

Project outcomes (please list specific objectives): *The project should address a specific need of the industry/industries and there should be clear expected outcomes from the project. It is expected that joint patents will result from this project.*

- Characterization of industry identified lignocellulosics (sugarcane tops and banana)
- Standardization of enzymatic delignification and saccharification
- Standardization of fermentation for maximum bioethanol production
- Strain improvement for ethanol yield
- Standardization of distillation process
- Pilot plant trials of the process
- Techno-economic analysis

Scope (not exceeding 1500 words): *The scope should clearly lay out the contributions of the academic partner and the industry partner.*

The world energy demand is increasing due to population growth and rising living standards. Global usage of petro-fuels was estimated as 85.7 million barrels per day in 2008 which is expected to reach 97.6 and 112.2 million barrels per day in 2020 and 2035 respectively. Thus, the world is progressively marching towards a serious energy crisis and needs a clean energy source that can be able to meet the progressing energy demand.

Utilization of natural and renewable resources for biofuel production can reduce the global dependency on fossil fuels. Continuous burning of fossil fuel is contributing immensely to environmental pollution. Renewable energy can supply a significant proportion of the country's energy needs, creating public benefits, including environmental improvement, increased fuel diversity and national security. The use of biomass for energy production as a substitute for fossil energy is often seen as an attractive option to reduce fossil-fuel dependency and help reduce greenhouse gas (GHG) emissions.

Lignocellulosic biomass is most abundant and sustainable energy source that includes agricultural residues, energy crops, wood residues, paper waste etc. It is a promising substrate for biofuel production as it does not interfere with the food chain. Due to these benefits, it is an appropriate feedstock for satisfying the energy demand in the developing countries. Chemically, lignocellulosic biomass consists of a complex heterogeneous mixture of polymers namely cellulose, hemicellulose and lignin along with some other biomolecules and inorganic composites. Cellulose and hemicellulose are storehouses of carbohydrates which can be utilized for biofuel production. Major liquid biofuels which can be produced from lignocellulosic biomass are bioethanol, biobutanol, biodiesel, bioalkanes, alkenes, alkynes etc. Among these fuels, bioethanol production technology is the most studied field and attracts a lot of investors.

Lignocellulosics are generally classified into hardwoods, softwoods and grasses. The terms softwood and hardwood are used to refer to the taxonomical classification that separates different plant species. Hardwood trees possess broad leaves and are deciduous in nature i.e., they shed their leaves towards the end of their growing season. Hardwoods include poplar, oaks, willow, cottonwood, maples, aspen, birches and fruit trees. Commercially important woody biomass is superior to other lignocellulosic biomass in terms of higher potential for bioethanol production and cost effective transportation to the production centers but they have many other market interests in terms of their utilization in construction industries etc., which comes in the way of their utilization as a sustainable feedstock for bioethanol production. Softwood trees are conifers and have needles or scale-like foliage. They have lower density in comparison to hardwoods and grow faster. Softwoods include pines, cedar, cypress, spruces, firs, redwoods, hemlocks etc.

Grasses are one of the most appropriate energy crops which have regeneration capability after their cut off, longevity, and effective potential to withstand drought conditions. Grasses include atratum, Bambusa bambos, Bermuda grass, dwarf napier leaves, king napier leaves, Miscanthus giganteus, napier leaves, purple guinea, pangola, ruzi, sugarcane, switchgrass, silver grass, etc. Softwoods and grasses constitute a major fraction of the agricultural residue and majority of them belonging to this category are having no commercial value and hence can be efficiently tapped for biofuel production. Further, the lignocellulosic biomass can be grouped into edible and non-edible lignocellulosics for getting the idea of the availability of biomass under each category.

Executive summary (not exceeding 500 words)

Increasing rates of petroleum products have compelled Government of India to implement E20 fuel by the year 2017. This policy of the government has suddenly created the demand for bioethanol and hence in order for this demand to be fulfilled, lignocellulosic biomass is looked upon as a sustainable raw material considering its abundant supply and reduced green house gas emissions.

