There are several reasons for the world in general and India in particular to initiate the development and use of ecofriendly option of biofuels to meet its ever growing energy demand. The world has already experienced oil-related tremors across its economy with major oil supply disruptions in the early 1970s that has become more frequent and evident in recent years. It is also likely that, with the current rate of consumption, some day we are likely to face an oil crisis that is not temporary. The other side of the picture is that with the ever increasing population and affluence, the waste production rates and hence the disposal costs are increasing with landfill sites getting diminished day-by-day. This is especially problematic for some segments of the society (mainly agricultural, domestic, and forest products industries) to get rid of their vast waste streams in an ecologically friendly manner. The highly perishable vegetable and fruit residues from such a fraction have an enormous potential for use as biofuels through various conversion technologies. This unfortunately, still largely remains unattended resulting in one of the major causes of health hazards and air pollution. A Biofuel technology, utilizing such wastes could be a very favoured option.

As the United States looks to alternate fuel sources, ethanol has become one of the front runners. Farmers have begun planting corn in the hope that its potential new use for corn will be a new income source. But they haven’t realized the potential of other crops besides corn, to provide an alternate energy source to fossil fuels. The agriculture industry already expects a massive increase in land planted with corn nationwide, based on recent demand for corn seed and fertilizer. Popularity of ethanol as an alternative fuel has reached all-time high. Earlier this year, President George W. Bush urged Americans to reduce gasoline consumption by 20 percent over 10 years by substituting alternative fuels, mainly ethanol. The ethanol would be in gasoline blends of 10 to 85 percent. The country's ethanol output was about 5 billion gallons till 2006 and is expected to double by about 2009, according to the Renewable Fuels Association, a trade group that promotes ethanol. Other countries are both producing and using ethanol in large quantities. They are providing incentives to expand ethanol production and use. In France, ethanol is produced from grapes that are of
insufficient quality for wine production. Brazil and Sweden are using large quantities of ethanol as a fuel. Prompted by the increase in oil prices in the 1970s, Brazil introduced a program to produce ethanol for use in automobiles in order to reduce oil imports. Brazilian ethanol is made mainly from sugar cane. Pure ethanol (100% ethanol) is used in approximately 40 percent of the cars in Brazil. The remaining vehicles use blends of 24 percent ethanol with 76 percent gasoline. Brazil consumes nearly 4 billion gallons of ethanol annually. In addition to consumption, Brazil also exports ethanol to other countries. Sweden has used ethanol in chemical production for many years. As a result, Sweden’s crude oil consumption has been cut in half since 1980. During the same time period, the use of gasoline and diesel for transportation has also increased. Emissions have been reduced by placing catalytic converters in vehicle exhaust systems which decrease carbon monoxide, hydrocarbon, and nitrogen oxide emissions. To address global warming concerns, the amount of carbon dioxide produced while burning fossil fuels must be reduced. Ethanol-blended gasoline and ethanol-blended diesel are being considered as viable alternatives to further lower emission levels.

India is becoming more susceptible regarding energy security with increasing world prices of crude oil and increasing dependence on imports. Energy plays a critical role in the development process, as a domestic necessity and its production cost directly affects the prices of other goods also. It affects all aspects of development, social, economic and environmental. Biofuels have potential to mitigate this vulnerability and other adverse effects of fossil fuels. In future, our energy system would need to be renewable, efficient, convenient, cost effective and safe. Biomass energy has always been considered an important factor in the energy planning of a tropical country like India. However, the energy planners are mainly focusing on agro residues. With a view to give boost to agriculture sector and reduced environmental pollution, Government of India has been investigating the supply of ethanol blended petrol in the country for quite some time. India is initiating the use of ethanol as an automotive fuel and it is mainly produced by using molasses. Based on experiments by the Indian Institute of Petroleum, a 10% ethanol blend with gasoline is being considered for use in vehicles in at least one state and it will be mandatory for all oil companies to blend petrol with 10% ethanol from October 2008. In view of the above, the Government has already started supply of 5% ethanol blended petrol from 2003 in the following nine states and four contiguous Union Territories: Andhra Pradesh, Daman and Diu, Goa, Dadra and Nagar Haveli, Gujarat, Chandigarh,
Haryana, Pondicherry, Karnataka, Maharashtra, Punjab, Tamilnadu and Uttar Pradesh. Currently, fuel ethanol is produced mainly from molasses, corn, wheat and sugar beets. The production cost of ethanol from these agro-feedstocks is more than twice the price of gasoline. The high feedstock cost poses a major obstacle to large scale implementation of ethanol as a transportation fuel. Molasses could be in short supply due to the implementation of 10% blending norm. A reduction in import duty for industrial alcohol from 7.5% to 5% has been suggested to sort out this problem. The use of lignocellulosic energy crops, and particularly low cost biomass residues, offers excellent perspectives for application of ethanol in transportation fuels (Ridder, 2000). These materials will increase the ethanol production capacity and reduce the production cost to a competitive level. There is a huge demand (500 million litres) of ethanol to meet the 5% blending in India. With the present infrastructure, only 90 million litres of ethanol was produced till November 2006 and could reach up to 140 million litres (around) till October 2007. Bioethanol from these materials provides a highly cost effective option for CO2 emission reduction in the transportation sector. The aim of the present investigation was to evaluate the potential of biomass as feedstock for ethanol production.

