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Production of Bioethanol and Biodiesel from Water Hyacinth

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ABSTRACT
Biofuels made from biomass are being investigated as a result of the growing need for renewable and sustainable energy sources.The invasive aquatic weed water hyacinth (Eichhornia crassipe, which is distinguished by its high lignocellulosic content and quick growth, offers a biomass that may be used to produce both bioethanol and biodiesel.In order to produce bioethanol and biodiesel from water hyacinth, this study explores a holistic strategy that combines physical, chemical, biological, and enzymatic pretreatment techniques.Prior to fermentation with Saccharomyces cerevisiae to create ethanol, the dried and powdered biomass was subjected to alkali pretreatment and enzymatic hydrolysis with cellulase to liberate fermentable sugars.In order to produce biodiese, lipids were simultaneously removed from the dried biomass using Soxhlet extraction with n-hexane and transesterified using methanol and NaOH.Approximately 8.1 mL of ethanol was produced for every 100 g of biomass, according to experimental results, which showed a glucose yield of 25 g/L.Eight grams of oil were obtained through lipid extraction, and 90% of that was successfully transformed into biodiesel.FTIR, UVVis, and HPLC are examples of analytical methods that verified the successful transformation of biomass into valuable biofuels.The findings underline the feasibility of employing water hyacinth as a costeffective and ecologically acceptable feedstock for integrated bioethanol and biodiesel synthesis, offering a sustainable solution to both energy needs and aquatic weed management.

Keywords: *Bioethanol, Biodiesel, Water Hyacinth, Pretreatment, Fermentation, Transesterification, Renewable Energy*

1. INTRODUCTION

The global dependency on fossil fuels has led to several critical challenges, including environmental degradation, greenhouse gas emissions, and resource depletion. In response, renewable energy sources have gained significant attention, with biofuels such as bioethanol and biodiesel emerging as viable and sustainable alternatives. Bioethanol, typically derived through the microbial fermentation of sugars, and biodiesel, produced via the transesterification of lipids, are biodegradable, eco-friendly, and compatible with existing fuel infrastructure.First-generation biofuels, derived from food crops like sugarcane, corn, and vegetable oil, have raised serious concerns regarding food security and land use. This has driven interest in second-generation biofuels, which utilize lignocellulosic biomass such as agricultural waste, forestry residues, and invasive plant species. One such biomass of interest is water hyacinth (Eichhornia crassipes), an aquatic weed with considerable ecological and economic impact.Water hyacinth is one of the most invasive aquatic plants worldwide. It proliferates rapidly, doubling its biomass in less than two weeks under favorable conditions. It forms dense mats on water surfaces, disrupting aquatic ecosystems, depleting oxygen, blocking sunlight, and hampering biodiversity. It also impedes fishing, irrigation, and navigation, while increasing the cost of water management and disease control.

2. MATERIALS AND METHODS

Materials

Water hyacinth from Ramsagar talab Raipur Chhattisgarh, Sulphuric acid from Loba Chemie pvt.Ltd, Sodium hydroxide from Loba Chemie Pvt. Ltd, Potassium Hydroxide from from Loba Chemie Pvt. Ltd, Phenopthalin from Loba Chemie Pvt.Ltd, Citric acid from BLULUX Laboratries, PDB from Himedia Laboratries, Yeast from Pagariya food products Pvt.Ltd, N-hexane from Loba Chemie pvt.Ltd, Methanol from Himedia pvt.Ltd,

2.1 Collection and Preparation of Water Hyacinth Biomass Collection site

Water hyacinth (*Eichhornia crassipes*) was collected from [insert location], a site known for its invasive proliferation. The biomass was harvested manually, ensuring minimal ecological disturbance, and immediately transported to the laboratory in sterile polyethylene bags.

Upon arrival, the biomass was washed thoroughly with tap water followed by distilled water to remove debris and epiphytic organisms. Cleaned samples were shade-dried for 72 hours and subsequently oven-dried at 60 °C until constant weight was achieved. The dried biomass was milled using a mechanical grinder and sieved (1 mm mesh) to obtain a fine, homogenous powder, which was stored in airtight containers for subsequent analysis.

2.2 Pretreatment Methods

A) Physical pretreatment

An essential first stage in processing water hyacinth biomass for a variety of uses, including the creation of biofuel, composting, or the extraction of goods with added value, is the physical pre-treatment of the biomass. Physical pre-treatment's primary objectives are to increase surface area, break up the plant's structure, and decrease particle size in order to improve the effectiveness of subsequent chemical, biological treatments. This include the collection of water hyacinth from the site then roots were separated from leaves. Washed with tap water multiple times to remove dirt and debris. Then kept in 2to 3 days for sun dry for further process.

Chopping/Shredding:

Mechanical choppers are used to cut large stalks and leaves into smaller bits. Grinding/Milling: Using ball mills or grinders, further size reduction is achieved to produce finer biomass particles. It has advantage of expanding the surface area, improves interactions with chemicals or microorganisms in subsequent procedures.Sun drying: Low-cost, eco-friendly method; biomass is spread out and dried under sunlight Oven drying: Controlled drying at 60–105°C for faster moisture removal.

B) Chemical Pretreatment

1. Acid Pretreatment

Preparation of 1% w/v NaOH solution by mixing 1gms of NaOH in 100ml of distilled water. Soak the 10gms dried powder of water hyacinth biomass into the solution in 250ml of conical flask. Stir the solution on magnetic stirrer for 2hrs continuously uniform for mixing of the solution by heating at 60℃ then kept at incubation for 3-4 days for proper breakdown of lignocellulose. Then treated biomass was washed multiple times around 3-4 times with distilled water to remove excess of alkali in the solution at every wash the pH of solution was checked by filtering the washed water by filter paper from the biomass till the solution becomes neutral. Then the treated biomass was dried out to be analysed in FTIR and SEM.

2. Alkali Pretreatment

Preparation of 1% v/v H_2SO_4 solution by mixing 1ml of H_2SO_4 in 100ml of distilled water in 250ml of conical flask. Soak the 10gms dried powder of water hyacinth biomass into the solution. Stir the solution on magnetic stirrer for 1hrs 30 mins continuously uniform for mixing of the solution by heating at 90℃ then kept at incubation for 3-4 days for proper breakdown of lignocellulose. Then treated biomass was washed multiple times around 3-4 times with distilled water to remove excess of acid in the solution at every wash the pH of solution was checked by filtering the washed water by filter paper from the biomass till the solution becomes neutral. Then the treated biomass was dried out to be analysed in FTIR and SEM

C) Biological Pretreatment

1. Fungal Pretreatment

Firstly, the Preparation of PDA by mixing 3.9gms of PDA powder added to 100ml distilled water close the conical flask with a sterile cotton plug or aluminium foil to remove contaminants and uniformly mixing on magnetic stirrer and sterilized in autoclave for 15 min at 121℃, 15psi. Then a fungal strain was inoculated in the solidified media in aseptic condition by working in LAF and kept in incubator for 2-3 weeks for proper growth of fungi. Secondly the preparation of 250ml PDB broth to be prepared by adding 6gms of PDB powder to be added in 250ml of distilled water in 500ml of conical flask by uniformly mixing on magnetic stirrer and sterilized in autoclave for 15 min at 121℃, 15psi. After this the 10gms dried water hyacinth biomass was added to the solution and the fungal strain was inoculated from the cultured plate for the proper degradation of lignocellulosic component while keeping the conical flask in shaking incubator at 150rpm & temperature 25-30℃ for 1 week. After this the solution was centrifuged at 5000rpm for 10 mins to collect supernatant for analysis in FTIR and HPLC.

