CHAPTER – 1

GENERAL INTRODUCTION
1. General Introduction

1.1 Ethanol

Ethanol which is also called ethyl alcohol, pure alcohol, grain alcohol or drinking alcohol is a volatile, flammable, colorless liquid. In common usage, it is often referred to simply as alcohol or spirit. Ethanol is a straight-chain alcohol and the molecular formula of ethanol is C₂H₅OH. Its empirical formula is C₂H₆O. An alternative notation is CH₃-CH₂-OH. In ethanol, the carbon of a methyl group (CH₃-) is attached to the carbon of a methylene group (-CH₂-) which is attached to the oxygen of a hydroxyl group (-OH). It is a constitutional isomer of di-methyl ether. (Osunkoy and Okwudinka 2011)

The addition of even a few percentage of ethanol to water reduces the surface tension of water. This property partially explains the “tears of wine” phenomenon. Mixtures of more than 50% of ethanol in water are flammable and easily ignited. (Osunkoy and Okwudinka 2011)

![Chemical Structure of Ethanol](image)

**Figure1.1: Chemical Structure of Ethanol**
1.2 Energy and ethanol

The population of world will increase to 11 billion in this century and the expectancy of life has increased 2 times in the last two centuries and the energy demand has increased 35 times during the same period. For scene of resource for alternative energy, we have to visualize various parameters. Mainly with the increase in population, living standard also increased that forced us to make new alternate energy options. Another factor forcing to search alternate energy sources is the climate change which is threatening the existence of life.

The population growth, economy, technology and agriculture are the main drivers of the alternate energy search. (An introduction to energy sources 2006). Switching from petroleum to renewable sources could also cause effects on the national economy such as agricultural and forestry sector and in creating new jobs (Lin and Tanaka, 2006).

In the past, the energy sources to be adopted had to meet the varying needs of different countries and at the same time enhance the security of each one against the energy crisis or energy shortage. The factors that need consideration for the search for new energy sources should include:

- The situation and demand of global energy
- The availability of fossil sources
- The efficiency of the energy sources
- The availability of renewable sources
- The options for nuclear fission and fusion. (An introduction to energy sources 2006)

In recent years, there has been increasing demand of sustainable energy resource such as bio fuels due to the rise in petroleum prices and environmental problems related to greenhouse gas (GHG) emissions (Prasad et al., 2007).
The use of ethanol as an automobile fuel is not a new invention. In 1908, Ford’s model T could be adjusted to run on either gasoline or alcohol. However, after World War II the interest in using ethanol as a fuel declined because cheap gasoline made from petroleum was available. In the 1970’s, the demand of fuel ethanol was increased due to the oil crisis (Di Pardo, 2000).

Bioethanol has advantage as energy source. It can be easily blended with gasoline and can be widely used for transportation purpose worldwide. The use of biofuels will significantly lower the emission of exhaust gases thereby resulting (Demirbas, 2007) in a clean and eco-friendly environment. The first problem for the more widespread production of energy from biomass feedstock is the general absence of low-cost technology in converting these materials (Himmel et al., 2007, Lynd et al., 2005)

1.3 Bioethanol and air pollution

The main environmental advantages of fuel ethanol are its sustainability in using a renewable resource as a feedstock, thus promoting independence of fossil fuel and maintaining the level of greenhouse gas (CO₂). (Mabee and Saddler, 2010, Alvira et al., 2010) Carbon dioxide in the atmosphere is assimilated through photosynthesis and metabolized to be a building block of plants. The energy of sunlight is used to make carbohydrates stored in crops and in the whole plant body. Combustion of these fuels produces CO₂ gas which would be assimilated again by plants. In total, almost no net CO₂ is produced by using fuels generated from biomass (Luo et al., 2010). How effectively ethanol reduces greenhouse gas emission has been widely discussed. The issues are mainly related to the net energy content in ethanol and it depends on the assumption of ethanol production routes. A number of life-cycle assessments have been studied and they show
that a change from fossil fuel to biofuels could reduce CO2 emission (Bernesson et al., 2006, Hu et al., 2004, Kadam, 2002, Rosillo-Calle and Walter, 2006).

