ABSTRACT

The big goal of biotechnology is shifting of world economy from fossil resources to renewable ones. It will provide the economically optimized chemicals for modern use; heat and power through processing of the bio-based raw materials. A major outcome of the biorefinery is bioethanol, shaped from the lignocellulosic materials through second generation process. Lignocellulosic material has recalcitrant nature with huge reservoir of carbohydrate macromolecules, available for fermentation after converting into monomeric sugars through different processing steps which are:

- Pretreatment step involves hydrolysis of hemicellulose, along with minute quantity of cellulose which is made more accessible for further conversion into simpler sugars through lignin removal and reduction in its crystallinity.
- Enzymatic step involves the hydrolysis of amorphous cellulose and hemicellulose into fermentable sugars through the action of cellulase enzyme which produces pure sugars.

The present project was conducted for optimization of pretreatment process used for enzymatic hydrolysis of lignocellulosic biomass i.e., water hyacinth, corncob, bagasse, banana pseudo-stem and rice husk, for the production of bioethanol and lactic acid having decentralized availability in the world. Response surface methodology (RSM) was employed for the optimization of catalyst concentration, temperature (^oC) and time (hr). Seven different catalysts including mineral and organic acids i.e., maleic acid, sulfuric acid, phosphoric acid and hydrochloric acid and alkali such as NaOH, NaOH catalyzed Na_2S and Na_2SO_3 were employed for the pretreatment of above substrates. Eighteen experimental runs were performed with each catalyst at different predefined conditions. Each catalyst showed different outcomes and drawbacks at different conditions which vary with the change in biomass material. These catalysts hydrolyzed the hemicellulose and removed the lignin content from each biomass substrate depending on the reaction conditions and substrate composition which resulted decrease in biomass weight. When biomass pretreated with acids, proton breakdown the glycosidic bond present between the hemicellulose and provided monomeric sugars such as glucose, arabinose and xylose. The amount and type of monomers depend on the composition of substrate and type of acid catalyst as well at reaction conditions. Water hyacinth, banana stem and rice husk acid hydrolyzate provided C-6 sugars as major constituent while corncob and bagasse showed C-5 sugars as key component during pretreatment.

Maleic acid and phosphoric acid hydrolyzed the hemicellulose effectively with limited amount of furan inhibitors. These hydrolyzates were detoxified with activated carbon and $Ca(OH)_2$ at pH 10.0 which decreases 6.12-11.58% of sugars along with 96.0% of furan. The detoxified hydrolyzates were fermented with *Pichia stipitis* which produced 13.0-16.62 gL^{-1} of ethanol. Sodium hydroxide resulted in disruption of hydrogen bonding between cellulose and hemicellulose, cessation of the ester bond between xylan and lignin and deprotonation of phenolic groups. This leads to the solubilization of hemicellulose, lignin and swelling of the cellulose.

A quadratic model was proposed to predict the enzymatic hydrolysis yield of each catalyst for each biomass substrate, which had high coefficient of determination ($R^2>0.9$) along with a low probability value (p), indicating the reliable predictability of the model. The pretreated substrates were hydrolyzed enzymatically with cellulase and xylanase enzymes having multiple enzyme activities. Each substrate has different response against each catalyst providing different amount of fermentable sugars ranging from 37.0 – 44.0gL^{-1}. Mixture of sugars was obtained during dilute acid pretreatment with glucose being the most prominent sugar as compared to xylose and arabinose. At optimized conditions, alkali, Na_2S, Na_2SO_3 catalysts removed up to 98.0% of lignin, while acids hydrolyzed hemicellulose up to ~95.0% along with removal of lignin which increased the amount of cellulose in remaining residue. Due to less sever catalytic property of phosphoric and maleic acid, less amount of furan derivatives was present in their hydrolyzate. Saccharification efficiency of rice husk, water hyacinth, bagasse and corncob after treatment with 1.0% Na_2S at 130.0 °C for 2.3- 3.0 hr was 79.40, 85.93, 87.70 and 88.43% respectively. Water hyacinth treated with Na_2SO_3 showed higher hydrolysis yield (86.34%) as compared to Na_2S while other biomass substrates showed 2.0 – 3.0% less yield with Na_2SO_3. At optimized conditions, the amount of sugars obtained from water hyacinth, corncob, bagasse, banana stem and rice husk was 76.41, 78.3, 76.27, 75.35 and 68.6 gL^{-1} respectively from both steps. Resulting sugars were evaluated as substrate for production of bioethanol and lactic acid. Glucose was obtained during enzymatic hydrolysis especially from water hyacinth and rice husk, which was finally fermented in to ethanol with 95.0% conversion yield (theoretical yield: 0.51g/g glucose) by using commercial baker's yeast (*Saccharomyces cerevisiae)*. There obtained 26.48, 25.36, 31.73 and 30.31 $gL^{-1}$of lactic acid with 76.0, 76.0, 86.0, 83.0% conversion yield from corncob, bagasse, water hyacinth and rice husk hydrolyzate using *Lactobacillus* respectively.