2. Bacterial Pretreatment

Firstly, the Preparation of NA by mixing 3.9gms of NA powder added to 100ml distilled water then close the conical flask with a sterile cotton plug or aluminium foil by uniformly mixing on magnetic stirrer and sterilized in autoclave for 15 min at 121℃, 15psi. Then a bacterial strain was inoculated in the solidified media in aseptic condition by working in LAF and kept in incubator for 2-3 weeks for proper growth of bacteria. Secondly the preparation of 250ml NB broth to be prepared by adding 6gms of NB powder to be added in 250ml of distilled water in 500ml of conical flask by uniformly mixing on magnetic stirrer and sterilized in autoclave for 15 min at 121℃, 15psi. After this the 10gms dried water hyacinth biomass was added to the solution and the bacterial strain was inoculated from the cultured plate for the proper degradation of

lignocellulosic component while keeping the conical flask in incubator & temperature 28-35℃ for 1 week. After this the solution was centrifuged at 5000rpm for 10 mins to collect supernatant for analysis in FTIR and HPLC in UV spectroscopy at 254nm to check the microbial growth.

D) Enzymatic Hydrolysis

Weigh 10.5 g of sodium citrate & 5.25 g of citric acid using an analytical balance. Transfer both chemicals into a 1000 mL beaker. Add 800 mL of distilled water and stir with a magnetic stirrer until fully dissolved. Measure the pH using a pH meter to adjust to pH 4.8 using 1N NaOH (if pH is too low) or 1N HCl (if pH is too high) by adding dropwise while continuously stirring. Once pH is stable at 4.8, make up the volume to 1000 mL with distilled water. Transfer the buffer solution into autoclavable glass bottles (250 mL and 500 mL capacity) to be autoclave at 121°C for 15 min at 15 psi then kept the buffer to cool at room temperature before use. Weigh 10 g of dried, physically pre-treated water hyacinth biomass using an analytical balance. Transfer the biomass into a sterile 250 mL conical flask and add 100 mL of sterile citrate buffer (50 mM, pH 4.8) into the conical flask containing the biomass. Swirl the flask gently to ensure complete wetting of the biomass. Add 1.5 mL of cellulase solution to the conical flask. Close the conical flask with a sterile cotton plug or aluminium foil. Swirl the flask gently to ensure uniform enzyme distribution in the buffer-biomass mixture. Verify pH remains stable at 4.8 after enzyme addition using a pH meter then ensure enzyme-buffer mixture is homogeneous and no biomass settling occurs. The solution was then centrifuge at 5000 rpm for 10 mins to collect the supernatant as hydrolysate to be analysed in FTIR and HPLC.

2.3 Bioethanol Production

2.3.1 Hydrolysis (Acid/Enzymatic)

Hydrolysis is the initial step aimed at depolymerizing complex carbohydrates (cellulose and hemicellulose) into fermentable monomeric sugars.

a. Acid Hydrolysis

Dried and milled biomass (e.g., water hyacinth) was treated with 1.5% H_2SO_4 in a 1:10 ratio (w/v). The mixture was autoclaved at 121°C for 30 minutes. After cooling, the slurry was filtered, and the supernatant containing hydrolysate was neutralized with $Ca(OH)_2$ to pH 5–6.

b. Enzymatic Hydrolysis

Pre-treated biomass was incubated with cellulase (15 FPU/g substrate) in 50 mM citrate buffer. Incubation was carried out at 50°C for 72 hours with shaking (150 rpm). The hydrolysate was centrifuged, and the supernatant collected for sugar and fermentation analysis.

2.3.2 Fermentation (Using Saccharomyces cerevisiae)

Yeast was activated in 2% glucose medium for 12 hours. The hydrolysate was supplemented with yeast extract (0.5%), peptone (1%) and adjusted to pH 5.0. Fermentation was conducted at 30°C for 72 hours under anaerobic conditions. Samples were drawn at 24-hour intervals and analyzed via DNS assay for reducing sugars and ethanol via GC or UV-spectrophotometry.

2.3.3 Distillation

Distillation separates ethanol from the fermentation broth based on its lower boiling point (78.4°C).Fermented broth was filtered to remove cells. The clear liquid was distilled using a distillation setup or rotary evaporator. Ethanol was collected and analyzed for purity and volume.

2.3.4 Ethanol Yield Calculation

Ethanol yield was calculated based on the initial sugar concentration and final ethanol concentration using the formula:

a. Theoretical Yield:

Theoretical Ethanol = (Glucose (g) × 0.51) / Volume of hydrolysate

b. Actual Yield (%):

Ethanol Yield (%) = (Actual ethanol obtained / Theoretical ethanol) × 100

Example:

Initial glucose: 10 g/L

Actual ethanol: 4.5 g/L

Theoretical ethanol: 5.1 g/L

Yield = (4.5 / 5.1) × 100 = 88.2%

FERMENTATION

Fermentation is a crucial step in bioethanol production, where fermentable sugars obtained from hydrolysis of biomass are converted into ethanol by microorganisms, primarily Saccharomyces cerevisiae (yeast). This process occurs under anaerobic conditions and is optimized for temperature, pH, sugar concentration, and yeast inoculum size.

Step 1.1: Preparation of Yeast Culture Medium

Dissolve the ingredients in 500 mL of distilled water in a 1000 mL conical flask. Use a magnetic stirrer to mix until fully dissolved. Adjust the volume to 1l using additional distilled water.Use a pH meter to measure the pH. The final pH should be between 5.0 and 5.5, as this is optimal for Saccharomyces cerevisiae growth.Transfer the medium into sterile 250 mL or 500 mL Erlenmeyer flasks. Cover the flasks with cotton plugs or aluminium foil to prevent contamination. Autoclave at 121°C, 15 psi for 15 minutes to kill all unwanted microorganisms.

Step 1.2: Yeast Inoculation & Growth

Cool the sterilized medium to room temperature (25-30°C) before inoculation.Take a fresh Saccharomyces cerevisiae culture (freeze-dried or from a yeast slant). Suspend 1 g of yeast cells in 10 mL of sterile distilled water. Shake gently to disperse the cells evenly.Transfer 10 mL of yeast suspension (1% v/v inoculum) into 500 mL of yeast culture medium using a sterile pipette.Place the inoculated flasks in an incubator shaker at 30°C with 150 rpm shaking for 24 hours. This promotes aerobic growth, allowing the yeast to multiply rapidly.

Step 1.3: Yeast Viability & Growth Monitoring

A. Optical Density (OD600) Measurement Using Spectrophotometer

Take a 1 mL sample of the yeast culture and dilute it with 9 mL of sterile distilled water. Measure the optical density (OD600) using a spectrophotometer at 600 nm. A well-grown yeast culture should have an OD600 between 0.8 - 1.2 after 24 hours.