In addition, Farrell et al. (2006) developed the “Energy and Resources Group (ERG) Biofuel Analysis Meta Model” (EBAMM) to predict net energy and GHG emission of ethanol produced from cellulosic material was reported, that those values are 23 MJ/L and 10 g CO$_2$ emission/MJ-ethanol energy respectively. Although it is still a theoretical calculation, the results give a promising future. (Scordia et al., 2011)

1.4 Bioethanol and soil & water pollution

Ethanol is harmless to the environment. In ground water and soil mixtures, ethanol can be rapidly degraded both aerobically (100 ml/l in 7 days) and anaerobically (100 mg/l in 3-25 days) (Armstrong, 1999). Ethanol in surface water is also rapidly degraded and thus it is not harmful to the biotope as long as it is not present in concentrations directly toxic to microorganisms. The half-time of ethanol in surface water is 6.5 to 26 hours, while ethanol releases volatile organic compounds (VOC) due to its low vapor pressure; degradation of ethanol in the atmosphere is also predicted to be rapid.

1.5 Bioethanol and humans health

Exposure of humans to ethanol is harmless. The exposure may be carried out mostly by inhalation of ethanol vapor as VOC and by body contact or rarely through ingestion from either blended fuel or denatured fuel. Biological exposures and responses to ethanol are typically evaluated in terms of blood ethanol concentrations (BEC). The endogenous level is 0.02-0.15 mg/dl while the legal limit for vehicle drivers is 80-100 mg/dl (Armstrong, 1999).
The occupation standard for ethanol in the air is 1000 ppm (1900 mg/m3) on an eight-hour basis.

1.6 Economics of bioethanol production

The interest in fuel ethanol on a global scale has been growing in the past few years due to a combination of factors including environmental, social and energy security issues. Brazil and the USA are responsible for 70% of world production. In addition, more than 30 countries have already introduced for fuel ethanol programs (e.g. Australia, Canada, Colombia, China, India, Mexico and Thailand) (Rosillo-Calle and Walter, 2006, Chandel et al., 2011).

The recent spike in the price of global crude oil has made maize-based ethanol production very profitable. The situation is similar for biodiesel production (Hill et al., 2006). Despite these potential profit margins, traditional crop-based biofuel production has led to direct competition between food and energy and as a result, the recent crude oil price increase has already led to the global inflation of the cost of food and associated products. There is an imminent need to move to lignocellulosic biomass-based platforms. The lignocellulosic ethanol price is still high. However, as processing technologies mature and biomass crops are modified for higher yield and lower recalcitrance, the cost of lignocellulosic based ethanol production is expected to decrease to rival maize grain platforms. (Hill et al., 2006, Kumar et al., 2011)

The demand for ethanol is predicted to increase greatly in the future due to the rise of energy demand in transportation (Rosillo-Calle, 2006). The main ethanol feedstock will be shifted from crops to lignocellulosic materials. (Zhu et al., 2011, Scordia et al., 2011)
1.7 Xylitol

Xylitol is an acyclic five-carbon sugar alcohol. The structure of xylitol is presented in Fig 1.3. molecular formula of xylitol is \( \text{C}_5\text{H}_{12}\text{O}_5 \) and xylitol molar mass is 152.15 g/mol. Xylitol occurs in nature in fruits and vegetables, in nature the yellow plum and greengages have the highest xylitol content i.e. nearly 1% of dry weight (Anne Usvalampi 2013, Makinen and Soerling, 1980).

![Figure 1.2: Structure of xylitol (Anne Usvalampi, 2013)](image)

Table: 1.1: Xylitol content in some fruits and vegetables (Washuttl et al., 1973)

