2G ETHANOL FROM FOREST WASTE (PINE NEEDLES) – GREEN INNOVATION FOR BIOREFINERIES

Sponsored by

NATIONAL MISSION ON HIMALAYAN STUDIES (NMHS), ALMORA, UTTRAKHAND

Devised at

Dr Y S PARMAR UNIVESITY OF HORTICULTURE & FORESTRY, NAUNI, SOLAN, H.P., INDIA

By

Dr Nivedita Sharma

Dr. Nisha Sharma

Microbiology section, Department of Basic Sciences
Dr Y S Parmar university of Horticulture & Forestry, Nauni-Solan, Himachal Pradesh-173230- India

2G Ethanol- A Cost Effective Technology

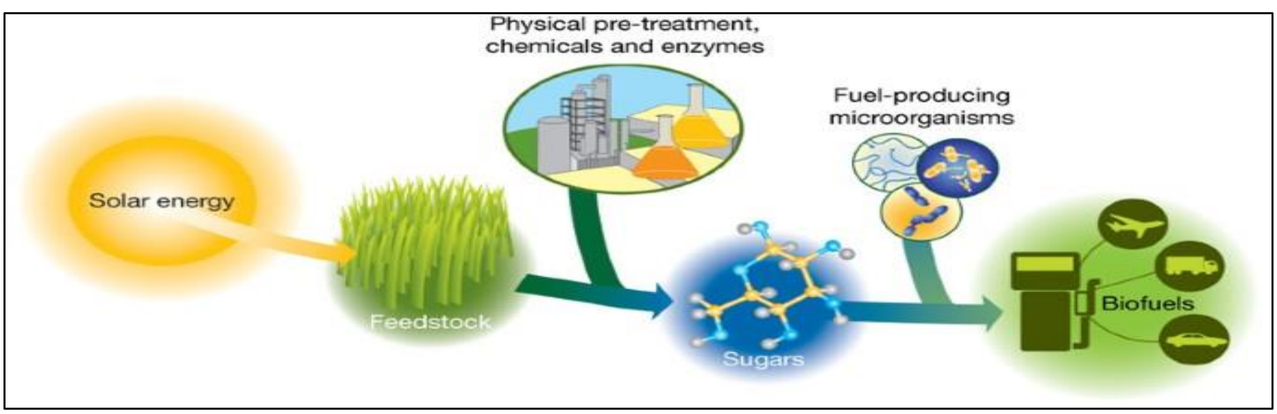
The world's energy needs are leading to rapid depletion of natural resources and simultaneous increase in environmental pollution. The transportation sector is one of the major sectors to sap the energy and accounts for 25% of global greenhouse gas (GHG) emission. There is a clear need to decarbonise transportation by using biofuels in place of conventional fuels. Thus, Biofuels are viewed as one of the solution to this problem. Liquid petroleum blended with bioethanol (20- 30 % Gasohol) is a latest proposed policy of GOI. Lignocellulosic biomass is a promising renewable resource for attaining value added products. Lignocellulosic biomass is mainly composed of cellulose, hemicellulose and lignin distributed within the cell walls. Pine needles are major recalcitrant lignocellulosic softwood biomass rich in cellulose and hemicelluloses along with lignin that cannot serve as fodder. These do not even decay like any other biomass and piled up pine needles are a major cause of wild forest fires and adversely affecting biodiversity as well as soil fertility. Forest fires deteriorate the fertility of soil and top layer of soil left with pine needle litter prevents absorption of rain water by soil thus resulting in depletion of ground water and thus demolishing livestock of important food. Furthermore, dry pine needles fallen from the tree act as a barrier between the sunlight and the ground, thus slopping the growth of grasses. The specific objective of this work was:

"Bioconversion of pine needles into ethanol by using suitable microbial technology and scale up of the process for commercialization."

Bioconversion Technology: Two step process

Step1. Enzymatic Saccharification and Solubilization to sugars

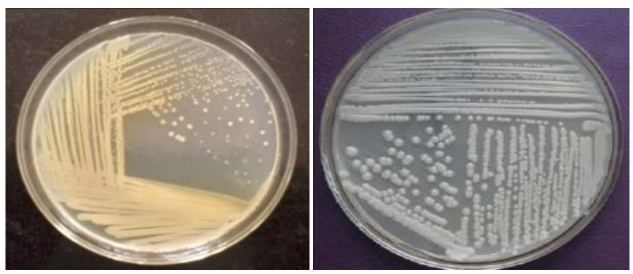
Step2. Fermentation of sugars to ethanol



