1.0. INTRODUCTION

India is the second foremost grower of vegetables and fruits and ranks next to Brazil, in the world. It contributes 12 percent of the world fruit production and 14 per cent of the world vegetable production. Fruits and vegetables are easily get spoiled than grains due to their nature and constitution, and this spoilage happens at the time of harvesting, handling, carrying, storage, marketing and processing. Well-organized management of these wastes can aid in protecting vital nutrients of our foods and feeds, and carrying down the production cost of processed foods, apart from minimizing pollution hazards.

As per a survey published in India Agricultural Research Data Book 2010 (http://www.iasri.res.in/), the losses in vegetables and fruits are due to spoilage nearly 30%. The expected production of vegetables and fruits in India stands at 150 million tones. The total waste accounts to 50 million tons per annum. Betterment in post-harvest technologies for preservation and processing of agri products would help in improving the returns to the farmers, which will aid in regulating the market infrastructure. Reclaiming of vegetable and fruit waste is one of the most significant ways of utilizing it in a number of resourceful ways for producing novel products.

The present fossil fuels, i.e., petroleum, coal and natural gas will run out in a not too distinct future. Biomass energy can be used to generate heat and electricity through direct combustion or liquid fuels for motor vehicles. One such fuel source is the production of
ethanol from carbohydrate (biomass). Therefore, the bio process technologies have the potential to generate alternate fuel sources in developing countries (Johansson, 1996; Aristidou and Penttila, 2000; Spetdel et. al. 2000; Chynoweeth et. al. 2001; Ulgrati, 2001).

Biomass is generally described as all plant and animal material, which can be transformed into energy (Slapack et. al. 1987). Biomass, on the other hand, is the easy term for all organic material that stems from plants, has consistently a major source of energy for mankind. In the past 10 years, there has been a renewed interest for utilization of biomass as an energy source. Technological developments have enabled the use of low cost technologies for biomass conversion with improved efficiencies (Larson et. al. 1992, Kumar et. al. 2005a; Kumar et. al. 2005b). Besides, the products of biomass processing are typically biodegradable and non toxic (Lynd et. al. 1996) and the future bio industry will utilize the wide availability of biomass (Koutinas et. al. 2004).

The manufacture of alcohol is an art, which has been exercised for thousands of years (Slapack et. al. 1987). It can be used for warming, cooking, lightening and as a motor fuel. In reality, alcohol is a high energy, hygienic burning and totally renewable liquid fuel. Ethanol can also be produced from ethylene, acetylene and carbon monoxide extracted mainly from various fossil fuels or from carbohydrates. Enormous quantities of renewable biomass are ready for use for conversion to liquid fuel, ethanol (South and Lynd, 1994; South et. al. 1995; Lynd, 1996; Gong et. al. 1999; Krishna and
Chowdary, 2000). Ethyl alcohol can be produced by fermentation of cereals, grains or agri wastes (Mathewson, 1980; Shirai et. al. 1998; Underwood et. al. 2002; Zaldivar et. al. 2005). Low priced agricultural residues which can readily be converted to fermentable sugars (Converti et. al. 1989; Jesse et. al. 2002). By-products of starch industry like potato pulp is an attractive resource for economic production of ethanol.

Large amounts of renewable biomass are available for conversion to liquid fuel, ethanol (Balls et.al 1944, Johansson et.al. 1996, Aristidon et.al. 2000, Anderson et.al. 2005). Ethyl alcohol can produced by fermentaton of feedstock, starch, grass or even agri wastes (Krishna et.al 2000, Kumar et.al.2005a,2005b). Low value agricultural residues which can easily be converted to fermentable sugars, agricultural by-products of starch industry such as potato pulp and from raw starch hydrolyate are attractive resources for economical production of ethanol(Kumar et.al 2005b, Larson et.al 1992, Sandstedt. R. M et.al. 1937 & 1954, Taguchi. M et.al 1981).

