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Bioethanol Production from Shorea Robusta Seeds by Using Micro Wave Pretreatment

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Abstract: *Bioethanol Is The Most Potent And Best Alternative To Traditional Fuels. Production Of Ethanol Form Lignocellulosic Biomass Is At Rising Demand. Shorea Robusta Seeds Have Rich Carbohydrate Content That Makes Them Suitable For The Production Of Bioethanol. In This Study, Shorea Robusta Seeds Were Pretreated Using Microwave Before They Were Subjected To Separate Hydrolysis And Fermentation (Shf). Microwave Power Of 80 W For 5min Was Found Best For The Treatment. Hydrolysis Was Done By Aspergillus Niger And Fermentation By Klebsiella Pneumoniae Strain Ut3 (Isolated Bacterial Sp. From Shorea Robusta Seeds). The Optimum Ph And Temperature For The Fermentation Was 6 And 37°C. The Results Show That Microwave Treatment Ruptures The Crystalline Structure And Increases The Surface Area For Further Hydrolysis And Fermentation Process. Shorea Robusta Seeds Can Be Efficiently Utilized For The Production Process.*

Keywords: *Aspergillus Niger, Bioethanol, Klebsiella Pneumoniae, Shorea Robusta*

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I. Introduction:

Sal Tree Occurs In Northern And Central Indian Region In The States Of Up, Mp, Chhattisgarh, Bihar, Orissa, Bengal, And Assam As A Principal Tree In Both Moderately Dry And Moist Conditions. Bastar District Of Chhattisgarh Has A Rich Depository Of *Shorea Robusta* (Sal Seeds) And Is Often Referred As "Island Of Sal Forest". Forest Residues Are Among The Major Sources Of The Total Biomass That Can Be Utilized As Food, Feed And Fuel. Biofuels Like Bioethanol Is The Best Alternative To Fossil Fuels. Bioethanol Has A Higher Octane Number, Higher Flame Speeds, Broader Flammability Limits And Higher Heat Of Vaporization Which Gives It A Higher Compression Ratio And Shorter Burn Time, Which Lead Advantages Over Gasoline In Internal Combustion Engine (Balat Et Al. 2008). A Variety Of Substrates Are Used For The Production Of Bioethanol. Sal Seeds Are Lignocellulosic, Renewable Source Of Green Energy As It Doesnot Emit Green House Gases Making The Fuel Produced From Them Ecofriendly. Pretreatment Constitutes The Major Step In Ethanol Production From Lignocellulosic Biomass. Digestibility Of Cellulosic Biomass Has Been Enhanced By The Use Of High Energy Radiation Methods Like Microwave Heating (Kitchaiya Et Al. 2003; Zhu Et Al. 2005). Microwave Pretreatmet Is A Feasible Method Which Uses The High Heating Efficiency Of A Microwave Oven And It Is Also Easy To Operate (Bjerre Et Al.1996). Separate Hydrolysis And Fermentation (Shf) Method Was Used For The Present Study. In This Process Each Step Can Be Processed At Its Optimal Operating Conditions And Separate Steps Minimize Interaction Between The Different Steps. Hydrolysis Is The Critical Step For Bioethanol Production Where Complex Carbohydrates Are Converted To Simple Monomers. The Method Less Energy And Mild Environment Conditions (Ferreira Et Al. 2009). *Aspergillus Niger* Is One Of The Efficient Cellulase Producing Organisms. It Hydrolyzes The Complex Structure By Breaking The Bonds Present Between The Units Thus Liberating Simple Sugars For The Fermentation Process. *Klebsiella Pneumoniae* Ut3 Was Used For The Fermentation Process. Fermentation Step Utilizes The Simple Sugars And Converts It To Ethanol. The Ethanol Produces Is Recovered By The Process Of Distillation.

