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Optimized Extraction and Performance Evaluation of Hydrilla-Based Third-Generation Biofuel in a Single-Cylinder Diesel Engine

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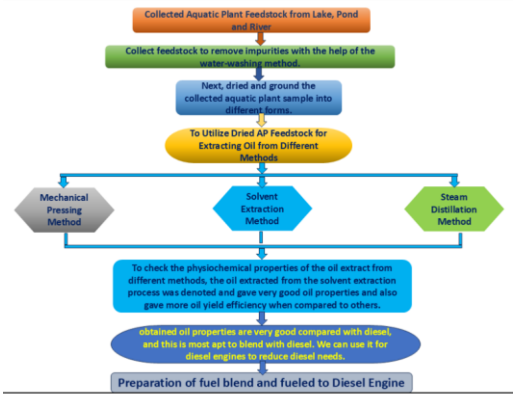
Graphical abstract



Abstract

Aquatic plants are the one of largest and fast-growing biomass resources that have substantial potential for diesel production. One of these, Hydrilla verticillata, is a submersed aquatic plant that can prosper in warm lakes and rivers at depths of several meters and was therefore studied as a potentially new feedstock for biofuel. This study demonstrates “third-generation biofuel”, for the first time, the viability of Hydrilla-based biofuel as a sustainable third-generation feedstock. The research was on oil extracted from Hydrilla using various methods with same weight of feedstock for three different methods: steam distillation, Soxhlet extraction and mechanical press. Yields of extraction were greatly affected by the particulate size and biomass moisture content. A maximum yield of 13% oil was obtained by mechanical pressing, suitable for biodiesel production, under the optimized conditions in case of steam distillation, we could get about 34.2% oil (the similar quantity as in crushed seed). Maximum yield of 63.8% was observed in the case of solvent extraction with fully dried aquatic plant feedstock using n-hexane solvents and finely powdered biomass gave a low viscous oil suitable for fuel applications by production method itself as solvent extraction, we’ll all levels of properties results very closer to diesel fuel compared to other two process so that obtained oil from solvent extractions process has been taken for further blend with diesel blending and also fuelling to CI engine level. Physical and chemical properties analysis of the Hydrilla oil revealed similar characteristics with a relatively acceptable range in comparison to diesel, and it could be utilized as an engine fuel. Performance was evaluated by using Hydrilla biofuel blends and diesel in a single cylinder, four stroke Compression Ignition engine. Engine trials showed that a blend of 20% biofuel and 80% diesel felt similar to neat diesel and better compared with already existing aquatic plant of (water hyacinth, Spirogyra) in terms of the key findings from BTE investigation using the Hydrilla B20 blend reveals that it complies up to 31.13% of normal diesel fuel and a small drop of 1.70%, in cylinder pressure was reduced, the energy released during power stroke is 0.87 percent lower and heat release rate of the engine was decreased 2.02% when compared to standard diesel. Beside emission part CO 15% and HC emission 1.38% increased due to the slightly improper combustion, NO_x and CO_2 emissions are decreased due to low temperature occurred in combustion chamber. These findings demonstrate the potential of Hydrilla-based biofuel as an ecofriendly partial replacement to diesel with greater versatility and lesser dependence on fossil fuels in favour of cleaner energy sources.

Keywords: Aquatic Plant, Solvent Extraction, Bio-fuel, Biodiesel, Low viscous fuel, Emission Reduction.

1. Introduction

The worry about what continued use of fossil fuels is doing to our environment and human health mandates that sustainable energy options must be explored. Amidst the renewable energy resources, biofuels like bio-oil, biodiesel, bioethanol, biogas and biohydrogen are becoming the potential alternatives of conventional fossil fuels (Brindhadevi *et al.* 2021). The depletion of fossil fuel reserves has further accelerated the search for alternatives.

