Summary

There are established technologies for ethanol production from conventional feedstock such as sugar cane, sugar beets and different grains. High feedstock cost and limited availability of conventional feedstock presents a great obstacle to large scale implementation of ethanol as transport fuel. Generally, substrates that are used for food production have high prices because of quality demands and therefore could not be considered as substrates for ethanol production. Therefore, in the present study alternative feedstocks were explored for ethanol production. However, presently there are limitations with respect to the substrate preparations and the process to make ethanol from renewable sources. Currently, the research activities have been focussed in the direction of ethanol production using an economically feasible technique. The problems, such as feedback inhibition of the enzymes in the hydrolysate and inhibition by fermentation products are still to be answered clearly. However, technological advances in the process development could well give a solution. The main aim of the research work was to develop a feasible technology for ethanol production from abundantly found agro-industrial waste material in India.

Collection of fruit and vegetable wastes was done either from fruit and vegetable market or fruit processing industries. Another waste substrate, thippi was then obtained from the Sree Lakhsmi Starch Industry, Salem, Tamilnadu. It is a rich source of starch and contains pectin and other polymeric substances like cellulosics in small amounts. The characterization of thirteen different fruit and vegetable wastes indicated the variability in the amount of reducing sugars. The mixed fruit and vegetable waste extract indicated an average value of reducing sugars was up to 12%. The characterization of thippi had shown high starch content (57.8%). Thippi is one of the rich fermentable substances for the ethanol production.

An ethanologenic strain, TERI SH 110, was isolated from raw honey and identified as Candida tropicalis by standard procedure. Cells of this strain were found to assimilate glucose, galactose, maltose, xylose and trehlose. Comparison
of ethanol production efficiency of three strains, *Z. mobilis* DSM473, *Z. mobilis* MTCC 92 and indigenous strain *C. tropicalis* TERI SH110 was done by growing them on M21 medium prepared by using fruit, vegetable and thippi hydrolysate. The reducing sugar utilization by *Z. mobilis* DSM473 was observed to be comparatively slower than the other two cultures. All the three cultures have shown significant difference in their ethanol production efficiencies. *Candida tropicalis* has shown biphasic growth curve when it was grown in presence of ethanol. The hyphae formation in the growing culture was triggered due to ethanol, temperature and age of the culture while deviation from the optimum temperature seemed to induce the mycelia formation.

Hydrolysis of the different feedstocks has shown significant release in reducing sugars. All the three, acid, alkaline and enzymatic hydrolysis of agricultural crop wastes were compared for yields of total reducing sugars. In case of thippi, a tremendous increase in reducing sugars yield was observed when it was steam pre-treated prior to hydrolysis process.

Mild acid hydrolysis for mixed fruit and vegetable waste and thippi was optimized and highest amount of reducing sugars were obtained at 0.75% sulphuric acid concentration. Further increase in acid concentrations had affected the hydrolysate quality and resulted in charring of sugars. Fruit waste mixture yielded 40.33 g l$^{-1}$ reducing sugars, vegetable waste mixture yielded 36.44 g l$^{-1}$ reducing sugars while thippi has shown comparatively a greater release of reducing sugars in the hydrolysate i.e., 78 g l$^{-1}$. Further optimization of acid hydrolysis of fruit waste yielded 84 g l$^{-1}$ reducing sugars, vegetable waste has yielded 61.8 g l$^{-1}$ reducing sugars and thippi has yielded 113 g l$^{-1}$ reducing sugars. Acid hydrolysis has few limitations like, the hydrolysate had a very low pH and it was required to neutralise it before fermentation. Also, toxic by-products produced during the hydrolysis have acted as inhibitors to the fermentation process.

Alkaline hydrolysis for these substrates has not yielded significant amount of reducing sugars, but the trend was similar to dilute acid hydrolysis. It has shown the maximum reducing sugars yield at 0.75% alkaline concentration. Since, sugar yields were very low, alkaline hydrolysis was not further studied and optimized.
The enzymatic hydrolysis of the selected fruit and vegetable wastes have shown a significant increase in reducing sugars as compared to other two hydrolysis processes. The maximum yield of reducing sugars from apple and carrot pomace was in the hydrolysate treated with pectinase (5 U enzyme g\(^{-1}\) substrate) however the pineapple, mango and sapota hydrolysate yielded maximum reducing with xylanase (5 U enzyme g\(^{-1}\) substrate) treatment. There was not much significant increase in reducing sugars yield with increasing concentrations of enzymes.