II. Materials And Methods:

Sample Preparation
Shorea Robusta Seeds Milled To Increase Surface Area

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Pretreatment
Milled Sample Was Pre-Treated In Microwave At 60, 80, 100w For 5min

↓

Hydrolysis / Saccharification
Pre-Treated Sample Undergoes Hydrolysis To Convert Into Fermentable Sugar

↓

Fermentation
Sugars Are Fermented To Ethanol

↓

Distillation

***Shorea Robusta* Microwave Pretreatment**

The Flasks Were Put Into The Microwave Oven And Power Was Set At 60, 80, 100w For 5 Min, Respectively. After The Pretreatment Was Completed The Mixed Liquor Was Collected To Undergo Hydrolysis.

Separate Hydrolysis And Fermentation (Shf)

Enzymatic Hydrolysis Of The Sample Was Carried Out Using *Aspergillus Niger* And Ethanol Fermentation By *Klebsiella Pneumoniae* Ut3. For The Preparation Of Inoculum Of *Aspergillus Niger* The Cultures Were Maintained On Pda Plates. Inoculum Was Prepared In 250 Ml Erlenmeyer Flasks Containing 100 Ml Potato Dextrose Broth By Inoculating The Fungal Spores From The Pda Plates. The Flasks Were Incubated For Another 7 Days At 30 ^{0}c Till The Mycelial Mat Develops. This Mycelial Mat Was Used As Inoculum In Saccharification Experiments. After The Hydrolysis Process Is Completed The Flasks Were Sterilized By Autoclaving At 121°C For 30 Min. Fermentation Was Carried By Adding Inoculum *Klebsiella Pneumoniae* (2% V/V). Fermentation Was Done For 3 Days And The Ethanol Content Was Measured After Every 24 Hours.

Glucose Estimation

Glucose Content Was Determined By Dns (Dinitro-Salicylate Method), Miller (1959). The Absorbance Value Was Determined At 540 Nm By A Spectrophotometer. The Reducing Sugar In The Samples Was Calculated With Standard Curve.

Fractional Distillation And Estimation: The Fermented Sample Was Transferred Into Round Bottom Flask Fixed To A Distillation Column With Running Tap Water For Cooling. A Conical Flask Was Fixed To A Distillation Column At The Other End To Collect The Distillate. The Ethanol Content Was Measured Using Specific Gravity Method (Pharmacopoeia Of India, 1985). The Percent Of Ethanol Was Calculated Using Following Formula:

$$P^{t0} = \frac{W_3 - W_1}{W_2 - W_1} \times \text{Density Of Water At } T^0 C$$

Where P^{t0} = Specific Gravity, W1 = Weight Of Empty Specific Gravity Bottle, W2 = Weight Of Empty Bottle + Distilled Water, W3 = Weight Of Empty Bottle + Fermented Liquid (Yadav, 2003).

III. Results And Discussion

Effect On Ethanol Production After Pretreatment: Microwave Assisted Pretreatment Have Shown A Positive Effect On The Yield Of Ethanol Production. It Utilizes Thermal And Non-Thermal Effects Generated By Microwaves In Aqueous Environments. In The Thermal Method, Microwave Radiation Generates Internal Heat In The Biomass That Results From The Vibrations Of Polar Bonds In The Biomass And The Surrounding Aqueous Medium. This Creates A Hot Spot Within The Material. This Heating Results In An Explosion Effect Among The Particles And Disrupts The Recalcitrant Structures Of Lignocellulosic Biomass. In The Study It's Found That Microwave Assisted Treatment Gave Maximum Sugar Release At 80w At 5min. Further It Has Been Found In Literature That, Pretreatment Provides An Acidic Environment For Auto Hydrolysis By Releasing Acetic Acid From The Lignocellulosic Materials. Microwave Treatment Results In More Changes In Cellulosic Biomass That Includes Increase In Surface Area, Decrease Of Degree Of Polymerization And Crystallinity Of Cellulose. Separate Hydrolysis And Fermentation (Shf) Results In Enhanced Ethanol Production. The Highest Ethanol Production Was 7.49% (V/V) On 2 Day Of Incubation Period. The Amount Of Sugar Released Before And After Fermentation Estimated By Dns Method. Further, Parameters Like Temperature And Ph Were Optimized For The Production Process. It Was Found That, Temperature 35^{o}c Was Optimum For Maximum Ethanol Production Of 8.30% (V/V) And 7.22% (V/V) At Ph 7.