Step 3: Fermentation Process

Incubate the inoculated flask in a shaking incubator at 30°C for 48-72 hours. Maintain anaerobic conditions by using an airlock or sealing the flask with parafilm. Observe bubble formation (CO_2 evolution), indicating fermentation activity. The solution is then centrifuged at 8000rpm for 10mins for analysis of FTIR and DNS ASSAY.

Preparation of DNS Reagent

Dissolve 1 g of 3,5-Dinitrosalicylic acid (DNS) in 20 mL of 2 M NaOH. Add 30 g of sodium potassium tartrate (Rochelle salt) and dissolve completely. Make up the volume to 100 mL with distilled water. Store in a dark bottle at 4°C.

Preparation of Standard Glucose Curve

Prepare glucose solutions in 0.1 M citrate buffer (pH 4.8) ranging from 0.1 to 1 mg/mL. Take 1 mL of each standard glucose solution in test tubes.

Performing the DNS Assay

(A) For Standard Glucose Curve

Take 1 mL of each standard glucose solution in separate test tubes. Add 1 mL of DNS reagent to each test tube. Vortex or mix well.

(B) For Hydrolysed Biomass Sample

Take 1 mL of hydrolysed sample in a separate test tube. Add 1 mL of DNS reagent and mix well.Place all tubes in a boiling water bath at 100°C for 5 mins. A reddish-brown colour develops, indicating the presence of reducing sugars.Cool the tubes to room temperature. Measure absorbance at 540 nm using a UV-Vis spectrophotometer.

TRANSESTERIFICATION OF BIODISEL

Biodiesel is a renewable energy source produced through the transesterification of oils and fats using alcohol and a catalyst. Water hyacinth, an aquatic weed with high biomass yield, contains lipids that can be extracted and converted into biodiesel. This experiment details the materials, methods, and procedures required for biodiesel production from water hyacinth biomass

Step 6.4.1) Lipid Extraction

6.4.1.1) Preparation of Biomass for Extraction:

To ensure that the dried water hyacinth biomass is finely ground and suitable for effective lipid extraction.

Sample Preparation:

Weigh 20 g of dried and ground biomass powder using an analytical balance. Ensure the biomass has a uniform particle size

of 200–500 µm to increase surface area for lipid extraction.If larger particles are present, sieve the sample through a 100-mesh sieve.Wash all glassware (Soxhlet apparatus, round-bottom flask, condenser) with distilled water and dry in a hot-air oven at 105°C for 1 hour before use. Rinse with n-hexane to remove residual impurities.

6.4.1.2 Solvent Extraction Using a Soxhlet Extractor:

To extract the maximum amount of lipids from the biomass using a continuous solvent reflux process.

Assembling the Soxhlet Extraction Setup:

Place 20 g of the biomass powder inside a thimble or folded filter paper.Load the thimble into the main chamber of the Soxhlet extractor.Attach the Soxhlet extractor to a 500 mL round-bottom flask containing 200 mL of n-hexane (used as the extraction solvent).Connect a reflux condenser to the Soxhlet extractor.Place the entire setup on a heating mantle or water bath at 70°C.Heat the system to initiate solvent evaporation.The n-hexane vapors rise into the condenser, where they cool and condense into liquid.The condensed solvent drips into the biomass thimble, soaking the powdered sample and dissolving lipids.When the Soxhlet chamber fills to a certain level, the solvent siphons back into the round-bottom flask, carrying dissolved lipids.This cycle repeats continuously for 6 hours, ensuring thorough lipid extraction.

FIG 1: DISTILLATION TO EXTRACT BIOETHANOL

FIG 2: SOXHLET FOR LIPID EXTRACTION

6.4.1.3 Solvent Recovery and Oil Collection:

To separate the extracted oil from the solvent n-hexane using a rotary evaporator. After 6 hours of extraction, allow the system to cool to room temperature. Transfer the n-hexane-lipid mixture into a pre-weighed glass beaker. Filter out any residual solid particles using a Buchner funnel with vacuum filtration.Transfer the filtered lipid-hexane solution into a 250 mL round-bottom flask.Attach the flask to a rotary evaporator set to 40–50°C under reduced pressure.The rotating motion increases the evaporation surface area, while the vacuum system removes n-hexane vapours.The hexane vapours condense and are collected for reuse.The residue in the flask is crude lipid extract.Transfer the extracted lipid into a weighed glass vial.Place in a vacuum desiccator containing anhydrous sodium sulfate to remove residual moisture.Store at 4°C in an airtight container for further analysis.

3. PRE-TREATMENT (IF FREE FATTY ACID (FFA) > 2%)

3.1 Determination of Free Fatty Acid (FFA) Content

Before proceeding with pre-treatment, the FFA content of the extracted oil must be measured.

Take 10 mL of isopropyl alcohol in a conical flask.Add 1 mL of oil sample to the alcohol and mix well.Add 2–3 drops of phenolphthalein indicator.Titrate the solution with 0.1 N KOH solution until a persistent pink color appears.Record the volume of KOH used.

3.2 Procedure for Pre-treatment (Esterification of FFAs) Measure the extracted oil to be treated (100 g of oil).Calculate the required methanol using a 1:6 molar ratio (oil to methanol). Transfer both the oil and methanol into a 250 mL round-bottom flask.Add 1% (by weight) sulfuric acid (H_2SO_4) as a catalyst. Example: If using 100 g of oil, add 1 g of H_2SO_4. Stir the mixture vigorously for uniform catalyst distribution.Heat the mixture to 60°C while stirring continuously for 1 hour. Use a magnetic stirrer with hot plate to maintain constant mixing.Maintain a reflux setup with a condenser to prevent methanol evaporation.Monitor the pH and viscosity change, indicating conversion of FFAs to esters.After 1 hour, turn off the heat and allow the mixture to cool to room temperature.Transfer the reaction mixture to a separating funnel.

Step 1: Removal of Excess Methanol & Acid

Carefully decant the top layer (esterified oil + methanol) into another container. Wash with warm distilled water (50°C) 3–4 times to remove residual acid. Use anhydrous sodium sulfate (Na_2SO_4) to dry the oil and remove any remaining water.

Step 2: Recovery of Excess Methanol

- The excess methanol can be recovered using a rotary evaporator at 50°C under vacuum for reuse.

TRANSTERIFICATION PROCESS

Measure 100 g of extracted oil (or adjust accordingly based on available oil).In a separate conical flask, dissolve 1 g of KOH or NaOH (1% by oil weight) in 20 mL of methanol.Stir continuously for 5–10 minutes until the catalyst completely dissolves.The solution should turn clear—this is methoxide solution ($CH_3O^- + Na^+/K^+$).Transfer the 100 g of pre-treated oil into a 500 mL round-bottom flask or conical flask.Slowly add the methoxide solution to the oil while stirring continuously.Set the molar ratio of oil to methanol at 1:6 (for 100 g oil, use 60 mL of methanol).Place the flask on a magnetic stirrer with hot plate and heat the mixture to 55–65°C.Maintain a constant temperature of 60°C ± 5°C (optimum for reaction).Stir the mixture at 600 rpm for 90 minutes to ensure complete reaction.During the reaction, triglycerides are converted into biodiesel (methyl esters) and glycerol:After 90 minutes, turn off the stirrer and allow the reaction mixture to cool to room temperature.Transfer the mixture into a separating funnel (1L capacity).Let it stand undisturbed overnight (8–12 hours) to allow gravity-driven phase separation.