The promise of algae-based biomass compared with other forms of biomass has generated significant interest in recent times. Algae provide a far superior oil potential as well as utilizing wastewater for growth and emitting less carbon dioxide. For instance, the algae may yield around 37–131 tonnes of oil per hectare per year (equivalent to 18,927–75,708 pounds) which is over 31% more than palm oil. (Barry *et al.* 2016; Demirbas and Demirbas 2011). Additionally, some types of algae can produce three times the amount of oil per unit area that terrestrial crops do and can grow at a much faster pace. Due to these properties, algae are an ideal source for biofuel production. Raceway open ponds and photobioreactors are the most commonly used systems in large scale to produce microalgae (Arun *et al.* 2022).

Use of effective oil extraction strategies is necessary for biofuel production following cultivation. For example, Aravind *et al.* (2021) Drape and Şenkan (Shi *et al.* 2019) on the other hand modified an Soxhlet extraction method with Chlorella vulgaris and n-hexane at 75 °C were time for obtaining maximum recovery of oil was 3 hours. Similarly, Mani Yuvarani *et al.* (2017), who obtained 18% oil yield from Cladophora glomerata using methanol-chloroform mixture in a Soxhlet apparatus, underlining that some factors like biomass dryness, solvent type and extraction time need to be addressed during optimization.

Chinese research on aerobic treating of waste grown fuel used to get biodiesel, the effect on environment using (Jain *et al.* 2023). Rui Shi *et al.*, studied several harvesting and extraction technologies that were developed under the National Alliance for Advanced Biofuels and Bioproducts (NAABB) project effort with regards to effects it may have on environment (Shi *et al.* 2019). Acoustic harvesting and acoustic oil extraction resulted in 45% reduction of greenhouse gas (GHG) emissions, based on their study.

In the present study, oil from Spirogyra algae was extracted using n-hexane as a solvent. According to the results, reduction of biomass particle size, increase in contact time and solvents/biomass ratio were effective for improving oil recovery (Baig *et al.* 2018). In addition, the potential use of other algae for biodiesel has been studied by others. An assessment and comparison of method of cell disruption and biodiesel production for the macroalgae Enteromorpha flexuous with microalgae such as Spirulina platensis, Nannochloropsis oceanic have been reported (Elnajjar *et al.* 2023).

Jun Cheng *et al.* (2014) also found that the yields of FAMEs could be improved six-fold and nitrogen contents would be halved, as well as with lower extraction of polar compounds if biodiesel is extracted by hexane after microwave-assisted transesterification (EHMT). This process is promising for commercialisation, but it needs a miniaturised microwave apparatus.

Algal feedstock is an efficient resource in the production of biofuel because of its high oil conversion rate to approximately 70%. After all, the best chemical process to turn alga into a quality biodiesel is still transesterification. While it meets with ASTM requirement, algal biodiesel is considered as a viable diesel engine alternative (Ijaz *et al.* 2023; Kale *et al.* 2021).

Moreover, macrophytes such as Spirogyra porticalis and Nymphaea alba showed high cellulose content, implying that can make possible the production of bioethanol and biodiesel also from plants instead of marine macroalgae (Anvanwu *et al.* 2022). Jain *et al.* (2023) emphasised Eichhornia crassipes (water hyacinth) as an ignored, but promising biodiesel feedstock. Studies have previously demonstrated that blending its biodiesel with traditional diesel can also decrease tailpipe emissions.

Finally, extensive study has also investigated oil yield from seed kernels of Calophylluminophyllum (beauty leaf) confirming its viability as biodiesel component that generalizes the applicability of plant- and algae-based biofuels (Arun *et al.* 2022) and This organism's adaptiveness and excellent lipid producing ability make it a good candidate for all-purpose strain in terms of biodiesel generation. Due to its efficiency and safety, Soxhlet extraction is proposed. In the study, 92.2% biodiesel yield was obtained for scenedesmus parvus oil, a result that is comparable to related studies when using KOH as catalyst. The physio-chemical properties of the biodiesel were tested in order to compare it with international quality standards. Based on this work, Scenedesmus parvus is a suitable candidate as feedstock to produce biodiesel (Bhuiya *et al.* 2020; Rash *et al.* 2024).