The two important factors that are to be considered for conversion of carbohydrates to ethanol are the cost of product and availability of the substrate. It is worthwhile to develop an economical process which permits the use of cheap substrates, such as starch and cellulose into fermentable sugars and subsequent conversion into ethanol. Cellulosic materials are cheaper substrates but require a preliminary processing step before the ethanol fermentation. Starchy materials, nevertheless, have been suggested and have proven feasible
as a substrate for the manufacture of ethanol (Lee et. al. 1985). In the conventional alcoholic fermentation of starchy materials, a cooking procedure is necessary for saccharification. This procedure requires a huge amount of heat energy, which accounts for 30-40% of the total energy input. In order to reduce the energy costs, alternate methods like use of amylolytic enzymes for saccharification is being carried out. Raw starch digesting enzymes isolated from different sources are being extensively used (Higashihara and Okada, 1974; starch Taniguchi et. al.1988 Mizokami et. Al.1978;Latham et. al. 1977). The maximization of amylase productivity at reduced cost is possible either by improving the strain or by the optimization of fermentation conditions. Nutritional studies on the production of raw starch digesting enzymes in liquid cultures were also carried out by Kainuma et. al., 1984, Tanie et. al. and Nishie et.al.1988. This amylo-process does not need cooking and the sugars thus produced will be economically converted into alcohol.

1.1. ETHYL ALCOHOL

Ethanol is a colorless liquid also called as ethyl alcohol. In general practice, it is often referred to simply as alcohol. Its empirical formula is C₂H₆O and its formula is C₂H₅OH. It is also can be written as CH₃-CH₂-OH. In this (CH₃-) indicates the methyl group and it is attached to the methylene group (-CH₂-), which is attached to the hydroxyl group (-OH). Ethanol fuel is regularly used as a motor fuel, generally as a bio additive for gasoline. Bio ethanol is a form of renewable energy that can be produced from agricultural feed stocks.
It can be synthesized from very common crops such as corn, sugar cane, manioc and potato. According to the International Energy Agency, cellulosic ethanol could allow ethanol fuels to play a much greater role in the future.

1.2. CHEMISTRY OF ALCOHOL

**Fig:1.1 Structure of Ethanol**

Glucose (a simple sugar) is created in the plant by photosynthesis.

\[6 \text{CO}_2 + 6 \text{H}_2\text{O} + \text{light} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{O}_2\] (1)

Ethanol is produced by incomplete oxidation of glucose

\[\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2 \text{CH}_3\text{CH}_2\text{OH} + 2 \text{CO}_2 + \text{heat}\] (2)

During combustion ethanol reacts with oxygen to produce carbon dioxide, water, and heat:

\[\text{CH}_3\text{CH}_2\text{OH} + 3 \text{O}_2 \rightarrow 2 \text{CO}_2 + 3 \text{H}_2\text{O} + \text{heat}\] (3)

After doubling the combustion reaction, two molecules of ethanol are produced for every glucose molecule, and summing all three reactions together, there are identical numbers of each type of atom on each side of the equation, and the net reaction for the overall production and utilization of ethanol is just \(\text{light} \rightarrow \text{heat}\). The heat of the combustion of ethanol is soiled to drive the piston in the engine by expanding heated gases. It can be said that sunlight is used to jog the
engine. Glucose itself is not the solely substance in the plant that is fermented. The simple sugar fructose also stands fermentation. Three other compounds in the plant can be fermented after breaking them up by hydrolysis into the glucose or fructose molecules that create them. Starch and cellulose are molecules that are successions of glucose molecules, and sucrose is a molecule of glucose bonded to a molecule of fructose. The energy to produce fructose in the plant eventually comes from the metabolism of glucose created by photosynthesis, and so sunlight also supplies the energy aroused by the fermentation of these other molecules. Ethanol may also be manufactured industrially from ethylene. Inclusion of water to the double bond converts ethylene to ethanol. This is completed in the presence of an acid which catalyzes the reaction, but is not used. The ethylene is manufactured from petroleum by steam cracking.

1.3. SOURCE OF ETHANOL

Formation of ethanol starts with photosynthesis leading to a feedstock, such as sugar cane or corn, to grow. These feed stocks are then converted into ethanol. About, 5% of the ethanol manufactured in the world in 2003 was actually a petroleum product. It is manufactured by the catalytic hydration of ethylene with sulfuric acid as the catalyst. It can also be acquired via ethylene or acetylene, from calcium carbide, coal, oil gas, and other sources. Two million tons of petroleum-derived ethanol are manufactured annually. The principal suppliers are plants in the United States, Europe, and South Africa. Petroleum derived ethanol is chemically identical to bio-ethanol.
and can be identified only by radiocarbon dating.

1.4. ETHANOL PRODUCTION PROCESS

The basic steps for the production of ethanol are microbial fermentation of sugars, distillation, dehydration and denaturing. Preceding fermentation, some crops need saccharification or hydrolysis of carbohydrates such as cellulose and starch into sugars. Saccharification of cellulose is called cellulolysis (Green Car Congress, 2008).