<table>
<thead>
<tr>
<th>Fruits and vegetables</th>
<th>Xylitol (mg/100 g dry substance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greengage</td>
<td>935</td>
</tr>
<tr>
<td>Strawberries</td>
<td>362</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>300</td>
</tr>
<tr>
<td>Raspberries</td>
<td>268</td>
</tr>
<tr>
<td>Endives</td>
<td>256</td>
</tr>
<tr>
<td>Egg plant</td>
<td>180</td>
</tr>
<tr>
<td>Lettuce</td>
<td>131</td>
</tr>
<tr>
<td>White mushrooms</td>
<td>128</td>
</tr>
<tr>
<td>Spinach</td>
<td>107</td>
</tr>
<tr>
<td>Pumpkins</td>
<td>96.5</td>
</tr>
<tr>
<td>Onions</td>
<td>89</td>
</tr>
<tr>
<td>Carrots, fresh</td>
<td>86.5</td>
</tr>
<tr>
<td>Bananas</td>
<td>21</td>
</tr>
</tbody>
</table>
1.8 D-xylose

D-xylose is the second most abundant sugar in nature. In structure if plant D-xylose is a part of hemicellulose polymer. Hemicellulose is one of the main constituents of the Lignocellulose. Lignocellulose substrates are potentially attractive for value added products like xylitol and ethanol. Xylose is an aldopentose which is found in embryos of most edible plants and lignocellulosic materials (hemicellulose).

Table 1.2: Physical properties of xylitol (Counsell JN, 1978, Bar, 1991)

<table>
<thead>
<tr>
<th>Physical properties of xylitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
</tr>
<tr>
<td>Molecular Weight</td>
</tr>
<tr>
<td>Appearance</td>
</tr>
<tr>
<td>Odor</td>
</tr>
<tr>
<td>Solubility at 20 °C</td>
</tr>
<tr>
<td>pH in water (1 g/10 mL)</td>
</tr>
<tr>
<td>At 60% relative humidity</td>
</tr>
<tr>
<td>At 92% relative humidity</td>
</tr>
<tr>
<td>Density (specific gravity) of aqueous solution (20 °C)</td>
</tr>
<tr>
<td>10%</td>
</tr>
<tr>
<td>60%</td>
</tr>
<tr>
<td>Melting Point (°C)</td>
</tr>
<tr>
<td>Boiling Point (at 760 mmHg)</td>
</tr>
<tr>
<td>Density (bulk density) (15 °C)</td>
</tr>
<tr>
<td>Caloric value</td>
</tr>
<tr>
<td>Moisture absorption (%) 4 days, 20-22 °C</td>
</tr>
<tr>
<td>Heat of solution,</td>
</tr>
<tr>
<td>Relative sweetness</td>
</tr>
<tr>
<td>Specific rotation</td>
</tr>
</tbody>
</table>
Using xylitol as a sweetener is better due to its anticariogenicity, tooth rehardening and remineralization properties. Xylitol is suitable as sugar substitute for diabetics and it limits the tendency to obesity when it is continuously supplied in diet (Salgado et al., 2010).

1.9.1 Xylitol application in food and confectionery

There are so many reports for using xylitol in food industry (Table1.3). Hyvonen and Slotte, (1983) reported beneficial effects of xylitol in yogurts either as a sole sweetener or combined with other sweeteners. Xylitol is applied for preparation of jams, jellies, marmalades, desserts and relishes (Emodi, 1978). Xylitol is used in different products for infants and adults. It is applied solely or in combination with other sugar substitutes in the manufacture of sugarless chocolates, chewing gums, hard caramels, licorice sweets, wafer fillings, chocolate, pastilles, and other confectioneries for diabetics (Bar, 1991).
Table 1.3: Xylitol applications in food and confectionery

<table>
<thead>
<tr>
<th>Food</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yogurts</td>
<td>Hyvonen and Slotte (1983)</td>
</tr>
<tr>
<td>Jams</td>
<td>Emodi, (1978)</td>
</tr>
<tr>
<td>Desserts</td>
<td>Emodi, (1978)</td>
</tr>
<tr>
<td>Marmalades</td>
<td>Emodi, (1978)</td>
</tr>
<tr>
<td>Jellies</td>
<td>Emodi, (1978)</td>
</tr>
<tr>
<td>Hard caramels</td>
<td>Bar, (1991)</td>
</tr>
<tr>
<td>Chewing gums</td>
<td>Bar, (1991)</td>
</tr>
<tr>
<td>Licorice sweets</td>
<td>Bar, (1991)</td>
</tr>
<tr>
<td>Wafer fillings</td>
<td>Bar, (1991)</td>
</tr>
<tr>
<td>Chocolate</td>
<td>Bar, (1991)</td>
</tr>
<tr>
<td>Pastilles</td>
<td>Bar, (1991)</td>
</tr>
</tbody>
</table>

