Final Completion Report (2016-2020)
Indo-Brazil project ((DBT- India and MCTI-CNPq-Brazil)

Title: Integrated Biorefinery Approach towards production of sustainable fuel and chemicals from Algal biobased systems.

Participating Institutions (India):

1. **Department of Energy**
   Tezpur University
2. **Department of Chemical Engineering**
   Indian Institute of Technology, Guwahati
3. **Department of Biotechnology**
   Gauhati University

Submitted to

Department of Biotechnology,
Govt. of India, New Delhi

Submitted by

Professor D. Deka
Lead Principal Investigator-India
Indo-Brazil Project
Department of Energy
Tezpur University

DEPARTMENT OF BIOTECHNOLOGY, MOST, INDIA
&
MCTI, BRAZIL

Submission of Final Report

1. **Title:** "Integrated Biorefinery Approach towards Production of Sustainable Fuel and Chemicals from Algal Bio-based Systems"
2. **Project Investigators and address:**

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3. **Date of sanction:** 15.06.2016
4. **Date of completion:** 14.12.2020
5. **Approved cost:** Rs. 244.17751 lakhs
6. **Budget released:** Rs. 192.87546 lakhs
7. **Objectives**
    a) To select and screen the microalgae strains having large growth rate and high biomolecules productivity from previously collected microalgae species.
    b) To investigate the morphological characteristics and chemical composition of the selected microalgae species.
    c) To develop innovative culture technique for the selected microalgae species *in vitro* and *in vivo* mass production.
    d) To develop innovative harvesting methods for algal biomass production.
    e) To integrate bio-refinery approaches for the production of liquid/gaseous biofuel and high added-value compounds from algal mass.
    f) To utilize waste algal biomass and deoiled algal mass for production of bio-oil, bio-char and development of bio-based catalyst.

Tezpur University Final Report

1. **Title:** "Integrated Biorefinery Approach towards Production of Sustainable Fuel and Chemicals from Algal Bio-based Systems"
2. **Project Investigators and address:**

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3. **Date of sanction:** 15.06.2016
4. **Date of completion:** 14.12.2020
5. **Approved cost:** Rs. 102.3383 lakhs
6. **Budget released:** Rs. 82.92356 lakhs
7. **Objectives**
   a. DoE in association with MBBT will culture the already selected microalgae strains at Gauhati University for mass production in control laboratory condition and natural water systems.
   b. The Department of ECE will design and develop robotic algal harvest systems to collect dehydrated algal mass. The Algal biomass will also be collected from GU and IITG as well as from Brazil collaborator.
   c. The algal biomass after collection will be further investigated for their oil content, biochemical constituents and hydrocarbon content. Characterization of fatty acid will also be done in the laboratory of DoE and MBBT. Algal mass thus produced will be sent to the laboratories of other partner institutions in India and abroad for further laboratory analysis.
   d. Algal mass produced in this process will further be processed for biodiesel and bio-oil production in the Biomass Conversion and Biofuel laboratory of DoE. Biofuels as produced from this process will be analyzed with the help of latest analytical techniques.

Biofuel samples will also be sent to the laboratories of the Dept. of Chemical Engineering, IITG and Federal University of Rio de Janeiro, Brazil for further analysis.

e. Dewatered microalgae or biomass left after extraction of lipid would be used as raw material for preparation feed for dark fermentation in the Department of MBBT. Appropriate saccharification methods would be used to convert the carbohydrates from the lipid-extracted residual biomass into sugars for bioethanol fermentation. Dark fermentation (batch) for ethanol production will be conducted in the microbial laboratory of MBBT. Ethanol concentrations in the fermentation broth would be determined using gas chromatograph (GC) equipped with a flame ion detector (FID).

f. Deoiled and waste algal mass will also be further processed in DoE and MBBT for development of renewable catalyst that will be employed against esterification/transesterification/saccarification/fermentation and chemicals production route from biomass.

8. Progress made against targets including methodologies, discussions, etc.

8.1. Photobioreactor Design

Experiments were carried out to find the effect on the growth characteristics of algae using different LED light conditions. In the first experiment 12 conical flask of 500 mL capacity were taken. The flasks were separated into 4 groups consisting of 3 flasks each. Each group was illuminated using different LED lights. In the 1st group LED of White, Blue and Red colour was used, in the 2nd group only White LED were used, in the 3rd group Red LED were used and in the 4th group only Blue LED were used. The LED and the flask were placed inside cardboard box lined with silver foil for reflecting the light. The setup is shown in Fig 1. The open faces of the boxes were then covered using glass mirrors, to conceal the chamber from external light. Readings of Light intensity, Optical Density (OD) at 560nm, temperature and pH were taken at 12hour interval. The light intensity measured are given in Table 1.

Table 1: Light intensity of the different LED arrangements.

LED Color	Intensity (center)(in Lux)	Intensity (frontal)(in Lux)
Red-Blue-White	5010	3450
White	14760	6430
Red	1580	700
Blue	210	120

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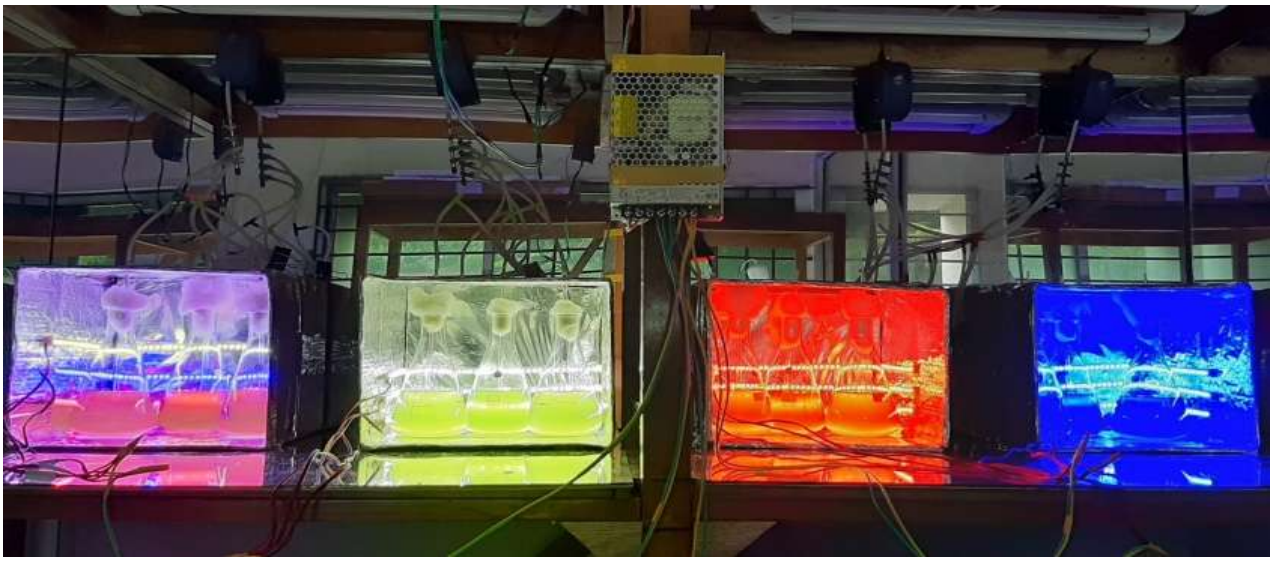


Figure 1: Micro algae culture using different LED setup.

The light intensities were measured using a LUX meter. The lux meter was placed in two positions, in the first position the light sensor was placed in the centre of the box facing upward (the readings are shown in the 2nd column of Table 1) and in the second position the light sensor was placed directly facing the light (the readings are given in 3rd column of Table 1).

Figure 2: Optical Density (OD) of different culture at 560 nm wavelength.

The optical density (OD) of the cultures is shown in Figure 2. The OD of the culture with Red-Blue-White (RBW) showed the highest rate of growth. Large variations were observed in the OD readings within the same groups. This was suspected due to difference in the air flowrate to the cultures.

Based in the experimental findings, LED based Internally Illuminated Photo-bio Reactor (IIPBR) was designed and developed. The IIPBR system have a tank made of opaque plastic. This type of material is selected to reduce the cost of the system. It is a cylindrical PBR tank as shown in Figure 3, with internal lighting and stirring system. Figure 4, 5 and 6 shows the assembly of the lighting and stirring mechanism and Figure 7 shows the total assembly of the lighting and stirrer system without the top of the tank for better understanding.

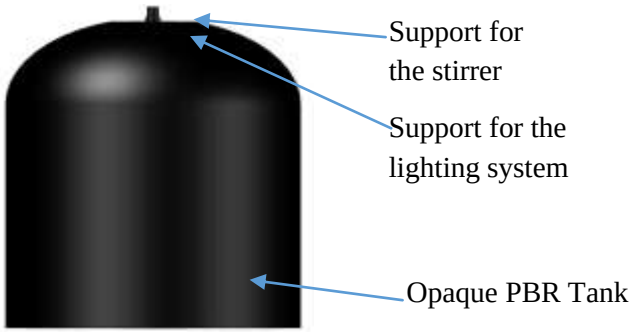


Figure 3: The opaque photo bio reactor

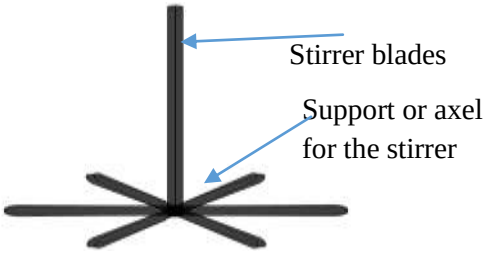


Figure 4: The stirrer assembly

Apart from the lighting and the stirring system, there will be other auxiliary systems like gas delivery system at the bottom of the tank for carbon dioxide or ambient air input, electrically controlled inlet and outlet valves to fill and drain the tank, various sensors like pH sensor, temperature sensor, turbidity sensor and temperature sensor. These auxiliary systems will be discussed in details in the later part. In this section we will primarily focus on the lighting and the stirring system as they are the majority power consumers of the system.

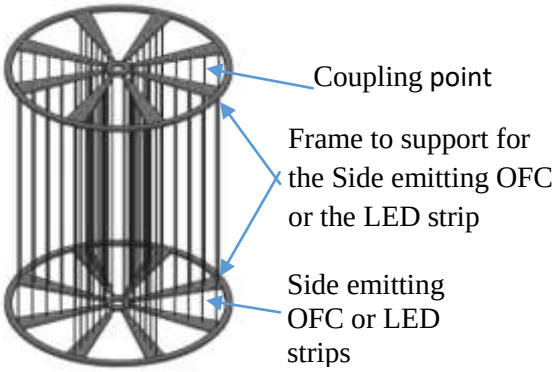


Figure 5: The internal lighting assembly

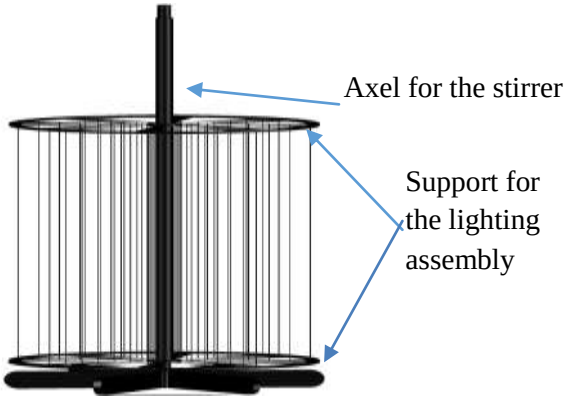


Figure 6: The stirrer and the lighting assembly with their central supports

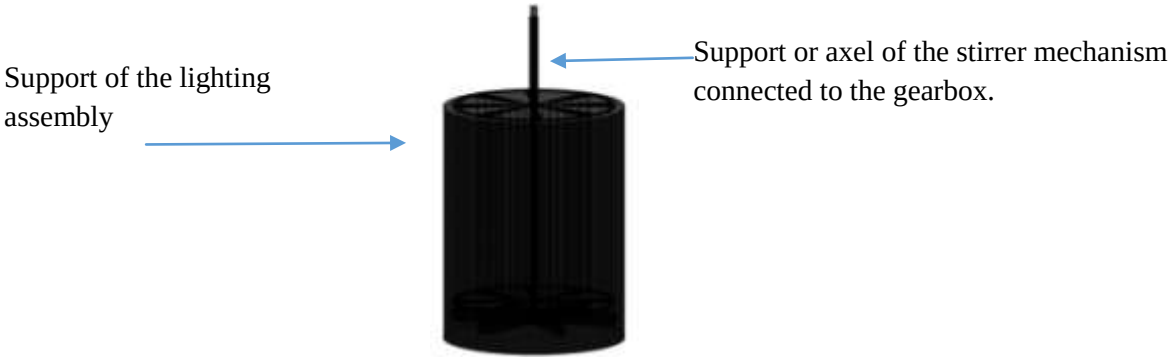


Figure 7: The PBR with stirrer and the lighting assembly

The stirrer blades are connected to the bottom of the axel and an electrical motor at the top. The motor rotates the axel which in turn rotates the blades to create turbulence. The turbulence created keeps the solution homogenous, preventing the algae from settling down to the bottom. It also ensures the algae receives the light efficiently.

The internal lighting assembly for the PBR is as shown in Figure 5. The frame consists of two aluminum/plastic rings or rims. The rims are connected to the centre hub with flat spokes. The rims are fixed to the central vertical support with the help of the central hub and are separated from each other. LED strips are fixed vertically in between the spokes as shown in figure 5. The number of spokes and the number of side emitting LED strips per spoke is calculated based on the optimum lighting requirements. The LED are powered by coupling electrical wires connected at the coupling points located at the top of the lighting assembly.

Figure 6 shows the lighting and the stirring mechanism along with their central support system for a better understanding. The stirrer support or the axel runs through the hollow support of the lighting mechanism. The lighting structure is stationary while the axel of the stirrer is connected to a motor and gearbox at the top. The total assembly of the lighting assembly, the stirrer mechanism and the PBR tank is shown in Figure 7. The top dome of the PBR is removed for better understanding.

LED's are semiconductor devices which emit light when powered using an electrical source. There are various reasons to opt for LED lighting like, low cost, energy efficient, compact, can emit light at preferred wavelength.

The cell count of micro algae cultivated under red light increases 1.5 fold when cultivated under red light compared to cultivation under blue or white lights. The cell diameter increases up to 60% when cultivated under blue light compared to red or white.As we can vary the light spectrum using LED, we can use LEDs for designing more energy efficient systems. Constructed a 100L capacity internally illuminated PBR using commercially available plastic water containers. IP-68 rated waterproof LED strips were used for illumination. The LED strips were supported utilizing an aluminium frame mounted on an axel made of CPVC pipe. The aluminium frame also acted as fins to circulate the algae culture, preventing the algae from settling down. Air was pumped from the bottom of the tank supplying carbon dioxide to the algae as well as aiding to the mixing of the culture. The fin and the LED arrangement were supported from the top using a ball bearing assembly allowing the entire structure to rotate freely inside the container. Figure 8(a-c) illustrates the fabricated PBR. Scenedesmus sp. algae were cultured in the constructed PBR and readings were noted. The Optical Density readings of the experiment are given in Figure 9. We stopped the culture after seven days, due to unsatisfactory growth and instrument malfunctioning. It was found that the inadequate growth was due to insufficient light supply. The thorough study revealed the distance between the LED strips were large, resulting in insufficient light supply, hence the design is still being modified.

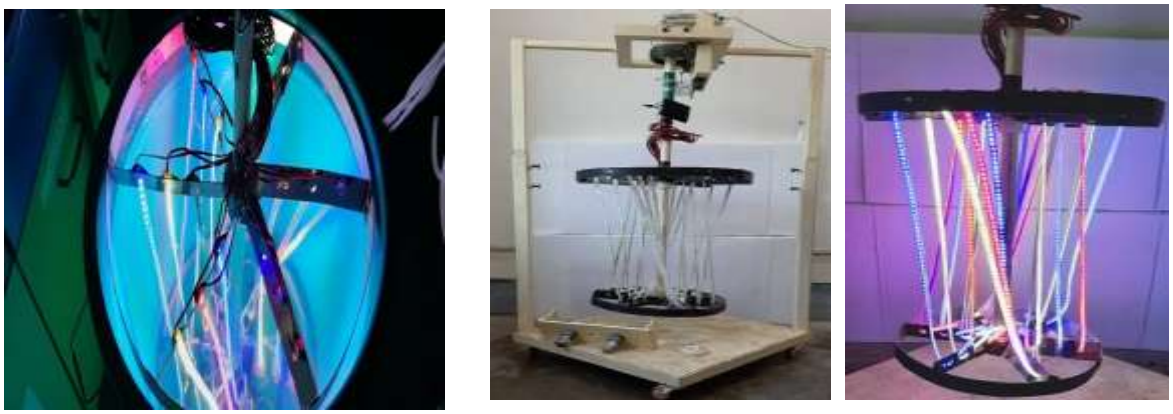


Figure.8a.(left): The PBR illumination setup inside the 100L PBR tank; **8b.(centre):** The illumination setup and the rotation mechanism; **8c.(right):** The illumination arrangement powered up.

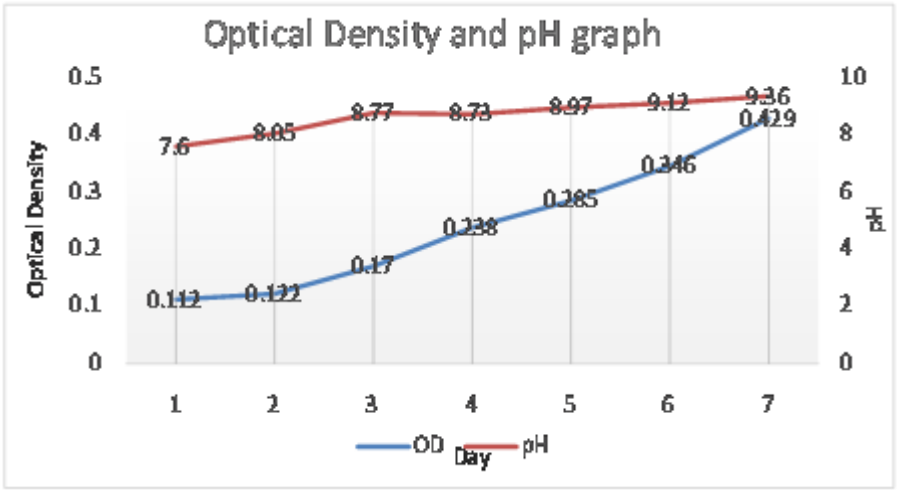


Figure 9: Optical density (OD) of algae culture measured at 560 nm using UV-Vis Spectrophotometer for a period of 7 days and their corresponding pH values.

8.2 Design and Development of a Robotic Algae Harvesting (RAH) System

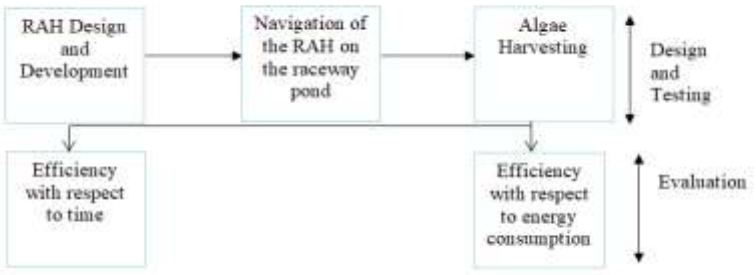


Figure 10: Conceptualization of the RAH

For the current research work, a raceway pond of the Department of Biotechnology, Gauhati University, Assam was selected where the developed robotic algae harvesting system could harvest the algae. Figure 10 shows the conceptualization of the RAH from design to evaluation.

8.2.1. Mechanical design

The microalgae were cultivated in an oval shaped concrete raceway pond of length, breadth and depth 240 cm, 80 cm and 30 cm respectively. Investigating the dimentions of the raceway pond where the robot will harvest algae, the design dimensions were determined for the robotic algae harvester (RAH). The design of the RAH assembly consisted of three structural units: mobile unit, algae collection unit and the control unit. The ruggedness of metal makes it a viable candidate among wood, plastic, metals and composites for designing the outer chassis of the RAH. The frame was constructed with light iron sheets (2mm thickness) of length and breadth 20 and 17 cm respectively. Iron sheets are cheaper, easily available and provide good rigidity to the structure of the robot without making it heavier. The chassis was supported by mild steel threaded bolt of 5 inches and lock nut. The mobile unit of the RAH consisted of four screw mount wheels of 35 mm radius directly connected to the shaft of 4 geared DC motors attached to the frame with motor clamps; powered by an on-board DC source. This unit aided in the linear movement of the RAH in the raceway pond. Two algae collection units were designed on each side of the RAH with plankton nets of size 5 micron and hollow tube plastic pipe for support. The collection units were controlled by two servo motors which facilitate in the collection of the algae. A wireless switch control system was designed for controlling the robot locomotion while collecting the algae from the raceway pond. At the initial stage, the RAH moved from one end of the raceway pond to the other end in a straight line. During this linear movement of the RAH, the algae collecting units which were submerrged in the algae water mixture harvested the algae allowing the water to pass through the units; thus retaining the microalgae on its surface. In the second stage, the algae collecting units changed its position from vertical state to horizantal state by an angle of 90 degree with the help of servo motors. The microalgae harvested by the RAH was seperated with the help of a spatula manually. The same procedure was repeated until all the microalgaes were harvested. The mobile unit of the RAH is presented in Figure 11.

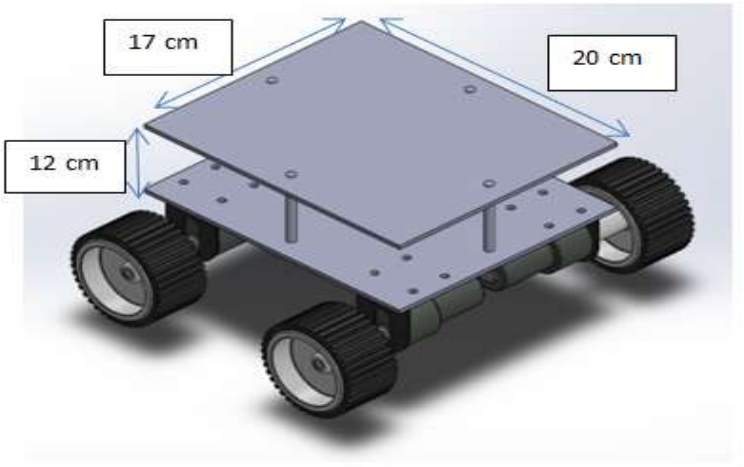


Figure 11: Design of the RAH mobile unit

8.2.2. Design of algae collection unit

During the preliminary design phase of the algae collection units, three designs were conceptualized and tested physically for algae collection in a laboratory scale environment. The first design was constructed in the shape of a cylindrical shaped hollow filter made up of fine mesh. Figure 12.1 shows the computer aided design of the initial design. During its testing for algae collection in a photobioreactor, it was observed that majority of the microalgae attached to the rear end of the algae collection unit thus making it difficult for removing them manually after the harvesting process. This was a major drawback in the conical algae collection units. To ovecome this bottleneck, a cubical algae collection unit was designed. The idea was to seperate the harvested algae from the sides of the cubical unit with the help of a spatula manually. It was observed that initially the microalgae started accumulating on the inner surface of the algae collection unit. But gradually the harvested microalgae were pushed away from the collector due to the water currents produced by the inner walls of the collection unit; thus resulting in loss of the total harvested microalgae quantity. Figure 12.2 shows the design of the cubical algae collection unit. Finally, a square flat plate type algae collection unit was designed. It was observed that the microalgae were easily attached to the filter material of this design. Moreover, the removal of the harvested microalgae from the algae collection unit was easier and less time consuming as compared to the other two designs. Based on these preliminary laboratory testings and observations, the third design (Figure 12.3) was adapted for the RAH algae collection unit.

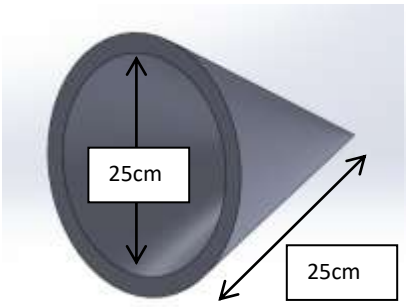


Figure 12.1: Conical unit

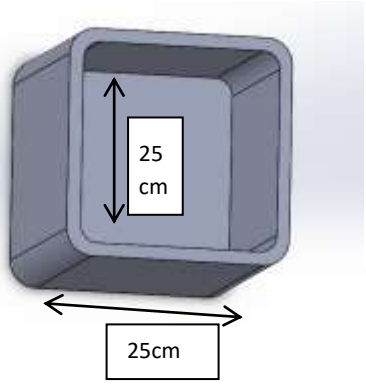


Figure 12.2: Cubical unit

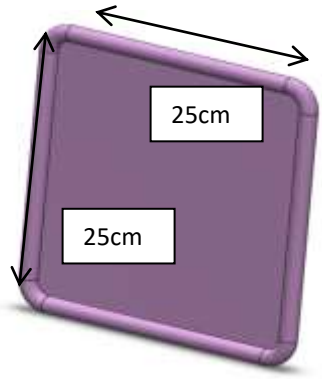


Figure 12.3: Flat plate unit

Final assambled design of the RAH along with the algae collection units is presented in Figure 13.

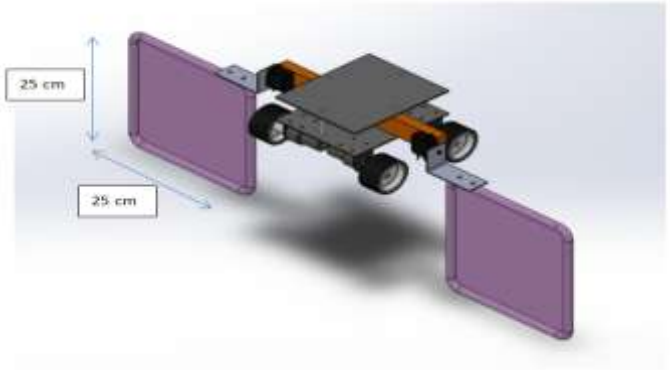


Figure 13: Computer aided design of the assembled RAH

8.2.3. Torque calculation of the RAH

The torque required by each of the robotic wheel i.e. the torque of the DC motors to drive the algae harvesting robot was derived from the total resistive force acting on the RAH. The RAH being partially submerged under water is influenced by both underwater forces and above surface forces acting upon it. Rolling resistance due to frictional force dominates the above surface forces, while hydrostatic force and drag force due to movement of the submerged algae collection units are the major underwater forces to be considered in this design. Thus, in order to drive the RAH, the actuation force must be greater than the total resistive force.

