the following steps: (i) adsorption of enzymes randomly onto the surface of the granules, (ii) hydrolysis of these starting points, (iii) radial progression hydrolysis from the surface to the center of the granules, and (vi) trapping of enzymes inside the granules, so that they can only hydrolyze the substrate within their limited range of diffusion, leading to centrifugal hydrolysis [23]. The enzymatic erosion of the surface and deepening of pores into the interior part of corn, tapioca and sweet potato starch granules was observed after 24 h of hydrolysis using the commercial raw-starch degrading preparation Stargen™ 001 by Yussuf et al. [24]. Cinelli et al. [25] used a hydrolytic enzyme preparation produced in house by solid-state fermentation and showed scanning electron microscopy photographs of native starch granules in water (control) and progressively digested granules after 24 h, 48 h and 72 h of hydrolysis.

Shrestha et al. [26] compared structural changes in maize starch granules with different amylose contents during enzymatic hydrolysis. The authors observed that all starches were digested by a