

Cellulase immobilization on magnetite nanoparticles applied on biomass (orange bagasse) hydrolysis for ethanol 2G production

Giovanni M. Varotti*, Stephanie F. F. Silva, Verônica Queiroz, Daniela Cypriano, Ljubica Tasic.

Abstract

Orange bagasse is a low cost agroindustrial residue that possesses high carbohydrate levels, high susceptibility to enzymatic hydrolysis, and it does not compete with food production; such characteristics make this bagasse a promising source of carbon for the production of second-generation ethanol. The cellulases are enzymes able to hydrolyse cellulosic substrates, such as lignocellulosic biomass, producing monosaccharides - used in posterior fermentation. We propose the immobilization of a recombinant cellulase from *Xanthomonas axonopodis* pv. *citri* in the surface of magnetic nanoparticles aiming the enzyme reuse and process cost reduction.

Key words: Enzymatic hydrolysis, magnetic iron nanoparticles, ethanol-2G.

Introduction

Brazil is the biggest world producer of oranges and their residues, obtained after juice extraction, composed from bark, seeds and membranous residues, which are rich in carbohydrates.¹ Thus, orange bagasse (OB) can be employed in the second-generation ethanol production. However, the carbohydrates available should, firstly, be transformed in monosaccharides, which are susceptible to fermentation using yeast cells, for example, *Saccharomyces cerevisiae*. The carbohydrates hydrolysis proposed is an enzymatic approach using a cellulase from *Xanthomonas axonopodis* pv. *citri* (*Xac*).² Since *Xac* infects oranges, provoking citrus canker using an arsenal of hydrolytic enzymes, cellulases as well, we believe that this bacterium cellulase might be more effective in OB hydrolysis.

Aiming the reuse of the target enzyme, and the cost reduction of the process, we propose the immobilization of the cellulase on magnetic nanoparticles (MNP), constituted from a magnetite core and an inert capping of tetraethyl orthosilicate (TEOS) and surface modification with (3-aminopropyl) trioxysilane (APTES).^{3,4}

Results and Discussion

A cellulase from *Xac* (PDB ID Q8PJK9) was expressed in Origami 2 cells in a pET28a(+) vector, using kanamycin as a selective antibiotic. It was purified with Nickel Affinity Chromatography and Gel Filtration. According to the ProtParam tool from ExPasy,⁵ the enzyme has a molecular mass of 63715 Da and a pI of 6.47. This cellulase is part of the hydrolases family 9 that perform the β -(1 \rightarrow 4) bond cleavage with an inversion mechanism as shown in Image 1.⁶

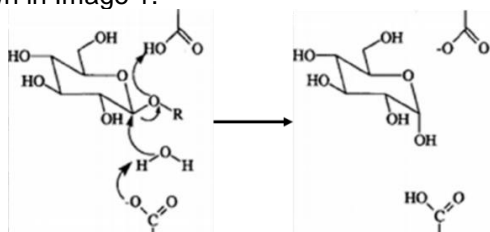


Image 1. Representation of the enzymatic reaction with inversion mechanism.

The cellulase structure was predicted by I-Tasser tool⁷ and is shown in Image 2a. The enzymatic reaction was optimized using a commercial substrate, sodium carboxymethylcellulose (CMC), and best results were obtained in 30 min reactions at 30 °C and pH 6.5 in buffer Na₂HPO₄/citric acid 50 mmol L⁻¹.

On the other hand, the MNP with 351 ± 7 nm in the hydrodynamics diameter measured by Dynamic Light Scattering and Zeta potential of +26 ± 1 mV was employed as support for the cellulase and a Scanning Electron Microscopy (SEM) is shown in the Image 2b. The immobilized enzyme was found active after immobilization.

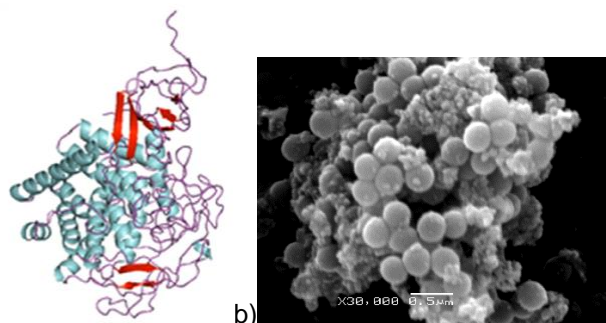


Image 2.a) Representation of the cellulase structure predicted by I-Tasser. **b)** SEM micrograph of the MNP.

Conclusions

The immobilization of cellulase on nanometric inert magnets enables the cost reduction of the hydrolytic step in lignocellulosic exploit for 2G-ethanol production. It allows the recycling of the enzymes in a practical and economic way.

¹ Awan, A. T.; Tsukamoto, J.; Tasic, L. *RSC Adv.* **2013**, 3, 25071.

² Ferreira, C. B., et al. *Adv. Microbiol.* **2017**, 7, 22-46.

³ Mascolo, M.; Pei, Y.; Ring, T. *Materials.* **2013**, 6, 5549.

⁴ Khatiria, R.; et al. *ICNS4.* **2012**, 1456-1458.

⁵ <http://web.expasy.org/protparam/> (accessed on 05/07/17)

⁶ McCarter, J. D.; Whitters, G. S. *Curr. Opin. Struct. Biol.* **1994**, 4, 885-892.

⁷ <http://zhanglab.ccmb.med.umich.edu/I-TASSER/> (accessed on 05/07/17)