In the words of Manoj Shrivastava *et al.* (2021), possibly due to its rapid growth and aquatic nature, H. verticillata is an important resource in the ecological restoration process. The plant has the ability to take in and filter out toxic material from polluted water because it is capable of accumulating metals and metalloids using ligands such as thiols. Hydrilla biomass may be used for composting, pyrolysis, and biogas manufacture. In order to fully understand the practical application of Hydrilla, further research is needed (Shrivastava and Srivastava 2021; Zhong *et al.* 2025; Liu *et al.* 2020; Gong *et al.* 2025; Qiu *et al.* 2025; Ellappan and Rajendran 2021; Rajendran 2021; Rajendran and Ganesan 2021; Rajendran *et al.* 2023; Bangari *et al.* 2024). The contamination of hydrocarbons on aquatic environment due to petroleum discharge and effluent releases is considered to be among the crucial ecological hazards; it demands for appropriate remediation methods. Bioremediation strategies, particularly bioaugmentation and bio stimulation, have been proven to improve TPH degradation in water. Bioreactor basins Washing and optimization palms oil mill effluent as a bio stimulant have shown positive results on the rate of hydrocarbon degradation in seawater when applied to floating oil spill containment booms (Sayed *et al.* 2022, 2021a, 2021b, 2021c).

Oil was obtained from the species Hydrilla verticllata for this study. Extraction The one-litre solvent extraction, steam distillation and mechanical pressing method. Hydrilla verticillata, water plant commonly found growing in lakes, ponds and rivers but not cultivated It grows naturally We can get it freely for this purpose. The harvested aquatic plants are then dried under direct sun for 3 days with no auxiliary source of heating. After drying the dried plants were grinded to fine powder and ordinary distilled water and solvents was used. The reaction gave the highest populated percent yield of oil from an aquatic plant (Hydrilla verticillata). Physiochemical properties of the raw Hydrilla verticillata oil-diesel blends should also be evaluated using ASTM standards. The oil was finally bio-test for its potential to be developed as a substitute fuel in IC engines. The novelty of this study lies in the combined approach of:

- Utilizing *Hydrilla* biomass as a non-conventional biofuel feedstock,
- Employing optimized transesterification parameters (temperature, catalyst concentration, and reaction time), and
- Using zirconia-coated piston surfaces to enhance combustion efficiency when blended with diesel.

These innovations collectively contributed to the higher oil yield and improved engine performance observed in this study. The revised text explicitly discusses these factors to better highlight the advancement over prior research.

2. Materials and Methods

2.1. Collection of Aquatic plant sample for oil extraction process.

Swedish scientist Carl Linnaeus described the first-known aquatic plant Hydrilla verticillata in species in 1753 publication Plantarum. Linnaeus developed the pantomime, or binomial, method of naming species still used today. Hydrilla verticillata has nevertheless been the focus of later taxa and botanists who helped us to better understand H. verticillata, in particular its invasiveness and wide world distribution in aquatic ecosystem. We can study this plant without any permission because it is a waste biomass and largely available everywhere such as lake, pond and river etc. we also grow up small pot in front of our home so, somewhere it was growing and became harmful for human being.