Phase Separation:

1. Bottom layer (denser, darker layer) → Glycerol (by-product).
2. Top layer (lighter, yellowish layer) → Biodiesel (FAMEs).
3. Carefully drain the glycerol from the bottom by opening the stopcock of the separating funnel.
4. Collect biodiesel in a separate clean container for further purification.

FIG 3: SEPERATION OF GLYCEROL AND BIODISEL

Post-Treatment & Purification of Biodiesel

Transfer biodiesel into a clean conical flask.Add an equal volume of warm distilled water (50°C) to biodiesel.Gently mix the contents for 2–3 minutes, allowing soap and residual catalyst to dissolve in water.Let the mixture settle for 30 minutes—water (impurities) will settle at the bottom.Carefully drain the bottom water layer and repeat the washing process 2–3 times until the water is clear.Remove any remaining water by adding anhydrous sodium sulphate (Na_2SO_4) (drying agent) to biodiesel.Stir gently for 10–15 minutes until biodiesel appears clear.Filter out the sodium sulphate using a Buchner funnel with vacuum filtration.

RESULT AND DISCUSSION

1. **FTIR RESULT**

A) ACID PRETREATMENT

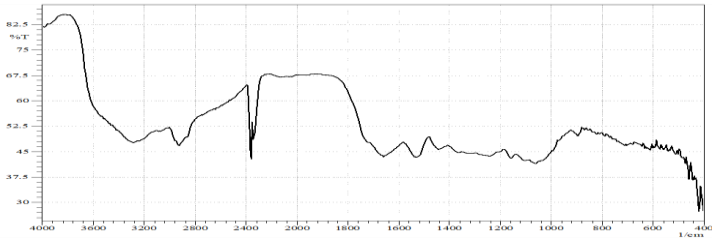

FIG 4: FTIR ANALYSIS OF ACID PRETREATMENT

Wavenumber (cm^{-1})	Peak Assignment	Functional Group
~3420	O–H stretching	Hydroxyl groups
~2920	C–H stretching	Aliphatic CH (–CH_2–)
~1730	C=O stretching	Ester/Carboxylic acid
~1620	C=C or C=O stretching	Aromatic skeletal vibration
~1510	Aromatic skeletal vibrations	Lignin
~1420	CH_2 bending	Cellulose/hemicellulose
~1375	C–H bending	Cellulose
~1240	C–O stretching	Lignin/hemicellulose
~1240	C–O stretching	Polysaccharides
~890	β-glycosidic linkages	Cellulose

B) ALKALI PRETREATMENT

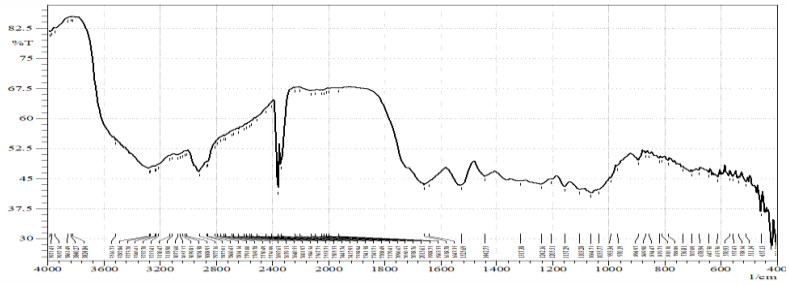

FIG 5: FTIR ANALYSIS OF ALKALI PRETREATMENT

Wavenumber (cm^{-1})	Peak Assignment	Functional Group
3425	–OH stretching	Hydroxyl group (alcohols, phenols)
2925	C–H stretching	Aliphatic –CH_2 groups
2850	C–H symmetric stretch	Aliphatic chains
1730	C=O stretching	Ester groups in hemicellulose or waxes
1625	C=C aromatic stretching	Lignin aromatic rings
1510	Aromatic skeletal vibration	Lignin-specific bands

1420	CH_2 scissoring	Cellulose and lignin
1375	C–H deformation	Cellulose and hemicellulose
1240	C–O stretching	Syringyl ring or C–O in lignin
1035	C–O–C or C–O stretching	Polysaccharide backbone
890	β-glycosidic linkages	Cellulose fingerprint region
600–500	Aromatic ring deformation	Lignin-derived vibrations

SEM RESULT

A) ACID PRETREATMENT

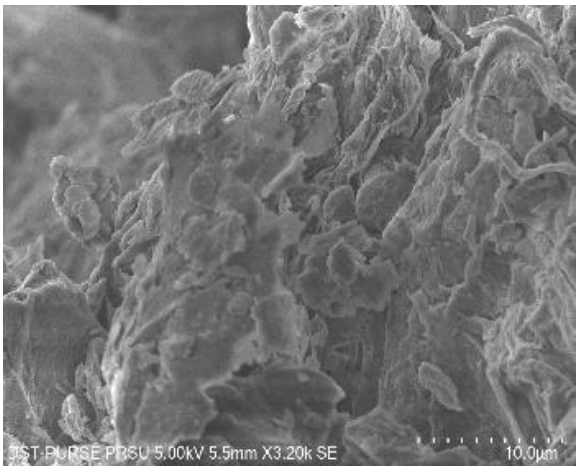

FIG 6: SEM ANALYSIS OF ACID PRETREATMENT

The SEM micrograph of acid-pretreated *Eichhornia crassipes* (Figure X) at 1920× magnification (scale bar: 30 μm) demonstrates pronounced morphological disruption. The fiber structure appears highly irregular with evident **surface roughening, cracks, and fibrillation.** The compact cell wall matrix seen in untreated samples is replaced by a **loosened, porous structure,** indicating **extensive breakdown of hemicellulose and partial delignification.**

B) ALKALI PRETREATMENT

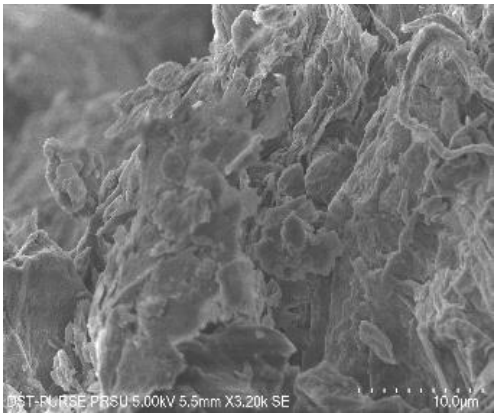

FIG 7: SEM ANALYSIS OF ALKALI PRETREATMENT

This scanning electron microscope (SEM) image shows a material surface at a magnification of 1.90k times. The scale bar indicates that 30.0 μm is the length of the horizontal bar at the bottom right of the image.

