5.0 CONCLUSION AND FUTURE STUDIES

5.1 Concluding remarks

In the present study, cotton stalk used as lignocellulosic feedstock for bioethanol production by using co-culture of *Saccharomyces cerevisiae* and *Pachysolen tannophilus*, through both acid and enzymatic hydrolysis. The main conclusions of the research findings are listed below:

- The physically pretreated debarked cotton stalk contains approximately 65.23% holocelluloses, which mainly comprises of 42.40% glucan and 23.20% xylan. Klason lignin was found to be 24.18% while moisture and ash was recorded as 3.05% and 0.95% respectively.

- Attempts have been made to optimize two stage acid treatments including concentrated acid decrystallization followed by dilute acid hydrolysis along with steam explosion and heat treatment to obtained maximum sugar yield. The data reveals that, the optimum condition for converting lignocellulosic biomass of cotton stalk to fermentable sugar is decrystallization with 75% H$_2$SO$_4$ under specific sample acid ratio of 1:2 (by weight) followed by dilution up to 1N then steam explosion at 121°C for 30 minutes and four hour heat treatment with reflux in water bath yielded maximum fermentable sugar and specifically D-glucose of 0.49 g/g and 0.36 g/g of biomass respectively, While the byproduct of hydrolysis such as furans and phenolics were also formed with a concentration of 1.971 g/L and 4.909 g/L respectively.

- Detoxification of acid hydrolyzate was the next key step in series after hydrolysis. Since no separate delignification prior to acid treatment; and acid hydrolysis itself generates toxic compounds which negatively impact on fermentation process. To
overcome these inhibitors, detoxification with over liming followed by charcoal treatment was applied on hydrolyzate. An optimal strategy of detoxification process should be considered, since chemical detoxification also degrade valuable sugars, cost extensive and produces waste. Optimization study shows that over liming up to pH 10 for an hour followed by filtration and by maintaining pH 6; 4% charcoal treatment for half an hour gives maximum reduction in inhibitors including 92.69% furans and 88.89% phenolics while 19.84% sugar losses were also reported during process. The detoxified hydrolyzate is then exposed to fermentation for ethanol production.

- Parametric optimization of detoxified hydrolyzate of cotton stalk fermentation was carried out by using co culture *Saccharomyces cerevisiae* MTCC 36 and *Pachysolen tannophilus* MTCC 1077. The key parameters optimized during process were temperature, pH, aeration, agitation, inoculum development and optimum time period required for fermentation; the resultant data shows that fermentation in semi aerobic mode of aeration (250 mL Erlenmeyer flask containing 150 mL of fermentation medium), having pH 5.5, inoculated with 10% inoculum (6% *Saccharomyces cerevisiae* and 4% *Pachysolen tannophilus*) and was allowed to agitate with 120 rpm for first 24 hours and then kept in static mode at 30°C for 48 hours utilized 93.84% total available sugars and produced ethanol of 4.96 g/L with fermentation efficiency of 87.52%. The obtained yield was recorded as 0.179 g/g of biomass, 0.278 g/g of holocelluloses and 0.446 g/g of sugar available for fermentation. Moreover, comparative study was also carried out in between monoculture and co culture of *Saccharomyces cerevisiae* and *Pachysolen tannophilus* to explore the importance of co culture fermentation since the cotton stalk hydrolyzate comprises of both 6C and 5C sugars, while native
Saccharomyces cerevisiae can only convert 6C sugars to ethanol during fermentation.

- Similarly, attempts also have been made to standardize alkaline delignification and enzymatic hydrolysis for exploring cotton stalk for bioethanol production. In this regards, various concentrations of NaOH were applied for various time period at constant temperature of 121°C and delignified biomass was further treated with various concentration of enzymes for optimizing enzyme hydrolysis and resultant data showed that 2% NaOH for 60 minutes at 121°C was suitable for maximum delignification which removed 0.201 gram of lignin per gram of biomass and 100 CMC unit of enzyme per gram of dry biomass was found to be optimum concentration for enzyme hydrolysis which yielded total sugar of 0.49 g/g of biomass. Furthermore, when it goes to fermentation using co-culture of Saccharomyces cerevisiae and Pachysolen tannophilus, it gives an ethanol concentration of 9.56 g/L, corresponds to a fermentation efficiency of 76.85%. The yield of ethanol was recorded as 0.191 g/g of biomass, 0.298 g/g of holocelluloses and 0.392 g/g of fermentable sugar.

- Final attempt was made to immobilize the cell of Saccharomyces cerevisiae and Pachysolen tannophilus and make a comparative account with free cell fermentation. The results showed that, ethanol yield and fermentation efficiency of immobilized cell fermentation was 0.142 g/g biomass, 0.221 g/g of holocelluloses, 0.355 g/g of fermentable sugar and 69.53% respectively. Comparative analysis reveals that, free cell fermentation was found to be dominated over immobilized cell fermentation. As for as recycling of immobilized cell is concern, no significant change in concentration, yield and fermentation
efficiency was observed in between first two batches of ethanol fermentation and after second recycling of immobilized cell, beads became disintegrated.

5.2 Future study

The current work is only a part of efforts throughout to find the best alternative in overcoming society’s fuel demand. The continuity of this work is necessary for development of ethanol production at commercial level. Therefore, following few suggestions may be useful for continuing the work:

- The main outcome of current study is the concept of two stage acid hydrolysis of lignocellulosic biomass for ethanol production. It gives a possibility to carry out rapid cellulose conversion by overcoming separate delignification process at batch level. But these findings still leave some problems and questions unresolved, including acid recovery and increase in sugar concentration after hydrolysis, minimization of sugar loss and precipitate production after detoxification.

- Isolation of potential laccase and cellulases producers and there scale up for biological delignification and efficient enzyme hydrolysis.

- The economics is the major driving force for the adaptation of any research and practicability of technology. The investigation in the present study will evaluate for their economic returns.

- Lastly, ethanol may not be the only ultimate product from any lignocellulosic biomass. It opens opportunities to alter the ultimate product such as biogas, biodegradable plastics and enzymes etc.