Rolling resistance was found to be 8.32 N.

Total hydrostatic force acting on each submerged algae collection unit of the system was derived using the thrust force (F) acting on the algae collection unit by water and was found to be153 N.

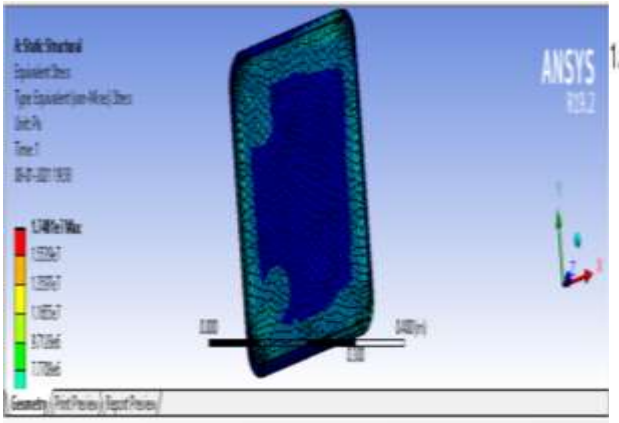
Further, the drag force (F_d) was 1.76 N

Torque required by a single wheel of the RAH was calculated as 14.7 kg cm.

Torque required for each servo motor was found to be 15.6 kg cm.

8.2.4. Structural analysis of the RAH design

Structural analysis enables to solve complex structural engineering problems and make better, faster design decisions. Ansys R19.2 was used for the structural analysis of the algae collection unit along with the assembled RAH design. Ansys structural analysis is used across industries to help designers analyse the product designs before hardware fabrication thus reducing the costs of physical testing. Figure 14 (a) shows the graphical results of the stress on the algae collection unit when 80 N force is applied. Figure 14 (b) shows the graph between stress and time. The design material used for the algae collection unit is thermoplastic polymer.



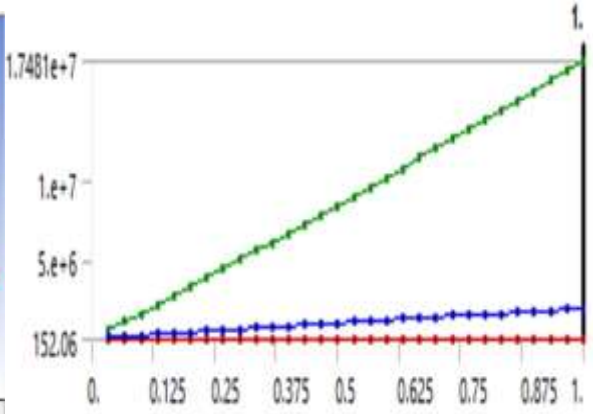
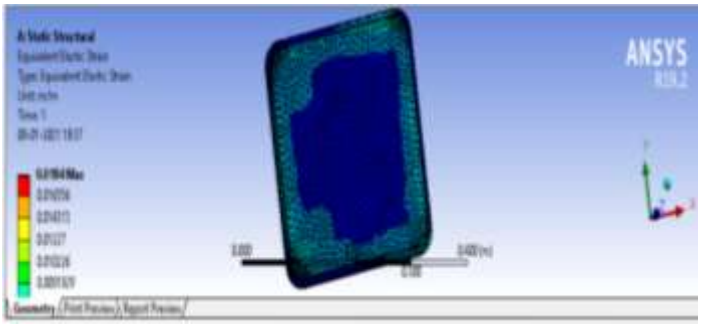


Figure 14 (a): Stress exerted on algae collection unit **Figure 14 (b):** Stress verses time graph

Figure 15 (a) shows the graphical results of the strain on the algae collection unit when 80 N force is applied. Figure 15 (b) shows the graph between strain and time.



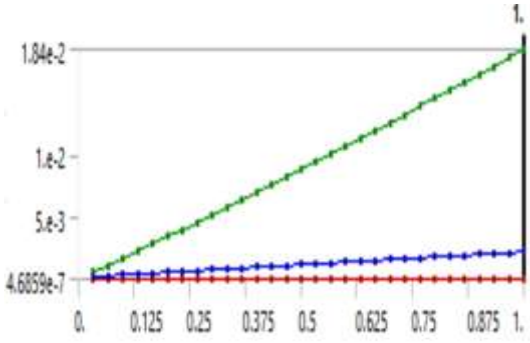
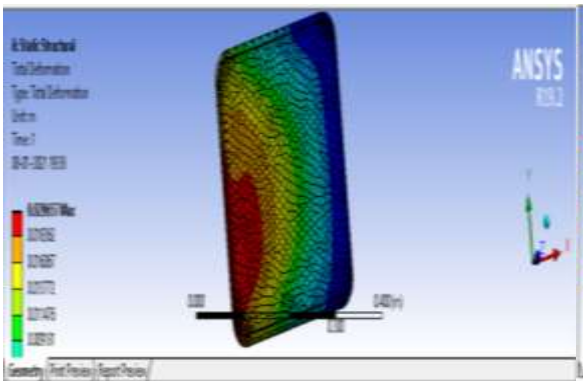


Figure 15 (a): Strain on algae collection unit

Figure 15 (b): Strain verses time graph

Figure 16 shows the total deformation of the algae collection unit. The maximum value for stress, strain and deformation are respectively 1.7481e+007 Pa, 1.84e-002 m/m and 2.0657e-002 m. The average value for stress, strain and deformation are respectively 1.9652e+006 Pa, 2.114e-003 m/m and 9.3419e-003 m.



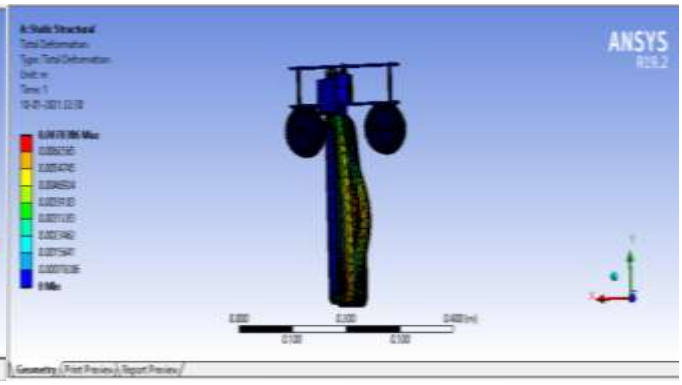


Figure 16: Total deformation

Figure 17: Total deformation of the RAH

Figure 17 shows the total deformation on the assembled RAH designs. A very small deformation of 7.0386e-003 m was observed. Design materials used are iron sheet plates for the chassis and virgin plastic for the wheels. From the above structural analysis, it was observed that the developed RAH design could bear the force without failure. From the stress versus strain curve it is clear that the material for the algae collection unit is capable of easily bear the force during the algae collection.

8.3. Thermochemical conversion of *Scenedesmus Obliquus (S. obliquus)* for biofuel production

8.3.1. Thermochemical conversion

Pyrolysis was performed in a fixed bed, vertical tubular reactor of length 30 cm and internal diameter 2.47 cm. An electrical furnace with thermocouple made of Ni-Cr placed in the centre was used for the temperature control. For each run, 10 g of the *S. obliquus* have been fed at the beginning of the test to the vertical reactor with 40 °C/min heating rate to final temperatures of 350 °C, 400 °C, 450 °C, 500 °C and 550 °C at a constant N_2 flow rate of 110 ml/min. 40 °C/min heating rate was selected for the present experiment as at a lower heating rate there is a resistance to heat transfer inside the pyrolysis feedstock. The reactor outlet was attached with a condenser to collect the vapours using water as a coolant. The condensed vapours were stored in a glass beaker as liquid product. The gases which were non-condensable were allowed to escape to the environment. The product yields of the biooil and biochar

were calculated by weighing in a digital balance scale whereas the gas quantity was calculated by mass balance. The liquid faction produced from the pyrolysis process was a mixture of the aqueous and organic phase which was later separated using standard techniques. Using diethyl ether as a solvent, the aqueous portion was carefully alienated from the oil portion by using a separator funnel. Drying of the oil over anhydrous sodium sulphate was carried out followed by filtration and evaporation in a lab scale rotary evaporator at 40 °C. After the solvent have been removed, the liquid part was collected in a glass container which was categorized as biooil and studied for various physicochemical properties.

8.3.2. Biooil analysis

The FTIR spectroscopy of the biooil at temperature range 350 °C to 550 °C is presented in Figure 18. The FTIR spectra show the changes of the functional groups of the biochar produced at different temperature. The biooil contains a very wide range of organic chemicals, which is evident from the presence of functional groups identified from FTIR spectrum. The strong absorption at 3426 cm^{-1} indicates the presence of phenolic and alcoholic compounds and moisture. The presence of peaks at 2845 cm^{-1} and 2921 cm^{-1} indicated the presence of methylene groups in the biooil. The peak at 2345 cm^{-1} is due to the presence of alkynes or cyanide in the biooil. The absorbance peak at 1690 cm^{-1} indicates the presence of aldehyde and ketone. The characteristic bands at 1650 cm^{-1} – 1550 cm^{-1} are assigned to C=C stretching vibrations which is indicates the existence of alkenes and aromatics. 1458 cm^{-1} peak may be due to the bending and rocking vibrations of C–H. The peaks between 1050 cm^{-1} and 1300 cm^{-1} is due to C–O stretching and O–H bending, suggests the presence of primary, secondary and tertiary alcohols and phenols. The C-H plane bending vibration at 741 cm^{-1} further confirms the presence of aromatic compounds. Moreover, the absorption peaks between 900 cm^{-1} and 700 cm^{-1} and 1600- 1400 cm^{-1} represents mono, polycyclic and substituted aromatic groups. A sharp decrease in the band intensity at 3426 cm^{-1} (–OH) was observed at lower pyrolysis temperature (350 °C –400 °C), however the intensity of the other bands (C–O, O–H, C–H, C=C) at pyrolysis temperature of 500 °C –550 °C were decreased in magnitude but well-preserved.

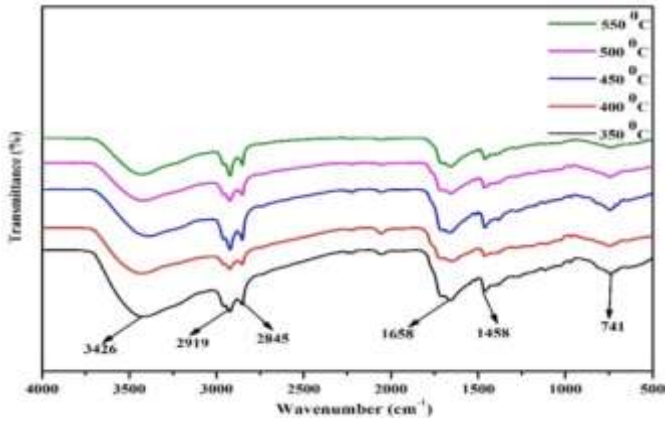


Figure 18: FTIR of biooil at temperatures of 350 °C, 400 °C, 450 °C, 500 °C and 550 °C

The Figure 19 shows the ^{1}H NMR spectroscopy of *S. obliquus* biooil. The ^{1}H NMR spectroscopy identifies the types of chemical functional groups present in the biooils based on the given chemical shift regions. As, highest biooil percentage was found at 500 °C, the biooil produced at this temperature was used for ^{1}H NMR spectroscopy. ^{1}H NMR spectroscopy shows the presence of various types of H in the biooil denoted by the appearance of different chemical shift. The region between chemical shift 6.0-9.0 ppm and 4.0-6.0 ppm corresponds to aromatic ring protons (2.62%) and phenolic and olefinic protons

(1%) respectively. Appearance of the chemical shift at 3.0–4.0 ppm and 0.5–1.5 ppm are related to aromatic ring-joining methylene groups (13.67%) and protons of alkane groups present in the compounds (82.69%) respectively.

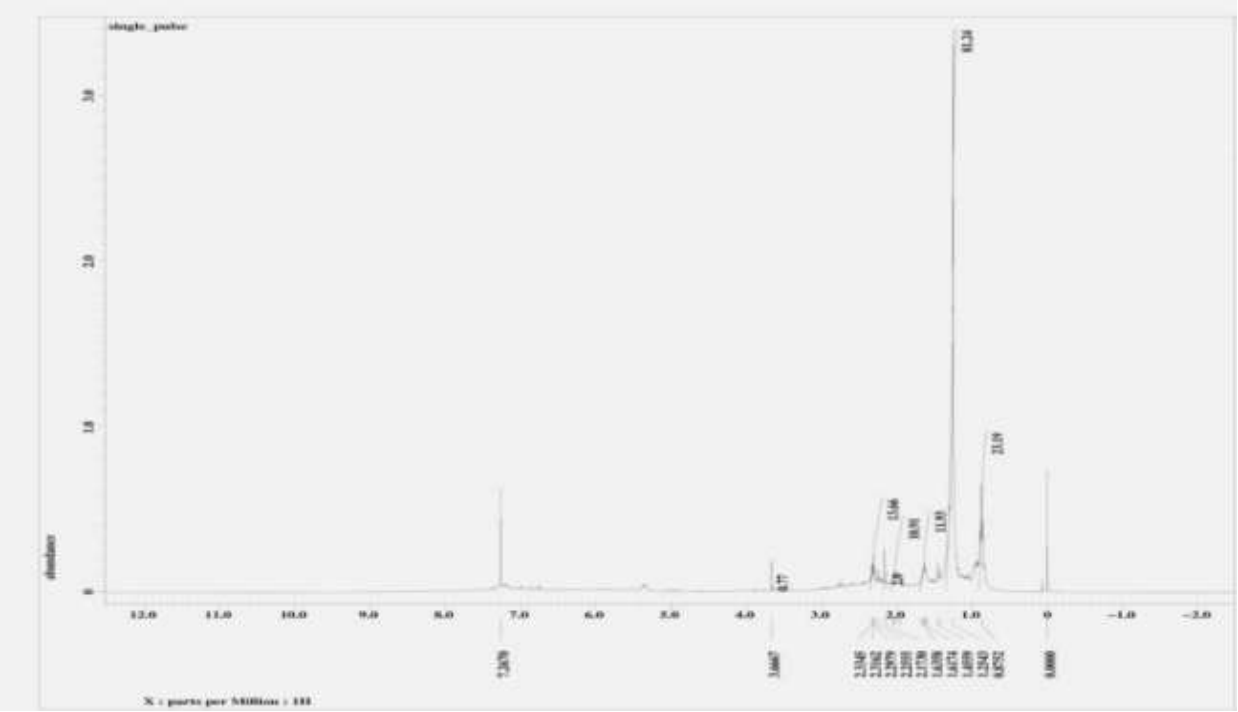


Figure 19: ^{1}H NMR spectroscopy of biooil

Table 2 shows the C, H, N and O percentage along with HHV data of the produced biooil. Amount of O percentage in the biooil (16.12–21.54 wt%) was found to be less than that of the *S. obliquus* (41.88 wt%) which favours the production of transportation fuel. The C (65.59–71.41 wt%) and N (4.38–5.27 wt%) contents were found to be higher than those of *S. obliquus* feedstock (49.52 wt% for C and 2.17 wt% for N). This indicates that the biooils have higher energy density. A decrease in the O content and increase in the C and N content was observed with increase in pyrolysis temperature. Higher percentage of N in the biooil may be due to breakdown of proteins, as proteins decompose easily at higher temperature. Higher percentage of C, H and N in the biooil also indicates higher energy density of the biooil. Moreover, the increase in HHV of the biooil with the increase in pyrolysis temperature can be attributed to higher values of C with low O level. The outcome justifies the high pH value of the biooil (5.21–6.18). The HHV of the biooil (32.97) was found to be higher than the feedstock *S. obliquus*. This could be due to the elimination of oxygenated compounds from the biooil. It can be seen that *S. obliquus* has a higher percentage of C (71.41%) amongst other microalgae samples reported in literatures. The HHV is highest for *S. obliquus* (32.97 MJ/kg) and lowest for *Scenedesmusdimorphus* (28.52 MJ/kg). The high HHV for *S. obliquus* may be due to higher values of C as compared to the other microalgae. This result is supported by findings reported in literatures elsewhere. The O content for *S. obliquus* biooil is 16.12% which is most likely in the mid-range as compared to the other microalgae. A higher O content of the biooil suggests that it could potentially be used as production goods for catalytic steam reforming.

Table 2**:** Elemental and calorific analysis of biooil.

Component (wt%)	**350 °C**	**400 °C**	**450 °C**	**500 °C**	**550 °C**
pH of biooil	5.21±0.07	5.36±0.10	5.84±0.15	6.13±0.15	6.18±0.03
C	65.59±0.35	66.73±0.15	68.11±0.06	70.84±0.05	71.41±0.03
H	8.49±0.05	8.21±0.09	7.96±0.15	7.64±0.05	7.20±0.03
N	4.38±0.09	4.61±0.04	4.94±0.04	5.13±0.08	5.27±0.05
O	21.54±0.25	20.45±0.08	18.99±0.05	16.39±0.03	16.12±0.12
HHV (MJ/kg)	31.15±0.03	31.66±0.08	32.19±0.04	31.80±0.02	32.97±0.06

*The reported values are average of triplicate determinations with standard deviations.

8.3.3. Biochar analysis

The proximate, elemental and calorific analysis of the biochar is shown in Table 3.

Table 3:Biochar proximate, elemental and calorific analysis

Properties	**Biochar**				
	350 °C	**400 °C**	**450 °C**	**500 °C**	**550 °C**
pH	7.1±0.046	7.19±0.053	7.47±0.080	7.82±0.060	7.96±0.061
Proximate analysis (dry basis wt %)					
Moisture Content	0.09±0.002	0.08±0.004	0.07±0.004	0.06±0.003	0.06±0.001
Ash Content	39.67±0.39	40.71±0.41	42.16±0.18	44.80±0.50	46.73±0.53
Volatile Content	19.00±0.42	17.30±0.56	16.20±0.24	14.70±0.33	12.60±.49
Fixed C (by difference)	41.24±0.12	41.91±0.27	41.57±0.03	40.44±0.14	40.61±0.05
Ultimate analysis (dry basis, wt %)					
C	39.23±0.31	40.67±0.32	42.76±0.14	44.83±0.07	46.41±0.12
H	8.36±0.04	8.98±0.06	9.24±0.04	9.80±0.11	10.21±0.05
N	8.27±0.11	7.42±0.04	6.90±0.06	6.28±0.44	5.90±0.29
O (by difference)	44.14±0.05	42.93±0.54	41.10±0.17	39.09±0.09	37.48±0.06
HHV (MJ/Kg)	15.78±0.09	17.79±0.22	17.75±0.17	20.57±0.12	22.34±0.08

FTIR spectra of the biochar obtained at various pyrolysis temperatures are shown in Figure 20.

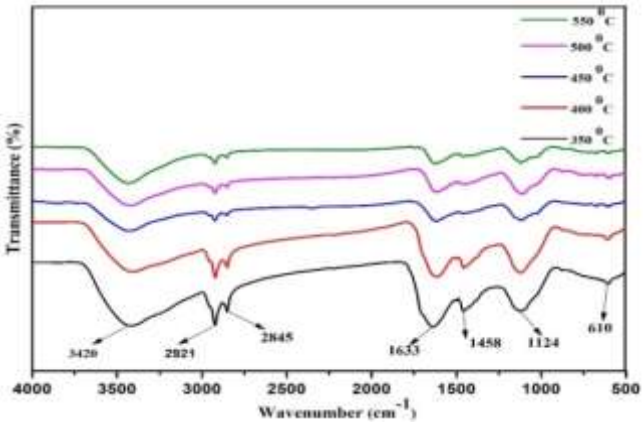


Figure 20: FTIR of biochar at temperatures of 350 °C, 400 °C, 450 °C, 500 °C and 550 °C

Figure 21 displays the SEM microgram of the biochar attained at various pyrolysis temperatures. SEM images of biochar exhibits rough surface and small holes and pits were observed. A few voids appeared on the surface of biochar pyrolysed at 550 °C as shown in Figure 21 e. The biochar obtained at 350 °C had thick cell walls and some were covered by agglomerated tar whereas biochar at higher temperatures (500 °C –550 °C) had a thin-wall surface since there was a fast volatile release during the thermal degradation process. Porous structure of biochar and holes on its surface provides more adsorption site for ions, nutrition and water retention.

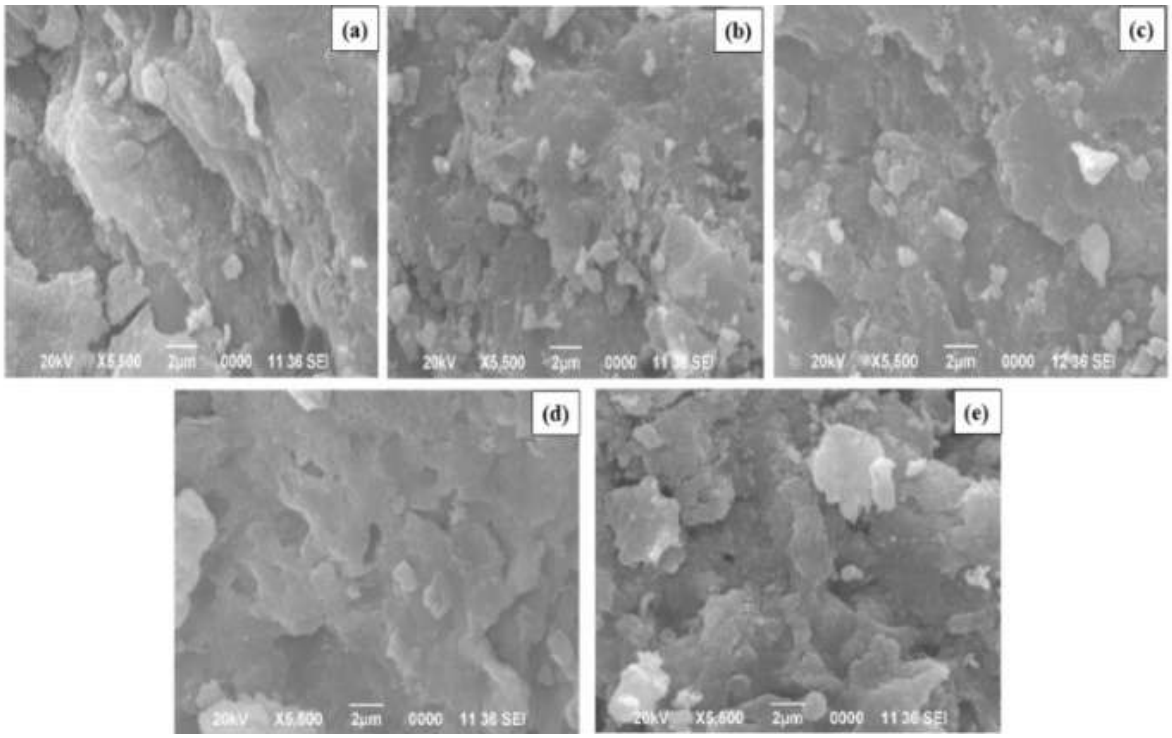


Figure 21: SEM of biochar at different temperatures (a) 350 °C (b) 400 °C (c) 450 °C (d) 450 °C (e) 500 °C

The elemental analysis of *S. obliquus* biochar obtained at 500 °C states the presence of C, O, Al, Si, S, K, Ca and Fe in the sample. The weight% and atomic% of all the elements were shown in Figure 22. Ca, K and S are essential inorganic nutrient which aids in soil fertility and production of crops. Hence, the biochar from *S. obliquus* may be used as a potential soil amendment.

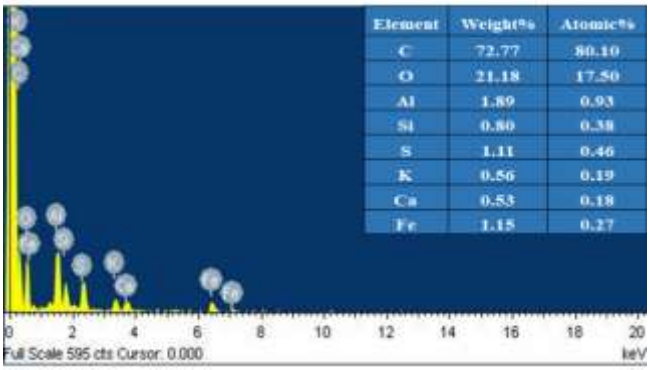


Figure 22: EDX analysis of biochar at 500 °C

8.4. Extraction of Algal lipid and its characterization

The extraction of lipid from *Scenedesmus obliquus* (SO) dried biomass was carried out by using Bligh and Dyer method. Dried biomass sample was weighed and homogenized in a mortar and pestle using 0.1-0.5 g of anhydrous Na2SO4 and 1-2mL of 2% butylated hydroxytoluene (BHT) (2.04 g of BHT in 100mL CHCl3). Total lipid was extracted from the homogenized powder with 5e10 mL mixture of $CHCl_3$: MeOH (2:1). The residue was extracted with chloroform until it became colourless. The extracts were mixed together, filtered and then transferred to a separating funnel. Then 0.9% NaCl solution (1/3 of the volume) and excess $CHCl_3$ was added to the separating funnel, mixed thoroughly and kept undisturbed overnight at room temperature. A clear biphasic layer was observed. The lower layer ($CHCl_3$) containing the lipid component was collected in a clean glass vial. The upper layer containing the methanol-water layer was washed twice with chloroform and collected similarly. All the collected chloroform layers were mixed together and were evaporated in a water bath at 60°C and finally dried in desiccator in presence of anhydrous Na_2SO_4. The physicochemical properties of SO lipid are shown in Table 4.

Table 4: Fuel properties of the feedstock

Property	**SO lipid**
Acid value (mg KOH/g)	1.4
Iodine value (g I_2/100 g)	130
Kinematic Viscosity (mm^2/s, at 40 °C)	30
Density (gm/cm^3at 15 °C)	0.9
Cloud point (°C)	5
Pour point (°C)	-1

8.4.1. Conversion of algal oil into its methyl esters and its characterization

In a typical process, 10 mL of lipid was poured to a 50 mL three-necked round bottom flask and heated (100 °C) to eliminate every trace of absorbed moisture. The WO was then cooled down to 60 °C. Catalyst, methanol, WO was then mixed to the reactor under reflux and the reaction continued for desired reaction parameters. To ensure proper mixing of MeOH and oil the agitation speed was maintained at 500 rpm. The reaction mixture was allowed to cool and transferred to a separating funnel where three distinct layers were clearly visible. The upper layer was mainly composed of FAME (fatty acid methyl ester), the middle layer of glycerine and catalyst was the bottom layer. The unreacted MeOH from the upper fraction was evaporated under reduced pressure. The mixture was then washed with distilled water (40 °C) to eliminate impurities followed by drying over Na_2SO_4 (anhydrous) to obtain methyl esters.