Ethanol is produced by microbial fermentation of the sugar, and it will work currently with sugars. Two main components of plants, starch and cellulose, are both made up of sugars, and can be transformed to sugars for fermentation. Currently, only the sugar and starch portions can be profitably converted. There is much pursuit in the area of cellulosic ethanol, where the cellulose part of a plant is broken down to sugars and eventually then converted to ethanol.

For the ethanol to be working as a fuel, the preponderance of the water must be removed. Most of the water is taken off by distillation, but the purity is limited to 95-96% due to the composition of a low-boiling water-ethanol azeotrope with the maximum 95.6% v/v ethanol and 3.5% v/v water. (Delphi South America Technical Center – Brazil, 2008).
1.5. PETROLEUM IMPORTS AND COSTS

Production of ethanol needs significant energy, but present U.S. production gains, mainly of that energy from coal, natural gas and other origins, rather than oil (Ethanol.org, 2011). Because 66% of oil used in the U.S. is brought in, compared to a net surplus of coal and exactly 16% of natural gas (Eia.doe.gov, 2011), the displacement of oil-based fuels to ethanol produces a net shift from foreign to domestic U.S. energy sources.

1.6. CRITICISM

There are different economic, social, technical and environmental issues involved with biofuel production and use the major one being the "food vs. fuel" debate. The others involve poverty minimization potential, carbon emissions levels, sustainable biofuel production, deforestation and soil erosion, demise of biodiversity, effect on water resources, as well as energy balance.

1.7. OTHER USES

Ethanol fuel could also be made use of as a rocket fuel. As of 2010, little amounts of ethanol are used in lightweight rocket-racing aircraft (Denise Chow et.al. 2010). There is a still broad use of kerosene for lighting and cooking in less-developed countries, and ethanol can play a role in reducing the use of petroleum products. A nonprofit organization named Project Gaia is trying to extend the use of ethanol stoves to replace wood, charcoal and kerosene (Project Gaia. 2009). 50% ethanol water blended has been tested in specially designed stoves and lanterns for bucolic regions.
1.8. EFFICIENCY OF COMMON CROPS

As ethanol yields mend or unusual feedstocks are introduced, ethanol production may become more economically workable in the US. At present, research on progressing ethanol yield from each unit of corn is afoot using biotechnology (Green Dreams J.K. Bourne JR, R. Clark, 2007).

1.9. ALCOHOL DEMAND

The percentage of ethanol in global gasoline use has increased from 3.7% to 5.4%. In 2009, worldwide ethanol fuel production reached 19.5 billion gallons. The world's highest ethanol fuel manufacturers in 2010 were the United States with 13.2 billion gallons (50 billion liters) and Brazil (26.2 billion liters), accounting with each other for 88% of world production of 22.95 billion US gallons (86.9 billion liters).

1.10. NEED FOR ALTERNATIVE PROCESS

In order to meet the demands of alcohol production there is an immediate requirement for alternative substrates. Many industries are producing ethanol from final molasses and corn. However, corn is the food supplement. In view of food demands, corn based production is not advisable. Therefore focuses on other alternative methods are required, which includes biodiesel from microalgae and bioethanol from waste's materials.

This study reports the production of ethanol from fruit and vegetable wastes of market yards. The motivation for the present study comes from the following figure.
1.11. **MOTIVATION AND IDENTIFICATION OF PROBLEM**

- India is the second major producer of fruits and vegetables in the world.
- Fruits and vegetables are more prone to spoilage than cereals due to their nature and composition.
- Due to lack of proper storage methods majority of vegetables are prone to spoil.
- Storage is expensive for farmers.
- Dumping huge amount of these organic compounds results, soil, water and pollution.
- A lot of research is in progress regarding the conversion of wastes to wealth.

**Figure:1.2** Dumping of spoiled vegetables into the environment
1.12. GOALS & OBJECTIVES

- Isolation, screening and selection of microbes, capable of producing amylase enzyme, from soil
- Collection of microbes from local soil and NCIM, which are responsible for producing α-amylase enzyme and ethanol production
- α - amylase production from isolated and collected cultures by solid state (S.S.F) and submerged fermentations (SmF)
- Optimization of α-amylase production by Response Surface Methodology (R.S.M)
- Development of neural network model and neuro fuzzy model for enzyme production and comparison
- Optimization of total protein and its activity by Genetic Algorithm and Particle Swarm Optimization
- Optimization of Enzyme mediated Saccharification of starch by using microbial amylases
- Conversion of sugars into Ethanol by employing *Saccharomyces cerevisiae* and *Zymomonas mobilis*.