CONCERN over global climate change and depleting fossil fuel resources has driven the search for environment friendly, renewable and sustainable energy resource. Use of ethanol as liquid fuel energy offers an alternative.

Ethanol can be blended with petrol or diesel (e-diesel, ethanol mixed diesel), it has higher octane number and higher heat of vaporization. The history of ethanol as a biofuel dates back to 1826 when it was used to power an engine. Later Henry Ford, in 1896, designed his first car, the Quadricycle to run on pure ethanol followed by the famous Ford Model T capable of running on ethanol, gasoline or a combination of both in 1908.

In modern times, ethanol first became a component of fuel as oxygenate and replaced methyl tertiary-butyl ether (MTBE), which causes environmental pollution and health hazards. Now ethanol has been seen as an alternative to liquid fossil fuel and as “green energy”. It was in Brazil in the 1970s that ethanol got a major boost as liquid fuel energy.

The problem associated with pure ethanol as fuel is that it cannot be transported in pipes, and engines cannot run on pure ethanol, because hydrophilic ethanol corrodes the metal. However, ethanol can be mixed up to 85% and the automobile companies are also making flex-fuel vehicles (FFV) that can use both petrol and E85 (85% ethanol & 15% petrol) and now even E100 dedicated vehicles are also being designed.

Fuel ethanol, also known as first-generation biofuel, comes primarily from sugarcane and starch-rich sources such as corn grains, potato, etc. The United States of America is the front-runner with about 52.6 billion liters, followed by Brazil with 21.1 billion liters of ethanol production in 2011.

In USA, the prime source of ethanol is corn grain, while sugarcane is being used in Brazil. The use of grain for biofuel production requires large cultivated land and competes with food, resulting in food price rise. Moreover, it has a high feedstock value and covers only a percentage of fossil fuel requirements.

This paved the way for the second-generation bioethanol known as lignocellulosic or cellulosic ethanol. Lignocellulosic ethanol is derived from biomass, which mainly consists of cell walls harvested from agricultural or forest residues such as stover from corn and sorghum, straw from wheat and rice, Bagasse from sugarcane and sweet sorghum, or dedicated bioenergy crops such as Switchgrass (*Panicum virgatum* L.), Elephant grass (*Miscanthus*).
Plant cell walls are dynamic and chemically complex structures surrounding the cells. Cell walls define the cell size, shape, and structural integrity of the plant and defend against biotic and abiotic stresses. Depending on the age and tissue type, the cells may have primary as well as secondary cell walls.

Cell wall composition varies with taxa, age, cell, and tissue type. There are three major components of a cell wall namely cellulose, hemicelluloses and lignin.

- **Cellulose** is a polysaccharide, a linear polymer of glucose in which glucose residues (2000-20,000) are linked by alpha 1, 4-glycosidic linkage. It exists in the form of microfibrils, which are several glucan chains bound together through hydrogen bond along their length. Cellulose is the most abundant molecule on earth. It is produced by all the living plant cell and constitutes 15-30% of the dry mass of the primary cell walls and up to 40% in secondary cell wall.

- **Hemicelluloses** are highly branched polysaccharides and cross-link the cellulose microfibrils. It is a heteropolymer of pentoses, hexoses and sugar acids and constitutes 20-40% of the cell dry mass.

- **Lignin** is a complex insoluble polymer of aromatic compounds called phenylpropanoids. It is a major component of the secondary cell wall and constitutes 10-25% of the total plant dry mass.

Lignocellulosic content of the cell walls is a rich source of solar energy trapped as carbohydrates and therefore could make a significant contribution to liquid fuel demand. The advantages of using lignocellulose include abundant renewable supply, eco-friendly, sustainable with limited conflict for cultivable land, food and feed production and raw material (biomass) is widespread, and it does not require import and thus can bring self-sufficiency.

Let’s take an example of rice and wheat that are the most widely grown crops in the world. As per FAO statistics, India’s annual production of wheat and rice in 2011 was about 156 and 87 million tons respectively. Assuming a residue per crop ratio of 1.3, a total of 315 million tons of rice and wheat straw was produced. Even if a part of this straw was kept as cattle feed, left on field or used for other activities, a major portion is usually burned which again is a source of environmental pollution. If this remaining biomass residue were used for making ethanol, it would not only become an ecological way of disposing waste but also provide fuel with minimal effect on environment.

In spite of so many advantages, lignocellulosic ethanol has not been able to come up front. The reason lies in its processing. A standard process for producing ethanol from lignocellulosic biomass includes four main steps:

1. Pretreatment — loosening and de-structuring of the complex lignocellulosic matrix
2. Enzymatic hydrolysis/saccharification — breaking down cellulose and hemicelluloses to simple sugars by enzymes
3. Fermentation — converting sugars to ethanol by yeast/bacteria/fungi
4. Distillation/rectification — separating and purifying the ethanol.
The genetic engineering approaches involve modulation of the lignin biosynthetic pathway to reduce the content or composition of lignin. Targeted expression of cell wall hydrolyzing enzymes has been carried out so that the cell wall hydrolysis can be programmed in plants and can be initiated at the desired time.

Besides, there are certain mutants known as brown midrib mutants, found in certain cereals such as maize, sorghum, pearl millet. These mutants are associated with altered lignin content/composition and increased in vitro digestibility and saccharification efficiency (glucose yields). In sorghum, these mutants are being crossed with high biomass sweet sorghum genotypes. This serves many purposes, the juice from sweet sorghum stalk can be directly used for fermentation, the bagasse and remaining biomass (stover) can be used for lignocellulosic ethanol and grain can be used for food or feed purposes.

Another variation and hurdle in lignocellulosic process is that unlike sugar or starch fermentation, lignocellulose hydrolysis yields mixed sugars containing six-carbon (hexoses) and five-carbon (pentoses) sugars as well as fermentation inhibitory compounds. The pentose sugars such as xylose and arabinose are not fermented by commonly used yeast (Saccharomyces cerevisiae) strains. Therefore, strains need to be modified or engineered to make them capable of utilizing various sugars or different microbes or fungi need to be identified and their optimum combination needs to be optimized for increasing the efficiency.

If feedstock is to be cultivated then they need to be more efficient for land, water and nutrient use. At present, sorghum and maize have the potential of being bioenergy crops. However, maize is a higher input crop than sorghum. Moreover, sweet sorghum fits more appropriately in the bioenergy crop model. There are other biomass crops such as the fast-growing perennial grasses (switchgrass and giant miscanthus), and woody crops (poplar and shrub willow). Among these, miscanthus and switch grasses gain points over poplar, as these are C4 crops with higher water and nutrient use efficiency, and can be grown on marginal lands. The C4 crops may be good for tropics and lands with low water availability while temperate regions can benefit from woody C3 plants like poplar.

Sugarcane still rules the biofuel chart because it has potential to be used as both sugar based and biomass based biofuel. As far as other crops are concerned, there is a need to maximize the biomass yield per hectare, bring sustainability with minimum input and pose least competition with food or feed. For overall success of lignocellulosic ethanol, all the disciplines including biology, chemistry to engineering to economics need to work together to make it an economically viable alternative to fossil fuel.

Ethanol is the prime candidate in the renewable liquid biofuel market and with all its advantages lignocellulosic ethanol holds the key for the future especially for a country like India that has a primarily agriculture-based economy.