BIOLOGICAL PRETREATMENT

A) FUNGAL PRETREATMENT (OD RESULT)

Time (hours)	OD_{600} Reading	Interpretation
0	0.10	Initial biomass suspension
24	0.25	Fungal growth begins
48	0.40	Increased fungal proliferation
72	0.35	Peak growth; onset of biomass degradation
96	0.20	Continued degradation; reduced turbidity
120	0.15	Further degradation; stabilization

FTIR RESULT

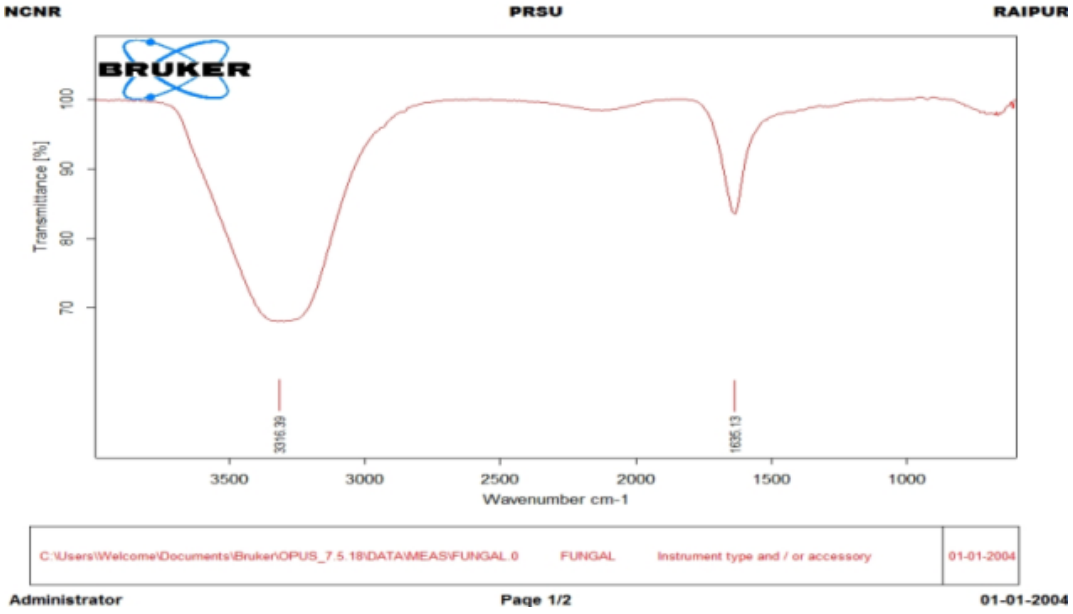

FIG 8: FTIR ANALYSIS OF FUNGAL PRETREATMENT

Peak Wavenumber (cm^{-1})	Approximate Transmittance (%)	Likely Functional Group(s) and Vibration Mode(s)
~3316	~68	**O-H stretching** (Alcohols, Carboxylic Acids, Water);
~1635	~82	**C=O stretching** (Amides - Amide I band);

HPLC RESULT

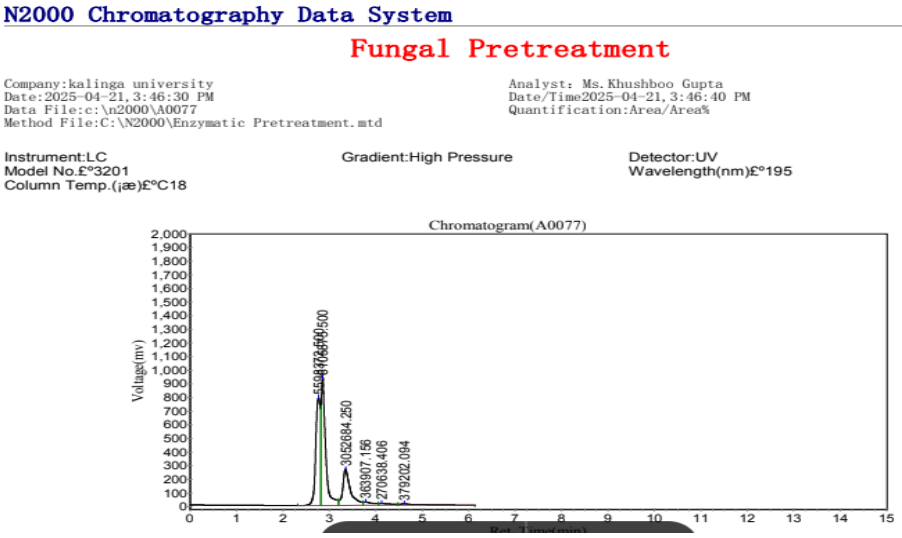

FIG 9: HPLC ANALYSIS OF FUNGAL PRETREATMENT

Peak Number (Approximate, from left to right)	Retention Time (min)	Approximate Peak Height (mV)
1	~2.5	~1850
2	~3.0	~600
3	~3.2	~400
4 (shoulder on peak 3)	~3.4	~250
5 (very small)	~3.6	~100 or less
6 (very small)	~3.8	~100 or less

B) BACTERIAL PRETREATMENT

OD RESULT

Bacterial mass with water hyacinth	Time of UV exposure	Reading
2ml	30 sec	2.06
2ml	1 min	1.31
2ml	3 min	1.76
2ml	5 min	1.17
2ml	10 min	1.34

HPLC RESULT

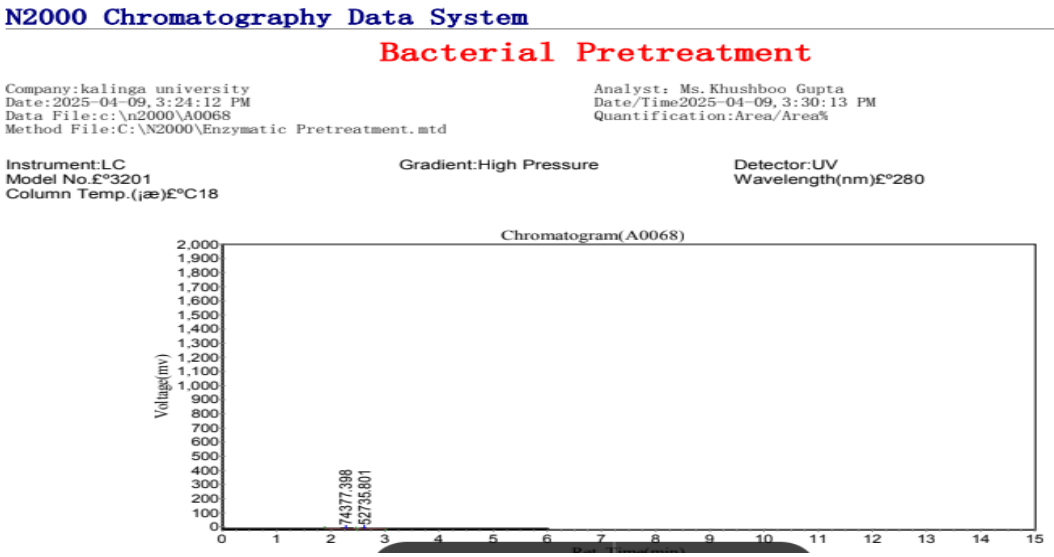

FIG 10: HPLC ANALYSIS OF BACTERIAL PRETREATMENT

Peak Number (Approximate, from left to right)	Retention Time (min)	Approximate Peak Height (mV)
1	~2.0	~1900
2	~2.5	~1800

