STUDIES ON SOLID-STATE FERMENTATION OF DEOILED JATROPHA SEED CAKE FOR REMOVAL OF PHORBOL ESTERS AND PRODUCTION OF INDUSTRIAL ENZYMES

by

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Submitted

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Dedicated to my Parents

CERTIFICATE

This is to certify that the thesis entitled "Studies on Solid-State Fermentation of Deoiled Jatropha Seed Cake for Removal of Phorbol Esters and Production of Industrial Enzymes" being submitted by **Ms. CHETNA JOSHI** to the Indian Institute of Technology, Delhi for the award of the degree of *Doctor of Philosophy* in Chemistry is a record of *bona fide* research work carried out by her. Ms. Joshi has worked under my guidance and supervision, and has fulfilled the requirements for the submission of the thesis which, to my knowledge, has reached the requisite standard.

The results contained in this dissertation have not been submitted in part or full to any other University or Institute for the award of any degree or diploma.

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Abstract

Jatropha sp. is a major source material for biodiesel production. Its seeds contain oil called biocrude which has properties akin to diesel. The biocrude is transesterified to make biodiesel, which is perceived as a future alternative of the fast depleting non-renewable petrochemicals. The biodiesel production generates large quantity of deoiled Jatropha seed cake. Although, it is wholesome in terms of protein, fibre, carbohydrates, array of vitamins and minerals, it cannot be utilized as cattle feed due to the presence of toxic phorbol esters. Its safe disposal or meaningful utilization is currently an important issue. The work encompasses the development of an eco-friendly solid-state fermentation (SSF) process for eliminating toxic phorbol esters from deoiled Jatropha seed cake. The cake has also been assessed as substrate for production of industrially important enzymes by SSF.

The deoiled *J. curcas* seed cake was efficiently utilized as substrate by thermophilic *Scytalidium thermophilum*, which secreted good amount of heat stable xylanase during SSF. Under optimized SSF conditions *viz.* deoiled seed cake moistened with Tris-HCl buffer (0.1 M, pH 9.0) at 1:3 (w/v) ratio, supplemented with 1% (w/w) oat-spelt xylan, inoculated with 1 x 10^6 spores per 5 g cake and incubated at 45 °C, produced 1455 U xylanase/g deoiled seed cake. The xylanase was found useful in bio-bleaching of paper pulp. However, the SSF by *S. thermophilum* did not degrade/ eliminate phorbol esters.

The SSF of deoiled *J. curcas* seed cake was further attempted by *Bacillus cereus* and *Pseudomonas aeruginosa* PseA with a viewpoint that these versatile strains may degrade the toxic esters during fermentation. The strategy was successful in degradation of phorbol esters. SSF by *B. cereus* under the conditions; seed cake: water ratio 1:1 (w/v),

inoculum size 3 ml/5 g seed cake, temperature 30 °C and relative humidity at 65% completely eliminated them in 12 days. During fermentation *B. cereus* produced an esterase, which reached to maximum level, 363 U/g cake on the 6th day of fermentation. Esterase activity may possibly be responsible for removal of phorbol esters.

SSF by *P. aeruginosa* PseA degraded the phorbol esters in 9 days. The conditions were: deoiled seed cake: water ratio 1:1 (w/v), inoculum size 1.5 ml/5 g seed cake, temperature 30 °C, and relative humidity at 65%. During fermentation *P. aeruginosa* secreted a lipase which reached to maximum level, 932 U/g cake on the 5th day of fermentation. Lipase activity was correlated with removal of phorbol esters. This was further confirmed by *in vitro* lipase treatment of the seed cake. The SSF seems a potentially viable approach for degradation of toxic phorbol esters from deoiled *J. curcas* cake.

The enzymatic studies were carried out for characterization of the *P. aeruginosa* PseA lipase produced during the SSF. It was purified to 33.5 fold with 48% yield by ultrafiltration and Sephadex® G-100 gel filtration chromatography. The relative molecular mass of enzyme was estimated to be ~60 kDa by SDS-PAGE. PseA lipase exhibited K_m 88.21 mM and V_{max} 2.11 µmol/min/mg towards *p*-nitrophenyl palmitate as substrate. The temperature and pH optimum of the lipase were found to be 40 °C and pH 8.0 respectively. PseA lipase preferred long chain fatty acid ester as substrate with maximum affinity towards C16 (palmitic acid esters).

The lipase was remarkably stable in presence of hydrophilic and hydrophobic solvents. The structural basis of the stability was investigated by analyzing the structure in presence and absence of solvents. The circular dichroism spectra and its analysis by K2D2

EMBL web server revealed that native PseA lipase is predominantly α-helical with 61.3% α-helix; 4% β-strand and 34.6% random coil. The secondary structure remained unchanged in presence of organic solvents. This stability/ rigidity of the structure is possibly the basis of solvent stability of PseA lipase.

The organic solvent stability of PseA lipase makes it potentially useful for ester synthesis in non-aqueous medium. PseA lipase (45 IU) synthesized 46 mM ethyl butyrate in 48 h in n-hexane medium. The reaction conditions were: butyric acid (100 mM) and ethanol (100 mM) as substrate, water content 1% (v/v), temperature 35 °C and constant shaking at 200 rpm. The application of lipase produced by SSF in ethyl butyrate synthesis further adds to its merit.

Overall, the work in the thesis demonstrated that SSF based bioprocesses may form an appropriate strategy for eliminating phorbol esters from deoiled *J. curcas* seed cake. The simultaneous enzyme production provides an added advantage along with the detoxification process.

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