1.9.2 Xylitol application in pharmaceutical industry

In the absence of insulin, xylitol can be metabolized and can replace sugar on a weight for weight basis (Cao et al., 1994). Xylitol is recommended as a sweetener for diabetic patients because it causes only limited increase in glucose and insulin levels in blood (Hassinger et al., 1981). On the other hand, xylitol finds use in post-traumatic states or post-operative when efficient glucose utilization is inhibited due to the induced resistance to insulin by excessively secreted stress hormones (Ritzel and Brubacher, 1976). Xylitol has anabolic effect with which catabolic disorders can also be corrected, when used regularly in diet, it limits obesity (Parajo et al., 1998). It is used for parenteral nutrition in infusion therapy (Beutler, 1984) as it is inert to amino acids. Chewing gums containing xylitol have been shown to prevent ear infection in children (Uhari et al., 1996).

For preventing denaturation of proteins during protein extractions, xylitol and other sugars are used as a stabilizing agent (Maloney and Amburdkar, 1989). Also xylitol finds use as a
stabilizer in fibrinogen pastes for human plasma when used at 80% weight/volume basis. Xylitol has anti-carcinogenic properties (Cheng et al., 2010) and reduces plaque formation (El-Baz et al., 2011) as carcinogenic bacteria cannot metabolize xylitol in its metabolism (Cheng et al., 2010). Hildebrandt et al., 2010 also reported it as an effective and safe tooth-decay-preventive agent; D-xylitol is used in chewing gums, mouth rinse and toothpaste.

Table 1.4 Xylitol applications in pharmaceutical industry

<table>
<thead>
<tr>
<th>Pharmaceutical properties</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweetener for diabetic</td>
<td>Emodi, (1978)</td>
</tr>
<tr>
<td>Anabolic disorders</td>
<td>Parajo et al., (1998)</td>
</tr>
<tr>
<td>Infusion therapy</td>
<td>Beutler, (1984)</td>
</tr>
<tr>
<td>Lipid metabolism disorder treatment</td>
<td>Aguirre-Zero et al., (1993)</td>
</tr>
<tr>
<td>Prevent ear infection</td>
<td>Uhari et al., (1996)</td>
</tr>
<tr>
<td>Prevent denaturation of proteins</td>
<td>Maloney and Amburdkar, (1989)</td>
</tr>
<tr>
<td>Anti-cryogenic</td>
<td>Cheng et al., (2010)</td>
</tr>
<tr>
<td>Reduces plaque formation</td>
<td>El-Baz et al., (2011)</td>
</tr>
<tr>
<td>Tooth-decay-preventive</td>
<td>Hildebrandt et al., (2010)</td>
</tr>
</tbody>
</table>

1.10 Lignocellulose

Lignocellulose biomass is a major source of biomass which will form the focus of researcher in energy research. Lignocellulose is particularly well suited for energy applications due to its large-scale availability, low cost, and environmentally benign production. In particular, many energy production and utilization cycles based on cellulosic biomass have near-zero greenhouse gas emissions on a lifecycle basis that makes Lignocellulose well suited (Demirbas, 2009).
In general, ethanol production from lignocellulosic feedstock is grouped as

- Crop residues (cane bagasse, corn Stover, wheat straw, rice straw, rice hulls, barley straw, sweet sorghum bagasse, olive stones and pulp);
- Hardwood (aspen, poplar);
- Softwood (pine, spruce);
- Cellulose wastes (newsprint, waste paper, recycled paper) and
- Municipal solid wastes (MSW).

In temperate regions, bioethanol production from lignocellulosic biomass is the best choice if key technical hurdles can be scaled (Taherzadeh and Karimi, 2007).

For conversion of lignocellulosic materials into bioethanol, the steps involved are procurement of lignocellulosic, pretreatment, hydrolysis to derive sugars from their fermentation bioethanol and its dehydration.