FTIR RESULT

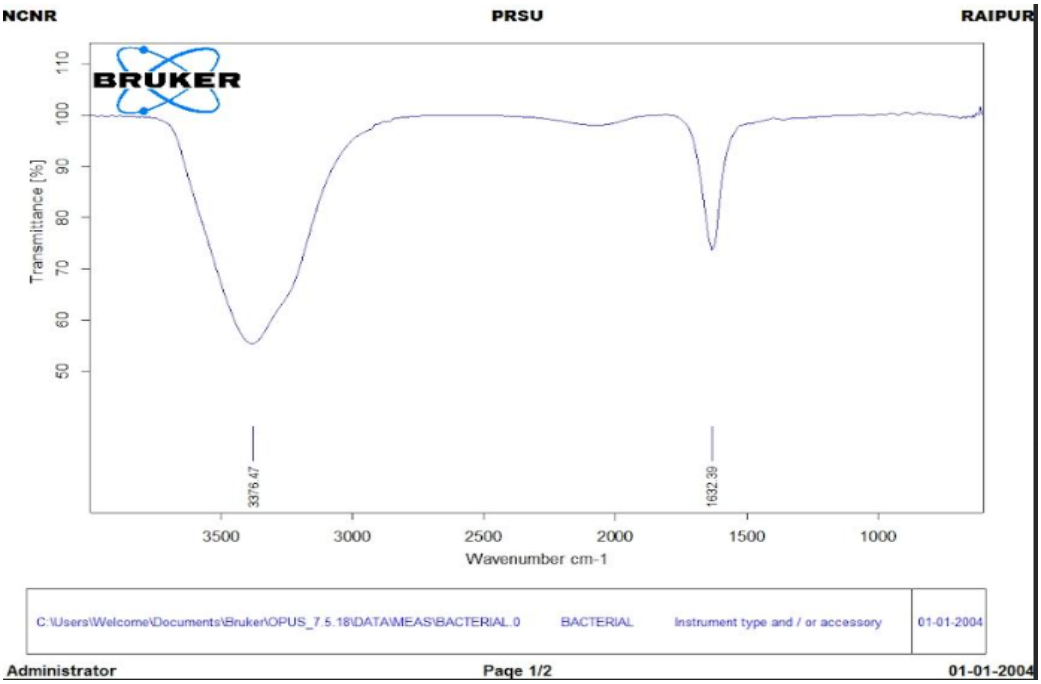

FIG 11: FTIR ANALYSIS OF BACTERIAL PRETREATMENT

Peak Wavenumber (cm^{-1})	Approximate Transmittance (%)	Likely Functional Group(s) and Vibration Mode(s)
~3376	~59	**O-H stretching** (Alcohols, Carboxylic Acids, Water);
~1632	~68	**C=O stretching** (Amides - Amide I band); possibly **C=C stretching** (Alkenes, Aromatic rings)**;**

ENZYMATIC HYDROLYSIS

S.N0.	Vol. of standard glucose (ml)	Concn of glucose	Vol. of Dis. Water (ml)	Vol. of DNS reagent	Absorbance
0	Blank	-	3	1	0
1	0.3	100	2.7	1	0.61
2	0.6	200	2.4	1	1.30
3	0.9	300	2.1	1	1.91
4	1.2	400	1.8	1	2.20
5	1.5	500	1.5	1	2.50
6	Test sample	-	-	1	2.90

HPLC RESULT

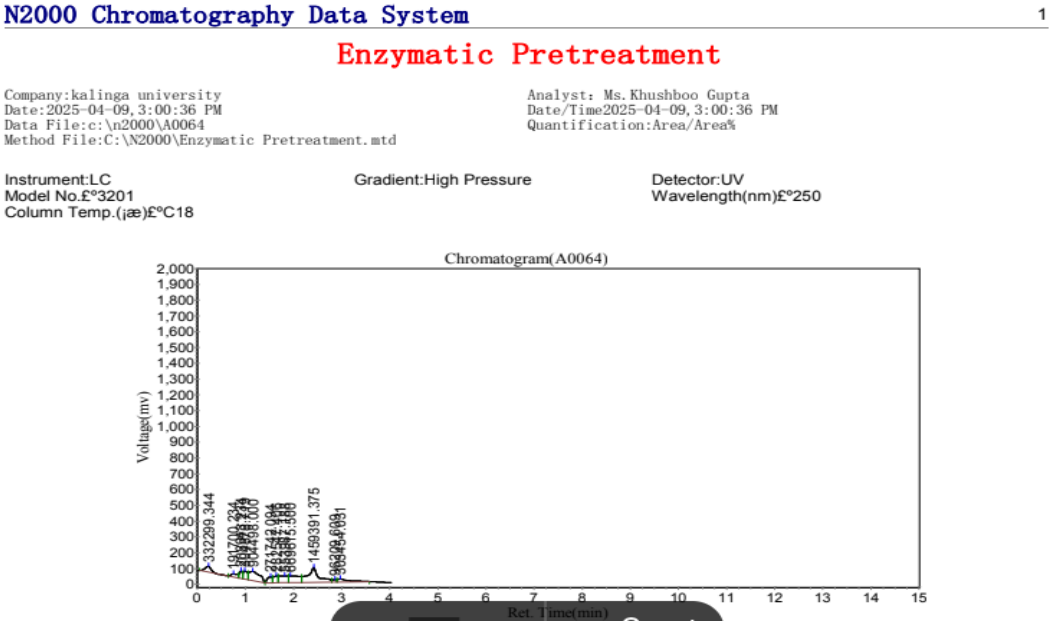

FIG 12: HPLC ANALYSIS OF ENZYMATIC HYDROLYSIS

Peak Number (Approximate, from left to right)	Approximate Retention Time (min)	Approximate Peak Height (mV)
1	~0.5	~350
2	~0.7	~250
3	~0.9	~450
4	~1.3	~550
5	~1.5	~300
6	~1.7	~250
7 (shoulder on peak 6)	~1.8	~150
8	~2.0	~200