Bioenergy is the application of natural and potential bioresources in order to produce renewable energy which contributes a lot towards the mitigation of the global issues associated with climate change, energy security, population growth and a global increase in per capita energy demand. In this context there has to be joint research collaborations between industry and institute in order to make "energy from biomass" a viable option for future energy use.

The staggering 8 percent average economic growth rate of India makes a huge demand on energy inputs. This growth in energy demand has resulted in increasing dependency up on fossil fuels. In rural India, energy consumption is mostly dependent upon biomass fuels accounting for over 80 percent of total energy consumed. Recently the Indian Institute of Science, Bangalore has prepared a "Biomass Resource Atlas", estimating total biomass generated from forests, agriculture and wasteland. The estimated biomass generated from forests and wastelands amounts to nearly 176 million tons per year which is a potential resource for a biorefinery. This potent field of research can not only provide a feasible energy source option for future use but can also develop the socio-economic status.

Thus the proposed project is an endeavour to bring the transformations, by involving biological components. It envisages the production of ligninolytic and holocellulolytic enzymes which would be consequently used for ethanol production from crop byproducts. It involves sustainable production of bioethanol by utilization of lignocellulosic residues available in surplus. The treatment process facilitates the production of utility by-products like biomethane, biobutanol, biomanure etc. Complex and seemingly recalcitrant biomass viz., whole banana plant and sugarcane tops will be subjected to enzymatic treatment for effective delignification and subsequent saccharification in optimized conditions. This enzymatic delignification step will replace the existing practice of utilizing physical/chemical/physico-chemical means of delignification which introduce intermediates like furfural/hydroxymethylfurfurals into the system and ultimately hamper the ethanol fermentation step. The delignified residues will then be hydrolyzed to hexose and pentose sugars at high substrate concentrations using cellulase and xylanase formulation, in an especially devoted bioreactor, and the hydrolysate will be fermented to ethanol using hexose and pentose fermenting yeast. The fermented broth will be subsequently distilled to fuel grade ethanol ready to be blended with gasoline.

This proposal has been formulated based on the preliminary work established at laboratory and pilot level with some non-edible lignocellulosics where 75-80% delignification and 5.9% (v/v) ethanol production was achieved during 20 h of processing time. The research team has the competence to utilize the two new substrates viz., whole banana plant and sugarcane tops which was identified by the company due to its plenty availability and thus can be efficiently channelized for bioethanol production.

Background and motivation (not exceeding 500 words)

The depleting fossil fuel sources and its harmful impact on the environment by generating greenhouse gases are a matter of great concern. These issues have fuelled a much-anticipated research towards the alternative fuels from biosources. Research on biofuel might be helpful for procuring the sources of fuels and overcoming world poverty by having more access to renewable energy. However, biofuel production from the sugar and starch based crops may threaten the food security, furthermore these crops have low sugar yield per hectare compared to the most prevalent forms of sugar in nature (cellulose and hemicellulose). Although countries like India have found non-food sources such as Jatropha oil for bio-diesel production, growing Jatropha also requires arable land, which could be utilized for other food production. India, being a second populous country in the world, really cannot afford the biofuel production from a source which is competing with food directly or indirectly. Gladly, India has large amount of lignocelluloses based biomass which are often major components of different waste streams from various industries, forestry, agriculture and municipalities, and lignocellulosic biofuels offer considerable reduction of Green House Gases (GHG) compared to fossil fuel. Some of the lignocellulosics viz., banana and sugarcane are cultivated almost all over India. Though, some pockets in India are a major hub for banana and sugarcane cultivation. A small state like Chhattisgarh plants 28-30 lakhs bananas per year and has three sugar mills which use local produce. Sustainability, availability and transport plays crucial role in cost effective ethanol production. Thus to meet the challenges, the present work will be carried out with the lignocellulosics identified by the company itself based on its plenty availability that includes whole banana plant and sugarcane tops.

A conversion process from lignocellulose based feedstock to biofuel is more complex than a conversion from sugar or starch based feedstock. The main component of lignocellulose is cellulose; the second most abundant component is hemicellulose; followed by lignin. Cellulose, hemicellulose and lignin form structures called microfibrils, which are organized into macrofibrils that mediate structural stability in the plant cell wall.