Isolation and Identification of Hyper Enzyme Producing Microorganisms

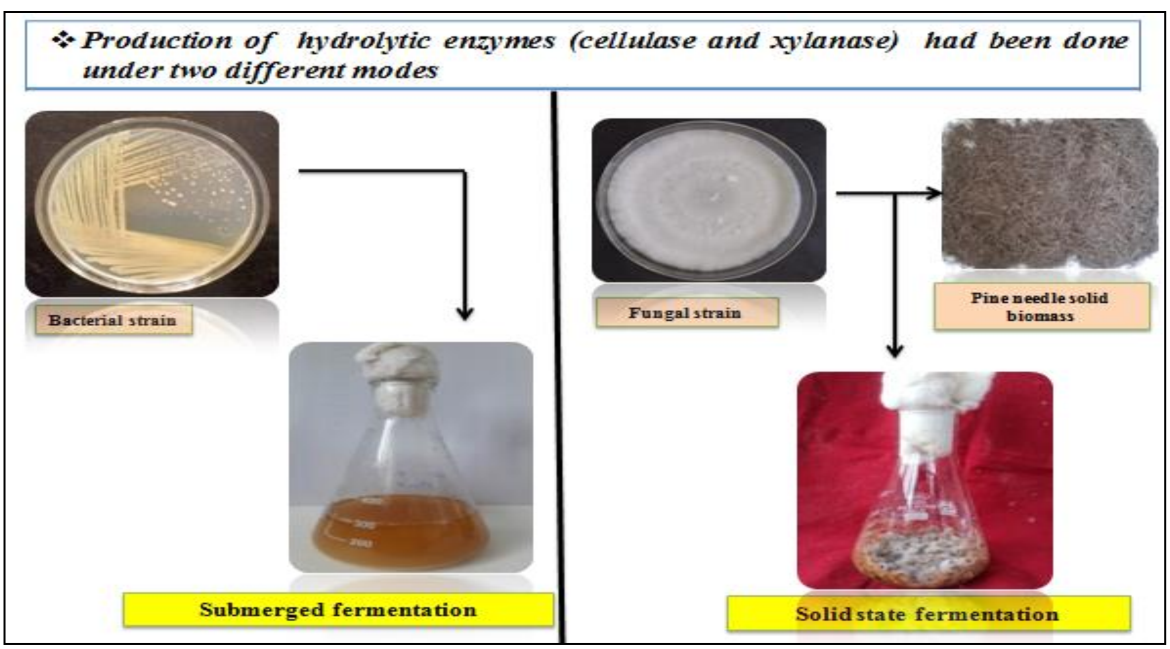
- Isolation of hyperligninolytic, hypercellulolytic and hyperxylanolytic microorganisms has been done from the rotten wood, soil and compost considering it a valuable niche of potential microorganisms for biodegradation.

Source of hyper enzyme producing microorganisms



Potential enzymes producing bacteria *Bacillus* sp. N12 and *Bacillus* sp. Kd1

Enzyme production and Purification



- Two potential bacterial strains i.e. *Bacillus* sp. N12 (M) and *Bacillus* sp. Kd1 (M) were used for the production and purification of hydrolytic enzymes (cellulase and xylanase) under submerged fermentation.

Collection and pretreatment of pine needles

- The lignocellulosic pine needles were collected from different coniferous forests of Himachal Pradesh, Uttarakhand and Jammu & Kashmir.
- Chipping of pine needles was done followed by microwave irradiation at standardized conditions.

Collected and pretreated pine needles biomass (forest waste)

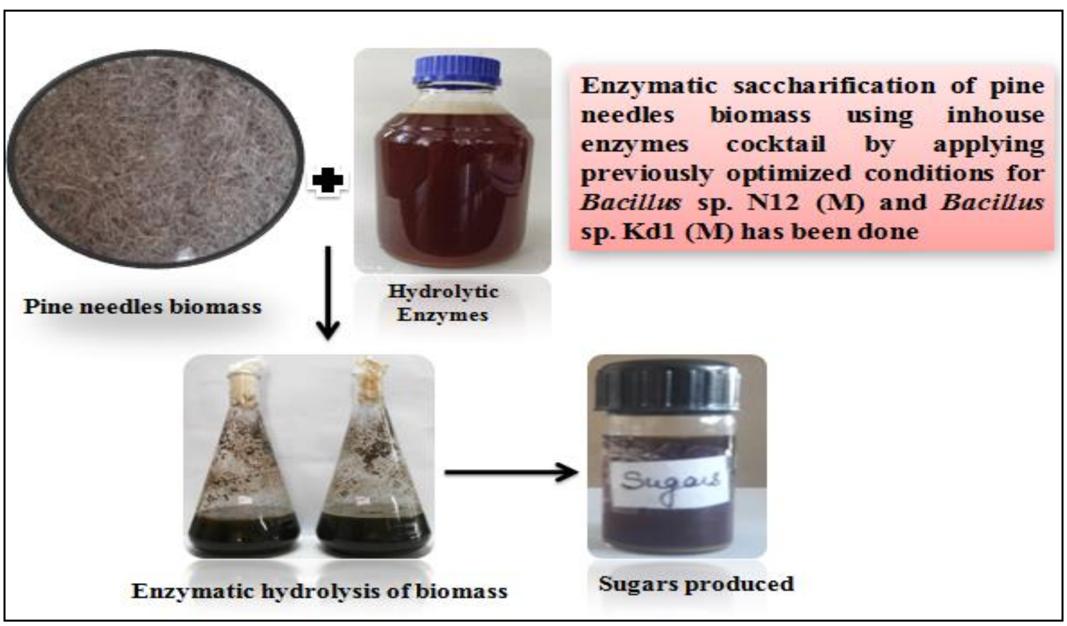
Saccharification and Production of Enhanced Reducing Sugars

