Evaluation of autohydrolysis process for cellulases production by Aspergillus niger using corncob biomass

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Lignocellulosic residues, such as corncob, are a complex matrix composed by cellulose, hemicellulose and lignin that can be used for different biotechnological applications (e.g. enzymes production). However, the applications of these crude residues as substrate for enzymes production are often inefficient.

An efficient hydrolysis of these residues requires a pretreatment (e.g. autohydrolysis) that lead to a more accessible structure for microorganisms attack. Recently, fungi have received significant attention as a source of new thermostable enzymes for use in many biotechnological applications, including biomass degradation (cellulases are key enzymes for efficient biomass degradation) [1]. The enzymatic degradation of cellulose to glucose is achieved by the cooperative action of endoglucanases (EC 3.1.1.4, hydrolyze randomly the internal glycosidic linkages), exoglucanases (cellobiohydrolases, CBH, EC 3.2.1.91, hydrolyze cellulose chains by removing cellobiose mainly from the non-reducing ends) and β-glucosidases (EC 3.2.1.21, cleave cellobiose to glucose) [2].

In this context, this work evaluates the inclusion of pretreated corncob in the nutrient media for cellulase production by Aspergillus niger van Tieghem in comparison with non-treated corncob. Autohydrolysis pretreatment conditions used were 180, 190 and 200 °C for 10, 30 and 50 min, and two fractions were obtained: solid and liquid fractions enriched by cellulose and hemicellulose, respectively. Three different mixtures (for each condition) were used as carbon source in Mandels medium [3] during the cellulase productions by the A. niger van Tieghem: a solid fraction (1% w/v in medium), a liquid fraction (100 % v/v in medium), and a mixture of the solid and liquid fractions (1% w/v + 10% v/v in medium). Fermentation conditions were at 30°C, 100 rpm, and the cellulases and β-xyllosidase were quantified by Miller [4] and Kersters-Hildebrand [5] methods, respectively, after 6 days of fermentation.

Interestingly, the results showed that the highest cellulases production was obtained when the microorganism grows in medium containing the hemicellulose fraction (or liquid fraction) as carbon source. The exoglucanase and endoglucanase production using the liquid fraction obtained at 200°C for 30 min, were three and twenty times higher, respectively, than the production obtained using corncob untreated, as carbon source. In relation to β-g production, the best autohydrolysis condition was 180°C for 30 minutes; this production was fifteen percent higher than the production detected with crude corncob.

This work shows the potential of autohydrolysis retread of lignocellulosic residues as a strategy to increase and add-value the cellulase production by filamentous fungi.

Keywords: autohydrolysis; cellulase, corncob.

References

Extracellular synthesis of selenium nanoparticles by Bacillus mycoides strain SfTE01

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Metal nanoparticles have been produced by using chemical and physical methods for many years. However, relying on strong reducing agents and very harsh conditions may lead to undesired environmental risks. It is, therefore, important to develop ecofriendly methods. Recently biosynthetic methods which make use of microbes have emerged as a procedure for the production of metal/metalloid nanoparticles in alternative to conventional techniques which invariably involve hazardous waste generation.

Selenium is a trace element commonly found in the earth’s crust. It belongs to the Group 16 (chalcogens) of the periodic Table and occurs, in the environment, in a variety of oxidation states. In particular, the predominant Se species in oxic conditions are the oxyanions selenite (SeO₃²⁻) and selenate (SeO₄³⁻), with the latter exerting the highest toxicity. Interestingly, the ability to reduce the toxic SeO₃²⁻ species into non-toxic elemental forms is widespread among microorganisms. A bacterial strain (SfTE01), isolated from the rhizosphere of the selenium hyperaccumulator legume Astragalus bisulcatus [1] and identified as Bacillus mycoides, was studied for its ability to efficiently reduce selenite to elemental selenium in aerobic conditions and consequently to produce elemental selenium nanoparticles. The isolate exhibited significant tolerance to selenite (SeO₃²⁻) up to 30mM in oxygen concentration. SfTE01 was incubated with both 0.5 and 2mM Na₂SeO₃, performing the complete reduction of selenite respectively within 12 and 24 hours. The strain converted 91% of the initial selenite added to the culture medium into elemental selenium with cultures developing a deep red color characteristic of crystalline Se⁰. Characterization of red Se⁰ precipitate by using transmission electron microscopy (TEM), scanning electron microscopy (SEM) and UV-Vis spectroscopy revealed the presence of extracellular spherical nanoparticles. The sizes of these nanoparticles range from 200 to 250 nm in bacterial cultures after 24 hours of exposure to selenite. Moreover, after 48 hours, the nanoparticles reach a diameter of 1μm. EDX analysis of the same particles revealed the characteristic peaks of selenium absorption at 1.37 keV (SeL₃) and 12.49 keV (SeKβ), respectively. Selenium reduction activity was observed mainly in membrane proteins and even in the exoenzymatic fraction. Therefore, a hypothetical mechanism for the synthesis of selenium nanoparticles has been proposed.

Keywords: selenium; metal nanoparticles; Bacillus mycoides SfTE01, nanobiotechnology

References