A general process for bioconversion of lignocellulose includes four main steps: (i) Pre-treatment, (ii) Enzymatic hydrolysis, (iii) Fermentation, and (iv) Distillation. The objective of pre-treatment is to alter the structure of biomass in order to make the cellulose and hemicellulose more accessible for enzymatic hydrolysis responsible for generating the fermentable sugars which can then be efficiently converted to bioethanol.

Edible lignocellulosics generally have a sizeable demand in the market as a food source and hence, its utilization as a source for bioethanol production invites a lot of controversies. On the contrary, non-edible lignocellulosics does not have the same demand because they suffer mostly with the presence of toxic compounds. Lantana camara, Ricinus communis and Jatropa contains toxin such as lantadene, ricin and forbol toxin respectively. Non-edible lignocellulosics need to be majorly focussed owing to their no competition in market and sustainable nature.

There are various challenges which are to be overcome in order to produce biofuels from lignocellulosic biomass. Use of lignocellulosics as substrates for biofuel production has many advantages over the use of other edible substrates namely:

- Cheap and easy availability of large variety of lignocellulosics
- Crops grown in adverse conditions: on poor soils, agricultural and forest wastes could also be utilized
- They are non-edible and hence food, fodder vs. fuel issue is resolved

In India, banana ranks first in production and third in area among fruit crops. It accounts for 13% of the total area and 33% of the production of fruits. Production is highest in Maharashtra (3924.1 thousand tonnes) followed by Tamil Nadu (3543.8 thousand tonnes). Within India, Maharashtra has the highest productivity of 65.70 metric tonnes /ha. against national average of 30.5 tonnes/ha. The other major banana producing states are Karnataka, Gujarat, Andhra Pradesh, Assam, West Bengal, and Kerala. The huge production of banana accounts for substantial amount of banana waste which constitute about 70-80% of cellulosic fibers that can be utilized for biofuel production such as ethanol.

In India, sugarcane is grown as a Kharif crop. It needs hot and humid climate with an average temperature of 21°C to 27°C. 75-150 cm rainfall is favorable for sugar cane cultivation. Sugarcane is grown on around 2.8% of gross cropped area of India. India produced around 350 million tonnes of sugar in 2013-14. As per 3rd Advance Estimate (2014-15) of DAC released on 13/5/2015, India is expected to produce 356 million tonnes of Sugarcane and 25 million tonnes of sugar in 2014-15. Looking into the figures it is estimated that there is a scope for large amount of sugarcane top which is either buried or burnt in the field itself. Sugarcane top constitutes a large reserve of carbohydrates which can be easily converted to bioethanol if proper technology and management practices are employed.

IIT Kharagpur is engaged in the production of liquid and gaseous biofuels since a long time and already several lignocellulosics have been explored for bioethanol production. Maximum bioethanol obtained through SSF from Ricinus communis and Lantana camara was 5.72% (v/v) and 5.14% (v/v) respectively. Moreover, 7-8% ethanol was obtained by utilizing a newly isolated yeast strain using Kans grass and mixture of lignocellulosic feedstocks. Pilot plant run of 550 kg biomass handling capacity for bioethanol production has been established based on lab scale results. The outcome of the pilot plant trials is encouraging which can be translated to commercial scale production. Thus the scientific team involved is highly competent to utilize the two feedstocks viz., whole banana plant and sugarcane tops which have been identified by the industry for bioethanol production.

The USP of ethanol production from lignocellulosic feedstock are:

- i. Eco-friendly biotechnological process
- ii. Enzymatic delignification by surpassing physical/chemical/physico-chemical pretreatment techniques
- iii. Enzymatic saccharification for improved hexose and pentose sugar production
- iv. Simultaneous utilization of hexose and pentose sugars for enhanced ethanol production
- v. A modified yeast strain with improved ethanol tolerance

IIT will standardize the process for bioethanol production using the two identified feedstocks upto pilot scale and support the industry in implementing the process at the commercial level.

The industry partner is a highly experienced biotechnology based company and will take up the process after standardization at the pilot level and will proceed for commercializing the product.