The dedicated energy crops would require thorough support as well as planning efforts such as assessing resources, availability and utilization. Furthermore, applied research is needed to develop environmentally and socially acceptable low-cost, high quality crops and cropping systems for producing sufficient quantities of value added biomass feedstock on substantially larger areas. This would require taking a look at environmental implications and economic assessments as over 70% of Indian population directly or indirectly depends on agricultural income sources. In other words, a long term strategy of intensive research would be required to get the desired level of acceptance both by the researchers and the farmers. This would mean long term field trials with the newly developed energy crops, awareness creation, and demonstration of visual benefits to farmers leading to change in mind-set towards greater flexibility for cropping patterns. This holds enormous promising research and development opportunities, but substantially longer period might be required to achieve these goals.

The petroleum industry is now committed to the use of ethanol as fuel, as it is expected to benefit sugarcane farmers as well as the oil industry in the long run. Production of ethanol from agricultural and biodegradable wastes provides a
viable solution to multiple environmental problems simultaneously creating a sink for waste and renewable energy production as well. Using ethanol-blended fuels for automobiles can significantly reduce petroleum use. Ethanol is one of the best tools to fight vehicular pollution, contains more oxygen that helps complete combustion of fuel and thus reduces harmful tailpipe emissions. It also reduces particulate emissions that pose a health hazard. Currently, fuel grade ethanol is produced from sugarcane, corn, wheat and sugar beets but the ethanol production cost from these substrates is very high as compared to gasoline. This high feedstock cost is the biggest hindrance in large scale implementation of ethanol as a transportation fuel. To counter the high feedstock costs, use of lignocellulosic materials, such as crop residues, grasses, sawdust, wood chips etc., can be promoted, which presents an inexpensive and abundant renewable source for ethanol production. Also there is an enormous production of fruits and vegetables in India and a very huge amount goes waste due to post-harvest losses and a large quantity of unused portion is also generated from processing industries. These substrates can be used as a potential source for ethanol production.

These substrates are complex and are required to be broken down into simple sugars by acid, alkaline or enzymatic treatment. Two common methods for converting complex substrates to fermentable sugars are dilute acid hydrolysis and concentrated acid hydrolysis, both of which use either HCl or H$_2$SO$_4$. Since, acid hydrolysis has few disadvantages enzymatic hydrolysis was explored and found to be a better and more economic option. After substrate selection and its hydrolysis, it is very important to optimize the fermentation parameters and scale up the process. Different agro-industrial substrates were explored for this process.

**The present study**

In the present study, an efficient method of combined steam pre-treatment and enzymatic hydrolysis process was employed to obtain high conversion efficiency and ethanol production from agro-industrial waste materials. The feedstock selected for this study was collected form fruit and vegetable market as well as food processing industries, which generate a huge amount of waste. Selected wastes contained fruits and vegetables and thippi (starchy waste material from tapioca processing industry). The substrates were characterized in terms of reducing sugars followed by optimisation of the hydrolysis process to obtain maximum yield of reducing sugars. Acid, alkaline and enzymatic hydrolysis
processes were studied in detail and were compared on the basis of reducing sugars yield and conversion efficiency. Since, commercially procured strain, Zymomonas mobilis had fermented only hexoses like glucose and fructose we have isolated other ethanol producing strains from rotten fruits, molasses and honey. For complete conversion of the complex sugars and starch (thippi) to ethanol, mixed culture fermentation was done using Z. mobilis and a culture isolated in our laboratory Candida tropicalis TERI SH110. Following the optimization studies, fermentation of the selected substrate, thippi hydrolysate was scaled up to 10 L fermentor.

This work includes feasibility study for fruit and vegetable residues and thippi as low cost substrate for ethanol fermentation by mixed culture Z. mobilis and C. tropicalis. The research work was executed with following objectives:

**Objectives**

♦ Collection and storage studies of agro-industrial waste material from different sources.

♦ Characterization of the feedstock.

♦ Enrichment and isolation of ethanol producing microorganisms from ripen fruits, honey and molasses.

♦ Characterization and identification of the potential strains.

♦ Assessment and estimation of ethanol production capability of isolated and commercially procured strains.

♦ Comparative study of the agro-industrial waste material hydrolysis, using acid, alkaline and enzymatic treatments.

♦ Evaluation of nutrient profile by qualitative and quantitative analysis of the hydrolyzed substrate for selected strains.

♦ Standardization of fermentation conditions of waste hydrolysate by using selected strains.

♦ Scale-up of ethanol fermentation using thippi hydrolysate from 1 to 10 L fermentor.
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♦ Assessment of the selected strains and their ethanol production capability in high sugar medium (sugar tolerance of the selected strains).

The thesis contains seven chapters as described hereunder.
Chapter 1 includes the information, which addresses the need for focus on the main objectives of work, ethanol production from agro-industrial wastes. Basic background information of energy demand and existing and potential fuel supply scenario vis-à-vis energy demand and potential substrates and use of microorganisms for renewable energy. The chapter also provides the state of the art of biofuels, current techniques employed for ethanol production and their limitations.
Chapter 2 reviews the available literature on following aspects,
• Alternative transport fuels- Bioethanol
• Biofuels and their need
• World ethanol scenario
• National ethanol scenario
• Substrates and their potential for ethanol production
• Microorganisms explored for ethanol production
• Ethanol production process
Chapter 3 delineates the material used and methods followed to address the objectives of the present study. It also contains the experimental procedures followed for search and study feedstock that is inexpensive and renewable.
Chapter 4 describes the results obtained in the current research work. Results are summarised and with reference to similar studies conducted by other research groups on related aspects and given in chapter 5.
Chapter 6 discusses the summary of the selected research and its implications. This section presents detailed discussion of the results, inference and conclusions of the present study.
Chapter 7 provides all the reference material referred during the present study. Tables and Figures are provided in support of the findings. The entire study and the results thereof are summarised separately.