Thesis Title: Sequential separation and characterization of cellulose, xylan and lignin from alkali pretreated sugarcane tops and utilization of cellulose for higher scale bioethanol production based recombinant enzyme saccharification and fermentation.

Thesis abstract

The increasing use of non-renewable resources and their continuous depletion have become a major concern nowadays. The production of renewable fuels from agricultural lignocellulose waste is sustainable and economically feasible. Sugarcane top is one of the most abundant lignocellulosic biomasses in India. In the first methodology, the separation of cellulose (40 g) and xylan (25 g) was successfully carried out and characterized from 100 g of raw sugarcane tops (SCT) after delignification by following the alkaline pretreatment method and proven its commercial grade by analyzing using analytical methods such as FESEM, XRD, LCMS, HPSEC, DLS, TGA and enzymatic activity assay. In the second methodology, the sequential extraction of xylan (26.5 g) and lignin (8.5 g) was carried out by precipitation from the alkali-pretreated sugarcane tops (apSCT) hydrolysate after alkaline pretreatment of water-soluble extractives free SCT (100 g) without the delignification. The characterization of apSCTx and apSCTal was performed using various analytical methods such as HPSEC, LCMS, TGA, CHNS, FESEM and FTIR to prove their commercial grade. The alkali-pretreated SCT solids were optimized for its saccharification using recombinant and commercial cellulolytic enzymes which yielded about 265 mg (7.95 g/L) and 672 mg (18.6 g/L) of TRS per g of apSCT, respectively. The higher scale saccharification using commercial cellulases at optimized conditions at (3.6 L) resulted in 687 mg/g (18.8 g/L) TRS yield. The produced reducing sugars were further optimized for fermentation using hydrolysate containing 15 g/L TRS supplemented with 3 g/L yeast extract and 5% (v/v) consortium of fermenting microbes (*Saccharomyces cerevisiae*, *Pichia stipitis* and *Pachysolen tannophilus*, 1:1:1 from 107 CFU/mL) in 20 mL was carried out at initial pH 5, 100 rpm and 35°C for 48 h which resulted in 7.5 g/L of bioethanol. Under the same fermentation conditions, the higher scale bioethanol production in a 5 L bioreactor at 3 L working volume gave 7.43 g/L of bioethanol within 24 h with 96.9% fermentation efficiency. The biorefinery approach of sequential extraction of apSCTx and apSCTal from alkali-pretreated SCT hydrolysate waste can enhance the economics of the extraction process.