In enzymatic hydrolysis of thippi highest amount of reducing sugars were released in the hydrolysate treated with enzyme mixture amylase and pectinase, mixture of three enzymes and amylase alone. Since, much difference was not observed in mixture and individual amylase enzyme, amylase alone was utilized for the hydrolysis process in order to reduce the cost of process. Thippi hydrolysate obtained from amylase treatment has yielded 153 g l\(^{-1}\) reducing sugars. The optimization process showed that 1:5 ratio of thippi with water has yields maximum reducing sugars and conversion efficiencies. In the present study, the reducing sugars yield was 152.8 g l\(^{-1}\) and conversion efficiency was 92.53%.

Effect of temperature, pH, agitation, substrate concentration, inoculum size has shown different levels of variations in growth patterns and ethanol production as well substrate utilization by Z. mobilis and C. tropicalis. Optimal conditions for maximum ethanol production were found to be: temperature 30 °C, pH 6, agitation 200 rpm and 6 % inoculum size. Initially, optimum sugar concentration was found to be 12% but after further optimization, the selected strains showed 15% reducing sugar concentration for optimum ethanol and theoretical yield.

The process of media standardisation of ethanol fermentation of fruit and vegetable waste extracts indicated that there is no need of any additional mineral components to be added for the ethanol production or can be reduced further from the normal levels used in M21 medium while it was not observed in case of thippi. This will reduce the cost of ethanol production.

Medium prepared from enzymatic hydrolysate of fruit and vegetable waste have yielded 11-54 g l\(^{-1}\) ethanol while thippi hydrolysate has yielded 72.8 g l\(^{-1}\) ethanol using mixed culture (Candida tropicalis and Zymomonas mobilis).
fermentation. This yield was obtained when fermentation was done at 1 litre fermentor.

The strains, Z. mobilis and C. tropicalis have shown sugar tolerance. Zymomonas mobilis had shown more sugar tolerance as well as ethanol production as compared to C. tropicalis. Strains have tolerated sugars upto 180 g l⁻¹ but ethanol production was delayed in presence of high sugar media. Ethanol tolerance was found to be till 12% of ethanol but the growth of the culture was observed to be hampered to a maximum extent.

**Conclusion**

Our results demonstrate that among the fruit and vegetable wastes, apple, sapota, pineapple and mango yields high ethanol as compared to other substrates. Thippi yielded maximum reducing sugars and ethanol as well. The findings of this study give important information that the fermentation process from these substrates does not require pH maintenance and aeration during the batch, which may lead to cost reduction. The substrates explore in this study, showed a potential to be used as feedstock for ethanol production. These substrates produce enormous waste material due to post harvest losses and lack of proper storage/transport facilities in India. These substrates can be effectively used for ethanol production with above technology.

Acid hydrolysis process generates sludge after neutralisation and inhibitors of fermentation process therefore, can not be used as a promising hydrolysis process for these substrates. Enzymatic hydrolysis is proved to be the best method for maximizing reducing sugars in the fruit and vegetable wastes and thippi. Mixture of enzymes did not yield much higher reducing sugars as compared to single enzymes. Enzyme product inhibition seemed as the main cause for lower reducing sugars yield.

Fruit and vegetable waste hydrolysate have yielded high theoretical yields of ethanol. Mango and sapota have shown maximum theoretical yields of ethanol i.e., 97.39 % and 96.86%.

Results show that ethanol yield from thippi hydrolysate was 72.8 g l⁻¹, using mixed culture of Candida tropicalis and Zymomonas mobilis which was 93.16% of the theoretical yield of ethanol in 1 L fermentor. At the same level, Zymomonas mobilis alone yielded 65.3 g l⁻¹, which is 82.83% of the theoretical yield and Candida tropicalis alone yielded 61.2 g ethanol l⁻¹, reaching more than the published value for C. tropicalis with starch. This is 76.58 % of the
theoretical yield of ethanol. This finding provides useful information that mixed culture fermentation gives more ethanol yield and theoretical ethanol yield than the individual cultures. It was also found that mixed culture fermentation reduces the incubation time and fastens the batch.

The property of producing starch-decomposing enzyme by *C. tropicalis* can be utilised to obtain complete fermentation of thippi without hydrolyzing the substrate. Therefore, direct fermentation process can be developed on the basis of above information, which will be helpful in cost reduction of the ethanol production.

Since the agro-industrial wastes are low cost and widely availability they seemed to be suitable feedstock for ethanol production. Furthermore, the process development using these substrates could decrease energy use and capital costs of the ethanol production. Ethanol costs could be reduced effectively if efforts to produce ethanol from biomass are successful as these are available in large quantities and are relatively inexpensive.