FTIR RESULT

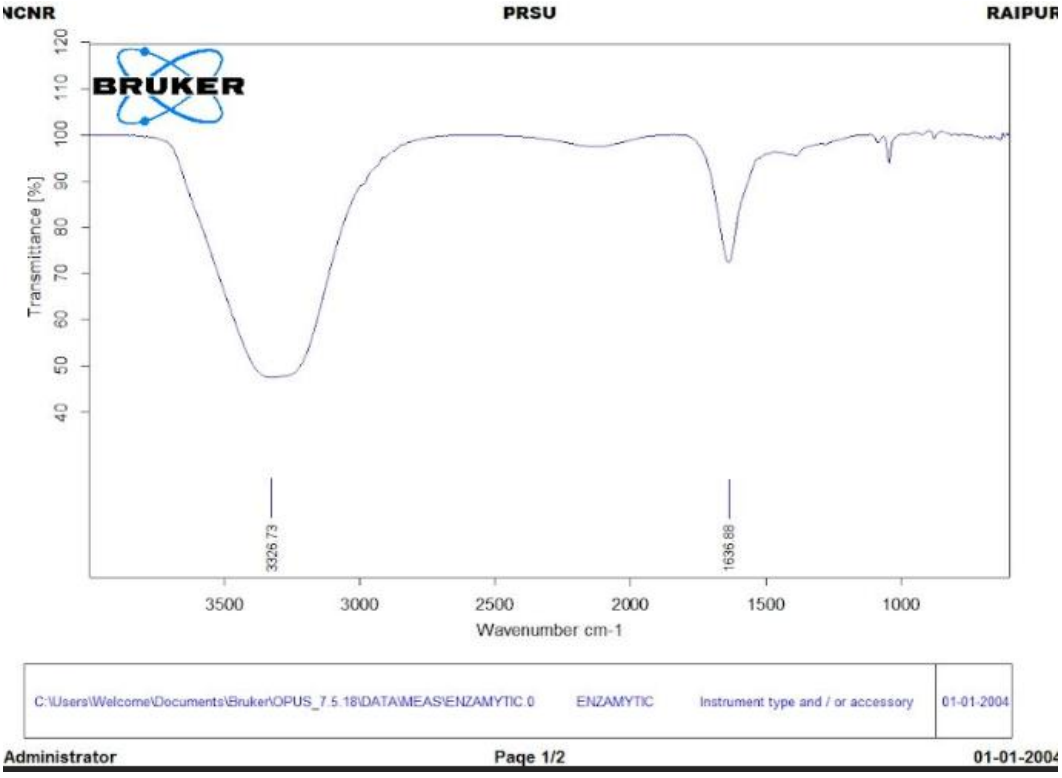

FIG 13: FTIR ANALYSIS OF ENZYMATIC HYDROLYSIS

Peak (cm^{-1})	Approx. Functional Group Range	Probable Functional Group	Type of Vibration
3237.13	3200–3550	–OH (hydroxyl) / –NH stretch	Hydrogen-bonded O–H/N–H stretch (broad)
1638.88	1600–1700	C=O (carbonyl) or C=C	C=O stretching (amide I /

			carboxylic) or C=C in aromatics

FERMENTATION (BIOETHANOL)

FTIR RESULT

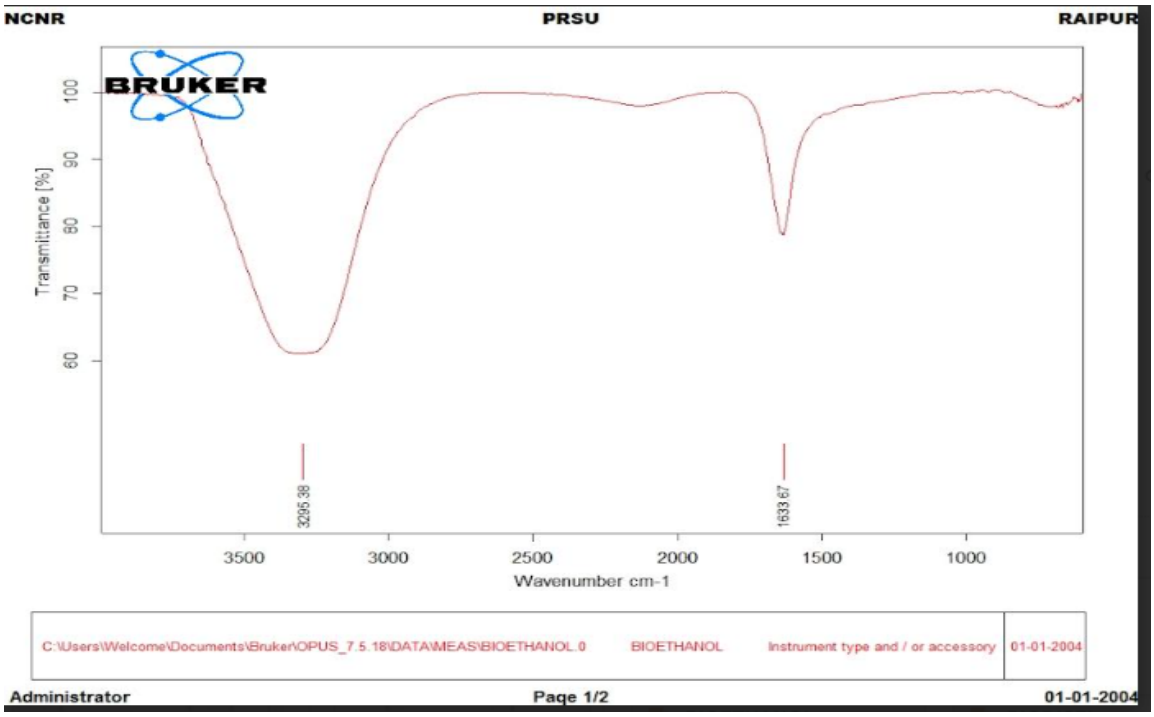

FIG 14: FTIR ANALYSIS OF FERMENTATION FOR BIOETHANOL

Peak (cm^{-1})	Approx. Functional Group Range	Probable Functional Group	Type of Vibration
3285.98	3200–3550	O–H (alcohol/hydroxyl) stretch	Strong, broad hydrogen-bonded O–H stretch
1637.91	1600–1700	C=O or C=C or O–H bend	Water deformation or unsaturated C=C

GC-MS RESULT

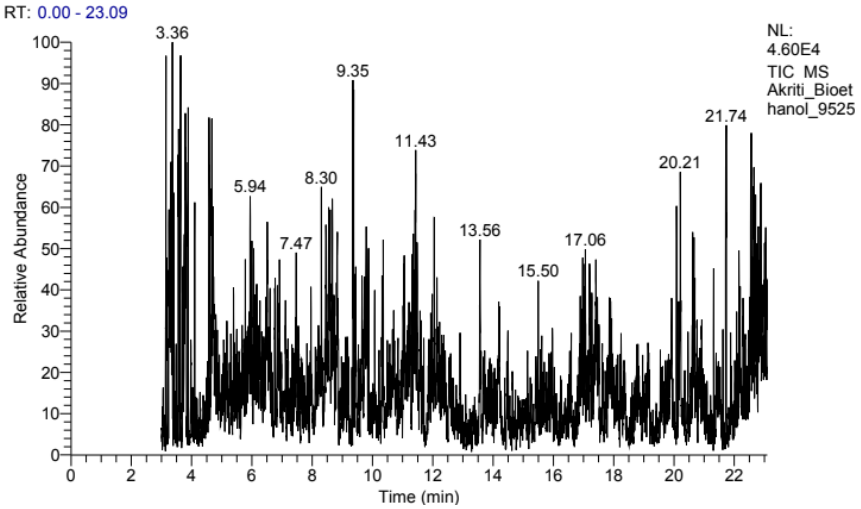

FIG 15: GC-MS ANALYSIS OF BIOETHANOL

S. No.	Retention Time (min)	Relative Abundance	Tentative Compound
1	3.36	~95	Ethanol
2	5.94	~50	Acetaldehyde
3	7.47	~45	Methanol / Isopropanol
4	8.30	~40	Ethyl acetate
5	9.35	~70	1-Propanol
6	11.43	~60	Isobutanol / 2-Butanol
7	13.56	~55	Acetic acid
8	15.50	~50	Furfural
9	17.06	~45	Levulinic acid
10	20.21	~65	Phenolic compound (e.g., guaiacol)
11	21.74	~60	Long-chain alcohol/ester