The ^{1}H and ^{13}C NMR spectra of oil and biodiesel were taken in a 500 MHz NMR spectrometer (Oxford, AS400, China). The GC-MS analysis was carried out in Agilent GC, 240 Ion Trap. The efficient conversion of oil to FAME was determined in terms of % FAME using nuclear magnetic resonance (NMR) techniqueusing deuterated chloroformas solvent (Eq. 1).

$$C_{MEster}(\%) = \frac{2\times X}{3\times Y} \times \quad 100 \qquad (1)$$

X = Integration value of methoxy protons ($-OCH_3$) at 3.6 ppm

Y = Integration value of α-methylene ($-CH_2-$) protons at 2.3 ppm

Successful FAME conversion of 93.33 % was achieved with SO using 9:1 methanol/oil molar ratio, catalyst loading of 2 wt % (bio-based catalyst) within 3 h. The ^{1}H NMR of *Scenedesmus obliquus* biodiesel (SOB) is shown in Figure 23.

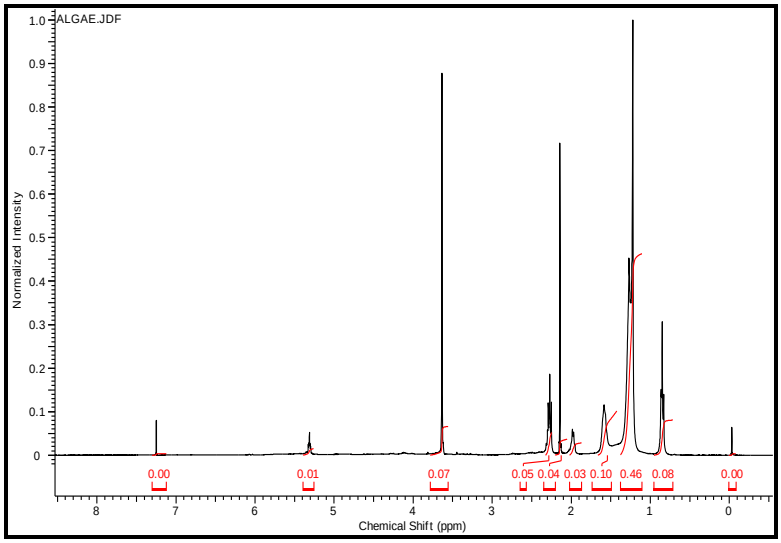


Figure 23: ^{1}H NMR spectrum of SO biodiesel

Two characteristic peaks of esters were observed for SO FAME [Fig. 1] i.e. a singlet at 3.6 ppm for methoxy proton and a triplet at 2.3 ppm for α - methylene proton of ester which did not exist in SO. Thus, it confirms the conversion of SO lipid into biodiesel. A multiplet was formed at 5.45 – 5.39 ppm due to olefinic hydrogens. Other characteristic peaks at 1.2 – 1.4 ppm and at 1.58 – 1.65 ppm is due to aliphatic hydrogen of carbon chain and methylene hydrogen of *β* - carbonyl respectively. Another peak at 0.89 – 0.9 ppm was because of terminal methylene proton.

Table 5: Fuel properties of FAME

Fuel Property	**SOB**	**ASTM D 6751**	**EN 14214**
Acid value (mg KOH/g)	0.2	<0.80	0.5 max
Kinematic Viscosity (mm^2/s, at 40 °C)	4.1	1.9-6.0	3.5-5.0
Density (gm/cm^3at 15 °C)	0.86	0.86-0.90	0.85
Cloud point (°C)	-8	---	---
Pour point (°C)	-12	---	---
Flash point (°C)	115	93 min	120 min
Carbon residue (% wt)	0.021	0.050 max	0.3
Calorific value (MJ kg^{-1})	40	---	35 min
Cetane Number	58	47 min	51 min

8.4.2.Deoiled algal biomass characterization

Calorific value (CV) was done with the help of an automatic adiabatic bomb calorimeter (Changsha Kaiyuan Instruments Co., 5E-1AC/ML). The sample i.e. *Scenedesmus obliquus* deoiled cake (SO) was oxidized by the process of combustion in an adiabatic bomb that contains 3.4 Mpsi oxygen under pressure. The reporting of mean values was done by performing the assays in triplicates. The CHN

analysis was done in a CHN analyser and the calculation of oxygen content was done by difference. The determination of moisture, volatile matter and ash content of SO were done according to ASTM protocols. Finally, the difference gives the fixed carbon content.

De-oiled *Scenedesmus obliquus* residue was subjected to compositional analysis following the gravimetric method. Extractives content was obtained using a Soxhlet apparatus and acetone as a solvent (For 2.5 g dried residue 150 mL of acetone was used). The mixture was boiled using the heating mantle at 70 °C temperature for time duration of 4 h. After extraction, the drying of the sample was done in an oven till the weight attained was constant. The weight difference between the raw-extractive laden residue and extractive-free biomass was expressed as % (w/w) of the extractive content. The hemicelluloses were estimated gravimetrically after treating with 0.5 M NaOH for 3 h. Acid-insoluble lignin was obtained similarly by drying the sample after treating with 72 % H_2SO_4 followed by autoclaving at 121 °C for a time duration of 1 h. The absorbance of the acid-hydrolyzed samples was measured at 320 nm for determining the acid soluble lignin fraction. The sum of acid-soluble and acid-insoluble lignin gives the total lignin content.

Total protein was estimated by Lowry's method. The FT-IR spectrum of algal deoiled residue was recorded on Nicolet IR spectrometer at room temperature. The blending of SO was done with potassium bromide (KBr) powder, and then for measurement these were pressed into tablets. The transmittance was recorded over a wave number range from 4000–400 cm^{-1}. The recording of powder X-ray diffractograms was done on a Rigaku miniflex diffractometer (CuKa radiation, λ = 1.5406 Å) in 2θ range 10-80° at a 2° scanning rate for crystalline phase determination. Thermo gravimetric analysis (TGA) was done in order to study the combustion behaviour of SO de-oiled residue. The thermo-gravimetric analysis of biomass was done in nitrogen atmosphere at 10 °C/min heating rate. 10 mg of each sample was heated from ambient temperature to 800 °C in a Pyris diamond TG/DT analyzer (PERKIN ELMER). For displacing air in the pyrolytic zone, feeding of pure nitrogen gas (99.99%) was done at 100 ml/min constant flow rate.

De-oiled algal biomass residue is generated as a leftover from biodiesel production process using microalgal lipids. Moreover, it is considered as a feedstock that is economic for biofuel production as it contains more than 70% of carbohydrates and protein by weight. *Scenedesmus obliquus* is a green alga which belongs to the class *Chlorophyceae* and it is used extensively lipid production.

8.4.2.1. FTIR analysis

The FTIR spectrum of SO is shown in (Figure 24). The 3300-3000 cm^{-1} region is characteristic for C-H stretching vibrations of C=C, C=C and Ar-H. The 3000 to 2800 cm^{-1} region is attributed to stretching vibrations of –CH, $–CH_2$ and $–CH_3$. The 1800 and 1500 cm^{-1} region signifies characteristic bands of proteins. The absorption at 1650 cm^{-1} showed the presence of C=O of carboxylic acid and derivatives. The 1600 and 1500 cm^{-1} region is characteristic of amide-II bands, which are indicative of NH_2 bending vibrations.

For bio-ethanol production, the presence of polysaccharide peaks is of interest as we are concerned with the conversion of complex carbohydrate molecules into the simple sugars. The sequence of bands in region 1200-900 cm^{-1} signifies C-O, C-C, C-O-C and C-O-P stretching vibrations of polysaccharides [45]. The presence of low lipid content (> 1% in SO) has been indicated by the declined intensity of absorption

between 3100-2800 cm^{-1}.

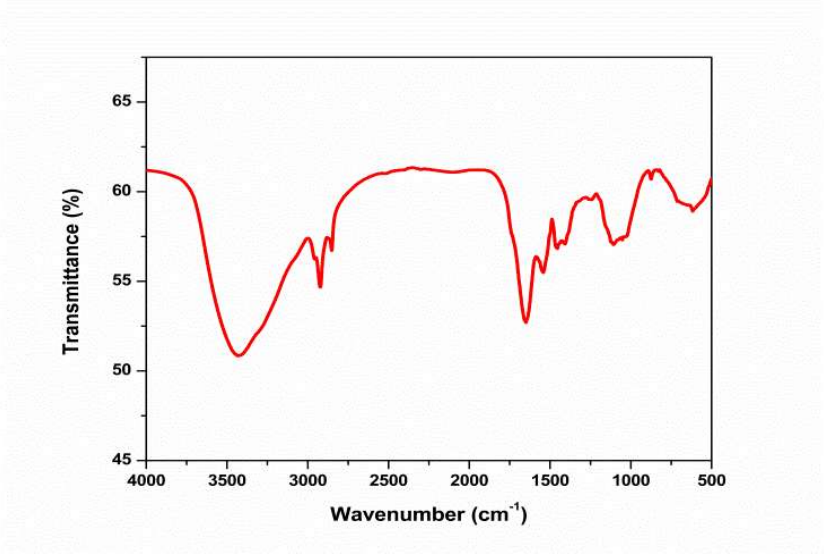


Figure 24: FTIR spectra of SO

8.4.2.2. TGA

The TGA profile of SO (Figure 25) reveals an initial loss of weight between ambient temperature and 160 °C possibly due to elimination of physically absorbed water. Subsequently, there was continuous loss of sample weight between 220-490 °C. This is the zone of active pyrolysis (where main degradation occurred). Passive hydrolysis occurred in between 500-700 °C. The weight loss is very slow until 800 °C signifying some further reaction involving char. The thermal degradation profile suggests suitability of SO for thermo-chemical conversion.

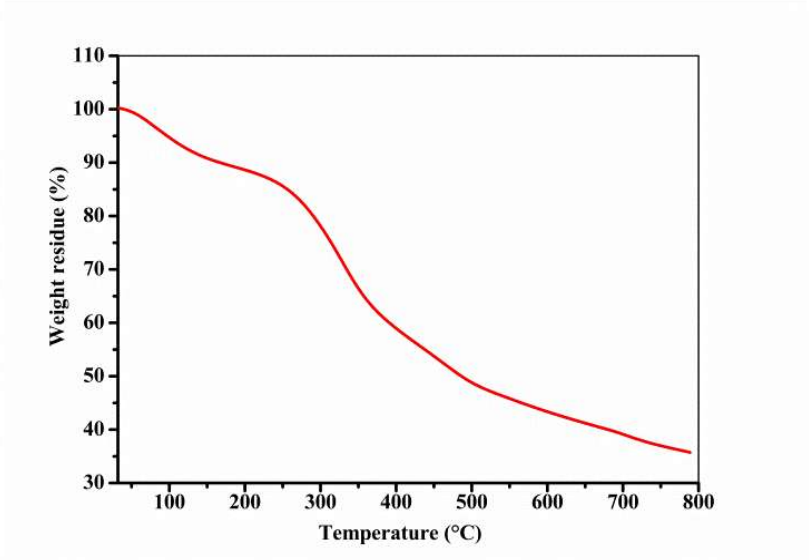


Figure 25: TGA of SO.

8.4.2.3. X-Ray Diffraction (XRD)

Figure 26 shows the XRD pattern for SO and SO after hydrolysis using calcined *Musa balbisiana* colla peels (CBPA), calcined water hyacinth (CWH), calcined *Carica papaya* stem (CCPS), calcined *Tectona grandis* leaves (CTGL) and *Rhodotorula mucilaginosa* deoiled cake activated carbon impregnated by using potassium hydroxide (K-RAC) as catalyst.

The intense X-ray diffraction peak was detected at 25° which characteristic of cellulose. From the Figure 26, it is well disclosed that the peak at 25° for the treated samples got reduced compared to the raw sample i.e. SO. This portrays release of cellulose and disruption of the cellulose lignin structure of SO. Breaking of the linkages between cellulose, hemicellulose and lignin can be done by the basic ions by penetrating the small pores present in the lignocelluloses. Evidence of similar results was found in number of studies. Hence, the prepared catalyst could be successfully utilized for hydrolysis reaction.

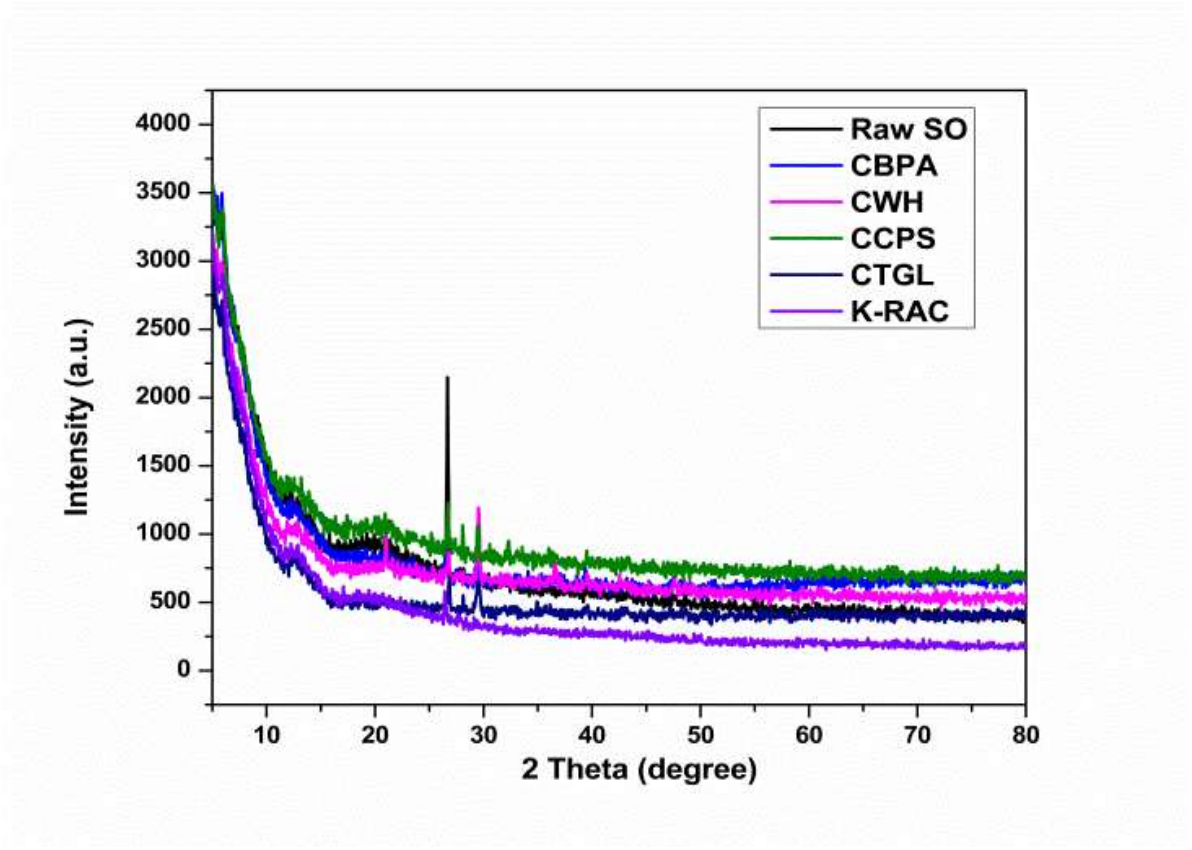


Figure 26: XRD pattern of (a) Raw SO and after utilization of (b) CBPA (c) CWH, (d) CCPS, (e) CTGL and (f) K-RAC.

8.4.3. Bioethanol production

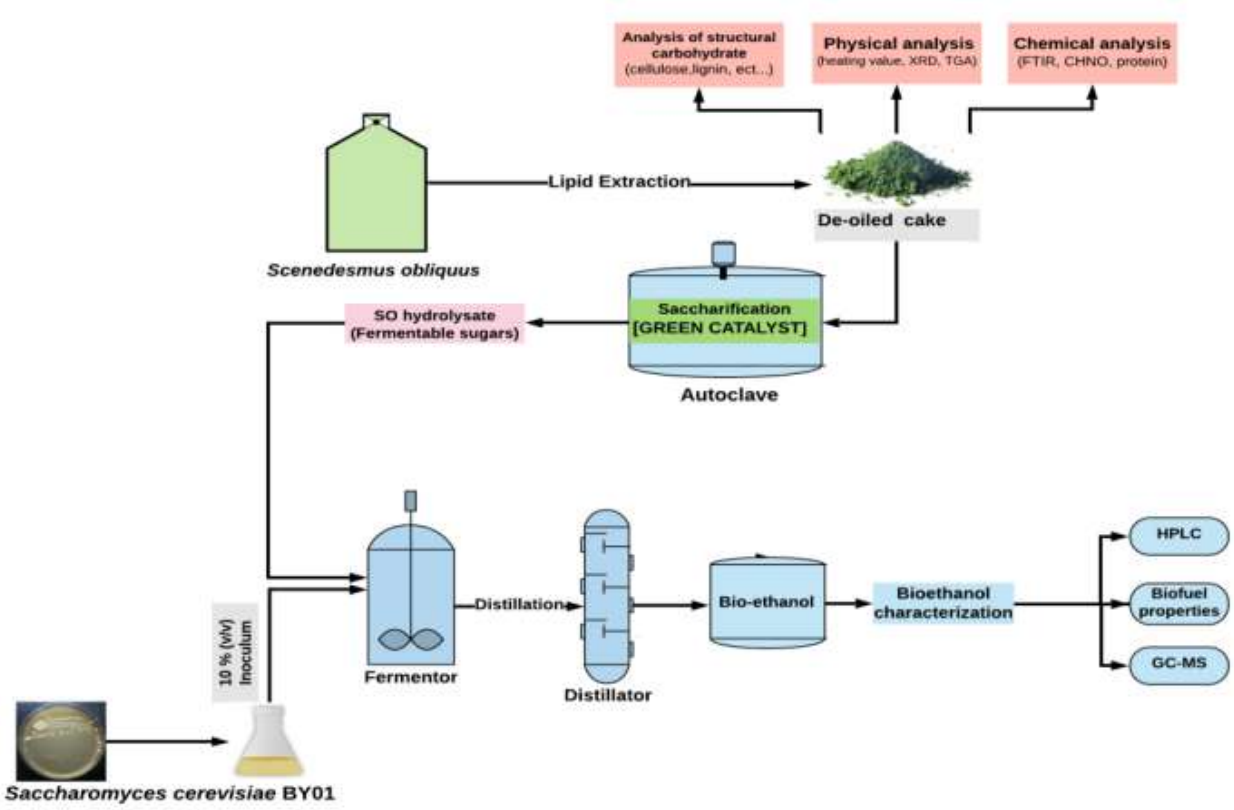


Figure 27: Schematic representation of bio-ethanol production from *Scenedesmus obliquus*.

For bioethanol production dilute alkaline pre-treatment of SO was done using the bio-based catalysts (2 wt. %). This alkaline sample mixture is then autoclaved at 121°C temperature for 30 minutes. The solid phase to the liquid phase ratio is maintained at 1:10. The analysis of total carbohydrate in the hydrolysate was done using a HPLC (Thermo Scientific) equipped with a refractive index (RI) detector according to the protocol described by NREL. Briefly, 20 µl of liquid hydrolysate (neutralized to pH 7) was injected. The column (Accucore 150-Amide-Hilic) temperature was set at 60 °C and the mobile phase was 5 mM H_2SO_4 at 0.6 ml/min flow rate.

The saccharification was calculated using Eq (ii).

$$\text{Saccharification (\%)} = \frac{Total\ sugar\ in\ the\ hydrolysate\ (g/L)}{Total\ sugar\ in\ raw\ sample\ (g/L)} \times 100 \qquad \text{(ii)}$$

After cooling, the treated biomass sample is then filtered for separation of solid and the liquid part. Then the hydrolysate was subjected to fermentation. Herein, *Saccharomyces cerevisiae* was used as the fermentative organism. Yeast inoculum was added to the hydrolysate and the fermentation process was done at 32 °C and pH 5 for 7 days at 150 rpm. Later, the fermentation broth was filtered in vacuum pump filter for the removal of solid and the filtrate is then distilled in the distillation unit. Bio-ethanol, which is the distillation product, is concentrated by using molecular sieve (pore size diameter of 3A). The yield of bio-ethanol was found out using the Eq. (iii).

$$\text{Ethanol yield (\%)} = \frac{Ethanol\,mass\,(g/L)}{Total\,carbohydrate\,mass\,(g/L)\times 1.11\times 0.511} \times 100 \quad \text{(iii)}$$

Where,

0.11 is the coefficient of cellulose being converted to glucose

0.511 is the theoretical conversion coefficient of glucose to ethanol.

8.3.4. Hydrolysis reaction of SO using Bio-based catalyst

The analysis of the total carbohydrate in the samples was done using NREL protocol and estimated from HPLC. For lignocellulosic biomass dilute acid or bases acts only as a pre-treatment and not as hydrolysis because of their complex structures as lignin is present. However, for *Scenedesmus obliquus* the cellulose present in the inner membrane is free from lignin, hence readily hydrolysable.

Availability of the active basic sites may affect the efficiency of base hydrolysis of SO for obtaining the fermentable sugars, due to the specific structure of *Scenedesmus obliquus*. The conversion of carbohydrates of the SO into fermentable sugars was done via catalytic saccharification for bio-ethanol fermentation. Catalytic hydrolysis of the biomass was done at 121°C, for 30 min. For the saccharification process, different catalysts were used as shown in the Table 6. From the table, it is evident that highest saccharification yield was exhibited by the CCPS catalyst while the other catalysts showed lower saccharification yield under the same condition. The hydrolysis gave saccharification yield of 60.39% for CCPS. Lowest saccharification was seen while using CWH. The reason behind it might be the lower basicity of CWH.

Table 6: Percentage saccharification using different catalyst

Catalyst	**Saccharification (%)**
CBPA	50.08
CWH	49.01
CCPS	60.39
CTGL	42
K-RAC	49.24

8.3.5. Fermentation

The hydrolysate obtained after the hydrolysis SO was subjected to fermentation using the fermentative microorganism *Saccharomyces cerevisiae*. For the fermentation process yeast inoculums was prepared maintaining a temperature of 30°C. The fermentation was performed using 10 % of yeast innoculum. Yeast should be capable of utilizing all monosaccharide present while withstanding potential inhibitors in the hydrolysate. In this study, the ethanol concentration obtained was 8.24 g/L which was comparable to bio-ethanol concentration obtained with other reported yeast strains like *Schefferssomyces stipitis* (8.2 g/L), *Saccharomyces cerevisiae* RL-11 (11.7 g/L) and *Pachysolen tannophilus* (11.92 g/L) using waste biomass such as giant reed, spent coffee grounds and green seaweed *Ulva rigida* respectively under

similar fermentation conditions. Furthermore, we performed separate hydrolysis and fermentation (SHF) that resulted in 68 % yield (ethanol produced/ g substrate consumed). The yield obtained in our study was found to be higher compared to simultaneous saccharification and fermentation (SSF) and continuous fermentation processes using sugars derived from cellulosic agro-residues like taro waste (43.55 %), wheat straw (24 %) and fruit peels (41 %). Moreover, SHF provides additional advantages like independent temperature optimization for both saccharification and fermentation, use of minimum quantity of hydrolytic enzymes and reduced risk of contaminating microbes. Our results indicate that *Scenedesmus obliquus* deoiled cake (SO) hydrolysate contains fermentable sugars that is quite high and has a great potential as a raw material for bio-ethanol production.

8.5.5.1. Bioethanol characterization

The obtained bio-ethanol was characterized using HPLC (Waters) equipped with UV-Vis detector and C18 column (5 µm × 250 mm). Sample aliquots were filtered through a 0.2 µm nylon filter. Acetonitrile/water in the ratio of 80:20 (v/v) was used as a mobile phase at 1 ml/min flow rate. The temperature of the column was set at 30 °C. GC-MS analysis was performed in a GC-MS 7890A (Agilent) equipped with FID and HP-1MS column (30 m × 250 µm × 0.5 µm). 1µl of sample was injected at an injection temperature of 230 °C, while the temperature of the column was held at 80 °C. Helium was used as carrier gas at 0.5 ml/min flow rate. The physico-chemical properties of bio-ethanol were compared with commercial ethanol, and have been shown in Table 7.

Table 7: The physico-chemical properties of bio-ethanol

Property	**Bio-ethanol**	**Ethanol**
Density (g/cm^3) @ 15°C	0.805	0.785-0.809
Kinematic Viscosity (cSt) @ 20 °C	1.28	1.2-1.5
Flash point (°C)	18	16.6
Flash point (°C)	37	26
Calorific Value (MJ/Kg)	24.82	29.7

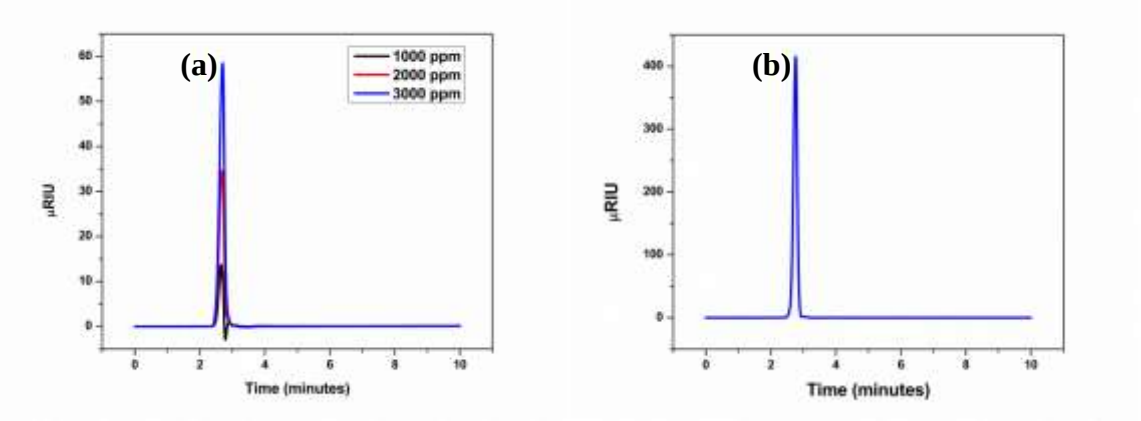


Figure 28: HPLC chromatogram of (a) standard sugar mixture and (b) total carbohydrate in SO

Figure 28 (a), shows a standard sugar mixture for comparison of the cellulose obtained from SO. The total carbohydrate in the SO was determined by the NREL protocol and estimated 21.263 g/L by HPLC [Figure 28 (b)]. The hydrolysate obtained after using CBPA, CWH, CCPS, CTGL and K-RAC was also analysed to detect the presence of sugar with the help of HPLC [Figure 29 (a)].