However, producing monomer sugars from cellulose and hemicellulose at high yields is far more difficult than deriving sugars from sugar- or starch-containing crops. Therefore, although the cost of lignocellulosic biomass is far lower than that of sugar and starch crops, the cost of obtaining sugars from such materials for fermentation into bioethanol has historically been far too high to attract industrial interest. For decreasing cost, it is crucial to solve the problems involved in the conversion of lignocellulose to sugar and further to ethanol. (Howard et al., 2003)

1.11 Composition of lignocellulosic materials

The term "lignocellulosic biomass" is used when referring to higher plants; softwood or hardwood. In lignocellulose there are three main components; cellulose, hemicellulose and lignin. The composition of lignocellulose highly depends on its source. There is a
significant variation of the lignin and (hemi) cellulose content of lignocellulose depending on whether it is derived from hard-wood, softwood, or grasses.

Cellulose is a major structural component of plant cell walls, and it provides mechanical strength and chemical stability to plants. Solar energy is absorbed through the process of photosynthesis and stored in the form of cellulose.

Hemicellulose is a copolymer of different C₅ and C₆ sugars that also exist in the plant cell wall. Hemicellulose is composed of a matrix of different polysaccharides such as xylan, arabinoxylan and xyloglucan.

Lignin is polymer of aromatic compounds produced through a biosynthetic process and forms a protective layer for plant walls. In nature, the above substances grow and decay during the year (Zha and Punt, 2013).

1.12 Water hyacinth (*Eichhornia crassipes*)

Water Hyacinth (*Eichhornia crassipes*) is a monocotyledonous freshwater aquatic plant, belonging to the family Pontederiaceae, related to the lily family (Liliaceae) and is a native of Brazil and Equator region. It is well known that ornamental plants found in water gardens and aquariums bear beautiful blue to lilac colored flowers along with their round to oblong curved leaves and waxy coated petioles (Fig1.4). There is no agreement among policy makers, environmental agencies and research scientists regarding the way to control this invasive species and practical benefits that can be gained (Lu et al., 2008).

It grows from a few inches to about a meter height. Stems and leaves are full of air filled sacs which help them to stay afloat on surface of water (Bhattacharya and Kumar, 2010).
In the developing world, Water Hyacinth is used in traditional medicine and even used to clean up toxic elements from polluted water bodies. Water hyacinths control is difficult because they reproduce both asexually through stolen as well as sexually through seeds which remain viable for up to 20 years (Center et al., 1999). Thus, in most parts of the world, this plant is considered a noxious weed because it grows very fast and absorbs nutrients and oxygen rapidly from water bodies. This adversely affects the flora and fauna. There have been cases where the water hyacinth closed waterway, making fishing and recreation very difficult. Water hyacinth is very difficult to remove physically, chemically or biologically and each year a significant amount is spent to control plants worldwide.

1.13 Objective of research:

- Analysis of chemical composition of Water hyacinth (*Eichhornia crassipes*) collected from different places at different times.
• Evaluation of chemical pretreatment for enzymatic saccharification of Water
Hyacinth
• Evaluation of chemical and enzymatic saccharification of water hyacinth
• Evaluation of chemical detoxification of Water Hyacinth acid hydrolysate to
minimize fermentation inhibitors
• Ethanol fermentation of water hyacinth hydrolysate: Selection of pentose
fermenting yeast and parameters optimization. Fermentation of water hyacinth
hydrolysate by mono-culture and co-culture using hexose and selected pentose
fermenting yeast
• Xylitol fermentation of water hyacinth hydrolysate: Screening and parameter
optimization of yeast, evaluation of xylitol production using water hyacinth
hydrolysate, scale up study of xylitol production.

1.14 Presentation of thesis
The thesis is presented in eight chapters; each chapter is divided into subchapters for easy
reference. Summary and conclusions are placed at end of the last chapter. The literature
consulted in the thesis is arranged at the end in the alphabetical order of names of the
authors, publication years, full title, journal title, volume number and initial page numbers.
A list of publications made from the present work, other publications and papers presented
at various symposia/ conferences are given at the end of the thesis.