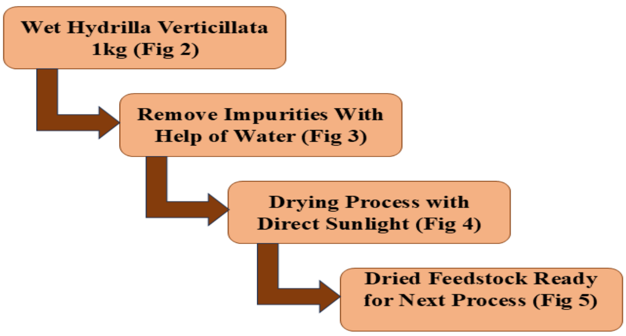


Figure 1. Flow of Aquatic plant Collection

From **Figure 2**. The samples of the plant material were taken from open lake water in Ponpathi village of Gingee taluk. We were able to harvest 0.8–1.5 kg per square feet we harvested wet hydrilla per cent within 5 days. The images of APs are shown in **Figures 2 and 4**. The samples collected were analysed and found to be Hydrilla verticillata. The plant was rinsed with deionized water to remove unwanted materials (**Figure 3**). The collected plant material was air dried for 5-7 days to avoid onus of moisture. **Figure 4** is a photograph of dried AP. **Figure 5** is grounded dried AP (0.5-10 µm mixture) and (10–80-micron mixture). A grinder (make- Butterfly, power: 750W, speed: 2000rpm; capacity: 0.75L) was used to pulverize dried algae to a talcum like powder and the schematic flowchart of the process of collecting hydrilla from pre-cultivation to post conversion for our use is presented in **Figure 1**.

Figure 2. Collected AP from open lake

Figure 3. Clean Impurities with water

Figure 4. Wet collected sample.

Figure 5. Dried collected sample

2.2. Mechanical Pressing oil extraction

Figure 5 depicts the steps to obtain oil from raw water hydrilla dried plant 1kg. It crushes oil out from the raw materials by the press of the mechanical pressures other than human. For the mechanical extraction of oil from aquatic plants like algae or water hyacinth, this method has to be specially adapted. The processes are developed taking into account the fact that composition of aquatic plant biomass is substantially different from that of typical oilseeds with better hydration level and lower structural rigidity. Biomass collection and preparation is the first step.

As shown in **Figure 6**, Aquatic plants or algae (1) are harvested from bodies of water (lake, pond, etc.) or cultivation systems and washed to remove contaminants such as dirt, stones or others. Aquatic plants need to be processed differently, compared to oilseeds since they contain a large amount of water and need substantial dewatering and drying. This section is important in efficient oil removal as the addition of excessive water may interfere with the mechanical pressure system. The material is dried down to 10% moisture and it is then ready for pressing. The conditioned material is then put through a press or expeller, which applies a great amount of pressure to the content that is squeezed by a spinning screw or piston. The oil is squeezed out through small openings and the pressed residue, which in turn is referred to as press cake or slurry, falls subsequently. The post dewatering filtration and separation steps are particularly notable. The oil that's extracted has water, small pieces of plant matter and other impurities which must be removed. Some of the more common methods to purify oil include centrifugation and multi-stage filtration. Special equipment may be needed to process the fine particulate found in microalgae and thereby produce oil of a high quality, but also is separated by additional particles an oil with very high density and viscosity.

Figure 6. Oil Extraction mechanical presser.

2.3. Steam Distillation Extraction process

The collected plant was shade dried for 5-7 days to deprive of moisture. **Figure 5** is a photograph of dried AP. Grounded dried AP (0.5–10micron mix) and (10–80micron mix) was employed to enhance the oil yield efficiency, (**Figures 7 & 8**). The dried alga was then milled to fine powder using grinder (make: Butterfly, power: 750W, speed: 2000rpm), capacity of the jar used here is 0.75L).

Figure 7. Grounded AP powder mixture of (0.5-10µ).

Figure 8. Grounded AP powder mixture of (10-80µ)

Figure 9 represented the to find the micron structure of the different powder particle size mixture of hydrilla verticillata.

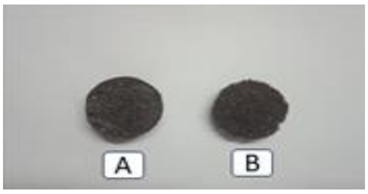


Figure 9. Test Samples for Microscope (*a*) 0.5-10 Micron Powder, (*b*) 10-80 Micron Powder

Figure 10 shows the microscopic view of (0.5-10) micron size mixture powder.

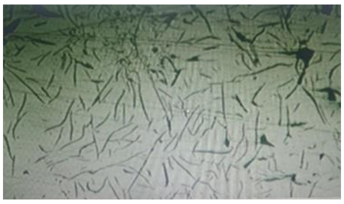


Figure 10. Microscopic view of (0.5-10) Micron Mixture AP Powder Size

Figure 11 shows the microscopic view of (10-80) micron size hydrilla powder mixture.