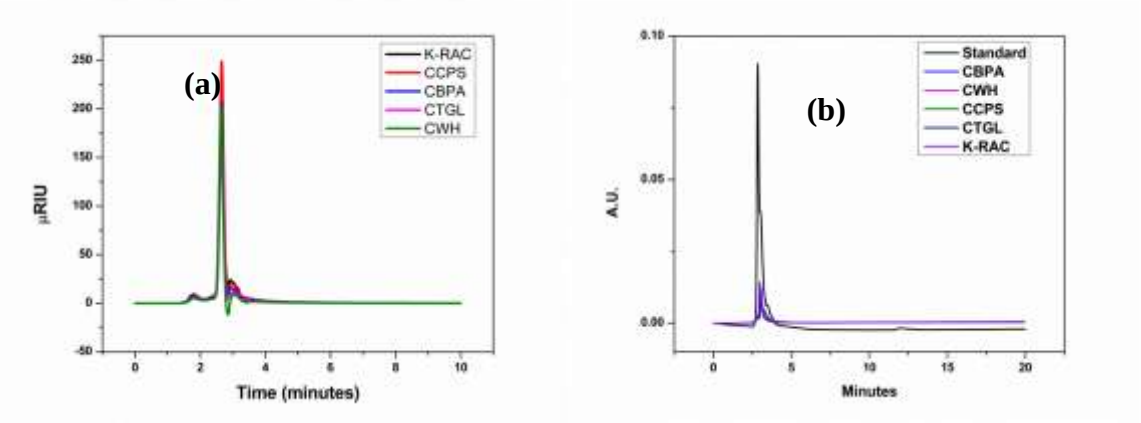


Figure 29: HPLC chromatogram of (a) Hydrolysate and (b) bio-ethanol

In this study, the HPLC analysis of bio-ethanol was estimated as a steep peak at retention time of 2.915, 2.893, 2.865, 2.903 and 3.024 for CBPA, CWH, CCPS, CTGL and K-RAC hydrolysate respectively, compared to commercial ethanol standard at retention time of 2.893 [Figure 7(b)]. Table 8 shows the bio-ethanol yield using different catalysts. Similar HPLC analysis of bioethanol produced by fermentation of agro-residue using *Streptomyces olivaceus* (MSU3) was estimated as a step peak at RT 2.055 min. Likewise, detected the bioethanol peak using HPLC (RT of 5.23 min against standard ethanol; RT 5.538) obtained from fermentation of green microalgal biomass *Acutodesmus obliquus* with *S. cerevisiae*.

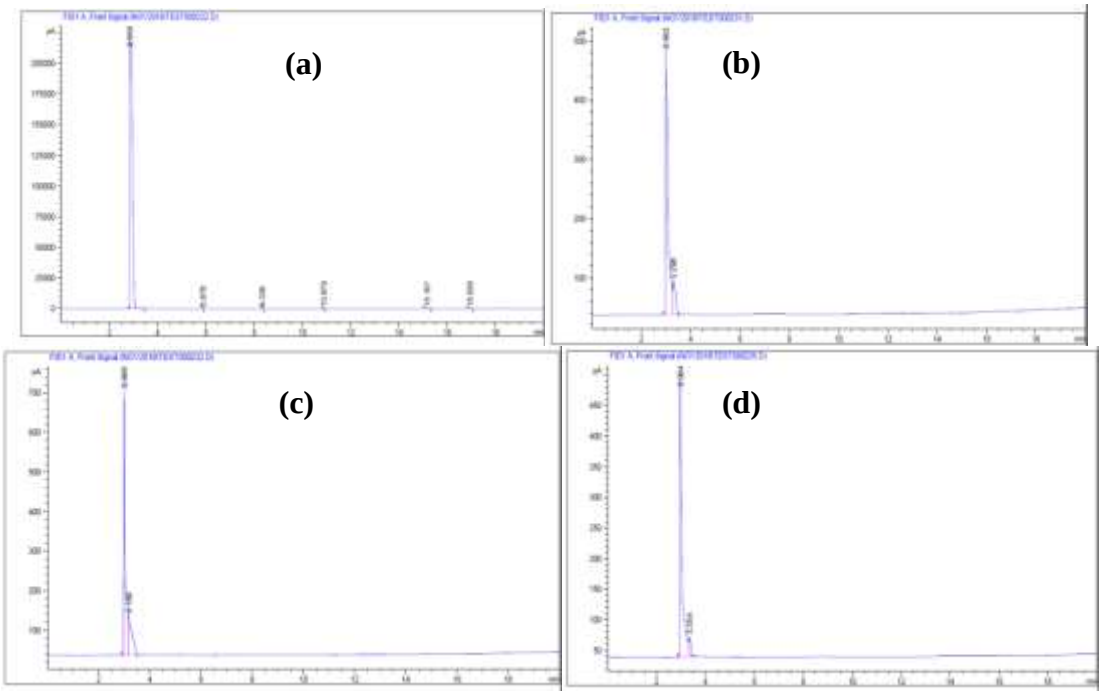
It is evident that the highest bio-ethanol yield [Table 8] was obtained from the CTGL treated biomass hydrolysate. The bio-ethanol concentration was found to be 8.24 g/L and yield of 68.32%. This indicates that the catalyst CTGL is more efficient for bio-ethanol production. Also the lowest ethanol concentration of 5.47 g/L with ethanol yield of 45.03% was obtained from the fermentation of CWH treated biomass hydrolysate. The results are comparable with other published articles, wherein researchers have reported an ethanol yield of 63 % (v/w) from rice straw pre-treated by a fungal strain of *Phanerochaete chrysoporium*under submerged cultivation. Again in another study, a 76% bio-ethanol yield has been reported, wherein agro-residues were utilized.

Table 8. Ethanol concentration and percentage ethanol yield

Catalyst	Ethanol concentration (g/L)	Ethanol yield (%)
CBPA	7.99	66.24
CWH	5.47	45.03
CCPS	8.10	67.16
CTGL	8.24	68.32
K-RAC	7.91	64.48

Gas chromatography (GC)

The GC retention time of the bio-ethanol was compared with the GC of the standard ethanol sample. The graphs as shown in the figures below (Figure 30) signifies that the bio-ethanol spectrum lies between the retention time of 2.99 min to 3.29 min, 2.99 min to 3.19 min, 2.98 min to 3.3 min, 2.97 min to 3.2 min, 2.86 min to 2.93 min for ethanol produced from CBPA, CWH, CCPS, CTGL and K-RAC treated sample respectively which matches with the retention time of the standard ethanol samples i.e. 2.8 min to 3.3 min. Similar GC-chromatogram for bio-ethanol (RT between 1.994 and 2.095 min) was obtained by while studying the effect of different sago waste and yeast type on bio-ethanol production levels. Likewise, have represented the GC of bio-ethanol obtained from *Acutodesmus obliquus* RDS01 and found that the RT of functional ethanol groups was around 4.00 min. In an earlier report, detected the presence of functional group of bio-ethanol produced from waste sugarcane residue in the GC with RT of 2.50 and 13.87 min.



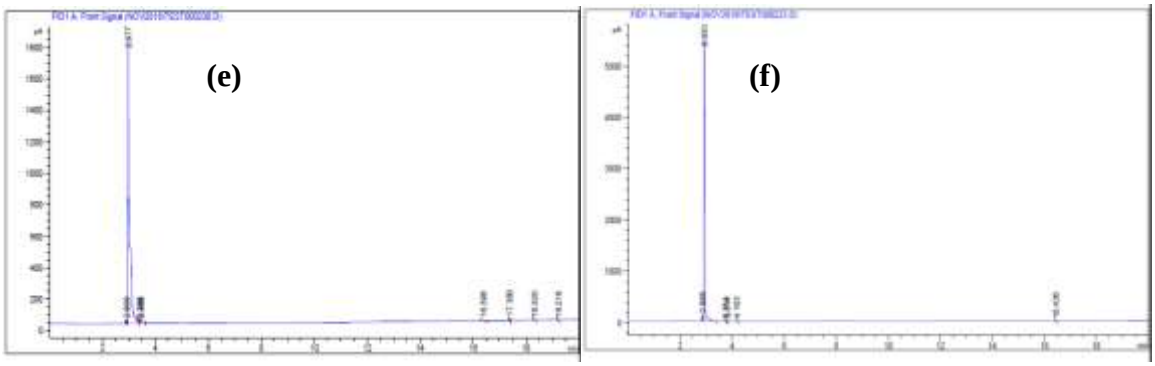


Figure 30: Gas Chromatogram of (a) standard ethanol and ethanol prepared by using (b) CBPA, (c) CWH, (d) CCPS, (e) CTGL and (f) K-RAC

8.5.6. Biooil, Biochar and Catalyst preparation from waste algal biomass

8.5.6.1. Heterogeneous catalyst has been prepared from *Scenedesmus obliquus* deoiled cake

In this study fatty acid methyl esters (FAME) was developed from *Scenedesmus obliquus* lipid using potassium hydroxide catalyst supported on *Scenedesmus obliquus* deoiled cake activated carbon (SAC) as heterogeneous base catalyst (K-SAC). The precursor and catalyst were characterized by powder XRD, FTIR, EDX, SEM, BET, TGA and the Hammett indicator analysis. Reaction parameters affecting FAME yield like methanol/oil molar ratio, catalyst loading and time were optimized. The highest FAME yield 95.21 was obtained at 1:9 oil to methanol molar ratio, 2.5 wt. % catalysts loading within 3.5 hrs at 65 °C. The ^{1}H NMR was used to confirm the formation of biodiesel. The K-SAC can be reused efficiently up to 5 cycles.

8.5.6.2. Activated carbon preparation

Typically, 20 g of finely powdered SO deoiled cake was immersed in 50 % ortho-phosphoric acid in 2:1 (w/w) impregnation ratio for 24 h. Then, it was placed in a silica crucible and calcined at 500 °C (1 h) in a muffle furnace. The produced black solid was initially washed with 0.01 molL^{-1} HCl followed by washing with hot double distilled water till pH of 6-7 was obtained. The product was dried at 110 °C to obtain SAC. The SAC was ground to powdered form using a mortar and pestle, sieved and stored until further use.

8.5.6.3 Catalyst synthesis

The catalyst was synthesized by wet impregnation technique. Typically, 150 ml of KOH solution with initial concentration of either 0.100 - 0.600 g/ml was added to 10 g portions of SAC. The mixture was then agitated at 500 rpm (25 °C) for 24 h and dried at 110 °C (5 h). Finally, it was calcined for 2 h at 500 °C to obtain K-SAC. Gravimetric analysis was performed to determine the potassium loading on SAC.

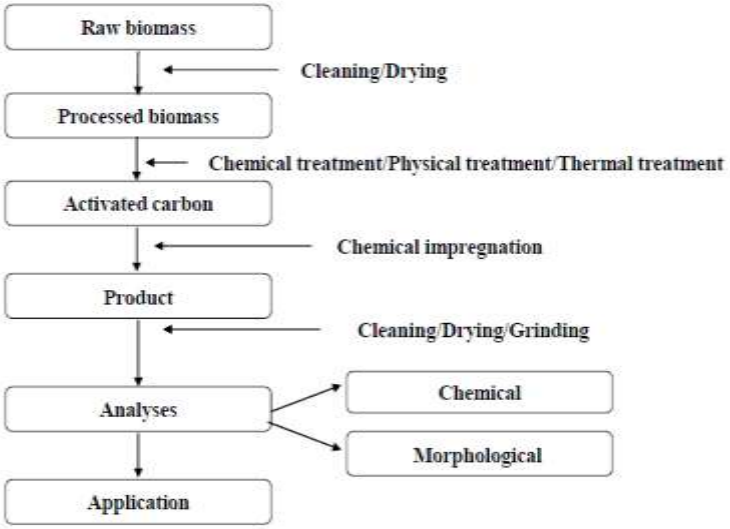


Figure 31: Synthesis of deoiled *Scenedesmus obliquus* cake waste catalyst

8.5.6.4. Catalyst characterization

The XRD pattern of K-SAC was evaluated in a Rigaku miniflex diffractometer (CuKα radiation, λ = 1.5406 Å) for a 2Θ range of 10-80° at a scan rate of 2°. The FTIR spectrum was measured on using a Nicolet (Impact 410) FT-IR spectrophotometer for a wave number range of 4000-400 cm^{-1}. Scanning electron micrographs and surface elemental analysis were obtained on a Jeol, JSM-6290 LV scanning electron microscope. The Hammet indicator method was used to determine the basicity of K-SAC

.

9. Results

9.1. RAH results

The developed system was tested in a raceway pond of Gauhati University, Assam, India where it succesfully collected a total of 565.67 g algae in 200 test runs in 1 hour and 6 minutes. From the algae collection data, it was observed that collection of microalgae in 1 hour is approximately 515.71 grams. The estimated algae harvesting capacity for harvesting 1.5 kg algae by traditional method (by gravity sedimentation) is 12 hours. From these observations, it can be concluded that the developed RAH can harvest algae in less time in comparison to the traditional algae harvesting system.

9.2. Catalyst characterization

XRD

The XRD patterns of SAC and K-SAC are given in Fig. 32. Diffraction peaks for RAC were observed at 2θ = 15° and 23° for the carbon and at 2θ = 24°, 26°, 45° for the graphite. Whereas for K-SAC diffraction peaks at 2θ = 25.31°, 29.37°, 32°, 34°, 38°, 39°, 41.925°, 51.973° (JCPDS code # 77-2176) referred to K_2O. The XRD spectra revealed that the KOH has been successfully dispersed on the carbonaceous support.

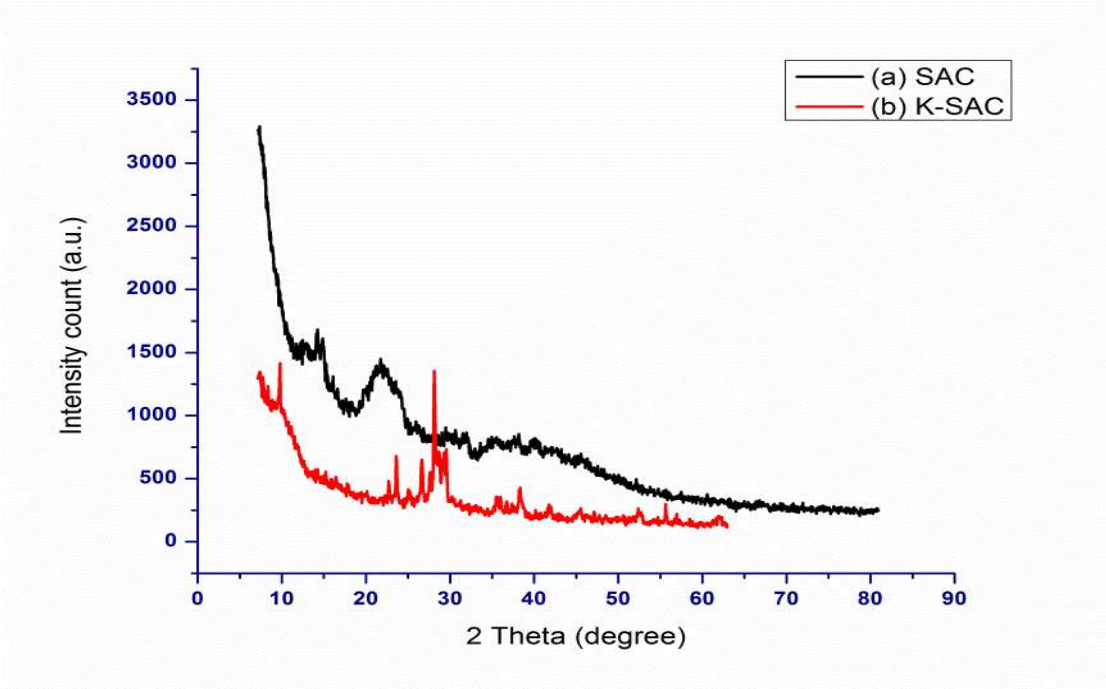


Figure 32: XRD pattern of (a) *Scenedesmus obliquus* deoiled cake activated carbon (SAC) (b) potassium supported SAC (K-SAC)

FTIR

The broad band at 3150 – 3500 cm^{-1} indicates stretching vibration of hydrogen-bonded hydroxyl groups. The band at 1637 cm^{-1} is associated with C = C stretching vibrations or aromatic ring, which assures the aromatization of the precursor. Broad band at 897 cm^{-1} corresponds to C – O stretching in acids, alcohols, phenols, ethers and/or esters groups. The formation of bands at 550 cm^{-1} and 1000 cm^{-1} was because of K – O stretching [Figure 33 (b)]

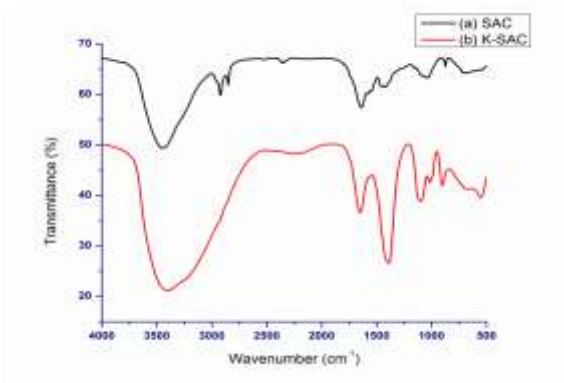


Figure 33: FTIR pattern of (a) SAC (b) K-SAC

SEM

The SEM image of K-SAC (Figure34) illustrates the spongy and porous nature of the catalyst. The high porosity of the RAC provides a large surface area for KOH impregnation. K-SAC catalyst exhibited larger and more consistent blocks, which can be attributed to K compounds on surface of RAC. The KOH was well dispersed on the surface of K-SAC (Figure 34).

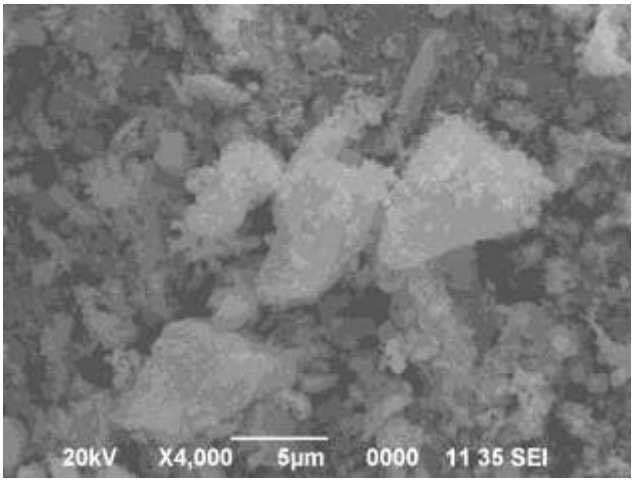


Figure 34: SEM image of K-SAC

EDX

High percentage of potassium was observed from the elemental composition analysis of K-SAC (Figure 35) which suggests that the loaded potassium dispersed properly on the catalyst support.

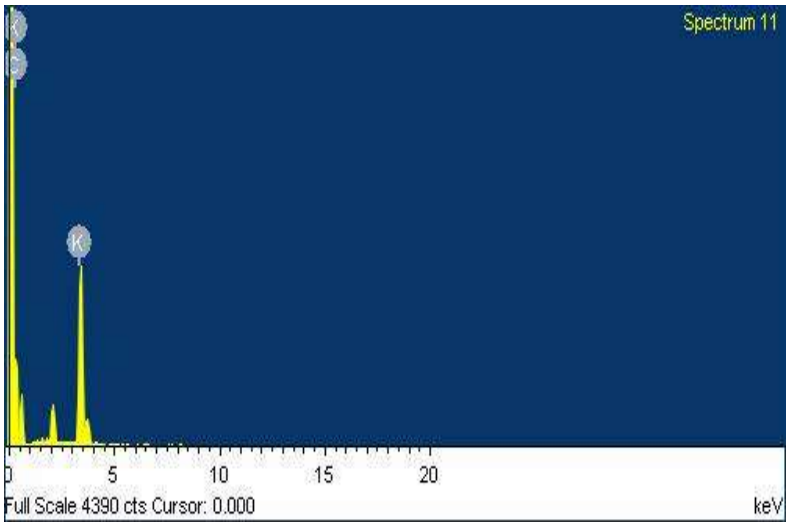


Figure 35: EDX analysis of K-SAC

9.3. Catalyst from waste algal biomass

Catalytic application of *Scenedesmus obliquus* deoiled cake waste has been studied for the first time as support for base catalyst preparation for the production of biodiesel from SO oil. The prepared catalyst exhibited high porosity, specific surface area as well as good reusability. The base catalyst showed higher biodiesel conversion due to high basicity. The obtained biodiesel meets all biodiesel standards. An

effective and straight forward method to transform algal bio-wastes to generate biodiesel and highly basic catalysts has been demonstrated. The reported catalyst shows remarkable potential as alternative and environmentally benign catalyst for biodiesel production.

10. Summary

From the developed Robotic Algae Harvester (RAH) it was observed that the total electrical energy consumed by the RAH to collect per kilogram of microalgae was 110.5 watt-hour which was less than the electrical energy requirement of traditional algae harvesting systems (200 watt-hour). The developed system was tested in a raceway pond where it successfully harvested a total of 515.71 g algae in 1 hour whereas microalgae harvesting using dissolved air floatation and electro flocculation was around 300 g and 500 g respectively for the same time period. The RAH could harvest algae without using chemical flocculants. So, the issues associated with algae biomass contamination were avoided by the use of RAH.

This study depicted the feasibility of producing bio-ethanol from Scenedesmus obliquus using various green heterogeneous catalysts. The highest saccharification efficiency of 60.39 % was achieved using CCPS as base without any pre-treatment step. Ethanol yield of 68.32% being the highest with ethanol concentration of 8.24 g/L was achieved using CTGL catalyst as base. Availability of potential active basic sites and high surface area of CTGL might have been the reason for its effective role. Thus, the bio-based heterogeneous catalysts could be successfully employed for large scale bio-ethanol production from carbohydrate rich microalgae.

11. Any new product process developed

a) Renewable heterogeneous catalyst has been developed for the first time from deoiled Scenedesmus obliquus biomass and utilized for biodiesel production from Scenedesmus obliquus lipid. The catalyst demonstrated a superior catalytic performance achieving fatty acid methyl esters (FAME) conversion of 97.1% within 3 h.

12. Any new lead

a) In this study, deoiled Scenedesmus obliquus (SO) was used for evaluating whether deoiled algal biomass residue is potential as an alternative energy resource for bio-ethanol production by using the newly synthesized heterogeneous catalysts from wastes. The successful hydrolysis of SO was performed employing different eco-friendly bio-based heterogeneous catalysts and hydrolysate thus obtained was then subjected to fermentation using Saccharomyces cerevisiaeand was analyzed through HPLC and GC which resulted in the production of bio-ethanol with the highest yield of 68.32 % at 8.24 g/L concentration without using any pre-treatment step.

b) The benefits of the RAH in terms of energy consumption, time of microalgae collection and quantity of harvested microalgae makes it a favorable technology over the existing algae harvesting techniques.

13. Any technology developed

a) Internally Illuminated Photobioreactor

b) Robotic Algae Harvesting System

14. Any patent taken: Not yet.

15. Publication from project work

List of publications Journal paper

1. Chutia, S., Gohain, M., Borah, M.J., Kakoty, N.M. and Deka, D. Thermal Degradation of Scenedesmus Obliquus for Biofuel Production. Biomass Conversion and Biorefinery. 1-13, 2020.

2. Gohain, M., Hasin, M., Eldiehy, K. S., Bardhan, P., Laskar, K., Phukon, H., Mandal, M., Kalita, D., and Deka, D. Bio-ethanol production: A route to sustainability of fuels using bio-based heterogeneous catalyst derived from waste. Process Safety and Environmental Protection, 146: 190-200, 2021.

3. Gohain, M., Laskar, K., Paul, A. K., Daimary, N., Maharana, M., Goswami, I. K., Hazarika, A., Bora, U., and Deka, D. Carica papaya stem: A source of versatile heterogeneous catalyst for biodiesel production and C–C bond formation. Renewable Energy, 147: 541-555, 2020.

4. Gohain, M., and Deka, D. Water hyacinth derived mixed-oxide heterogeneous catalyst for biodiesel production. IEEE Online Digital Library, 1-9, 2018.

5. Gohain, M., Chutia, S., and Deka, D. Microalgal biomass production and oil extraction for algae biodiesel production–A review. Journal of Energy Research and Environmental Technology, 4: 53-57, 2017.

List of publication Conference paper

1. Chutia, S., Deka, D and Kakoty, N.M. A Review of Underwater Robotics, Navigation, Sensing Techniques and Applications. In the proceedings International Conference on Advances in Robotics (AIR 2017), IIT Delhi, July 2017.

2. Chutia, S., Gohain, M., Deka, D., and Kakoty, N. M. A Review on the Harvesting Techniques of Algae for Algal Based Biofuel Production. Journal of Energy Research and Environmental Technology, 4:58-62, 2017.

3. Hasin, M., Gohain, M., and Deka, D. Bio-Ethanol Production from Carbohydrate-Rich Microalgal Biomass: Scenedesmus Obliquus. In Proceedings of the 7th International Conference on Advances in Energy Research (pp. 1215-1224). Springer, Singapore.

16. Financial progress. Viz. equipment's purchased, manpower, copies of UCs/SEs of various financial year, etc.

Submitted separately for the year 2019-20 and 2020-21.