Following process parameters were optimized:

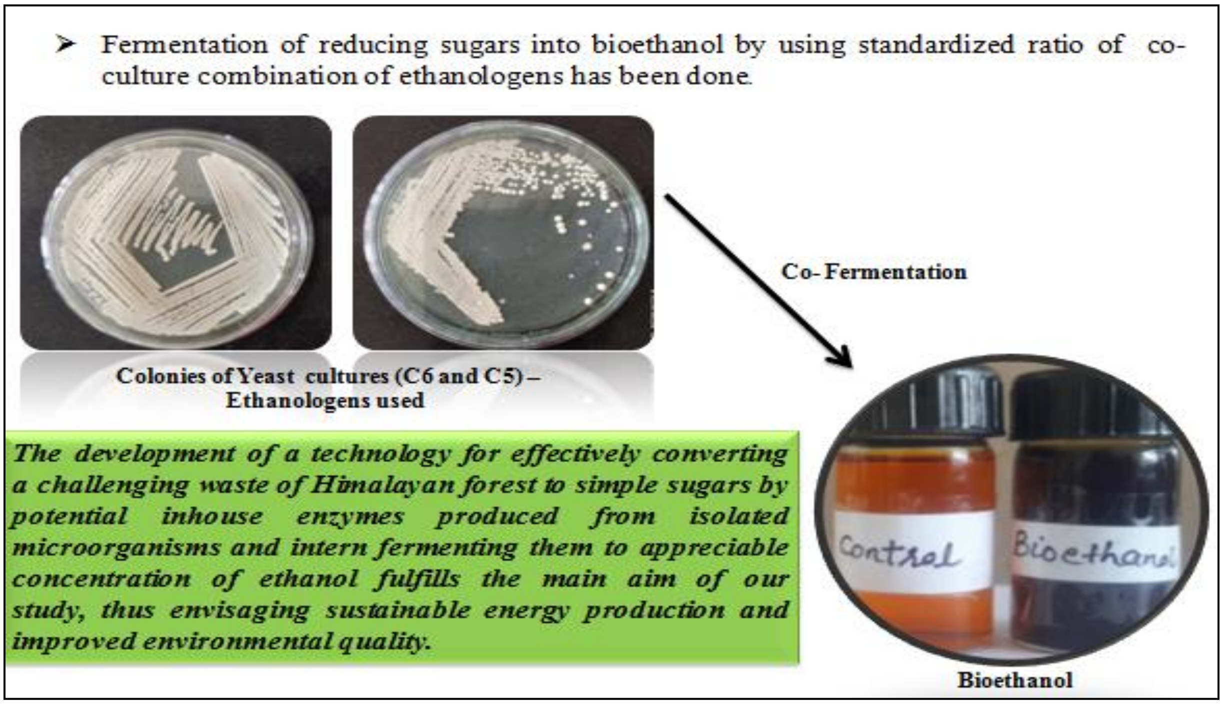
- Microwave dose, incubation period, enzyme dose, enzyme ratio and temperature

These conditions lead to 272.50 percent increase in sugars by optimizing process parameters by

- OFAT
- RSM



Bioconversion of sugars to ethanol: Fermentation



Sale up of the Fermentation Process: Bioreactor

Co-culture of Yeast used:

- ➢ *Saccharomyces cerevisiae*
- ➢ *Pichia stipitis*

Ethanol produced with fermentation efficiency of 71.50%

Scale up of the developed process

Comparative Chart of Yield and Efficiency of ethanol bioconversion process

Sr No.	1st Generation	2nd Generation
1.	Substrate: Sugarcane, corn, soyabean	Pine needles
2.	Requirement of water and land	No such requirement
3.	Water requirement: (i) Sugarcane:1800-2200 mm/ total growing period (ii) Maize: 500-800 mm (iii) Soyabean: 450-700mm	-
4.	Ethanol Production: (i) Maize (corn): 0.364 kg ethanol/kg dry corn Efficiency: 87.62% (Baustista et al., 2019) (ii) Sugarcane: 0.240 kg ethanol/kg cane Efficiency: 80.23 – 86.10% (Zuniga et al., 2013) (iii) Soyabean: 0.47g ethanol/ g dry soyabean Efficiency: 92.17 % (Nguyen et al., 2018)	Ethanol production : 0. 365 g/g of pine needles biomass Efficiency: 71.50%
5.	Cost of ethanol: Corn: Rs. 56.87/litre Sugarcane: Rs. 62.65/ litre One step process: Conversion of sugars to ethanol	Cost of Ethanol: Rs. 90.00/litre -Initially Cost will be drastically reduced after commercialization Two step process: (i) Saccharification by hydrolytic enzymes (ii) Conversion process

Recommendations

- Sustainable alternative for conventional energy source -Petroleum
- Improved environmental quality.
- Reduction in greenhouse gases
- Rural livelihood
- Reduction in forest fires in hilly states
- Prevention of soil fertility loss in hills
- Cost effective and ready to transfer commercial technology