

CHAPTER 36

MICROBIOLOGY

Doctoral Theses

01. CHAKRABORTY(Subhojit)
Production and formulation of cellulose(s) for saccharification of cellulose(s) to fermentable sugars.
Supervisor: Prof. R.C. Kuhad
Th24940

Abstract
(Not Verified)

Present investigation dealt with enhanced production and formulation of in-house cellulases by employing an efficient, indigenously isolated cellulolytic fungus, identified as *Trichoderma* sp. RCK65. It produced all three cellulase activities in good titre under both submerged and solid state fermentation (SSF). SSF proved better and further optimised by response surface methodology employing central composite design resulting in 2.4, 2.9 and 5.4 fold increase in FPase 47.4 U/gds, CMCase 154.2 U/gds and β -glucosidase 144.1 U/gds activities employing wheat bran 5 g, soybean meal 2.4% (w/v), solid to liquid ratio of 1:1 at pH 4.5 of medium. Enzyme production was successfully scaled-up from 5g to 100 g substrate and the estimated cost of cellulase Rs. 5.311/1000 U of FPase was quite low. LC MS/MS based proteomic analysis of fungal secretome revealed 284 proteins, 27.1% hydrolytic proteins out of total proteins, which might have played significant role during efficient hydrolysis of various cellulose(s). After optimization, saccharification of office paper waste resulted in sugar yield of 425.12 ± 0.02 mg/gds whereas that from other substrates, Avicel® PH-101, algal pulp and alkali treated mustard straw (ATMS) were 232.5, 276.7 and 546.4 mg/gds respectively. Cocktail of crude cellulases of *Trichoderma* sp. RCK65 and *Aspergillus niger* RCKH-3 further improved saccharification time (65% within 6 h) and sugar yield (519 mg/gds) from ATMS. The leftover wheat bran after SSF had enhanced antioxidant properties and could find applications in feed and food industries under biorefinery approach.

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02. KAUR (Amandeep)
Deconstruction of Rice Straw for Bioethanol Production.
Supervisor: Prof. R.C. Kuhad
Th24588

Abstract
(*Not Verified*)

The interest in development of alternative fuels has increased due to rapid decline in fossil fuel reserves. Second generation biofuel production is a promising solution this crisis. Rice straw being an abundantly available agricultural waste is rich in cellulose, hemicellulose and lignin. Sequential pre-treatment, dilute acid treatment lead to removal of 90% hemicellulose and dilute NaOH treatment removed 55% lignin from acid treated rice straw leaving the biomass with 830 mg/g holocellulose (mainly cellulose). Crystalline index of rice straw prior pre-treatment was 42%, which increased to 57% after dilute sulphuric acid treatment and decreased to 40% after alkali treatment. Two fractions (L2, L5) of lignin were recovered after gradient acidification of lignin liquor obtained after NaOH treatment and two fractions (L80, L95) were obtained by ethanol-water extraction. Lignin fractions were analysed by CHN analysis, UV absorption spectra, FTIR spectroscopy. These fractions were used to prepare hydrogels and the maximum swelling ratios for L2, L5 (8.8 and 6.7, respectively) and L80, L95 (8.3 and 7.2, respectively) were achieved in ethanol-water mixture. The acid hydrolysate obtained after the dilute sulphuric acid treatment was best detoxified with activated charcoal (97% phenolics and 92% furans). Hydrolysis of pre-treated rice straw with cellulases produced from *Trichoderma* sp. RCK65 produced saccharification yield of 674 mg/g which increased to 787 mg/g (87% saccharification efficiency) with statistical optimization (RSM). The fermentation of detoxified acid hydrolysate by *P. stipitis* NCIM 3499 produced 9.24 g/L ethanol and fermentation of saccharified hydrolysate using *Saccharomyces cerevisiae* HAU produced 33.12 g/L ethanol, which increased to 36.8 g/L ethanol with statistical optimization.

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