17. Manpower trained (Ph. D students)

Sl. No	Name	Topic	Current status
1	Minakshi Gohain	Synthesis and application of bio-based heterogeneous catalyst for bio-fuel production	Thesis submitted
2	Swagat Chutia	Design and development of a robotic algae harvesting system for efficient algae collection in biofuel production route	Thesis prepared
3	Doljit Borah	Design and Development of Photobioreactor for Mass Scale Algae Cultivation to Produce Biofuel	Ongoing

IIT-Guwahati Final Report

1. Title:

"Integrated Biorefinery Approach towards Production of Sustainable Fuel and Chemicals from Algal Bio-based Systems"

2. Project Investigators and address

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3. Date of sanction: 15.06.2016
4. Date of completion: 14.12.2020
5. Approved cost: Rs. 76.41321 lakhs
6. Budget released: Rs. 58.05746 lakhs

7. Objectives

1. Design and fabrication of hydrodynamic cavitation reactor for oil extraction from microalgae

2. Extraction of algae oil by hydrodynamic cavitation technique and screening for other biotechnological utilization. Exchange of faculty and students among collaborative institute in India and Brazil.

3 Conversion of algal oil into its alkyl esters by hydrodynamic cavitation, Thermo-chemical and supercritical processing of fresh and waste biomass into biofuel/chemicals and qualitative analysis of the product. Exchange of faculty and students.

4 Characterization of products, byproducts and production of some other value added products and Preparation and submission of Project Report

8. Progress made against targets including methodologies, discussions, etc.

A progress of research work made against the targets is discussed under each of the four objectives as following.

8.1 Design and fabrication of hydrodynamic cavitation reactor for oil extraction from microalgae

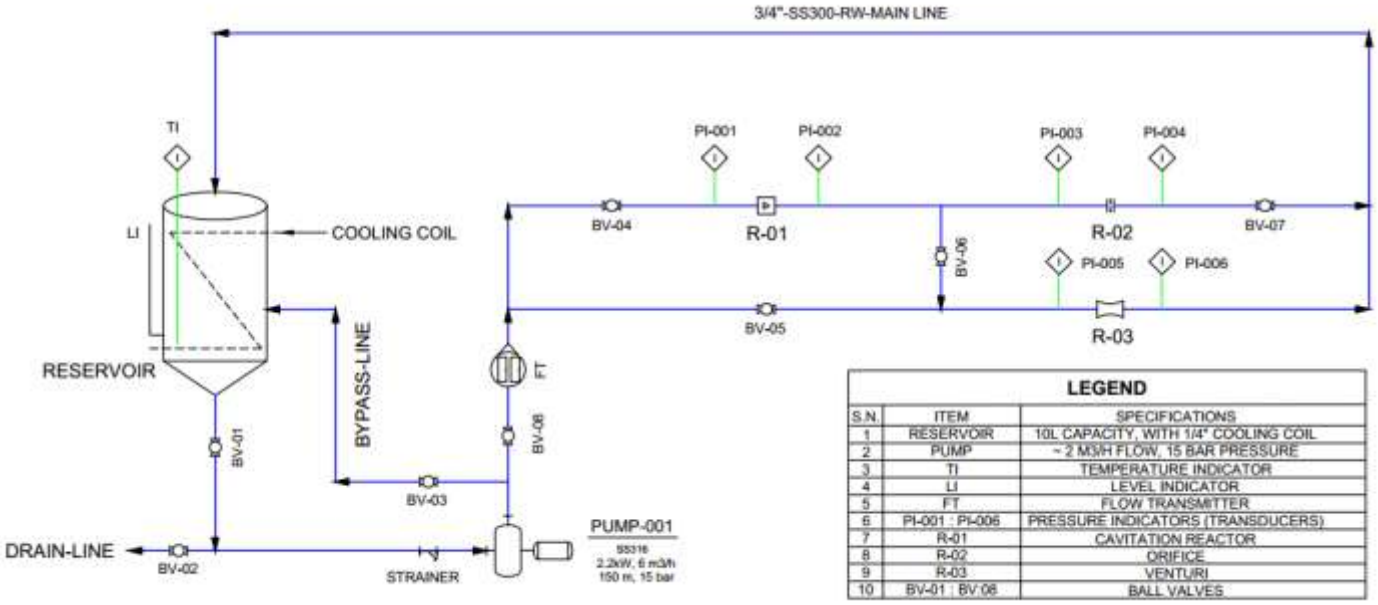

Figure 8.1.1. Hydrodynamic cavitation reactor design for oil extraction

To the date, the applications of hydrodynamic cavitation reactors are found in industrial wastewater treatment. In present research the hydrodynamic cavitation reactor was designed as a process intensification technology for the extraction of oil from aquatic biomass such as microalgae. A laboratory scale hydrodynamic cavitation setup designed for extraction of oil from microalgae with 10–20 L capacity, 15 bar g design pressure and 6 m^3/h pump capacity in SS 304 MOC (Figure 8.1.1). Provision made for comparison of performance of different cavitation reactors to achieve the highest yield of oil. The reactor is fabricated by Frontal Turnkey Solutions, Pune (India). Installation and commissioning work of the hydrodynamic cavitation plant was completed at IITG facility with physical inspection of materials, documentation, training of operator, and successful trial runs. The preliminary trials show the enhancement in oil extraction from microalgae in terms of oil recovery and reduced time of operation.

8.2. Extraction of algae oil by hydrodynamic cavitation technique and screening for other biotechnological utilization. Exchange of faculty and students among collaborative institute in India and Brazil

In present research work, the extraction of lipid from microalgae was investigated using a newer approach of hydrodynamic cavitation (HC) technique. An effective methodology was developed and optimized in this work for high lipid recovery from robust microalgae species.

Novel hydrodynamic cavitation (HC) process was applied to extract the lipid from wet microalgae biomass (MA). The motivation was to eliminate the cost of harvesting of microalgae before lipid extraction in conventional approaches. Using HC technique high lipid recovery of ~ 45% was achieved at optimized conditions. Investigations were made to standardize an efficient methodology to extract lipid directly from dilute culture of microalgae (0.5–1.25% wt.). Orifice plate of 9 numbers of 2 mm diameter holes equipped in pipeline of 20 mm internal diameter. The orifice device was designed with optimized cavitation parameters i.e. cavitation number, α and ß ratios (α = 2, ß = 0.09) for best compatibility with microalgae culture. Important operating parameters such as pressure drop (or flow rate), microalgae cell concentration and cavitation treatment time were studied for maximum lipid yield. Hydrodynamic cavitation pressure drop across orifice (ΔP = 1.5 bar, at 18 m/s flow velocity through orifice) was optimized based on cavitation number to result maximum cell disruption. In initial pilot scale experiments, about 5% of lipid recovery was obtained by treating 15 L of slurry of 0.5% (w/v) microalgae concentration (at ΔP = 1.5 bar, t = 30 min). To enhance lipid recovery several methodological strategies were studies such as: (Method 1) HC treatment of 0.5% MA slurry at ΔP = 1.5 bar, t = 30 min followed by recovery of lipid from water phase by centrifugation; (Method 2) treatment time was increased to 60 min and lipid recovered by heating aqueous phase followed by centrifugal separation; (Method 3) method 2 was repeated with some modification, such as increasing operating pressure drop to check extent of cell disruption; (Method 4) sequential treatment was given by increasing MA cell concentration from 0.5 to 0.75% to increase lipid concentration hence recoverability in effluent; (Method 5) n-hexane was mixed with MA slurry to the ratio 1:10 (hexane:water) by volume to improve extraction of lipid; (Method 6) Sequential extraction cycles of 5 min each were introduced for further improvement in lipid recovery. Figure 8.2.1(a) represents an improvement in lipid recovery from these methodologies. The treatment time was significantly reduced in Method 6 due to simultaneous cell disruption and lipid extraction in hexane phase. 19% of lipid was recovered in first cycle of 5 min, whereas after 3 cycles the maximum of 28% lipid was recovered as represented in Figure 8.2.1(b).

Optimization of microalgae concentration is inevitable for the sake of harvesting cost, pumping cost and lipid extraction efficiency. By increasing MA cell concentration from 0.5% to 1% an increase of 190% was observed in lipid recovery as shown in Figure 8.2.1(c). A high up to 45% of lipid recovery was possible with 1.25% concentrated MA slurry, however, recovery was increased by only 10% by increasing concentration from 1% to 1.25%. Further increase of microalgae cell concentration to 1.5%, with 5 bar pressure drop for a 30 min cavitation time using 20% petroleum benzene as a solvent, achieved an improved lipid extraction efficiency high up to 82% and 94% of residual algal biomass was harvested from dilute (15 g/l) culture of *Scenedesmus obliquus* (Figure 8.2.1(d)). Additionally, the role of n-hexane in HC assisted lipid extraction was investigated. Hexane affect foaming during HC treatment of aqueous microalgae slurry making stable suspension of microalgae-hexane-water as shown in Figure 8.2.2(b). By this phenomena hexane found to play a synergistic role in extraction of intracellular lipid as well as assisting the recovery of lipid by breaking lipid-in-water emulsion as shown in Figure 8.2.2(c–d). The foam was settled to remove air and obtain the microalgae-hexane-water suspension. This suspension was centrifuged easily at 2000 rpm to obtain lipid-in-hexane phase and hexane was evaporated by vacuum evaporation to obtain pure lipid product.

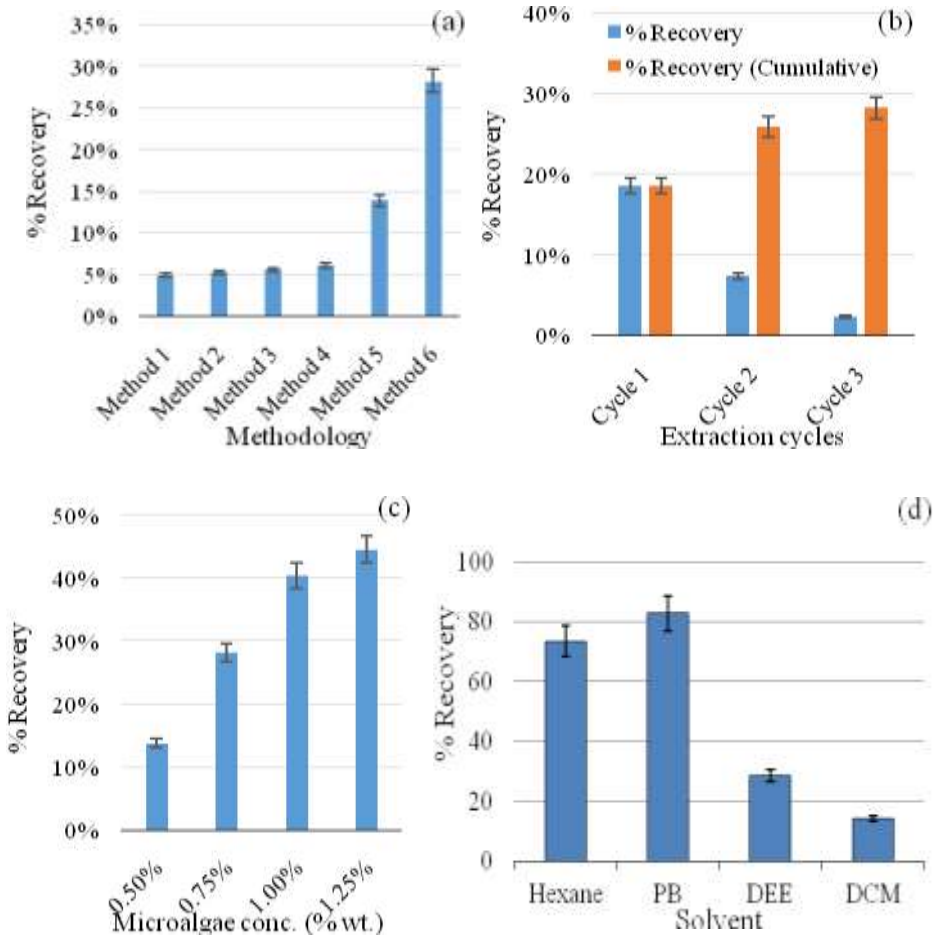

Figure 8.2.1. (a) Different methodologies developed to improve the lipid recovery from wet microalgae slurry using HC; (b) Effect of number of cycles on optimized yield in Method 6; (c) Effect of microalgae concentration on the lipid recovery. (PB = petroleum benzene, DEE = diethyl ether, DCM = dichloromethane).

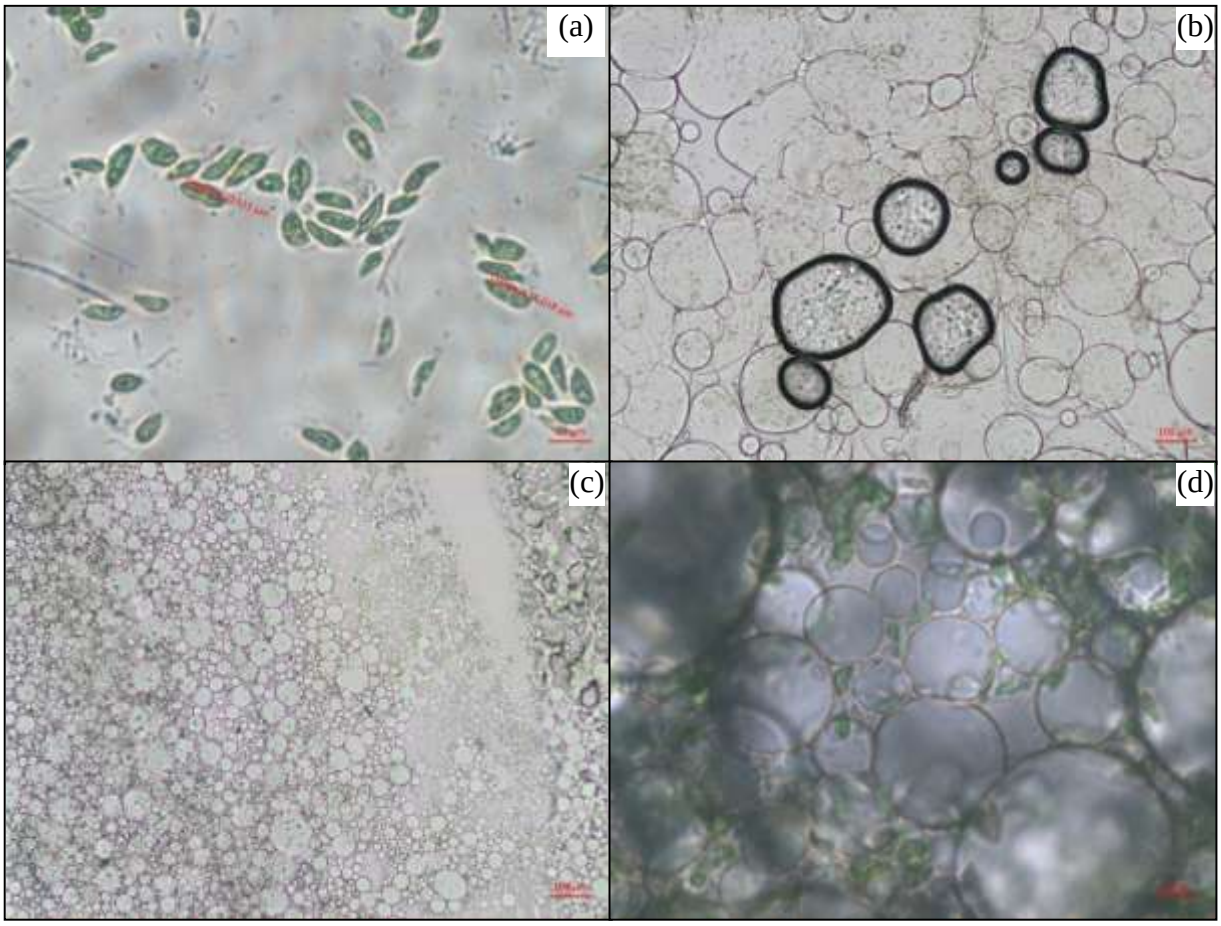

Figure 8.2.2. Microscope images of *Scenedesmus* sp.: (a) in aqueous culture before HC treatment – 100x magnification; (b) foam obtained after HC treatment with 10% n-hexane – 10x magnification; (c) emulsion formed after 24 h of settling – 10x magnification and (d) 100x magnification.

8.3. Conversion of algal oil into its alkyl esters by hydrodynamic cavitation, Thermo-chemical and supercritical processing of fresh and waste biomass into biofuel/chemicals and qualitative analysis of the product. Exchange of faculty and students

8.3.1. Conversion of algal oil into its alkyl esters

The algal oil extracted by hydrodynamic cavitation was converted to fatty acid methyl esters (FAMEs) by transesterification reaction. Methanol was used as reagent and BF_3 was used as a catalyst. The transesterification was carried out at 70 °C for 20 min with continuous mixing to the reaction mixture. After completion of transesterification step, n-hexane was added for the extraction of FAME from the product mixture, and 0.9% NaCl solution was added for the drying of the FAME containing phase, then mixed and allowed to separate the layers. Top n-hexane layer was collected which contains the FAME. The n-hexane + FAME solution was filtered and analyzed using GC-FID and NMR methods. The percentage conversion of the lipid to FAME was obtained to be 90% (w/w).

8.3.2. Thermo-chemical characteristics and kinetic analysis of thermo-chemical conversion of microalgae biomass

Thermogravimetric Analysis (NETZSCH – TG 209 F1 Libra®) was used to study the thermochemical behavior of SCR, heating in the temperature range from 25 to 1000 °C, with heating rates 10, 20, 30 and 50 °C/min under pyrolytic conditions (N_2 flowrate 30 ml/min). The thermogravimetric analysis (TGA) and its differential (DTG) plots of SCR are shown in Figure 2, and the data presented in Table 1 showing the thermal degradation of biomass under pyrolytic conditions. In the DTG plots in Figure 2, initial small peak at ~100 °C represents the dehydration stage. Major degradation stage in the range 163 – 550 °C yields 58% of volatile matter. This stage is considered as main pyrolysis stage, which involves the degradation of hemicellulose, cellulose and lignin. The first step of pyrolysis region in the range 160-400 °C corresponds to the decomposition of hemicellulose and cellulose, with mass loss up to 71% out of total volatile mass loss (58%) in pyrolysis region. Second step occurring in the region 400–550 °C represents relatively slow degradation of lignin with 29% of mass loss, which starts to decompose from about 300 °C along with cellulosic components. The third step in the region 550–780 °C represents the thermal degradation of volatiles from char formed in previous steps. Low heating rates lead to more char formation, whereas more liquid yield can be achieved at higher heating rates and low temperature in the range 200–520 °C. On the basis of careful consideration of the above results, process constraints determined for the pyrolysis of SCR to produce bio-oil.

Using TGA data, pyrolysis reaction kinetic analysis done in the range 200-550 °C. Different solid state kinetic models applied to determine the three essential kinetic parameters i.e. reaction model, activation energy (E_a) and Arrhenius constant (A). Kinetic data lead us for optimizing the pyrolysis reaction parameters and design of experiments to produce bio-oil from microalgae by pyrolysis process.

Table 8.3.1. Thermogravimetric analysis of SCR biomass. (Note: HR – heating rate (°C/min), T_i – initial peak temperature (°C), T_f – final peak temperature (°C), DR – maximum degradation rate i.e. $-(dm/dt)_{max}$ (%/min), T_p – peak temperature (°C) corresponding to $-(dm/dt)_{max}$, %ML – % Mass Loss).

	Step 1					**Step 2**				
	T_i	T_f	T_p	DR	ML	T_i	T_f	T_p	DR	ML
	163	231	256	2.9	56.5	231	246	258	1	7.7
	171	233	259	2.5	56.7	233	274	255	5	0.7
	181	236	252	2.5	56.6	236	235	254	8	0.7
	191	239	244	3.2	56.5	239	244	252	5	1.3

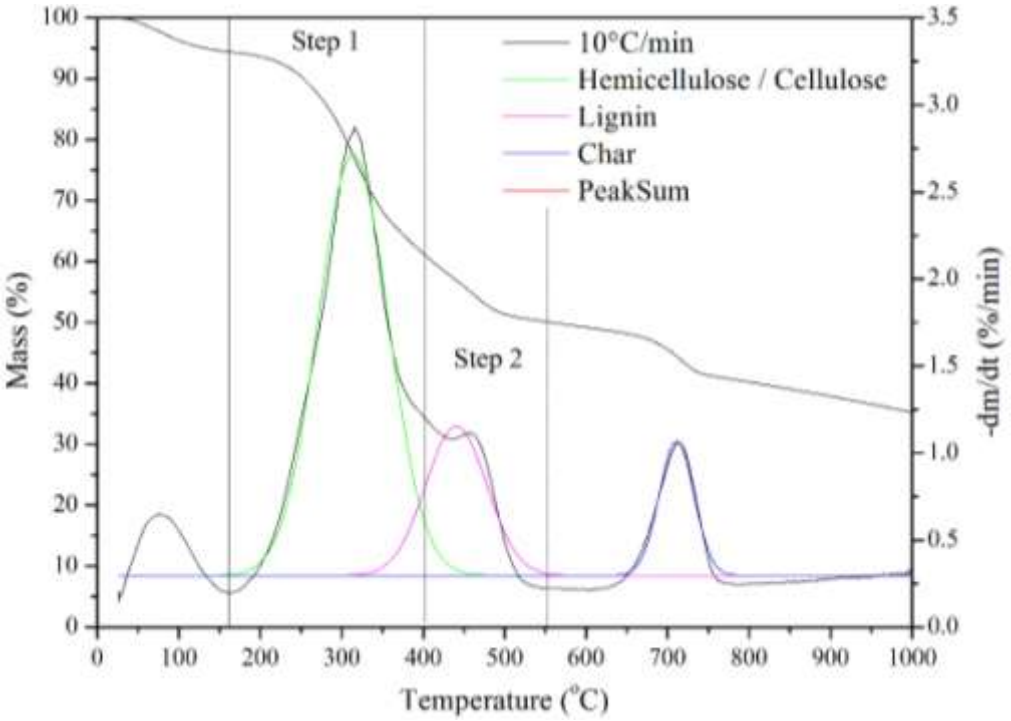

Figure 8.3.1. TGA & DTG analysis of SCR biomass at heating rate 10 °C/min

8.3.3. Thermo-chemical processing of fresh and waste biomass into biofuel / chemicals

Defatted microalgae residue remaining after extraction of lipid still has 72% of volatile matter content. Also, the microalgae residues generated from other biorefinery processes such as residue after extraction of bioactive compound contains considerable amount of fat and volatile carbonaceous matter. On the other side, lignocellulosic biomass (such as residue from agriculture and forestry) currently is one of the most abundant renewable source for biofuel and energy generation. Further utilization and management of such residue in eco-friendly manner could be a challenge on account of sustainable development. Pyrolysis, also known as thermal cracking is easy way for thermochemical decomposition of biomass and its residue to produce biofuel and range of valuable products. With this motivation a co-pyrolysis of microalgae residue (MAR) and rice husk (RH) was carried out produce bio-oil in bench scale tubular pyrolysis reactor in the range 300–600 °C under inert atmosphere of nitrogen gas 0.5–1.5 l/min for holding time of 6–73 min. The maximum of ~17% bio-oil yield was obtained at optimized operating conditions of 450 °C temperature, 1.15 l/min nitrogen gas flow rate, 40 min holding time in pyrolysis reactor. The dependence of operating parameters for the co-pyrolysis of MAR and RH represented in Figure 8.3.2.

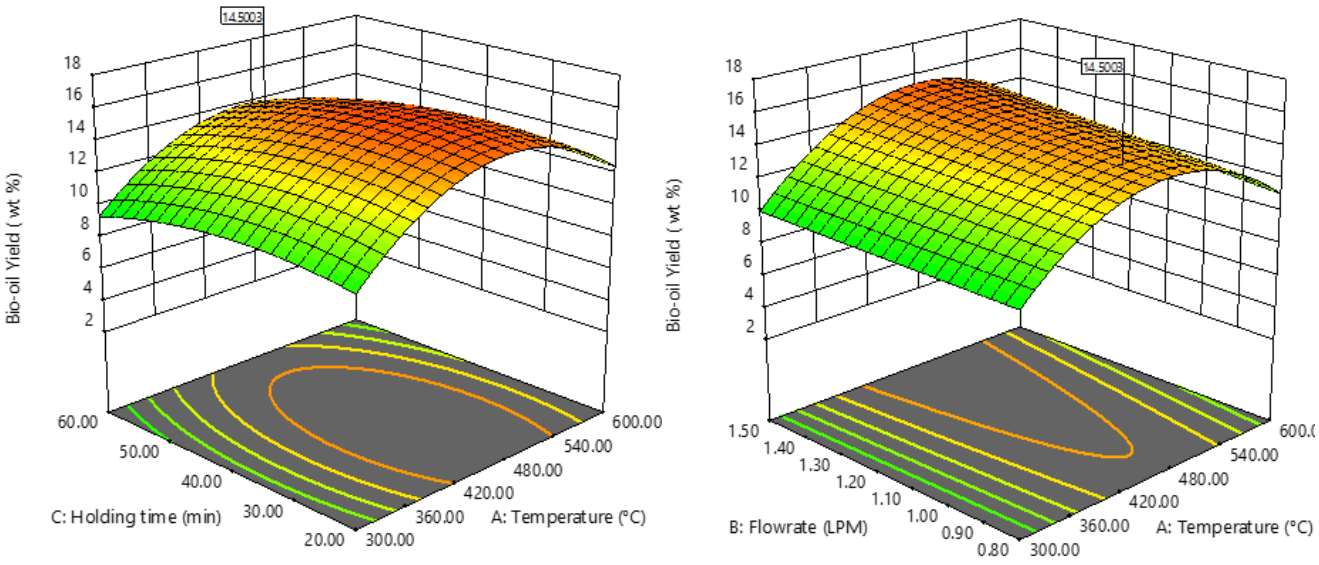

Figure 8.3.2. Optimizing process parameters for maximum yield of bio-oil from microalgae residue (MAR) by co-pyrolysis with rice husk

8.4. Characterization of products, byproducts and production of some other value added products and Preparation and submission of Project Report

8.4.1. Lipid and Biodiesel (FAME) analysis

It is a known fact that hexane extracts of the algal biomass possess higher percentages of lipids. The oil product of hydrodynamic cavitation induced extraction of wet microalgae is mainly the lipid. Therefore, the lipid product was characterized by ^{1}H NMR (600 MHz FT NMR- ASCEND 600, Bruker, United States) analysis. Deuterated chloroform ($CDCl_3$) was used as the solvent to prepare sample for NMR test. The fatty acid methyl ester (FAME) product (i.e. biodiesel) produced by the transesterification of lipid was characterized by ^{1}H NMR analysis and a gas chromatograph equipped with flame ionization detector (GC-FID)(Clarus 590, Perkin-Elmer, USA) coupled with a capillary column (50 m × 250 μm × 0.25 m) (BPX-70 column, SGE Analytical Science, Australia). The FAME composition was determined from respective peak area and presented as a percentile of total FAME content. The identification of the FAME components was performed by comparison to known standards of FAME components, and presented in Table 8.4.1.

