



INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: Fungal chitosan production using agricultural and industrial wastes and heavy metal removal using chitosan derived nano-biosorbents

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SHORT ABSTRACT

The above mentioned PhD thesis details chitosan production by fungi using different agricultural and industrial wastes as substrates. Initial experiments were carried out using batch systems to evaluate biomass and chitosan production using *Cunninghamella elegans* and *Penicillium citrinum*, a novel organism isolated from an infected bamboo shoot, with different industrial wastewater as the substrate. Domestic, paper mill, and dairy wastewater, were examined as substrates for chitosan production by *C. elegans* and *P. citrinum* which revealed that paper mill wastewater served as very good substrate for *P. citrinum*. Wastewater supplementation with mineral salt media (MSM) containing ammonium chloride as the N-source enhanced the COD removal from paper mill wastewater and chitosan production by *P. citrinum*. Addition of acetic acid at 50 mg/L resulted in a maximum chitosan production and COD removal from the wastewater. A similar trend was observed for phenolics present in the wastewater, and in which case ~70% removal efficiency was achieved due to acetic acid addition.. Among the various kinetic models analysed in the present study, Haldane model was found to accurately fit the data with a very high coefficient of determination value (R^2) greater than 0.9 for chitosan production rate by *P. citrinum*. The positive effect due to acetic acid addition on the utilization of xylose present in the paper mill wastewater was further confirmed by analysis of XR and XDH enzyme activities. Fed-batch experiments with the bioreactor resulted in a COD removal of ~70% during the initial 48 h followed by a drastic reduction in COD during the feeding stage. Fermentation using a bioreactor operated under controlled conditions of temperature, pH, agitation and aeration rate, enhanced COD removal efficiency upto 75% and maximum chitosan production upto 117 mg/L with *P. citrinum*. Chitosan production measured up to 160 mg/L during the fed-batch operation and a maximum COD removal of ~ 90% was achieved at the end of 72 h. Agro-industry wastes, viz. rice straw (RS), lime peel and paper mill waste sludge (PMWS) were evaluated as solid substrates for chitosan production by *P. citrinum*. Microwave alkaline pre-treatment further enhanced utilization of RS by the fungi. Chitosan production by the fungal isolate on rice straw was investigated by varying the moisture content (50-80%) and particle size. A lab scale tray fermenter with three trays (12cm×6.5cm) was later employed to carry out the solid state fermentation under controlled condition of relative humidity (65%),

which yielded a maximum chitosan production of 9 g/kg of rice straw. The results were similar with paper mill sludge as the substrate, and a maximum chitosan yield of 8.5 g/kg paper mill sludge was achieved at a relative humidity of 60%. MSM containing paper mill wastewater in different concentrations (5%, 10%, 15%, 20% and 25%) were added as a supplement to paper mill sludge further enhanced the chitosan yield up to 10.6 g/kg substrate. Application of biosorbent based on the fungal chitosan produced in the study was evaluated for heavy metal removal from aqueous solution. Magnetic iron (Fe_3O_4) nanoparticles were first prepared by the co-precipitation method, and later these nanoparticles were coated with chitosan and carboxymethyl chitosan (CMC) derived from the fungus. CMC was prepared by carboxymethylation of chitosan to form N-substituted CMC and tested for removing Cr(VI) and Pb(II) from aqueous solutions. Coefficient of determination (R^2) values for the pseudo-second order kinetics are comparatively high ($R^2 > 0.99$) for both the metals and with the two biosorbents. These results confirm that the rate limiting step for bio-sorption of Cr(VI) and Pb(II) species by CMC nanoparticles is governed by the pseudo second order model which is based on chemisorption. The Langmuir isotherm model gave the best fit for Cr(VI) and Pb(II) biosorption onto CNP at 303 K temperature with theoretical maximum sorption capacity (Q_{LM}) of 30 mg/g and 100 mg/g, for Cr(VI) and Pb(II), respectively. In the case of CMCNP as the biosorbent, Freundlich model gave the best fit with R^2 values of 0.991 and 0.998 for Cr(VI) and Pb(II). Based on the sorption-desorption results, it could be said that the nano biosorbents could be safely reused for up to 3-4 times without significant loss in metal removal efficiency. This study established an excellent potential of the cheaply produced biosorbent for heavy metal removal from wastewaters.