SUMMARY

Results reveal that pulverized peel of *Citrus sinensis var mosambi* contain 32 ± 0.36% cellulose, 25 ± 0.18% hemicellulose and 18 ± 0.08% of lignin on dry solid (DS) basis. The pulverized *Ananas cosmosus* peel was found to contain 25 ± 0.31% cellulose, 28 ± 0.18% hemicellulose and 8 ± 0.07% of lignin on dry solid (DS) basis. Pulverized peels of *Litchi chinensis* consists of 40 ± 0.38% cellulose, 28 ± 0.18% hemicellulose and 25 ± 0.31% of lignin on dry solid (DS) basis. The holocellulosic (hemicellulose + cellulose)/total carbohydrate content (TCC) content of peels of *C. sinensis var mosambi, A. cosmosus and L. chinensis* was found to be 56-58%, 52-54% and 67-69% respectively. Results elucidated that native wheat straw consists mainly of 32 ± 0.31% cellulose, 48 ± 0.37% hemicellulose and 17 ± 0.15% lignin. Results reveal that native bagasse consists mainly of 37 ± 0.29% cellulose, 28 ± 0.26% hemicellulose and 21 ± 0.28% lignin. The holocellulosic/TCC content of wheat straw and bagasse was found to be 79-81% and 64-66% respectively.

Insignificant amount of total carbohydrate and reducing sugars were detected in native substrates, which however improved after the pretreatment step. Maximum yield of reducing sugars, total carbohydrate and maximum weight loss of substrate was recorded after steam explosion pretreatment in preliminary studies, hence this pretreatment was preferred over the microwave and solar pretreatment for further studies. TLC analysis of hydrolysates of steam explosion pretreatment revealed the presence of arabinose, xylose, glucose, fructose and sucrose. A comprehensive account of the HPLC results indicate that 90.32 ± 0.55% saccharification (maximum) was obtained when *C. sinensis var mosambi* peels were steam exploded with 1% NaOH. 75.52% saccharification (maximum) was obtained when *A. cosmosus* peels were steam exploded with 1% H₂SO₄. 78.2%, 59.56%, 52.15% saccharification (maximum) was obtained when *L. chinensis* peels, wheat straw and bagasse were steam exploded with 1% HNO₃. Following these HPLC results, chemicals that were further used in steam explosion for pretreating peels of *Ananas cosmosus* and *Citrus sinensis var*
mosambi were 1% H₂SO₄ and 1% NaOH respectively, and 1% HNO₃ for pretreating wheat straw, bagasse and peels of *Litchi chinensis*.

Mostly hemicellulose sugars were liberated in hydrolysate after steam explosion pretreatment and this observation was strengthened when FTIR spectroscopy was conducted on the substrates left after optimized steam explosion pretreatment and compared with their native counterpart. FTIR spectroscopy data indicates the penetration of chemicals in the amorphous region of the biomass and degradation of hemicelluloses. There was an increase in crystallinity index of all the substrates after their pretreatment with respective chemical, which indicates the removal of lignin and hemicelluloses during pretreatment. It is observed that absorbance increased in the region between 3600–3000 cm⁻¹ after the pretreatment in all the substrates. Absorption around 1463 cm⁻¹ and 1733 cm⁻¹ were seen in all native and pretreated substrates. In case of peels of *Litchi chinensis*, apparent changes were also observed in the lignin-characteristic bands around 1606 cm⁻¹, and therefore, it is reasonable to state that the lignin was also degraded by the action of the 1% HNO₃. These observations could reflect that the lignin is not composed of percentages of p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units which makes it highly condensed and very resistant to degradation. Detoxification of hydrolysates was carried out cost effectively. Crude cellulase was used for further saccharification of pretreated substrates making the process economical again. Enzyme activity (U/ml) of crude cellulase produced by *Trichoderma reesei* NCIM 1052 was 311.1 µmole/ml/min with a hydrolysis efficiency of 71.65 ± 0.45%, 75.85 ± 0.47%, 30 ± 0.22%, 34 ± 0.24% and 70 ± 0.34% in 1% NaOH pretreated *C. sinensis var mosambi* peel, 1% H₂SO₄ pretreated *A. cosmosus* peel, 1% HNO₃ pretreated *L. chinensis* peel, wheat straw and bagasse respectively.