Figure 8.4.1. Identification and quantification of the fatty acids present in the fatty acid methyl ester (FAME) samples

Sl. No.	Fatty acid	Name of the component acid methyl ester	Retention time (min)	Percentage in FAME
	Saturated fatty acid (SFA)			
1	C10:0	Capric	11.612	2.74±0.02
2	C13:0	Tridecanoic	19.065	4.45±0.01
3	C14:0	Myristic	21.575	5.46±0.03
4	C16:0	Palmitic	26.646	30.49±0.02
5	C17:0	Heptadecanoic	29.028	2.88±0.02
6	C18:0	Stearic	31.339	0.44±0.01
7	C20:0	Arachidic	35.687	2.10±0.01
8	C22:0	Behenic	39.709	1.45±0.01
	Monounsaturated fatty acid (MUFA)			
9	C16:1	Palmitoleic	27.718	0.52±0.02
10	C18:1N9C	Oleic	32.235	10.10±0.02
11	C20:1N9	cis-11-Eicosenoic	36.495	3.72±0.01
	Polyunsaturated fatty acid (PUFA)			
12	C18:2N6C	Linoleic	33.743	16.72±0.04
13	C18:3N6	γ-Linolenic	34.715	0.15±.01
14	C18:3N3	α-Linolenic	35.516	18.78±0.02

8.4.2. Extractive value of the microalgae sample in different solvent by Soxhlet extraction method

Microalgae are the rich source of lipids, proteins, carbohydrates and variety of valuable chemicals of economic importance in food, medicine and public health. The nutritionally valuable chemicals that reported to be available in microalgae are fatty acids, steroids, carotenoids, polysaccharides, phycobiliproteins, lectins, amino acids, antioxidants, antibiotics, toxins, agar agar, alginic acid and

carrageenan. For commercial exploitation of these compounds it is necessary to isolate and extract those compounds in its naturally occurring form. In this present study successive solvent exaction of *Scenedesmus sp.* sample have been carried out in nonpolar and polar solvents to identify the phytochemicals. Further analyzed antioxidant activity with relation to phenolic and flavonoid content in *Scenedesmus* microalgae sample. Finally, the antibacterial activity was analyzed.

Extraction value added chemicals carried out using different solvents with varying polarity and percentage of the oil yield was determined (Table 8.4.2). It was found that water was having highest extractive value of 18% followed by methanol, chloroform, hexane, Ethyl acetate, and acetone which was found to be 17%, 9%, 6%, 6%, and 3%. Total 37 Phytochemical test were carried out including test for carbohydrate, hexose sugars, proteins, amino acids, cardiac glycosides, flavonoids, alkaloids and many more.

Table 8.4.2. Extractive values, antioxidant activity, and carotenoids, phenolics, and flavonoids content in the different solvent extracts of *Scenedesmus obliquus*. (± SD, n = 3)

Type of solvent extract	Extractive value (%)	IC_{50} value for DPPH (µg/ml)	IC_{50}value for ABTS (µg/ml)	Carotenoid content (mg/g DE)	Phenolic content (mg GAE/g EW)	Flavonoid content (mg QE/g EW)
n-Hexane	6±2.5	262±16	186±34	4.9±1.3	0.7±0.1	0.23±0.07
Chloroform	9±2.2	229±13	197±11	4.3±0.7	0.4±0.1	0.30±0.20
Ethyl acetate	6±1.2	180±18	156±19	3.4±0.9	7.3±0.7	2.65±0.35
Acetone	3±1.1	105±8	114±9	0.5±0.3	12.9±2.1	3.37±1.63
Methanol	17±3.3	68±7	64±10	1.1±0.2	18.3±0.7	5.88±2.12
Water	18±2.1	65±10	61±3	0.6±0.3	20.9±2.1	5.94±1.06
Ascorbic acid	-	2.35–2.79	9.09–11.03	-	-	-

*ABTS: 2,2'–azino–bis(3–ethylbenzothiazoline–6–sulfonic acid, DPPH: 2,2–diphenyl–1–picrylhydrazyl, DE: Dry extract, EW: Extract weight, GAE: Gallic acid equivalent, QE: Quercetin equivalent

8.4.3 Total Phenolic, Favonoid, and Carotenoid Content of Extracts

The total soluble phenolic compounds present in the extracts were determined colorimetrically using Folin-Ciocalteu reagent according to the method of Sen et al. (2013), with gallic acid as the standard. Similarly, the total flavonoid compounds present in the extracts were determined colorimetrically using aluminium nitrate reagent according to the method of Sen et al. (2013), with quercetin as the standard. Carotenoid content of the extracts was determined spectrophotometrically, according to the method reported by Maadane et al. (2015) using Lichtenthaler equation.

The total phenolic, flavonoid and carotenoid content of solvent various extraction products (extracts) of microalgae *S. obliquus* are presented in Table 8.4.2. The acetone, methanol, and water extracts comprised of maximum amount of highly polar phenolics (12–23 mg GAE/g EW) and flavonoids (2.7–8 mg QE/g EW). It can be seen that the hexane, chloroform, and ethyl acetate extracts comprised of maximum amount of carotenoids (3–6.2 mg/g of DE), which is due to the fact that carotenoids are only soluble in low polarity solvents. Phenols occurring in nature are of multiple applications (such as antioxidants, astringency, bitterness, browning reactions, color, oxidation substrates, protein constituents, etc.). Flavonoids are novel bioactive compounds of great interest in drug discovery. Flavonoids are secondary metabolites and have capacity to act as anti-oxidant, anti- bacterial, anti-inflammatory and anti-cancer agent.

8.4.4. Antioxidant Activity

The concentration of extracts (μg/ml) required for 50% scavenging of the DPPH (2,2–diphenyl–1–picrylhydrazyl) and ABTS (2,2'–azino–bis(3–ethylbenzothiazoline–6–sulfonic acid)) radicals (IC_{50} value) were determined and compared with the antioxidant activity of ascorbic acid (concentration= 1–5 μg/ml), following the standard method. The antioxidant activity of the various solvent extract products are presented in Table 8.4.2. Methanol and water extracts showed maximum antioxidant activity as deduced from their low IC_{50} values (60–75 μg/ml) for both the free radicals. Acetone extract also showed average antioxidant activity (102–123 μg/ml). But, the antioxidant activity of the hexane, chloroform, and ethyl acetate extracts was found to be very less as compared to the rest of the three solvent extracts. The IC_{50} values of the different solvent extracts followed the following trend: water > methanol > acetone > ethyl acetate > chloroform > n-hexane, which was statistically significant ($p < 0.001$), for both DPPH and ABTS radicals.

8.4.5. Antibacterial Activity

The Gram-positive and Gram-negative test bacteria such as *S. aureus, B. subtilis, M. luteus, E. coli, K. pneumoniae, P. aeruginosa,* and *E. aerogenes* were used for the evaluation of antimicrobial activity of the extracts. The inocula were prepared in Mueller Hinton broth according to CLSI recommendation, wherein the OD_{600} value was adjusted to the equivalent of 5×10^5 CFU/mL, which was determined from the calibration curve of each microorganism. The Resazurin Microtitre Assay (REMA) method was followed to determine the minimum inhibitory concentration (MIC) of the sample product to deactivate the bacteria (Figure 8.4.1). The zone of inhibition (ZOI) assay using Mueller Hinton agar plates was performed to determine the range of antibiotic activity of sample. The one-way analysis of variance (ANOVA) test was analyzed using SPSS® software to evaluate the statistical significant results. It was clearly seen from the results that the hexane, chloroform, and ethyl acetate extracts of *Scenedesmus obliquus* may possess many unique non-polar compounds apart from less amount of polar compounds like phenolics and flavonoids, resulting in its high broad-spectrum antibacterial activity against all the tested bacteria despite having low antioxidant activity (Figure 8.4.1).

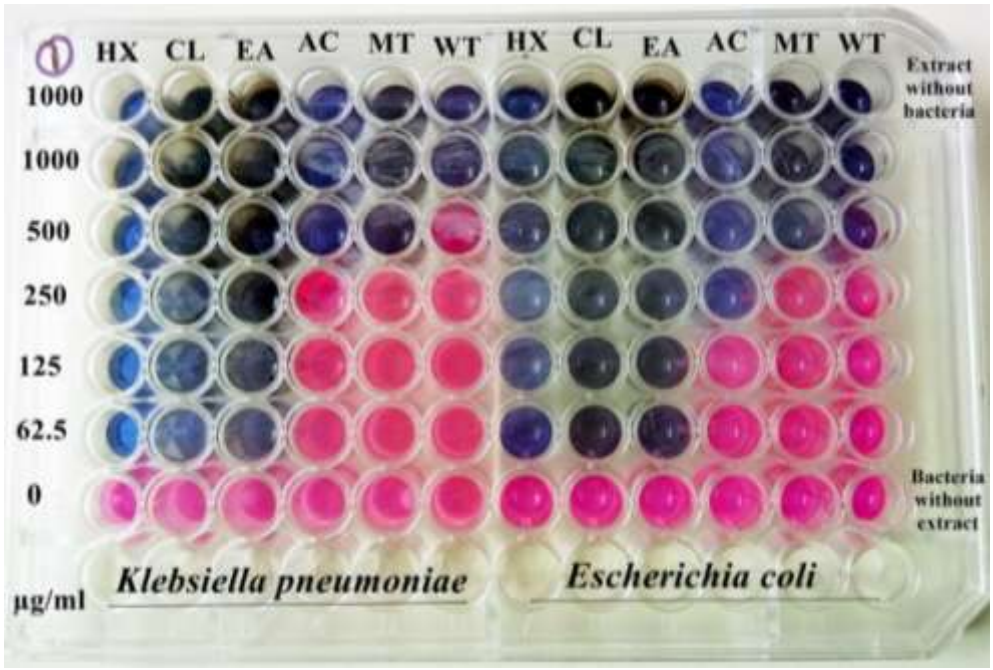

Figure 8.4.1. Microtiter plate showing minimum inhibitory concentration (MIC) of the different solvent extracts for Klebsiella pneumoniae and Escherichia coli. (Note: HX- hexane, CL- chloroform, EA- ethyl acetate, AC- acetone, MT- methanol, WT- water extract)

9. Results

9.1 Design and fabrication of hydrodynamic cavitation reactor for oil extraction from microalgae

A novel pilot scale hydrodynamic cavitation system was designed, fabricated, and a process was developed to intensify the extraction of oil or lipid from wet microalgae biomass and integrated harvesting of microalgae biomass for further utilization. The system and process was demonstrated under laboratory environment to process 15–30 L volume of 0.5–2% (w/w) dilute wet microalgae slurry upto 15 bar pressure to extract algal oil or lipids.

9.2 Extraction of algae oil by hydrodynamic cavitation technique and screening for other biotechnological utilization. Exchange of faculty and students among collaborative institute in India and Brazil.

The pilot scale hydrodynamic cavitation system was developed and demonstrated for the extraction of lipid from wet microalgae biomass. The oil or lipid recovery of 74–82% can be achieved in 30 min of cavitation time under 5 bar cavitating pressure. The oil or lipid product extracted from this system can be utilized for biofuel production as well as valuable chemical production.

9.3 Conversion of algal oil into its alkyl esters by hydrodynamic cavitation, Thermo-chemical and supercritical processing of fresh and waste biomass into biofuel/chemicals and qualitative analysis of the product. Exchange of faculty and students.

The algal oil or lipid was converted into fatty acid methyl esters (FAME or biodiesel) through transesterification reaction, where 90% w/w conversion of algal oil (lipid) into FAME was achieved.

The thermogravimetric analysis of dry algal biomass and its defatted residue revealed the favourable conditions for the thermo-chemical conversion of biomass to produce bio-oil to be in the range 300–600 °C. The co-pyrolysis of microalgae residue (MAR) and rice husk (RH) under produced a maximum of 17% bio-oil yield at optimized operating conditions of 450 °C temperature, 1.15 l/min nitrogen gas flow rate, 40 min holding time in pyrolysis reactor. The higher heating value of bio-oil was about 17 MJ/kg which is suitable for its use for the biofuel production

9.4 Characterization of products, byproducts and production of some other value added products and Preparation and submission of Project Report

The ^{1}H NMR analysis of lipid product of hydrodynamic cavitation revealed the presence of acylglycerols, mono-unsaturated fatty acids (MUFA), poly-unsaturated fatty acids (PUFA), and saturated fatty acids (SFA). From GC-FID analysis, it was observed that the FAME sample contained about 30% of palmitic acid among SFA, 10% oleic acid among MUFA, and 17% linoleic acid, and 19% linolenic acid among PUFA. The high content of total SFA & MUFA (~60%) proves the usability of the algal lipid product for biodiesel production. Also, the lipid contains about 36% total PUFA, which proved that the strain is useful for the production of valuable bioactive chemicals signifying pharmacological importance of the strain.

The microalgae extracts revealed significantly higher antioxidant and antibacterial activity. Significantly high content of Phenolic (18–23 mg GAE/g EW), Flavonoid (5–8 mg QE/g EW), Carotenoid (3–6 mg/g DE) were detected in methanol and water extracts of the *S. obliquus*. As a result, the extract shown the higher antioxidant activity with inhibitory concentration IC_{50} = 61–76 µg/ml DPPH, IC_{50} = 60–75 µg/ml ABTS. Hexane and chloroform extracts of *S. obliquus* showed a high antibacterial activity against Gram positive and Gram negative bacteria (MIC = 15.6–125 µg/ml, ZOI = 27–30 mm). The methanol and water extracts showed lower antibacterial activity (MIC = 250–1000 µg/ml) than the hexane and chloroform extracts (MIC = 15.6–125 µg/ml). Lipids were found as their main constituents because of their non-polar nature. However, all the extracts (polar as well as non-polar) showed high antibacterial activity against Gram negative bacteria which is a very promising fact and is being reported for the first time in *S. obliquus*.

10. Summary (in two hundred words only)

The holistic approach of this project was to integrate the biorefinery processes for the production of biofuel and high added-value compounds from algal biomass with maximum utilization of byproducts. To meet these objectives, a hydrodynamic cavitation system was designed and validated under laboratory environment to process wet microalgae slurry recovering upto 82% of lipid (oil). A high content of total SFA and MUFA was detected in the lipid which proved its usability for biodiesel production. The algal oil was converted into biodiesel or FAME at 90% transesterification conversion efficiency. It was demonstrated, that the waste microalgae residue can be utilized judiciously by applying thermo-chemical process *viz.* co-pyrolysis with locally available lignocellulosic biomass to produce bio-oil with about 17% yield. Also, 36% PUFA content was detected in lipids showing utilization potential of the strain for the production of valuable chemical. Therefore, sequential extraction process investigated to enhance the recovery of desirable chemicals. A significantly high broad-spectrum antibacterial activity, and important antioxidants, flavonoids like myricetin, rutin, and quercetin were detected for the first time. Overall, the systems and processes developed in this research to process microalgae biomass has high potential to be used industrially for the production of biofuels and valuable chemicals.

11. Any new product/ process developed

A system and process was developed for the extraction of lipid from wet algal biomass in biotechnological application.

12. Any new lead

The extracts of *Scenedesmus obliquus* microalgae showed higher antibacterial activity for Gram negative bacteria. The flavonoids like myricetin, rutin, and quercetin were detected for the first time in *Scenedesmus obliquus* stain.

13. Any technology developed

Hydrodynamic cavitation technology was developed for the intensification of the oil extraction from wet algal biomass.

14. Any patent taken

The patenting of the process / technology developed is in submission phase.

15. Publications from project work

1. Suryawanshi P.G., Goud V.V. (2019). Evaluation of pyrolysis kinetics and mechanism for thermal decomposition of defatted microalgae biomass. International Conference on Advances in Renowned Renewable Energy Technologies - 2019, VRSEC, Vijayawada (AP). (Conference Proceeding) (Best Paper Award)
2. Suryawanshi P.G., Das S., Borugadda V.B., Goud V.V., Dalai A.K. (2020). Process Improvements and Techno-Economic Feasibility of Hydrothermal Liquefaction and

Pyrolysis of Biomass for Biocrude Oil Production. Biorefinery of Alternative Resources: Targeting Green Fuels and Platform Chemicals, Springer, 221-248. doi: 10.1007/978-981-15-1804-1_10

3. Suryawanshi P.G., Goud V.V. (2021). Processing Thermogravimetric Analysis Data for Pyrolysis Kinetic Study of Microalgae Biomass. Springer Proceedings in Energy, Springer, 1415-1424. doi: 10.1007/978-981-15-5955-6_134
4. Mukherjee C., Suryawanshi P.G., Kalita M.C., Deka D., Aranda D.A.G., Goud V.V. (2021). Polarity wise Successive Solvent Extraction of Scenedesmus obliquus Biomass and Characterization of the Crude Extracts for Broad Spectrum Antibacterial Activity. Algal Research. (under review)
5. Suryawanshi P.G., Goud V.V. (2021). Pyrolytic degradation properties and kinetic analysis of microalgae waste residue, rice husk and their blends (Drafting).
6. Suryawanshi P.G., Kalita M.C., Deka D., Aranda D.A.G., Goud V.V. (2021). A process and system for extraction of lipid from wet algal biomass. (Drafting)

16. Financial progress. Viz. equipments purchased, manpower, copies of UCs/SEs of various financial year, etc.

Submitted separately.

Gauhati University Final Report

1. **Title:**

   "Integrated Biorefinery Approach towards Production of Sustainable Fuel and Chemicals from Algal Bio-based Systems"

2. **Project Investigators and address**

   (i) **Prof. M C Kalita (Ph.D.)**
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3. **Date of sanction:** 15.06.2016
4. **Date of completion:** 14.12.2020
5. **Approved cost:** Rs. 65.426 lakhs
6. **Budget released:** Rs. 51.89444 lakhs
7. **Objectives:**

   1. Laboratory scale cultivation and biomass production.
   2. Field scale cultivation and biomass production with experimental open-raceway pond.
   3. Field scale cultivation and biomass production with experimental low cost PBR.
   4. Exchange of faculty and students.

8. **Progress made against targets including methodologies, discussions, etc:**

During the reporting period (**June 2016- December 2020**) from the actual date of sanction of the project till the completion of this report we targeted the approved objectives within the time line of activities (A9.1). The project was officially executed from June, 2016 , soon after the appointment of the project staffs (one SRF, one JRF and Two field cum laboratory attendant) followed by purchasing necessary consumables as well as taking necessary steps to procure instruments as per the University system.The specific targets achieved during the reporting duration are listed below:

Objective 1 targeted:*Laboratory scale cultivation and biomass production.*

- As per the project objective, we have selected two fresh water microalgae species *viz.Scenedesmus obliquus* and *Haematococcus pluvialis* for culture optimization and bio

refinery production and further studies.

S.obliquus was selected based on our previous lab experiments, which showed the potentiality out of a number of species studied for bio-fuel production. Culture of *S. obliquus* was already standardized, so currently we are behind to augment and further optimization of the biomass production to the extent of *S. obliquus* using different media alternatives not carried out earlier and simultaneously also carrying out growth optimization studies for *Haematococcus pluvialis* which is the another microalga showing high possibility in fulfilling our biorefinery concept. Experiments for alternative sources of media input other than the conventional media, work on*Azolla pinnata* available in the state with rich nitrogen source , waste *Potato peeletc* have been already started based on our inquisitiveness of nutrient sources . Azolla is known as an aquatic green manure plant and it has the ability for fixing atmospheric nitrogen and in algal culture nitrogen is one of the key nutrients. We have already started mass cultivation of Azolla in large tanks of size 65cmx35cm (Fig 1).

H. pluvialis , which is a unicellular microalga belonging to class Chlorophyceae marks to be the best source of astaxanthin "the super antioxidant". Large scale cultivation of *H. pluvialis* can be practised either in closed photobiorector or in open raceway ponds. According to the work plan culture optimization of *H. pluvialis was* done to an extent. As per our pilot study *H. pluvialis* growth is significantly enhanced with the uniform circulation of media and surface area that is getting direct exposure to light as well as lesser depth of media volume. Therefore, we are trying to come up with different efficient options like cultivating *H. pluvialis* in 50 litre round plastic basins (Fig 2) , glass aquarium of capacity 30 litre (Fig 3). Moreover we are also trying to design plastic container with a capacity of 70litre for mass cultivation of *H. Pluvialis* (Fig 4)

Figure 1. *Azolla pinnata* growing in large water tanks.

Figure 2. 50 lit round Plastic basins for algae cultivation

Figure 3. *H. pluvialis* growing in aquarium of size (44cm x 30cm)

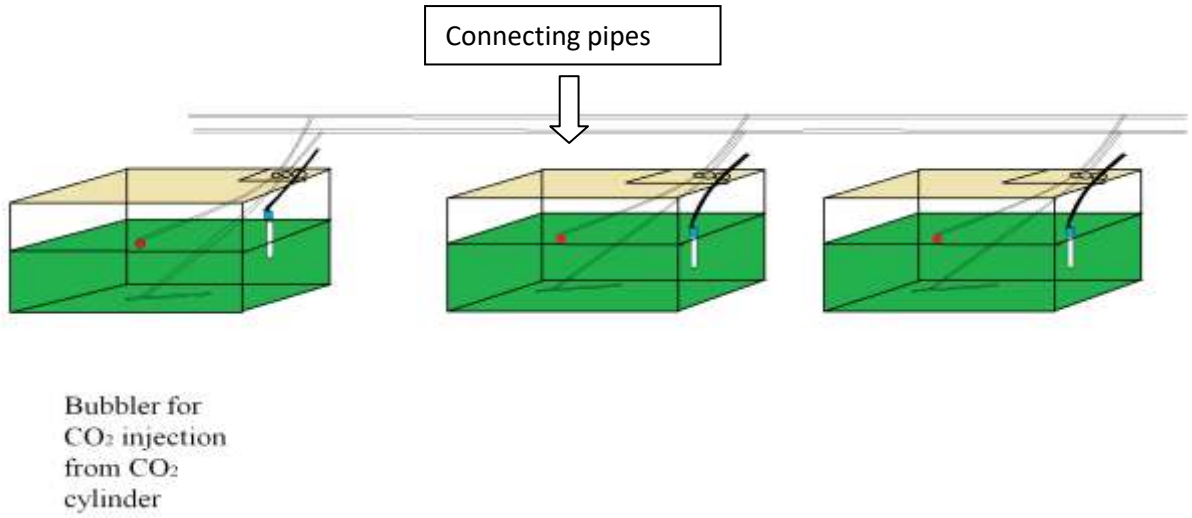
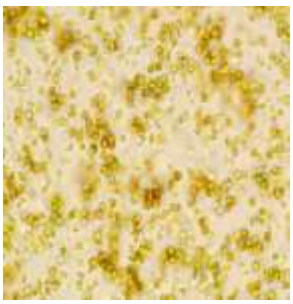
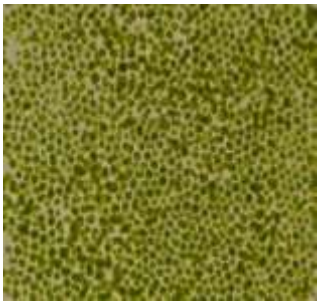


Figure 4. Design for construction of low cost plastic tank having capacity 70 litre for *H.pluvialis* culture

Isolation and establishment of monoculture: Isolation and axenic culture of *S. obliquus* and *H. pluvialis* were carried out using the standard practice (Kawai *et al.).* Serial dilutions were conducted up to 10^{-5} dilutions and 100µl of sample was taken out from each dilution , followed by repeated streaking on agar enriched plate (BG11 + 1% agar agar) and allowed to grow in controlled growth conditions of temperature 22°C and light intensity 35μ mole photons $m^{-2}s^{-2}$





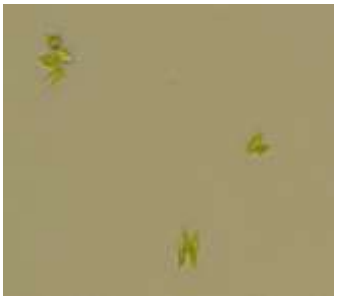


Figure 5a: Poly culture of algae **Figure 5b:** Axenic culture of *H. pluvialis***Figure 5c:** Axenic culture of *S.obliquus*

As per the project proposal our institute (Gauhati University) host the responsibility to supply biomass to IIT Guwahati (collaborative institute) as well as Tezpur University for further research and optimization as per the objectives. Therefore, currently we are engaged in production of biomass from *S.obliquus* using already established protocol and simultaneously undertaking experiments to optimize the culture conditions of *H.pluvialis*. In case of *S. obliqqus* , BG11 was found to be suitable media as per our lab standardised protocol. Keeping in mind, the need to supply algal biomass, *S.obliquus* was cultured in BG11 media with 20mM Urea in small scale as well as large scale cultivation undertaking in 500 litre open raceway ponds with organic enriched (OE) media i.e. sundried cow dung extract with filtered pond water and 5mM urea.



Figure 6a: Culture of *S.obliquus***Figure 6b**: Harvesting **Figure 6c**: Sun dried biomass

Growth parameters like suitable media, initial culture media pH, and alternative nitrogen sources of *H. pluvialis* was carried out under controlled laboratory conditions.Growth properties was inferred from direct cell count and biomass productivity was determined from dry cell weight (DCW). The growth conditions can be summarised as irradiance 35µ mole photons $m^{-2}s^{-1}$, temperature 22°C and 16:8 hr light and dark diurnal cycle. Specific growth rate (µ) and doubling time (T_2) were calculated by using equations (1) and (2) according to Levasseur *et al.* (1993).

$$\mu = \ln (N_2/N_1)/t_2\text{-}t_1 \quad \textbf{(1)}$$

$$T_2 = 0.6931/\mu \quad \textbf{(2)}$$

Where: N_1 and N_2 cell numbers at time (t_1) and time (t_2), respectively.