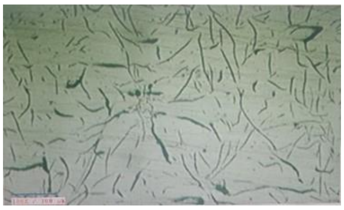


Figure 11. Microscopic view of (10-80) Micron Mixture AP Powder Size

2.4. Evaporating agent for steam distillation process.

Table 1 shows the physicochemical properties of distilled water for extract oil by the help of steam distillation process and **Figure 12** used an evaporating agent of steam distillation process.

Figure 12. Distilled Water

Table 1. Properties of Distilled Water

Physical Properties	Distilled Water
Boiling Point (°C)	100
Density(kg/m^3)	1000
Refractive Index(25°C)	1.333
Cost (INR/1L)	20

Apparatus

Figure 13. One liter capacity of Steam Distillation extraction apparatus

Figure 13 One liter capacity of Steam distillation extraction apparatus will have bought by India Mart MM LABCARE COMPANY (manuf. Lab. Glassware).

3. Experimental Setups

Steam distillation was used in the present study to extract oil from aquatic plants. The steam distillation apparatus is pictured in **Figure 14**. For the bio flask, a 500 gram mixture of raw aquatic plant leaves and stems was placed in the bio flask, one litter distilled water was introduced to it. The heater maintained the distilled water at 70 °C. Additionally, then water vapor was directed to the bio flask with an aquatic plant feedstock. Raw steam which is extracted from the raw feedstock by means of a steam extraction tube was converted to oil and delivered to the condenser using a steam extraction tube. The cloud was condensed into fluid by the condenser, and water and oil of hydrol were produced. The oil and water were separated using a separator. The oil was then water washed to remove any impurities such as dust and extra water. In this experiment we could recover oil 6.3% in 50 % dryness and 13.4% in 100% dryness. This same process was applied to powdered aquatic plant feedstocks of two different particle size blends: 0.5–10 and 10–80 µm. We finally attained an oil yield percentage of 34.2% with the (0.5-10) micron powder mixing. Afterwards, the same has been tested for its physio-chemical properties to determine density of aquatic plant oil, viscosity of Aquatic Plant Oil, Cetane Index and others.

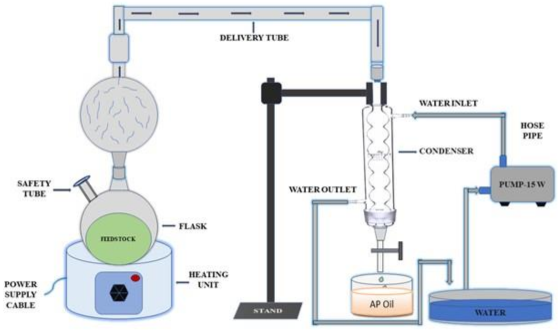


Figure 14. Entire Steam distillation extraction working process

Using the equations, the percentage of oil yield for the algae powder with various solvents was estimated. The percentage of oil yield % for the aquatic plant powder was calculated using these formulae. % is computed using the bellowed equation (Anyanwu *et al.* 2022).

$$\text{Percentage of oil yield form alge} = \frac{\text{Weight of Extracted Oil}}{\text{Weight of Sample}} \times 100 \quad (1)$$

Weight of the sample = Aquatic plant sample weight in g/kg, Weight of extracted oil = Obtained oil weight in g/kg form the extraction process,

A **Table 5, 6, and 7** indicates the amount of oil extracted by hydrilla powder dryness level and particle size with distilled water.

3.1. Solvent Extraction Process

Figure 15 shows the same circumstances as in the steam distillation method are employed in this procedure, but instead of distilled water, we use a Soxhlet extraction device to extract oil using three distinct solvents (isopropyl alcohol, n-hexane, and chloroform).

Figure 15. 1-litre capacity of Soxhlet Extraction kit

Solvents:

Figure 16 isopropyl alcohol has used as a first solvent to increase yield efficiency hydrilla oil

Figure 16. Isopropyl alcohol solvent

Table 2 represented as a Properties of Isopropyl alcohol.

