

ENHANCEMENT OF STARCH HYDROLYSIS BIOPROCESSES USING IMMOBILIZED ASPERGILLUS STRAINS ON OXIDIZED CARBONACEOUS MATERIALS

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ABSTRACT

The worldwide production of starchy crops is increasing every year, while starch is considered as a crucial renewable resource, which should be completely hydrolyzed during saccharification to enable its use as feedstock in industrial biotechnology applications^[1,2]. Among the processes employed for starch hydrolysis, enzyme hydrolysis could be performed in the presence of amylolytic enzymes, while biocatalyst immobilization on carbonaceous materials (e.g. biochar) has effectively enhanced productivity in different bioprocess^[3,4]. Adsorption of the biocatalyst (cells, enzymes) on the material is affected by the carrier's surface chemistry and morphology^[5].

Herein, char obtained from car tires, biochar produced using pistachio shells and oxidized biochar (OB) generated via application of HNO₃, H₃PO₄ and H₂O₂ were assessed as carriers for *A. niger* and *A. awamori* immobilization aiming to improve starch hydrolysis. EDS analysis applied on untreated and oxidized carbonaceous materials indicated enrichment of oxygenated groups on OB surface increasing up to 10% the content of oxygen on the material as compared to untreated biochar (UB). Furthermore, BET analysis demonstrated slight increase of OB's specific surface area using 1% (v/v) H₂O₂ (38 m² g⁻¹) and 0.2 M HNO₃ (44 m² g⁻¹) as compared to UB (30 m² g⁻¹), while the highest increase was observed on OB produced using 0.2 M H₃PO₄ (104 m² g⁻¹). Moreover, SEM images of the materials obtained prior and following immobilization confirmed formation of extracellular matrix by adhesion of cells on the surface of the material.

Starch hydrolysis was performed using char, UB and OB for biocatalyst immobilization. Results indicate that immobilized cells of *A. niger* on oxidized biochar using 1% (v/v) H₂O₂ achieved the highest yield 0.78 g_{glucose} g_{starch}⁻¹ demonstrating 70.3% of the theoretical yield. Thus, the combined effect of surface area and content of oxygen increase on the surface of OB obtained using 1% (v/v) H₂O₂ potentially resulted in substantial enhancement of glucose yield as opposed to untreated carbonaceous carriers. Further treatment of biochar using urea and sodium dodecylbenzene sulfonate surfactant will be performed to further improve starch saccharification. Oxidizing the surface of biochar and analyzing the properties of the carbonaceous biomaterials formed enabled, for the first time to our knowledge, tailoring and understanding the function of the immobilized biocatalysts developed towards enhancement of a major industrial biotechnology application.

KEYWORDS: Starch hydrolysis, *Aspergillus* strains, char, oxidized biochar, immobilization.

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