PRODUCTION OF CELLULASE AND XYLANASE *VIA* SOLID STATE FERMENTATION AND ITS APPLICATION IN THE ENZYMATIC DEINKING OF LASER PRINTED WASTE PAPERS.

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KS-04

ABSTRACT

The work deals with the production of cellulase and xylanase by local isolates *via* solid state fermentation (SSF) processes for the application in the enzymatic deinking of laser printed wastepapers. The production of cellulase and xylanase was carried out using *Aspergillus niger* USM AI 1 and *Trichoderma* sp. FETL c3-2. The production of cellulase and xylanase by *Trichoderma* sp. used sugar cane baggase (SC):palm kernel cake (PKC) as substrates. The optimized condition for cellulase production consist of 5 g SC: PKC, moisture content of 75% (v/v), pH of the moistening agent of 7.0, at 30°C and inoculum size of 1 x 10^8 spores/ml. Dextrin at 4% and yeast extract at 6% (w/w) acted as supplementary carbon and nitrogen sources with cellulose at 0.2% (w/w) as inducer. The maximum FPAse and CMCase production of 3.3 U/g substrate and 18.05 U/g substrate, respectively were obtained after 4 days fermentation. The cellulase production was growth dependent.

Xylanase production was maximum under the optimized conditions consisting of SC:PKC 90:10 (%,w/w), moisture content 75%, pH of moistening agent of pH 7.0, at 30°C and inoculum size of 1 x 10^8 spores/ml. Dextrin at 4% (w/w) and tryptone at 6% (w/w) were added as additional carbon and nitrogen sources with 0.2% cellulose as inducer. Under the optimized conditions, the xylanase production by *Trichoderma* sp. was 75.0 U/mg glucosamine after 4 days at 30°C. The production of the enzyme increased by 180% while the growth by about 40%.

Xylanase production *via* SSF by *Aspergillus niger* USM AI 1 was also carried out using palm kernel cake as substrate. The modification of the physical parameters of the SSF system indicated a production level of 23.97 U/g PKC at the moisture ratio of 1:0.75 of PKC : moistening agent with the inoculum size of 1x 10^4 spores/ml at ambient temperature (28±3°C). The supplementation of additional carbon and nitrogen sources in the PKC medium could enhance the enzyme productivity. The presence of $NaNO_3$ at 0.075% (w/w) as additional nitrogen sources further enhance xylanase production to 33.99 U/g PKC.

The effect of substrate amount, moisture content and temperature on the production of cellulase and xylanase by *Trichoderma* sp. FETL c3-2 and *A niger* USM AI 1 was examined in the tray system (20 x 30 x 5cm) using SC:PKC as substrates in SSF system. The maximum xylanase activity was observed when *A. niger* USM AI 1 was grown at 65% moisture content, of 50 g substrate at 30°C for 4 days with the activity of 77 U/g PKC. As for the cellulase, the maximum activity obtained when 50 g of PKC, 75% moisture content and 30°C was 0.57 FPU/g PKC. Enzymes production varies significantly with the change in cultivation conditions in the tray system suggesting the positive influences on enzyme production. Large substrate amount affected the fungal penetration ability into the substrates, while high moisture content caused low oxygen transfer and decreased porosity of the substrates. High cultivation temperature affected fungal growth as a result of heat accumulation within the substrates.

Enzymatic deinking on a laboratory scale using enzyme preparations consisting of cellulase and xylanase from laser printed wastepapers was performed. A maximum deinking efficiency of almost 73% was obtained using the optimized enzymatic hydrolysis conditions consisting of pulp consistency 1.0% with the pulping time of 3 min, temperature 50°C, pH 3.5, agitation rate 60 rpm, pulp concentration 4% (v/v), enzyme concentration of 2.5 U/g dried pulp and the enzyme ratio of 1:1. To further enhance the deinking efficiency, the flotation system must be optimized to enable effective detachment and dispersion of toner particles from the surfaces of the paper fibres. The results indicated that high deinking efficiency can be obtained under acidic condition in the presence of Tween 80 as the surfactant. Based on the optimized flotation system consisting of pH 6.0, Tween 80 of concentration 0.5% (w/w), air flow rate of 2.0 L/min and temperature of 45°C, an almost 100% deinking efficiency was obtained. Effective air flow rate is important in preventing redeposition or promote separation of ink or toner particles from the surface of the fibre network. When compared to the commercially prepared paper, the properties of the deinked paper were comparable suggesting that the effectiveness of the enzymatic deinking process. These properties include the drainage rate of 103.7 L/min, tensile strength 22.77 Nm/g, tear index 7.10 mN m^2/g and burst index of 4.71 kPa m^2/g. When compared to the control pulp, it was observed that the deinked paper showed either similar performance or better properties suggesting that the enzymatic deinking process was able to produce papers of comparable properties to that produced by chemical methods. However, lower tensile strength in the enzymatic hydrolysed papers is expected due to the enzymatic degradation of the fibres.