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Project Title

Process Optimization for Conversion of Paddy Straw into Ethanol and Xylitol

Objective

i. To study cellulase production by indigenous fungal strains under different fermentation conditions
ii. To study saccharification of pre-treated paddy straw using immobilized cellulases
iii. To study simultaneous saccharification and fermentation of paddy straw for ethanol production
iv. To standardize the xylitol production of hemicellulosic rice straw hydrolysate

Method

To study cellulase production by indigenous fungal strains under different fermentation conditions

Methodology: Two fungal strains will be obtained from Biodiesel laboratory, REE. Cellulase production from these strains will be carried out under solid state and shake flask conditions as per method of Singla *et al* (2018). The actively growing fungal colonies will be aseptically added to the production flasks and incubated at 28±2°C up to 144 h. The enzyme will be extracted from each flask at different time intervals (24, 48, 72, 96, 120 and 144 h) and analyzed for filter paper (Mandels and Sternberg 1976), endogluconase (CMCase) (Wood and Bhatt 1988), cellobiohydrolase (avicelase) (Wood and Bhatt 1988), β-glucosidase and xylanase (Bailey *et al* 1992) activities.

To study saccharification of pre-treated paddy straw using immobilized cellulases

Methodology: The cellulase produced by the promising fungal strain under solid state or shake flask conditions (selected from Experiment 1) will be used for immobilization studies. Chitosan coated Fe_3O_4 nanoparticles will be prepared and cellulase enzyme will be immobilized onto magnetic nanoparticles using glutaraldehyde coupling agent as per method of Zang *et al* (2014). The morphological and biochemical properties of the free and immobilized cellulase will be studied. The enzymatic hydrolysis of pre-treated paddy straw will be carried out by using immobilised cellulase as per method of Kaur *et al* (2020) and saccharification efficiency will be compared with that of free enzyme. XRD pattern, Transmission Electron Microscopy, Scanning Electron Microscopy, Thermogravimetric analysis and Fourier transform infrared (FT-IR) spectroscopy of immobilised enzyme will be performed. The optimum pH, temperature, Km, V_{max}, thermal stability and reusability studies of immobilized cellulase will be done. The reducing sugars and per cent saccharification of pre-treated paddy straw with immobilized enzyme will be recorded at different hours and compared with that of free enzyme.

To study simultaneous saccharification and fermentation of paddy straw for ethanol production

Methodology: The pre-treated rice straw solid residue will be subjected to simultaneous saccharification and fermentation as per method of Phitsuwan *et al* (2017). Pre-treated rice straw will be added in 500 ml flask and the suspension of yeast cells in YP medium along with cellulase enzyme will be added to the reaction medium. SSF will be carried out at 37°C and 150 rpm shaking speed for 96 h. The culture supernatant will be taken periodically after 24 h to determine the reducing sugar and ethanol concentrations in the fermentation broth as per method of Nelson (1944) and Caputi *et al* (1968), respectively. The kinetic parameters such as ethanol concentration (g L^{-1}), ethanol yield (g g^{-1}), conversion efficiency (%) and reducing sugar consumption (g L^{-1}) will be recorded at different hours of fermentation.

To standardize the xylitol production of hemicellulosic rice straw hydrolysate

Methodology: A pentose fermenting yeast strain (*Candida tropicalis*) will be procured from IMTECH, Chandigarh and paddy straw acid hydrolysate will be used for conversion of xylose into xylitol as per method of Baek and Kwon (2007). The acid hydrolysate will be subjected to neutralization, over liming, ion-exchange resin and activated charcoal adsorption. The compositional analysis of detoxified hydrolysate will be determined after various treatments. Fermentation of detoxified hydrolysates will be carried out at 30°C and 250 rpm. Xylitol concentration will be determined colorimetrically as per method of Sanchez *et al* (1998). The xylose, glucose, total phenols and furfural content will be recorded in detoxified hydrolysates. The xylitol concentration (g L^{-1}), yield (g g^{-1}) and volumetric productivity (g L^{-1} h^{-1}) of fermented hydrolysate will be recorded.

Outcome

The present research work will help to identify suitable fungal strains as well as the optimum fermentation process along with cellulases with high specific activity that could be produced and utilized for hydrolysis of paddy straw. Further, efforts to immobilize these cellulases on chitosan-coated magnetic nanoparticles for their repeated utilization and fermentation of xylose sugars into xylitol along with ethanol production may help to improve the cost competitiveness of ethanol production.