- **Experimental Findings**

Growth characterization of *H. pluvialis* in two media BG11 and Chu13: *H. pluvialis* was grown in both BG11 and Chu13 and growth pattern was studied for 14 days under controlled laboratory conditions. Parameters like Optical density, cell count by Neubauer haemocytometer

slide and photographs were also taken by cell imaging system Evos life technologies in 40x magnification after every 24hrs of regular interval.Chu13 was found to be the suitable growth media for the given strain compared to BG11 with specific growth rate of 0.347 and doubling time of 1.90

Table1: Compositions of BG11 and Chu13 media for microalgae cultivation.

Composition	**BG11**	**CHU (modified)**
Macronutrients (g/L)		
$NaNO_3$	1.5	_
KNO_3	_	0.371
KH_2PO_4	0.040	_
K_2HPO_4	_	0.080
$MgSO_4.7H_2O$	0.075	0.200
$CaCl_2.2H_2O$	0.036	0.107
NaCl	_	_
EDTA-FeNa	0.001	_
Na_2CO_3	0.02	_
Citric Acid	0.006	0.1
$NaHCO_3$	_	_
Ferric Ammonium citrate	0.06	_
Ferric Citrate	_	0.020
Urea	_	_
Micronutrients (mg/L)		
H_3BO_4	0.061	2.86
$ZnSO_4.7H_2O$	0.287	0.02
$CuSO_4.5H_2O$	0.003	0.08
$MnSO_4.5H_2O$	0.169	_
$Na_2MoO_4.2H_2O$	0.013	0.039
$Co(NO_3)_2.6H_2O$	_	0.05

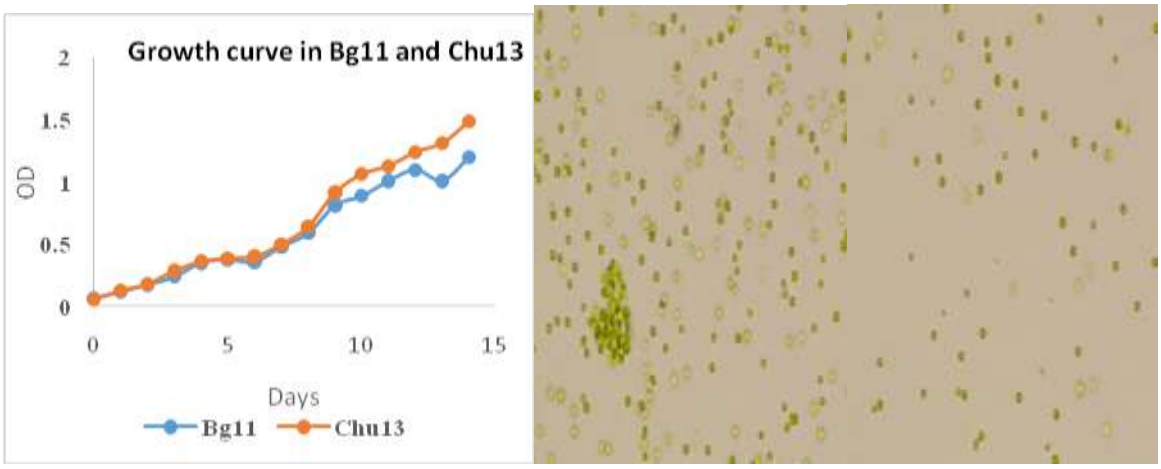


Figure 7a Growth curve in BG11 and CHU **Figure 7b**:10th day culture in CHU13 **Figure 7C:** 10th day culture in BG11 media

Test for alternative nitrogen sources for *H. pluvialis*

H. pluvialiswas grown in BG11 with different nitrogen sources like $NaNO_3$, NH_4NO_3, KNO_3 and Urea. $NaNO_3$ gives the best result as alternative nitrogen source with biomass yield of $0.65gL^{-1}$ (DCW) whereas biomass productivity was least for Urea $0.3gL^{-1}$ (DCW).

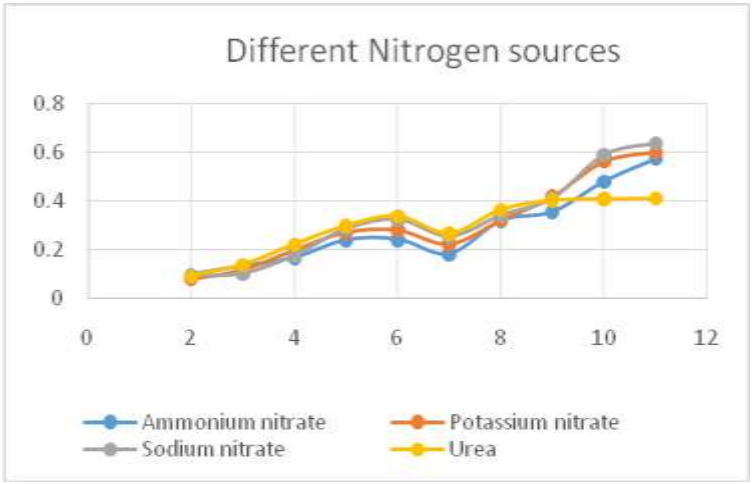


Fig 8:*H. Pluvialis* culture with different sources of nitrogen

Up scaling of microalga species *H. Pluvialis*:

From the small scale culture in culture flask , we tried to culture in round bottom 5 litre flask. Growth was checked by taking Optical density (Spectro 50 plus Analytik jena), cell count and

photos (Evos XL core life technologies) on everyday basis. Cell count was found to be 6.5x 10^5 in Chu 13 media compared to 6x10^5 in BG11 media at the end of 8^{th} day.

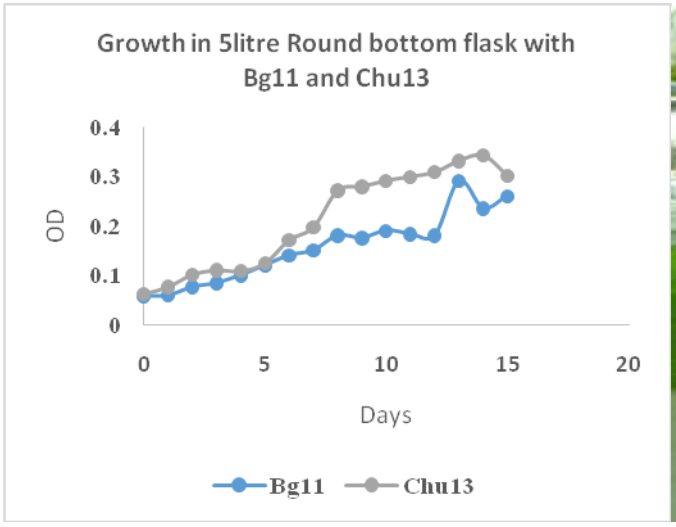


Fig 9a:*H. Pluvialis* growth curve in 5litre round bottom flaskround bottom flask

Fig 9b: Adding Urea to 5litre

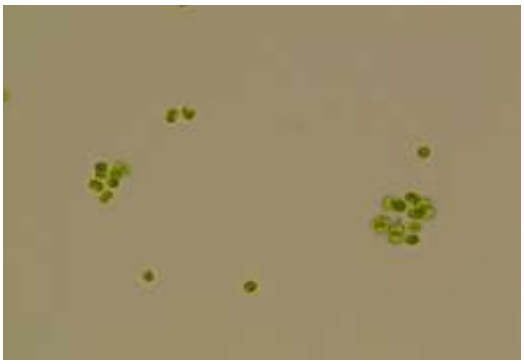


Fig 9c: Growth in Chu13 media on 8^{th} day

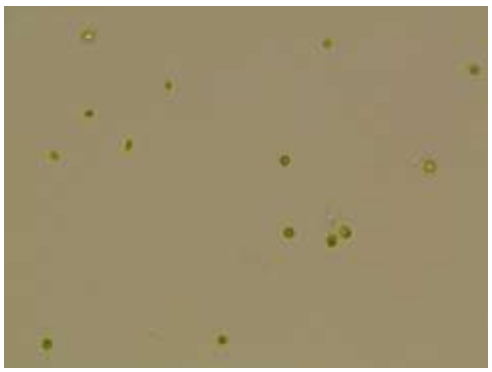


Fig 9d: Growth in BG11 media on 8^{th} day

Mass cultivation is one of our main project objectives, so we are trying to upscale of culture from indoor culture i.e. culturing in 250ml flask to first in 15 litre closed photobioreactor than cultivation of alga in open raceway ponds 1000litre x 3 Units.

Effect of different concentrations of Urea on growth of *Haematococcus pluvialis*:

The study was conducted for 13 days in batch mode by supplementing $NaNo_3$ of RM with urea as the sole nitrogen source ranging from 1mM to 9mM in an increasing order **(Table 2).** RMmedium was taken as the control medium to compare the growth performance of the strain. Nevertheless, there was a better growth performance of the strain in each of the urea treatment

than that of control medium (RM).Interestingly, the medium with 2 mM urea supported a constant growth of the strain, and yielded the maximum increase in cell numbers (10.3×10^5 cells / ml) at the terminal day of the experiment**.**

Effect of initial culture pH

The significant effect of initial culture pH on growth of *Haematococcus pluvialis* was monitored for 13 days, where it was observed that pH 7 provides better growth for the species with a specific growth rate of0.22(μ/d), **t**he cell density with pH 7 increased to 10.2×10^5 cells/ml, pH 8 also provided a good growth which is slightly lower than pH 7 with a specific growth rate of 0.207(μ/d),Where the cell number has increased to 9.6×10^5 cells/ml . It can be said the pH between pH 7-8 is best for a better growth of *H.pluvialis* as again in pH 9 the cell number dropped with a specific growth rate of 0.205(μ/d). The lowest growth was observed in pH 5 and pH 6 which concludes that acidic condition is not favorable for the growth of *H.pluvialis*

Table 2: Effect of urea on growth of *Haematococcus pluvialis*

Urea (mM)	**Specific growth (μ/d)**	**Doubling time (days)**	**Increase in cell number ($\times10^5$ cells/ml)**
1	0.212	0.31	9.8
2	**0.23**	0.31	10.3
3	0.205	0.29	9.4
4	0.207	0.29	9.6
5	0.200	0.28	9.2
6	0.203	0.29	8.8
7	0.197	0.28	8.1
8	0.195	0.28	8.4
9	0.196	0.28	7.7
Control	0.189	0.27	9.06

Effect of light on growth

Three levels of light intensity from 25, 40 and 75 μ mol/m^2/s (PAR) for 12 hours light and 12hrs dark were screened for selected microalgae strain**.** It was observed that the growth was maximum in 75 μ mol/m^2/s (PAR) with a specific growth rate of 0.203 (μ/d) where the cells increased 10.7×10^5 cells/ml followed by 40 μ mol/m^2/s (PAR) with a specific growth rate of 0.2198 (μ/d) where the cells increased to 10.1×10^5 cells/ml and the least growth was observed in 25 μ mol/m^2/s (PAR) where the cells increased ml to 9.6×10^5 cells/ml.

Effect of dissolved inorganic carbon (DIC) and CO_2 gas as carbon nutrientsource on

growth

C-source of the suitable media was supplemented with the suitable media (RM) in triplicates each having different concentrations of bicarbonate salt for *H.pluvialis*. Sodium bicarbonate salt concentration ranges from 15,30,45,60 and 75 mg/L (1mg/L=1ppm) was freshly added to each flask containing the media and the inoculums. The alga exhibited a good growth under the media supplemented with different concentration of bicarbonate salt. From the growth curve, it can be observed that the algal growth was found to be highest in 30ppm of sodium bicarbonate salt with a specific growth rate of 0.23(μ/d) where the cells have increased to 12.7×10^5 cells/ml as compared to control with a specific growth rate of 0.185(μ/d). The amount of lipid in 30ppm of sodium bicarbonate was found to be 22.66%. Gaseous CO2 is also supplied to culture of *H.pluvialis* in variable amounts (7929, 4758 and 4400 mg/L). From spectrophotometric analysis at 680nm it was observed that the highest growth was at 4758ppm of CO_2 gas with a specific growth rate of 0.25 (μ/d) and an increased cell density of 17.05×10^5 cells/ml and the lipid was found to be 26.6% . The growth rate in 4758ppm of CO_2 gas is much higher than 4400ppm and 7923ppm but control (RM) was found to be higher than 7923ppm but less than 4400ppm and 4758ppm.

Effect of salinity on growth

Salinity trial was conducted with the addition of NaCl at various concentrations (5.0 – 30.0 mM) **(Table 3)** in increasing order to the selected medium(RM) The strain was found to grow in all the six concentrations of NaCl (5 - 30 mM) . The highest specific growth rate (μ = 0.217/d) was observed with 5mM of NaCl .

Table 3: Effect of salinity on growth and lipid content of ***Haematococcus pluvialis***

Parameter	**Salinity (mM)**						
	Control (0)	**5**	**10**	**15**	**20**	**25**	**30**
Specific growth (μ/d)	0.214	**0.22**	0.206	0.204	0.19	0.208	0.202
Doubling time (days)	0.31	0.31	0.29	0.3	0.28	0.3	0.29

Standardization of efficient harvesting technique

For selection of a cost effective and energy efficient method of harvesting flocculation

efficiency was studied for *Haematococcus pluvialis* using different organic and inorganic chemicals such as $FeSo_4$, $ZnSo_4$, $MgCl_2$ and Chitosan at different concentration (0.05 to 0.3 gm with a difference of 0.05) for 270min. The highest (97.34% ± 0.66%) flocculating activity with a settling time of 210 min was achieved by **0.25gm of $ZnSO_4$**.

Organic extracts

Various organic extract treatments namely **Cow dung** extract (CD), **Goat Manure** (GM), ***Azolla pinnata*** compost (AC) and **Soap Nut** (Sapindus sp.) (SN) were studied with different concentrations each (2, 5, 8, and 10 %, v/v) in addition to this 2 mM urea was supplemented to each of the extract treatments separately (2, 5, 8, and 10 %, v/v). The concentration of urea was determined based on the result from previous experiment on growth studies with different concentrations of urea. The best growth among the three organic media was found to be in cow dung for *H.pluvialis* which was quite close to RM (inorganic media). For goat manure , the best growth for *H.pluvialis* among different concentrations was found to be 10% along with 2mM urea with a specific growth rate of 0.18(μ/d). Without urea the best growth was observed in 10% but the growth was less than 8%concentration of goat manure with 2mM urea**.** For Azolla Compost extract the best growth was observed in Azolla 10% with supplementation of 2mM urea with a specific growth rate of 0.19(μ/d) which is better than Goat manure extract. For cow dung manure extract the best growth was ovserved in 8% supplemented with 2mM of urea with a specific growth rate of 0.21(μ/d)**.** Soapnut extract experiment was aborted on 5^{th} day as flocculation of the algal cells was a major problem which may be due to the density of the extract**.** For *Scenedesmus obliquus* , the growth pattern from our previous standardized protocol with cow dung was by far the best as compared to Azolla compost extract and goat manure extract. But among goat manure and Azolla extracts the best was found to be in case of use of Azolla extract. Azolla (8%) with supplementation of 2mM urea with a specific growth rate of 0.404(μ/d)**.** Goat manure (8%) with supplementation of 2mM urea exhibited a specific growth rate of 0.37(μ/d)**.**

Table 4: Elemental Composition of Organic sources

Parameters	Amount (%)for cow dung powder	Amount (%)for Goat manure powder	Amount (%)for Azolla sp. compost	Amount (%)for Soapnut powder
Nitrogen	0.05	0.63	0.68	0.62

Potash	1.0585	0.56	0.75	2.44
Phosphorus	0.0197	0.23	0.13	0.1
Calcium	0.3485	1.88	1.14	0.18
Manganese	0.0395	0.0005	0.004	0.0005
Iron	0.9493	0.0027	0.0057	0.0039
Magnesium	-	0.14	0.12	0.05
Sulphur	-	0.069	0.060	0.058

Indoor open cultivation of *S. obliquus* and *H.pluvialis* using cow dung medium

For potential implication of the low-cost cultivation technique for the selected microalgae strain, the strain was cultivated in plastic storage container using treated tap water enriched with CD-EO medium (CD2%, v/v). Light intensity of ~4 Klux was provided with white florescent tube light. Culture was provided with continuous bubbling of membrane sterilized air. Culture medium pH was checked at 7.0 ±0.2 twice daily by bubbling gaseous CO_2 at the bottom from external source. The culture was run for 7 days for *Scenedesmus obliquus* and 13 days for *Haematococcus pluvialis* of culture duration and growth performance was measured by direct cell count. The average specific growth rate (μ/d) was 0.38/d with the biomass doubling time (T_2) of 0.41 day for 20L and he average specific growth rate (μ/d) was 0.36/d with the biomass doubling time (T_2) of 0.4 day for 40L. The biomass harvested during the late exponential phase of growth under the stated indoor open culture conditions, revealed a total lipid content 30.3% and 30.08% (*S.obliquus*) of dry cell mass. For potential implication of the low-cost cultivation technique for *Haematococcus pluvialis*, the strain was cultivated in plastic storage container (70L) using treated tap water enriched with CD-EO medium (CD EO 8%, v/v). The biomass harvested during the late exponential phase of growth under the stated indoor open culture conditions, revealed a total lipid content was found to be 19.8% of dry cell mass.

Objective 2 targeted:*Field scale cultivation and biomass production with experimental open-raceway pond.*

Outdoor mass cultivation

The cultivation was initiated with 15 L culture in PBR unit 1 with 0.2 g/L dry cell mass. the culture flow was regulated by fresh nutrient loading in to PBR 1 unit at a flow rate of 1 L/h to outflow the culture into PBR 2 unit. At the same time the media inflow to the PBR 1 unit and

culture outflow from PBR 2 unit to PBR 3 unit. The average cell biomass harvested during the study period was recorded at 0.6 – 0.7 g/L/d. The outdoor cultivation was initiated *H.pluvialis* in 70L plastic container where the specific growth rate was found to be 0.19 µ/d giving a biomass of around 0.4-0.6 g/L/d.

Outdoor pond

Outdoor culture parameters such as light intensity, air temperature at the culture site, pH of the culture, water level, and biological contaminations were monitored daily. It was noted that the daily average changes in culture pH in the range between pH 7.0 – 9.8. The pH change in the growing culture was controlled daily at pH 7.0 ±0.2 by supplying gaseous CO_2 from external source.

Table 5: Month and yield of lipid

Month	(g)	Yield of lipid (%, wt)	Month	(g)	Yield of lipid (%, wt)
April	650.0	23	October	420	19
May	575.0	20	November	395.0	31
June	390.0	32	December	305.35	32
July	455.0	25	January	180	20
August	570.0	32.6	February	365.0	29
September	305.0	19.68	March	621.4	28

Growth kinetics of *Chlorella homosphaera* on a few selected culture media:

Isolation of microalgae strains were done according to standard procedure (Kawai *et al*., 2005) using BG11 media. After the isolation *chlorella homosphaera* was explored in terms of its growth kinetic studies in three different culture media namely BG11 (Stanier *et al.* 1971), BBM (Kanz and Bold 1969) and CHU13 (Chu 1942; Yamaguchi *et al.* 1987) media.

Table 6: Specific growth (d^{-1}), Doubling /day, Doubling time (d), Increase in cells/ml/day/($X10^6$) ± SE and Biomass mg/ml/±SE of *Chlorella homosphaera* under different types of culture media

Culture media	Specific growth (d^{-1})	Doubling /day	Doubling time(d)	Increase in cells/ml/day/($X10^6$)±SE	Biomass mg/ml/±SE

BG11	*0.641*	*0.925*	*1.08*	*2.17*±0.02	*0.362*±0.01
BBM	*0.398*	*0.574*	*1.74*	*1.41*±0.05	*0.291*±0.001
CHU13	*0.202*	*0.291*	*3.43*	*0.71*±0.02	*0.156*±0.002

Effect of pH and salinity on the *Chlorella homosphaera*:

To determine the optimum pH, the growth of *Chlorella homosphaera* was carried out for different initial pH of 6,7,8,9 and 10 under BG11 culture media (Table 7). A growth study was conducted for 9 days to find out the optimum initial pH. The microalgae isolate showed maximum growth at pH 7. It showed maximum specific growth rate of 0.669 per day when cultured at pH 7. It has been found that along with the growth of microalgae strain, the pH value rises gradually and after 9^{th} day of culture the pH of the entire culture flask was found to be approximately 10 to 10.5.

To determine the optimum salinity for growth and lipid content *Chlorella homosphaera* was grown under different molar concentrations of sodium chloride i.e. 0.02 M, 0.04 M, 0.06 M, 0.08 M and 0.17 M. Results indicated maximum growth of *Chlorella homosphaera* at 0.02 M NaCl concentration. It was also observed that at higher concentration (<0.1 M) of salinity, the biomass productivity decreases and cells unable to grow which was followed by cell degradation.

Table 7: Specific growth rate (d^{-1}) of *Chlorella homosphaera* under different initial pH with BG11 media.

Microalgae isolate	**pH**				
	6	7	8	9	10
Chlorella homosphaera	0.414	0.669	0.552	0.61	0.414

Table 8: Specific growth (d^{-1}), Doubling /day, Doubling time (d), Increase in cells/ml/day/($X10^6$) ± SE and Biomass mg/ml/±SE of *Chlorella homosphaera* under different levels of salinity.

Salinity (Molar)	**Specific growth (d^{-1})**	**Doubling /day**	**Doubling time(d)**	**Increase in cells/ml/day/($X10^6$)±SE**	**Biomass mg/ml/±SE**
0.02	0.324	0.467	2.13	2.87±0.1	0.369±0.08

0.04	0.171	0.246	4.05	2.56±0.07	0.312±0.09
0.06	0.315	0.454	2.2	2.33±0.2	0.332±0.3
0.08	0.297	0.428	2.33	1.98±0.03	0.245±0.02
0.17	0.299	0.431	2.31	0.333±0.004	0.116±0.0001

*The values given in the table are mean of three replicates

Growth of *Chlorella homosphaera* under different Urea concentration:

Chlorella homosphaera was cultivated under different concentrations of urea as nitrogen source in BG11 media eliminating the nitrate source of media itself. For this purpose, urea (0.02, 0.04, 0.08, 0.1 and 0.2 g/l) was freshly weighed and added to the flask. In terms of specific growth rate and biomass production the optimum concentration of urea was found to be 0.1 g/l urea.

Table 9: Specific growth (d^{-1}) of *Chlorella homosphaera in different concentration of urea.*

Microalgae isolate	**Specific growth (d^{-1})**				
	0.02	0.04	0.08	0.1	0.2
Chlorella homosphaera	0.414	0.321	0.452	**0.567**	0.424

Growth of *Chlorella homosphaera* under different concentrations of inorganic carbon source (Sodium bicarbonate): It was observed that *Chlorella homosphaera* grows well under media composition supplemented with higher sodium bicarbonate concentration. It recorded best growth under highest bicarbonate concentration of 75 ppm which is equivalent to 1191.2 ppm of CO_2 gas.

Table 10: Specific growth rate and doubling/day calculated for *Chlorella* under different levels of sodium bicarbonate salt ($NaHCO_3$) concentration.

concentration	**Specific growth/day**	**Doubling/day**
15 ppm	0.744	1.07
30 ppm	0.738	1.046
45 ppm	0.487	0.702

60 ppm	0.551	0.794
75 ppm	**0.762**	1.11

Growth of *Chlorella homosphaera* under different concentration CO_2 gas s carbon nutrient source:

Chlorella homosphaera was grown in media supplemented with CO_2 gas as carbon nutrient source. The maximum Specific growth (d^{-1}) of 0.792/day was recorded. A very high concentration of CO_2 was found to be lethal for its growth due to formation of carbonic acid (H_2CO_3) in the media thereby lowering the pH upto a value of 5.

Table 11: Specific growth rate and doubling/day calculated for *Chlorella homosphaera*under different concentrations of CO_2 gas.

concentration	**Specific growth/day**	**Doubling/day**
7.93g/l	0.502	0.724
4.76g/l	**0.792**	1.142
4.44g/l	0.545	0.786

Lipid productivity:

Lipid production is an integral part of microalgae cultivation process, the quantity of intracellular lipid accumulated by microalgae cells depicts the growing condition and its performance in biofuel generation. Herein our study highest total lipid content of 20.5% was found in *Chlorella homosphaera* under normal culture media. Highest total lipid of 22%, 29% and 34% was recorded for bicarbonate, CO2 and salinity respectively.

Table 12: Difference in lipid production *Chlorella homosphaera* grown in different nutrient environment

Parameters	**Total lipid content (in terms of % dry cell weight ± SE)**
BG11 media	20.5 ±0.12
Bicarbonate (NaHCO3)	22 ± 0.1

CO2	29 ± 0.45
Salinity (NaCl)	34 ± 0.17

Comparative growth study of *Scenedesmus obliqus* and *Chlorella homosphera* using inorganic (BG11) and organic media (Cow and Goat dung extract):

It is known that *Scenedesmus obliqus* and *Chlorella homosphera* are potent microalgal strains for algal lipid generation as well as biofuel production. Here, we have studied their growth as well as lipid productivity in Cow and Goat dung extract. In figure 1, 2, 3 and 4 we have shown the obtained growth curves of *Scenedesmus obliqus* and *Chlorella homosphera* inCow and Goat dungextract. The nutrient compositions of both the extracts are shown in Table 8.