Table 1: Production Of Ethanol After Treatment

	Production Of Ethanol (%) V/V		
Samples/ Days	1day	2day	3day
Control (Without Treatment)	3.00	4.28	1.85

Sample (With Treatment + Shf)	5.35	**7.49**	6.28

Table 2: Optimization Of Temperature And Ph

Temperature (^{O}c)	Ethanol % (V/V)	Ph	Ethanol % (V/V)
30	5.41	5	5.52
35	**8.30**	6	6.12
40	6.68	7	**7.22**
45	4.88	8	6.28

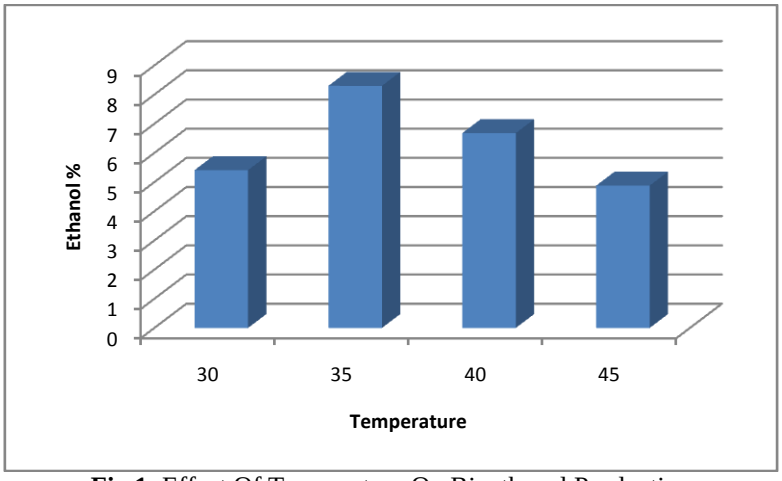

Fig 1: Effect Of Temperature On Bioethanol Production

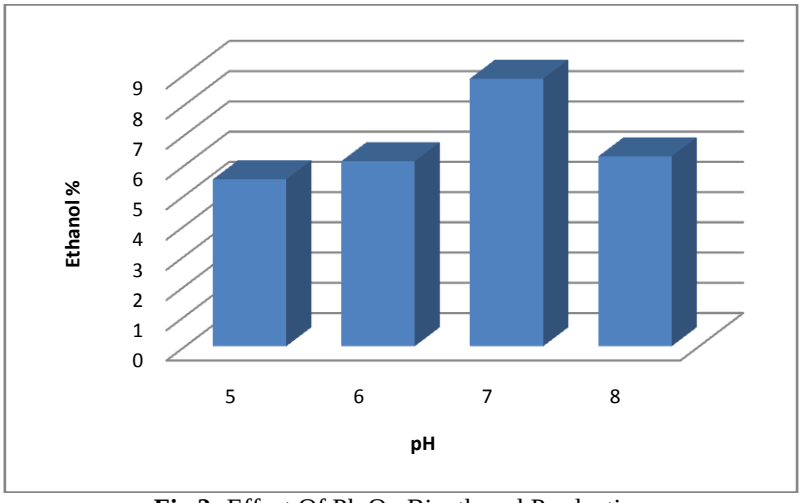

Fig 2: Effect Of Ph On Bioethanol Production

IV. Conclusions:

In Thus Study, Microwave Pretreatment Was Found Effective For Reducing The Polymerization Found In The Lignocellulosic Biomass. The Fermentation Process Was Optimized Considering Various Factors Influencing Fermentation Process And At Optimized Process The Production Was Found To Maximum With Temperature 35^{o}c And Ph 6. The Study Indicates That The Ethanol Can Widely Be Produced From The Lignocellulosic Biomass.

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