ABSTRACT

The steady increase in energy consumption and the depletion of fossil fuels has reawakened the interest in developing alternative energy sources that can compensate the growing energy demands. Ethanol production from lignocellulosic wastes is of growing interest around the world to replace the fossil fuels and the production of fuel ethanol from lignocellulosic materials eliminates the emission of greenhouse gases. Furthermore, lignocellulosics are available as a by-product of many agricultural and industrial processes.

The production of ethanol from lignocellulosics includes pretreatment which improves the accessibility of polysaccharides to enzymes, hydrolysis which breaks the polysaccharides to monosaccharides, followed by microbial fermentation which converts the fermentable sugars into ethanol. This research utilizes sugarcane bagasse (SCB) as lignocellulosic raw material for the production of ethanol and ultrasound was incorporated to improve the conversion processes.

Pretreatment of SCB was carried out by ultrasound assisted alkaline pretreatment, and the influence of particle size, liquid to solid ratio (LSR), NaOH concentration, temperature and sonication time on delignification and reducing sugar production was ascertained with Plackett-Burman design. The best combination of each significant factor was determined by a central composite design (CCD) and the optimum pretreatment conditions arrived for
the maximum reducing sugar yield (96.27%) were particle size of 0.27 mm, LSR of 25 (mL/g), NaOH concentration of 2.89% (w/v), temperature of 70.15°C and pretreatment time of 47.42 min. Under these conditions, 92.11% of theoretical reducing sugar yield was observed experimentally. The substantial reduction in pretreatment time and temperature with improved efficiency are the most attractive features of the ultrasound-assisted alkaline pretreatment.

The hydrolysis of pretreated SCB was carried out by ultrasound assisted acid hydrolysis and ultrasound assisted enzymatic hydrolysis. Ultrasound assisted acid hydrolysis was performed for the production of sugar monomers from SCB. In the pretreatment process, 99% cellulose and 78.95% hemicellulose were recovered in the solid content and lignin removal of about 75.44% was obtained. The solid content was subjected to ultrasound assisted acid hydrolysis. Under optimized conditions, the maximum hexose and pentose yields observed were 69.06% and 81.35% of theoretical yield, respectively. The hydrolyzate obtained was found to contain very less inhibitors, which improved the ethanol production significantly. The SCB hydrolyzate was subjected to fermentation and the yield obtained was 91.81% of theoretical ethanol yield.

The enzymatic hydrolysis was performed with *Cellulomonas flavigena* (MTCC 7450) and the effect of LSR, cell mass and pH were analyzed. The optimum reaction time, LSR, cell mass and pH were found to be 360 min, 15:1, 15 g/L and 6.0 respectively. At optimum conditions, the maximum glucose yield obtained was 91.28% of the theoretical yield and the
maximum amount of glucose obtained was 38.4 g/L. The enhancement in performance may be correlated with the swelling of cellulose and accelerated enzymatic saccharification due to the application of ultrasound. The hydrolysate obtained was fermented using *Saccharomyces cerevisiae* and about 90.66 % of the theoretical ethanol yield was observed in 48 h of fermentation.

The effect of ultrasound on enzymatic hydrolysis and simultaneous saccharification and fermentation of SCB was performed. The reducing sugar and ethanol production were investigated with different concentration of cellulase, % amplitude and treatment time. The ultrasound favours the reducing sugar and ethanol yields, significantly. Under optimized conditions, the maximum yield of 90.2% reducing sugar and 84.23% ethanol were observed with the power requirement of $1.71 \times 10^4$ J/g and $2.66 \times 10^4$ J/g, respectively. The hydrolysate obtained was found to contain very less inhibitors, which favored the ethanol yield. The circular dichroism (CD) spectroscopy analysis was performed to determine the effect of % amplitude on $\alpha$-helical structure of cellulase and the results showed that the % amplitude affected the $\alpha$-helical structure of cellulase.

Furthermore, the influence of SCB hydrolyzate composition on ethanol yield was studied using CCD method and the results showed significant individual and interactive effects of hexose, pentose, acetic acid and furfural on ethanol yield. The positive effect observed for hexose indicated the stimulation of ethanol yield while the negative effect observed for pentose, acetic acid and furfural indicated the repression of ethanol yield.
The optimum composition for the better yield of ethanol was found to be 24.96 g/L of hexose, 11.35 g/L of pentose, 2.66 g/L of acetic acid and 0.54 g/L of furfural. Under these conditions, the model predicted an ethanol yield of 0.405 g/g which is comparable with the experimental yield (0.409 g/g). The results concluded that the ethanol yield was not only regulated by substrate but also by other components. However, the hydrolyzate composition obtained from ultrasound assisted hydrolysis was not up to inhibitory level. The ultrasound enhanced both chemical and biological reactions and it would be a suitable method to improve the production of ethanol from lignocellulosic biomass.