Table 13: Nutrient Composition of Cow and Goat dung extract

Parameters	**Amount (%)for cow dung powder**	**Amount (%)for Goat manure powder**
Nitrogen	0.05	0.63
Potash	1.0585	0.56
Phosphorus	0.0197	0.23
Calcium	0.3485	1.88
Manganese	0.0395	0.0005
Iron	0.9493	0.0027
Magnesium	-	0.14
Sulphur	-	0.069

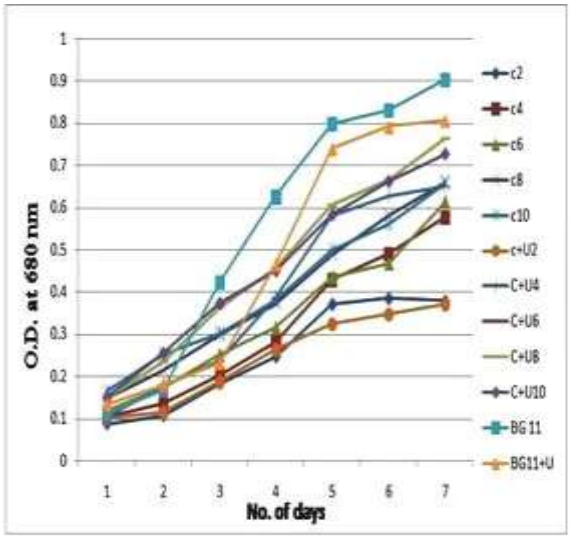


Fig 1: Growth study of *Chlorella homosphera* in cow dung extract

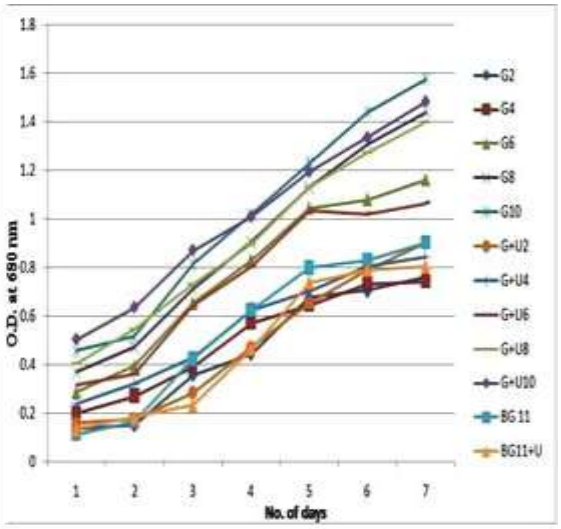


Fig 2: Growth study of *Chlorella homosphera* in Goat manure extract

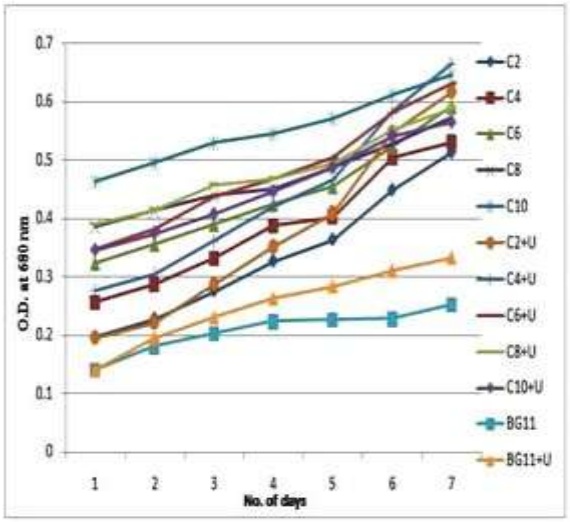


Fig 3: Growth study of *Scenedesmus obliqus* in cow dung extract

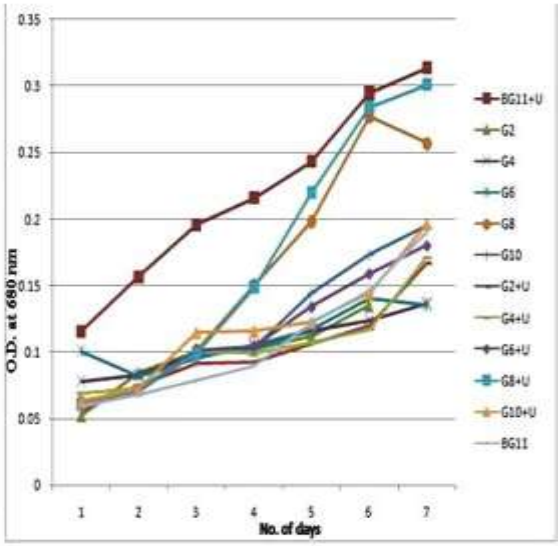


Fig 4: Growth study of *Scenedesmus obliqus* in goat manure extract:

Note: C2 to C10 indicates cow dung extract of 2%, 4%, 6%, 8% and 10% respectively. C+U2/ C2 + U to C+U10/ C10 +U indicates cow dung extract of 2%, 4%, 6%, 8% and 10% respectively with Urea (0.1g/litre) as a nitrogen source.

And

G2 to G10 indicates goat dung extract of 2%, 4%, 6%, 8% and 10% respectively. G+U2/ G2 + U to G+U10/ G10 +U indicates goat dung extract of 2%, 4%, 6%, 8% and 10% respectively with Urea (0.1g/litre) as a nitrogen source

From above graphs figure 1 it can be seen that *Chlorella homosphera* shows highest growth rate

in BG11 media than all the other media prepared with cow dung. Whereas in figure 2 *Chlorella homosphera* shows highest growth rate in G10 which is composed of 10 % goat dung extract prepared from the stock of 50 % goat dung. Here the growth rate of culture grown in G10 has exceeded the growth rate shown by BG11 media. From here we conclude that for *Chlorella homosphera* 10 % goat dung extract prepared from the stock of 50 % goat dung is suitable for its growth among all the prepared culture media. The highest specific growth rate shown by *Chlorella homosphera* in G10 is 0.701/day and 0.632/day is shown by BG11 which is less than G10.

From figure 3 it can be seen that *Scenedesmus obliqus* shows highest growth rate in C10 which is composed of 10 % cow dung extract prepared from the stock of 50 % cow dung. The highest specific growth rate shownby *Scenedesmus obliqus* grown in C10 is 0.496/day and 0.315/day in BG11 medium. Whereas from Figure 4 it can be seen that the growth of *Scenedesmus obliqus* in goat dung extract is poor in comparison with cow dung extract. *Scenedesmus obliqus* has shown highest specific growth rate in BG11+U.

Objective 3 targeted: *Field scale cultivation and biomass production with experimental low cost PBR.*

Outdoor mass cultivation

Outdoor culture of *Chlorella homosphera* was started with 15L culture in PBR unit 1 with 0.362±0.01 mg/ml /day. The culture flow was regulated through fresh nutrient loading into PBR 1 unit at a flow rate of 1L/ h to outflow the culture into PBR unit 2. The average biomass harvested during study period was recorded at 0.8-0.9mg/ml/day. Mass culture of *Chlorella homosphera* was initiated in the outdoor pond with 500 L of culture supplemented with 10% of goat dung extract. This concentration of goat dung extract was selected on the basis of our lab scale experiment. The highest specific growth rate shown by *Chlorella homosphera* in outdoor pond for 14 day study is 0.612/day shown in figure no 10. Lipid content in terms of %dry cell weight was found to be 31±0.24 for *Chlorella homosphera* grown in outdoor pond.

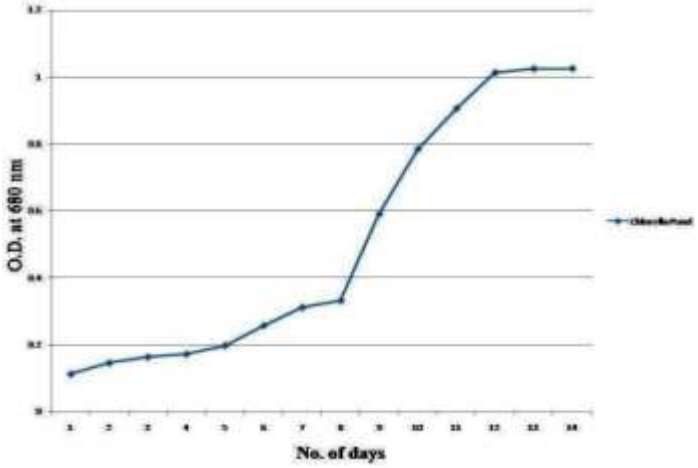


Fig 10: Growth study of *Chlorella homosphera* in outdoor pond

Fig 11: Field scale cultivation and biomass production of *Chlorella homosphera* with experimental low cost PBR

Fig 12: Field scale cultivation and biomass production of microalgae in outdoor raceway pond

Fig 13: Processing of harvested biomass

Standardization of efficient harvesting technique; finding alternative flocculating agent:

In continuation with the previous year's research work on flocculating agent we have studied efficient harvesting technique for *Scenedesmus obliqus* and *Chlorella homosphera* with different inorganic agents such as KAI $(SO_4)_2$ (potash alum), $MgSO_4$ (Magnesium sulphate), $FeSO_4$ (Ferrous sulphate) and organic agent such as Egg shell and tamarind. The experiment was carried out with different concentrations (0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, and 1.5) g/L of the selected agents. The study was carried out with 7, 14 and 21 days culture of both the species by taking Optical Density at 680nm in different time intervals of 60min, 120min, 180min, 300min and 1440min. The best of the results are given in table 9 and shown in figure 14. It is recorded that potash alum among the inorganic and egg shell among the organic flocculating agent have shown good harvesting efficiency. Tamarind has shown less efficiency as an organic flocculating agent as compared to eggshell.

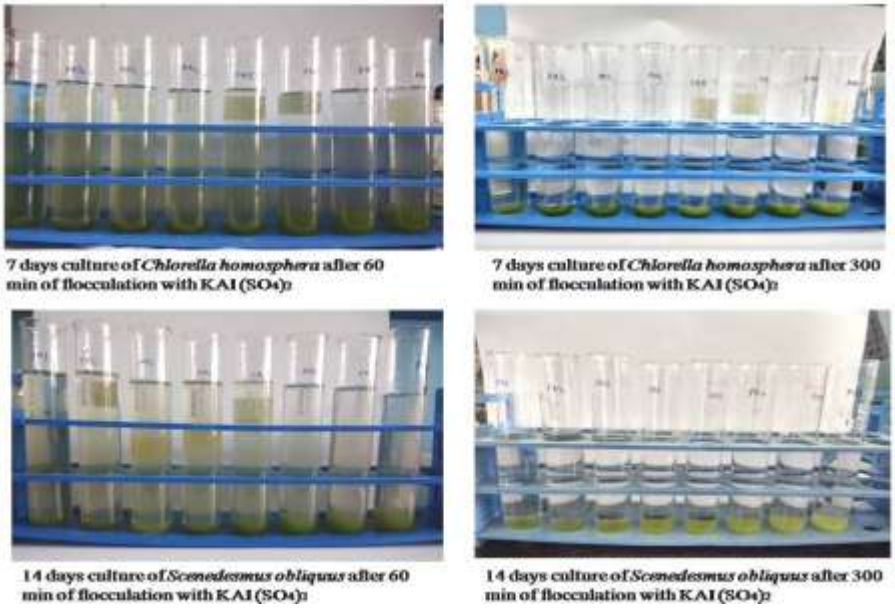


Fig 14: Flocculation experiments shown by the best flocculating agent in both the species

Table 14: Flocculation results shown by the best flocculating agents from the present study

ALGAL Species	Flocculating agent	Culture days	Flocculation time (minutes)	Concentration (g/L)
Scenedesmus obliquus	Potash alum	7	180	1.5
		14	300	1.5
		21	1440	1.5
	Egg shell	7	120	0.5
		14	300	1.5
		21	300	1.5
Chlorella homosphera	Potash alum	7	300	1.5
		14	1440	1.5
		21	300	1.5
	Egg shell	7	1440	0.9
		14	1440	0.7
		21	120	1.5

9. Results

- Culture technique for two microalgal species (*Scenedesmus obliquus* and *Haematococcus pluvialis*) in order to observe increased lipid content and to optimize the different parameters based on which the mass cultivation of the species can be carried out in the

raceway ponds and biomass can be generated thoroughly. Low cost PBR has been designed in a batch system for biomass production.

- High growth rate with high lipid was obtained for *Haematococcus pluvialis* using organic and inorganic carbon source. *Scenedesmus obliquus* has been successfully cultivated in open raceway ponds using cow dung enriched organic media. As far as cultivation is concerned, a long term continuous cultivation strategy was applied in this study, whereby the cultivation was continued with a multiple harvesting (2 times/month) to maintain a strategic growth rate of the culture. Biomass from open raceway pond ranges from 180 to 650gm/month and the lipid yield ranged from 19 to 32.6 % dry cell weight.
- Lipid production is an integral part of microalgae cultivation process, the quantity of intracellular lipid accumulated by microalgae cells depicts the growing condition and its performance in biofuel generation. Herein our study highest total lipid content of 20.5% was found in *Chlorella homosphaera* under normal culture media. Highest total lipid of 22%, 29% and 34% was recorded for bicarbonate, CO2 and salinity respectively.
- *Chlorella homosphera* shows highest growth rate in G10 which is composed of 10 % goat dung extract prepared from the stock of 50 % goat dung. Here the growth rate of culture grown in G10 has exceeded the growth rate shown by BG11 media. From here we conclude that for *Chlorella homosphera* 10 % goat dung extract prepared from the stock of 50 % goat dung is suitable for its growth among all the prepared culture media. The highest specific growth rate shown by *Chlorella homosphera* in G10 is 0.701/day and 0.632/day is shown by BG11 which is less than G10.
- *Scenedesmus obliqus* shows highest growth rate in C10 which is composed of 10 % cow dung extract prepared from the stock of 50 % cow dung. The highest specific growth rate shownby *Scenedesmus obliqus* grown in C10 is 0.496/day and 0.315/day in BG11 medium.
- It is recorded that potash alum among the inorganic and egg shell among the organic flocculating agent have shown good harvesting efficiency. Tamarind has shown less efficiency as an organic flocculating agent as compared to eggshell.
- For selection of a cost effective and energy efficient method of harvesting flocculation efficiency was studied for *Haematococcus pluvialis* using different organic and inorganic chemicals such as $FeSo_4$, $ZnSo_4$, $MgCl_2$ and Chitosan at different

concentration (0.05 to 0.3 gm with a difference of 0.05) for 270min. The highest (97.34% ± 0.66%) flocculating activity with a settling time of 210 min was achieved by 0.25gm of $ZnSO_4$.

- Reaserach work for production of value added products from microalgae like Bioethanol, astaxanthin have also been carried out. Ethanol production from microalgae via fermentation process traditionally used in assam is one of such initiative.

10. Summary (in two hundred words only) The results of the works completed within the time period are found to be satisfactory despite few constrains.Prior to the initiation of mass cultivation trials, the necessary set up was made. Within a few days mass cultivation of selected algal species was initiated. As far as cultivation is concerned, a long term continuous cultivation strategy was applied in this study, whereby the cultivation was continued with a multiple harvesting (2 times/month) to maintain a strategic growth rate of the culture. Since we got some interesting results with organic media, therefore characterization of organic media was carried out. In future prospective few more organic extracts study can be undertaken for augmentation of biomass production. We have studied low cost harvesting techniques. Low cost biomass production is a major thrust of the study.

11. Any new product/ process developed

12. Any new lead

13. Any technology developed:

- An indigenously developed low cost serial photobioreactor (LCSPBR) has been already undertaken for cultivation of *S. obliquus* and found successful with average cell biomass was found to be 0.6-0.8 $gL^{-1}d^{-1}$.
- The highest (97.34% ± 0.66%) flocculating activity with a settling time of 210 min was achieved by 0.25gm of $ZnSO_4$.
- *Chlorella homosphera* 10 % goat dung extract prepared from the stock of 50 % goat dung is suitable for its growth among all the prepared culture media. The highest specific growth rate shown by *Chlorella homosphera* in G10 is 0.701/day and 0.632/day is shown by BG11 which is less than G10.
- Low cost PBR has been designed in a batch system for biomass production.
-

14. Any patent taken: No

15. Publications from project work

Banasree Sharma, Priyanka Paul, Anuchaya Devi, Mohan Chandra Kalita, Dhanapati Deka and Vaibhab V. Goud; Cost Effective Biomass Production Of *ChlorellaHomosphaera* And Scenedesmus Obliquus-two BiofuelPotent Microalgae from Northeast India, *Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 22, No. (3) : 2020 : 486-490.* ***ISSN-0972-3005***

Anuchaya Devi[1], Janmoni Kalita[1] & Mohan Chandra Kalita[1] "Lipid augmentation of microalgae *Scenedesmus obliquus* and catalytic transformation of the algal oil into biodiesel using Eggshell catalyst" in Biofuels, Energy and Economy 2019 on July-17-18, 2019, Abu Dhabi, UAE.

16.**Financial progress. Viz. equipments purchased, manpower, copies of UCs/SEs of various financial year, etc.**

Submitted separately.

CONSOLIDATED UTILISATION CERTIFICATE

For three institutions viz. Tezpur University, Gauhati University and Indian Institute of Technology

(for the financial year 2020-2021 ending 31st March , 2021)

1.	**Title of the project/scheme**	:	Integrated Bio-refinery Approach towards production of sustainable fuel and chemicals from Algal bio-based systems
2.	**Name of the Organization**	:	(i) Tezpur University, Napaam, Tezpur 784028, Assam, India (ii) Gauhati University, Guwahati: 781014, Assam, India (iii) IIT Guwahati, Guwahati: 781039, Assam, India
3.	**Principal Investigator**	:	(iv) Dr. Dhanapati Deka, Professor, Department of Energy, Tezpur University, Napaam, Tezpur-784028, Assam, India (Lead PI) (v) Dr. M C Kalita, Professor, Department of Biotechnology, Gauhati University, Guwahati: 781014, Assam, India (vi) Dr. Vaibhav V Goud, Associate Professor. Department of Chemical Engineering, IIT Guwahati, Guwahati: 781039, Assam, India
4.	**Deptt. of Biotechnology sanction order No. & date of sanctioning the project**	:	DBT/IC-2/Indo-Brazil/2016-19/04 Date of Sanction: 15-06-2016
5.	**Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given**	:	i. Rs. 1.87605 lakh ii. Letter No: DBT/IC-2/Indo-Brazil/2016-19/04 iii. Date: 05/06/2020
6.	**Amount received from DBT during the financial year (Please give No. and dates of sanction orders showing the amounts paid)**	:	Rs. 25.63816 lakh
7.	**Other receipts/interest earned, if any, on the DBT grants**	:	Rs. 0.1 lakh
8.	**Total amount that was available**		

	for expenditure during the financial year (Sl. nos. 5, 6 and 7) :	Rs. 27.61421 lakh
9.	**Actual expenditure (excluding commitments) incurred during the financial year (statement of expenditure is enclosed)** :	Rs. 25.57995 lakh
10.	**Unspent balance refunded, if any (Please give details of cheque No. etc.)** :	Rs. 2.03426 lakh
11.	**Balance amount available at the end of the financial year (31st March, 2020)** :	Rs. 2.03426 lakh
12.	**Amount to be carried forward to the next financial year** :	Balance amount will be surrendered to DBT as the project is ended on 14th Dec 2020

1. Certified that the amount of **Rs. 25,57,995.00 (Rupees twenty five lakh fifty seven thousand nine hundred ninety five)** only mentioned against col. 9 has been utilized on the project / scheme for the purpose for which it was sanctioned and that the balance of **Rs 2,03,426.00 (Rupees two lakh three thousand four hundred twenty six)** remaining unutilized at the end of the year to be surrendered to Govt.

2. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled / are being fulfilled and that I have exercised the following checks to see that the money was actually utilized for the purpose for which it was sanctioned.

Kinds of checks exercised:

1. Sanction letter
2. Utilization certificate
3. Statement of Expenditure
4. Original voucher
5. Stock entry

(PROJECT INVESTIGATOR)
(Signed and stamped)

(FINANCE OFFICER)
(Signed and stamped)
Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)
(Signed and stamped)
Registrar
Tezpur University

For SURAJIT CHAKRABORTY &
CHARTERED ACCOUNTANTS

CA, SURAJIT CHAKRABORTY
(Proprietor)
Membership No.- 305054

Consolidated Statement of Expenditure for three institutions viz. Tezpur University, Gauhati University, and Indian Institute of Technology, referred to in para 9 of the consolidated Utilization Certificate

Showing grants received from the Department of Biotechnology and the expenditure incurred during the period from **1st April 2020 to 31st March 2021**

(Rs. in lakhs).

Item	Unspent balance Carried forward from previous year	Grants received from DBT during the year	Other receipts/ interest earned - if any, on the DBT grants	Total of Col. (2+3+4)	Expenditure (excluding commitments) incurred during the year	Balance (5-6)	Remarks
1	2	3	4	5	6	7	8
A. Non –Recurring							
1.Equipment							
a. Tezpur University	2.02684	(-) 2.02684	0	0	0	0	
b. Gauhati University	0.00061	(-) 0.00061	0	0	0	0	*Unspent balance of Rs 61 from non-recurring head is re-appropriated to Contingency Head in 4th year Grant as per DBT sanction letter.
c. IIT Guwahati	0.07897	(-) 0.07897	0	0	(-)1.18863	1.18863	
(i) SUB TOTAL	**2.10642**	**(-) 2.10642**	**0**	**0**	**(-)1.18863**	**1.18863**	
B. Recurring							
2. Manpower							
A. JRF							
a. Tezpur University	(-) 0.65808	0.65808	0	0	0	0	
b. Gauhati University	(-) 0.675	0	0	(-) 0.67500	0	(-) 0.67500	
c. IIT Guwahati	0	0	0	0	0	0	**No JRF position**
JRF Total (a+b+c)	**(-) 1.33308**	**0.65808**	**0**	**(-) 0.67500**	**0**	**(-) 0.67500**	
B. SRF							
a. Tezpur University	(-) 6.34696	9.62296	0	3.276	8.24465	(-) 4.96865	Fellowship of 3

							SRF till 14.12.2020 as per DBT approval.
b. Gauhati University	2.11544	3.99456	0	6.11	2.43329	3.67671	
c.IIT Guwahati	(-) 3.3667	3.69600	0	0.3293	4.21490	(-) 3.8856	
SRF Total (a+b+c)	**(-) 7.59822**	**17.31352**	**0**	**9.7153**	**14.89284**	**(-) 5.17754**	
C. RA							
a. Tezpur University	0	0	0	0	0	0	**No RA position**
b. Gauhati University	0	0	0	0	0	0	**No RA position**
c. IIT Guwahati	2.14903	1.31492	0	3.46395	2.55026	0.91369	
RA Total (a+b+c)	**2.14903**	**1.31492**	**0**	**3.46395**	**2.55026**	**0.91369**	
D. Project Assistant (PA)							
a. Tezpur University	0	0	0	0	0	0	**No PA position**
b. Gauhati University	(-) 0.4	1.093	0	0.693	1.09300	-0.4	
c. IIT Guwahati	0	0	0	0	0	0	No PA position
PA total (a+b+c)	**(-) 0.4**	**1.093**	**0**	**0.693**	**1.09300**	**-0.4**	
E. Project Technician (PT)							
a. Tezpur University	0	0	0	0	0	0	**No PT position**
b. Gauhati University	0	0	0	0	0	0	**No PT position**
c. IIT Guwahati	0.11775	0.69000	0	0.80775	0.68999	0.11776	
PT Total (a+b+c)	**0.11775**	**0.69000**	**0**	0.80775	**0.68999**	**0.11776**	
F. Stipend in Brazil							
a. Tezpur University SRF and PI or Co-PI	(SRF) 2.90315 (PI) 1.67	0 0	0 0	2.90315 1.67	0	2.90315 1.67	(No stipend for SRF in Brazil after 6th December,2018) (No stipend for PI or CO-PI in Brazil after 8th July,2018)
b. Gauhati University	0	0	0	0	0	0	**No grant**
c. IIT Guwahati	0	0	0	0	0	0	**No grant**
Stipend in Brazil Total (a+b+c)	**4.57315**	**0**	**0**	**4.57315**	**0**	**4.57315**	
ii) SUB TOTAL	**(-) 2.49137**	**21.06952**	**0**	**18.57815**	**19.22609**	**-0.64794**	
C. Consumables							
a. Tezpur University	0.00387	1.99613	0	2.00	1.76364	0.23636	
b. Gauhati University	0	1	0	1.00	1.00	0	
c. IIT Guwahati	0.07192	1.40668	0	1.47860	0.73002	0.74858	**Interest

							balance of Rs. 96370 and unspent NR balance of Rs. 7897 has been re-appropriated to consumable head as per sanction order
(iii) SUB TOTAL	**0.07579**	**4.40281**	**0**	**4.47860**	**3.49366**	**0.98494**	
D. Contingency							
a. Tezpur University	0.08755	0.91245	0	1.00	0.99944	0.00056	
b. Gauhati University	0	0.5	0	0.5	0.49939	0.00061	
c. IIT Guwahati	0.26110	0.36057	0	0.62167	0.60422	0.01745	
(iv) SUB TOTAL	0.34865	1.77302	0	2.12167	2.10305	0.01862	
E. Travel							
a. Tezpur University Domestic International Trainee (SRF 1) PI Local Hospitality to Visiting Scientist	0.2376 0.07665 0.05986 0.5	0.2624 0 0 0	0 0 0 0	0.5 0.07665 0.05986 0.5	0.11013 0 0 0	0.38987 0.07665 0.05986 0.5	
b. Gauhati University Domestic Local Hospitality to Visiting Scientist	0.0 0	0.5 0	0 0	0.5 0	0.5 0	0 0	**No grant for local hospitality to visiting scientist**
c. IIT Guwahati Domestic Local Hospitality to Visiting Scientist	0.26362 0	0.23638 0	0 0	0.5 0	0.07605 0	0.42395 0	**No grant for local hospitality to visiting scientist**
(v) SUB TOTAL Domestic International Local hospitality to visiting scientist	**1.13773**	**0.99878**	**0**	**2.13651**	**0.68618**	**1.45033**	

F. Overheads							
a. Tezpur University	0.2416	0.2584	0	0.5	0.7596	-0.2596	
b. Gauhati University	0.0	0	0	0	0	0	
c. IIT Guwahati	0.0	0.5	0	0.5	0.5	0	
(vi) SUB TOTAL	**0.2416**	**0.7584**	**0**	**1**	**1.2596**	**-0.2596**	
G. Total Interest earned							
a. Tezpur University	0.32053	-0.29425	0.00	0.02628	0	0.02628	
b. Gauhati University	(-)0.827	0	0	(-)0.827	0	(-)0.827	
c. IIT Guwahati	0.9637	-0.9637	0.1	0.1	0	0.1	
(vii) SUB TOTAL	**0.45723**	**-1.25795**	**0.1**	**(-)0.70072**	**0**	**(-)0.70072**	
TOTAL of all subtotals (i+ii+iii+iv+v+vi+vii)	**1.87605**	**25.63816**	**0. 1**	**27.61421**	**25.57995**	**2.03426**	

(Balance amount to be carried forward for the next financial year: Rupees Two lakh three thousand four hundred twenty six only)

7/6/2024

(PROJECT INVESTIGATOR)
(Signed and stamped)

25/6/2024

(FINANCE OFFICER)
(Signed and stamped)
Finance Officer
Tezpur University

24/6/22

(HEAD OF THE INSTITUTE)
(Signed and stamped)
Registrar
Tezpur University

For SURAJIT CHAKRABORTY & CO.
CHARTERED ACCOUNTANTS

CA. SURAJIT CHAKRABORTY
(Proprietor)
Membership No.- 305054

10/07/2024