TRANSTERIFICATION (BIODIESEL)

FTIR RESULT

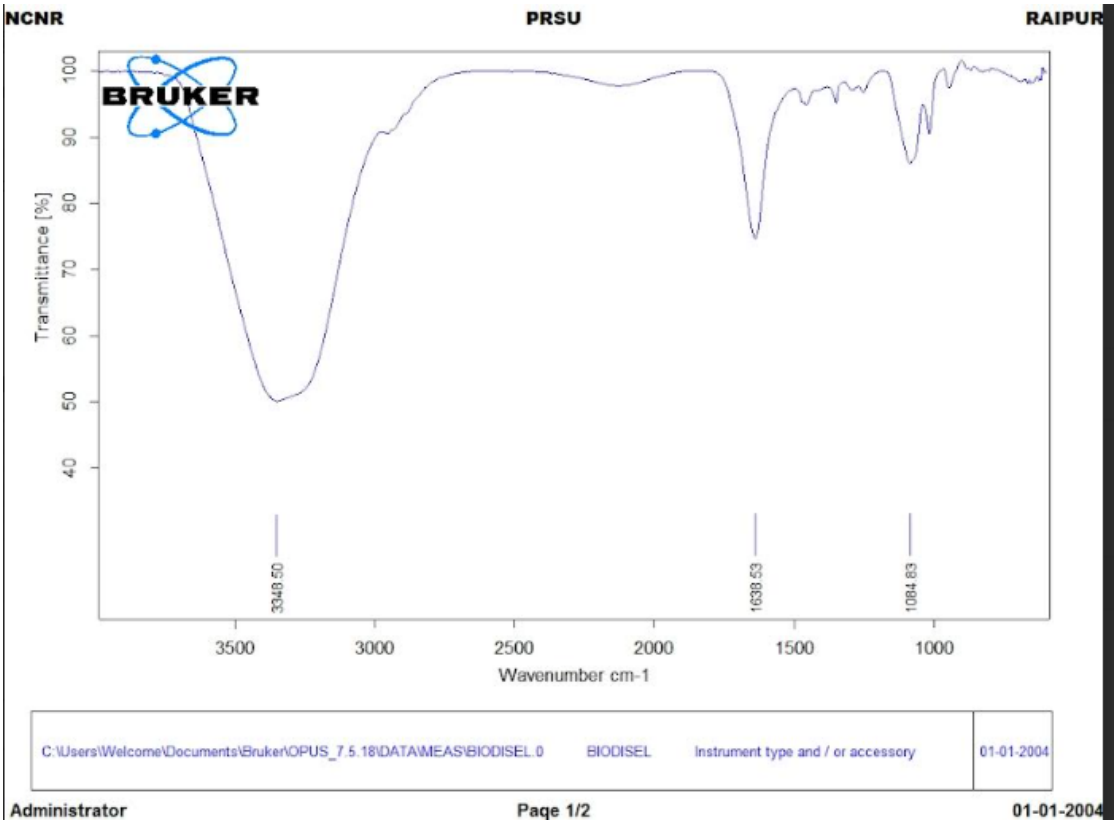

FIG 16: FTIR ANALYSIS OF BIODISEL

Peak Wavenumber (cm^{-1})	Approximate Transmittance (%)	Likely Functional Group(s) and Vibration Mode(s)
~3348	~48	**O-H stretching** (Carboxylic Acids, Alcohols
~2924 (Shoulder)	~88	**C-H stretching** (Alkanes - CH_2 asymmetric stretch).
~2853 (Shoulder)	~90	**C-H stretching** (Alkanes - CH_2 symmetric stretch)
~1743 (Not clearly labeled but a strong peak)	~42	**C=O stretching** (Esters).
~1638	~63	**C=C stretching** (Alkenes)
~1435 (Not clearly labeled but a noticeable bend)	~92	**C-H bending** (Alkanes - CH_2 bending).
~1195 (Not clearly labeled but a strong peak in the region)	~75	**C-O stretching** (Esters).

GC-MS RESULT

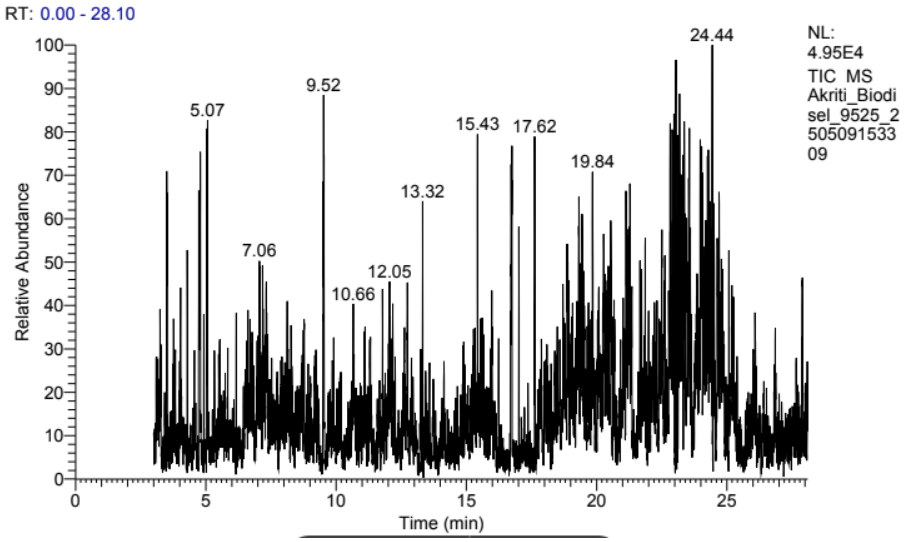

FIG 17: GC-MS ANALYSIS OF BIODISEL

Peak Number	Retention Time (min)	Relative Abundance (%)
1	5.07	~82
2	9.52	~85
3	13.32	~65
4	15.43	~78
5	17.62	~75
6	19.84	~70
7	24.44	~98

4. CONCLUSION

Water hyacinths were subjected to different pretreatments and among them H2SO4 pretreatment gave best results. The reducing sugar and glucose yields from enzymatic hydrolysis were maximum at high temperature (50 C) and acidic pH (5.0–5.5) with 5 % substrate and 30 FPU/g enzyme loading. The concentrated hydrolysate (with 5 % glucose) was subjected for ethanol production through response surface methodology. During co-culture, ethanol production was maximum (13.6 mg/ml) at optimum fermentation time of 37.7 h, fermentation pH of 6.41, and Saccharomyces to Zymomonas ratio of 1. Water hyacinth is one of the worst weeds in the aquatic ecosystem but it is also a potential resource of biomass available in many tropical regions of the world and with a proper technical knowledge can be used as feedstock for small-scale distributed production of fuel ethanol.Water hyacinth offers a promising and sustainable pathway for biodiesel production, presenting various advantages, including waste utilization, renewable energy generation, and environmental benefits. While some challenges exist, research and development efforts can further optimize the process and enhance the economic and environmental sustainability of water hyacinth-based biodiesel.

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