



Resuable Pectinase Nano Polymeric Particles

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ABSTRACT

Pectinase or pectinolytic enzymes are the group of enzymes that degrade the pectin substances. They have great importance in food industry especially for the assembly of clear fruit crush. During this study, a replacement method has been developed for hydrolysis of pectin by using nano pectinase particles in hydrolysis of pectin. At the primary step of the study, nano pectinase particles are synthesized consistent with aminoalkanoic acid (monomer) Decorated and lightweight Underpinning Conjugation Approach (ANADOLUCA) method and characterized by transmission microscopy (TEM) and zeta sizer. Within the second step, the hydrolysis of pectin has been investigated using newly synthesized nano pectinase particles and free pectinase enzyme separately and compared to every other. The various conditions that affect the hydrolysis of pectin like pH, thermal stability, storage stability and reusability are investigated. Then, the activity of nanopectinase particles and free pectinase enzyme has been analyzed in terms of Michaelis-Menten parameters comparatively. Additionally to those studies, the reusability data of nano pectinase particles has been showed an excellent potential as reusable catalyst in comparison to free enzyme. So, the newly synthesized nano pectinase particles are very attractive for hydrolysis of pectin substances and that they will have an excellent usage area in process. Magnetic-chitosan particles were prepared following three different protocols enabling the

preparation of particles with different sizes – nano (Nano-CMag, Micro (Micro-CMag) and Macro (Macro-CMag) – and used for pectinase immobilization and clarification of grape, apple and orange juices. The particle size had an excellent effect within the kinetic parameters, Nano-CMag biocatalyst presented the very best V_{max} value (78.95 mg. min⁻¹), followed by Micro-CMag and Macro-CMag, with V_{max} of 57.20 mg.min⁻¹ and 46.03 mg.min⁻¹, respectively. However, the very best thermal stability was achieved using Macro-CMag, that was 8 and 3-times more stable than Nano-CMag and Micro-CMag biocatalysts, respectively. Pectinase immobilized on Macro-CMag kept 85% of its initial activity after 25 batch cycles in fruit juice clarification. These results suggested that the chitosan magnetic biocatalysts presented great potential application as clarifying catalysts for the fruit crush industry and therefore the great importance of the chitosan particles preparation on the ultimate biocatalyst properties. Freshly pressed apple, orange and grape juices with none treatment were kindly provided by Vitivinícola Jolimont (Canela, RS, Brazil). Rohapect 10 L was from Amazon group (Brazil). Pectin from apple, polygalacturonic acid, galacturonic acid and chitosan were purchased from Sigma-Aldrich (St. Louis, MO). All other reagents and solvents were of analytical grade. A yeast strain secreting endopolygalacturonase was utilized in this work to review the likelihood of continuous

production of this enzyme. it's a feasible and interesting alternative to fungal batch production essentially thanks to the specificity of the sort of pectinase excreted by *Kluyveromyces marxianus* CCT 3172, to the lower broth viscosity and to the better downstream operations. so as to extend the reactors' productivity, a cellulosic carrier obtained from barley spent grains was tested as an immobilization support. Two sorts of reactors were studied for pectinase production using glucose as a carbon and energy source--a continuous stirred tank reactor (CSTR) and a packed bed reactor (PBR) with recycled flow.

the very best value for pectinase volumetric productivity ($P(V)=0.98 \text{ U ml}^{-1} \text{ h}^{-1}$) was achieved within the PBR for $D=0.40 \text{ h}^{-1}$, a glucose concentration on the inlet of $S(\text{in})=20 \text{ g l}^{-1}$, and a biomass load within the support of $X(i)=0.225 \text{ g g}^{-1}$. The results demonstrate the attractiveness of the packed bed system for pectinase production.

Keywords: Nano pectinase particles; ANADOLUCA method; Reusable catalyst; Photosensitive cross-linking