When studies on effect of operational parameters on Simultaneous Saccharification and Fermentation (SSF) was done at flask level, it was found that 6% of separate inoculum of *Saccharomyces cerevisiae*, *Pichia stipitis* and *Pachysolen tannophilus*, was found suitable for ethanol production from bagasse,
wheat straw and peels of *L. chinensis* and *C. sinensis var mosambi*, whereas 2% inoculum of *Mucor indicus* was found suitable for ethanol production from all the substrates. Similarly 4% inoculum of *Kluveromyces marxianus* and 2% *P. sipitis* was found suitable for ethanol production from bagasse and *A. cosmosus* peel respectively. Combination of nutrients like ammonium sulphate (0.3%), potassium dihydrogen phosphate (0.15%), yeast extract (0.5%), peptone (0.5%) i.e. Nutrient Factor 1 (NF1) was found best for the production of highest amount of alcohol by various organisms from all most all substrates except from bagasse. Combination of nutrients like Urea (0.3%), Sodium dihydrogen phosphate (0.15%), Meat extract (0.5%), Tryptone (0.5%) i.e. Nutrient Factor 2 (NF 2) was found unsuitable for ethanol production. Combination of nutrients like sodium nitrate (0.3%), di potassium hydrogen phosphate (0.15%), malt extract (0.5%), soya peptone (0.5%) i.e. Nutrient Factor 3 (NF 3) was found exceptionally supporting the caliber of *K. marxianus*’s ethanol production from bagasse and wheat straw. This combination of nutrients was also found suitable for *M. indicus* as far as ethanol production from peel of *A. cosmosus* is concerned.

Finally using the optimum inoculum concentration, optimum nutrient factor and the various temperatures (viz. 30°C, 32°C, 34°C) the maximum ethanol production from different substrates and their respective organism is as follow-

- **C. sinensis var mosambi** peel- *S. cerevisiae* at 6% inoculum concentration, NF 1 and 30°C (Ethanol yield = 10.3 g/l).
- **A. cosmosus** peel- *M. indicus* at 2% inoculum, NF 3 and 32°C (Ethanol yield = 11.1 g/l).
- **L. chinensis** peel- *P. tannophilus* at 6% inoculum concentration, NF 1 and 30°C (Ethanol yield = 10.5 g/l).
- **Wheat straw**- *P. stipitis* at 6% inoculum concentration, NF 1 and 34°C (Ethanol yield = 7.4 g/l).
- **Bagasse**- *P. tannophilus* at 6% inoculum concentration, NF 1 and 34°C (Ethanol yield = 10.2 g/l).
Before proceeding for modular fermentation, fermentation parameters for production of bioethanol were further optimized for the highest ethanol producing organism with their respective substrates using Response Surface Methodology. Model terms are significant because values of "Prob > F" was less than 0.0500 in all models. The $R^2$ value was in the range of 0 to 1.0 in all the models. The "Pred R-Squared" was in reasonable agreement with the "Adj R-Squared". The signal to noise ratio was found more than 4 in all the models studied. Response analysis revealed the maximum ethanol concentration (9.94773 g/l) from *C. sinensis var mosambi* peel by *Saccharomyces cerevisiae* could be achieved at the optimum process conditions. Response analysis revealed the maximum ethanol concentration (10.4293 g/l) by from *A. cosmosus* peel by *Mucor indicus* could be achieved at the optimum process conditions. Response analysis revealed the maximum ethanol concentration (9.25864 g/l) by from *Litchi chinensis* peel by *Pachysolen tannophilus* could be achieved at the optimum process conditions. Response analysis revealed the maximum ethanol concentration (7.06568 g/l) by from wheat straw by *Pichia stipitis* could be achieved at the optimum process conditions. Response analysis revealed the maximum ethanol concentration (10.1986 g/l) by from bagasse by *Pachysolen tannophilus* could be achieved at the optimum process conditions.

To the best of our knowledge we dint found any research on ethanol production from peels of *L. chinensis* at modular fermentor level, so we performed a single study at modular fermentor level for ethanol production from these peels. Optimized parameters (according to RSM study) were set for ethanol production in modular fermentor for 72 hrs. Gas Liquid Chromatography (GLC) results revealed that fermentation broth of modular fermentor had 9.15 g/l ethanol concentration after 72 hrs.

This research reveals that an integrated exploitation of these lignocellulosic wastes from agricultural production is economically feasible and highly advantageous for ethanol production utilizing suitable microorganism. It will indeed be a great help to the economy and the environment. It will benefit the
economy as this will use only wasted peelings, wheat straw and bagasse; it will not be in any way detrimental to the environment-friendly energy source and this will consequently lessen the pollution worldwide. This research answers the crisis of looking for a clean, alternative source of energy. The researcher would recommend the people to not throw away these fruit peelings whenever they consume the fruit, and also these fruits-made product manufacturers to store the peelings of the fruits they make use of. The researcher would also recommend local waste management committees to religiously collect these fruits peelings from citizens and submit them to laboratories.