Table 2. Properties of Isopropyl Alcohol solvent

Physical Properties	Isopropyl alcohol
Boiling Point (°C)	82.5°C
Density(kg/m^3)	0.786 g/cm³ at 20°C
flash point 11.7°C	11.7°C
Cost (INR/1L)	65

Figure 17 N-hexane has used another solvent to extract oil from hydrilla oil.

Figure 17. N Hexane solvent

Table 3 represented as a Properties of N Hexane

Table 3. Properties of n-hexane solvent

Physical Properties	Isopropyl alcohol
Boiling Point (°C)	68.7°C
Density(kg/m^3)	0.659 g/cm³ at 20°C
flash point 11.7°C	-22°C
Cost (INR/1L)	50

Figure 18. Chloroform solvent

Figure 18 chloroform solvent has used our third option for extract the oil yield efficiency.

Table 4 shows the Properties of Chloroform.

Table 4. Properties of Chloroform Solvent.

Physical Properties	Isopropyl alcohol
Boiling Point (°C)	61.2°C
Density(kg/m³)	1.49 g/cm³
flash point 11.7°C	Non-Flammable
Cost (INR/1L)	50-60

Soxhlet is a very effective method of fluid extraction; n-hexane, and water as the solvent for aquatic plants are commonly used in Soxhlet extraction to recover fat-soluble substances such as oils, fats and waxes from plant materials. With this approach, the plant sample is dried and powdered to increase surface area and then placed in a porous paper thimble and inserted into Soxhlet extractor. A non-polar N-hexane is added to a boiling flask, when it evaporates and ascends into the condenser. Vaporized n-hexane is condensed as it passes the thimble onto the plant sample. The plant material is thus denied of oils and other fat-soluble materials by the solvent. After complete percolation through the sample, the solvent collects in a bottom chamber and is drawn again to boiling flask. This repetitive series of solvent vaporization, recondensation, and siphoning ensures continuous contact between the n-hexane and plant material for better extraction efficiency. The n-hexane with extracted chemicals is removed from the plant residues after several cycles. The subsequent step is to evaporate off the solvent, typically under reduced pressure or by means of a rotary evaporator, leaving behind the pure extracted oils or lipophilic compounds from the aquatic plants. For the isolation of hydrophobic compounds, common in most aquatic plants: the same must be performed with isopropyl alcohol and chloroform.

3.2. Experimental Setup for testing obtained biofuel to the CI engine.

The experimental investigation was carried out on a four-stroke single-cylinder water-cooled direct injection diesel engine. The power of the engine was rated at an output of 3.7 kW at 1500 rpm. Being of 80 mm bore and 110 mm stroke. Fitted with a 200–bar, programmed injection system which injected fuel at 23° before TDC, the engine was rated at 17.5:1 compression ratio. A calibrated eddy current dynamometer was attached to the engine for loading and a digital data acquisition system recorded all performance and emission data as presented in **Figure 19**.

The engine was run on regular diesel before testing to obtain baseline values. Following cleaning of the gasoline lines to remove cross contamination, the Hydrilla Veticillata 20% and Diesel 80% (HV20D80) mix was introduced. The engine was operated at steady-state conditions with an engine speed of 1500 rpm and five load levels: 0%, 25%, 50 %, 75% and 100%. The engine was permitted to stabilize for 5 min at each load point before obtaining performance and emissions data.

The EGT, BSFC and BTE had been considered as important performance measurement parameters during the study. Emission parameters consisting of nitrogen oxides (NO_x), unburned hydrocarbons (HC), carbon monoxide (CO), carbon dioxide (CO_2) and smoke opacity were monitored with the help of a five-gas analyser and a smoke meter. All test conditions were performed in triplicate to assure homogeneity, and the results were all presented as means.

Properties of Blend is almost very close to diesel when compared diesel fuel like the density 832.6 (kg/m³), Viscosity 3.01 (mm²/s), latent heat of evaporation 264 (kJ/kg), calorific value 43.6 (MJ/kg) and flash point 57.2˚C.

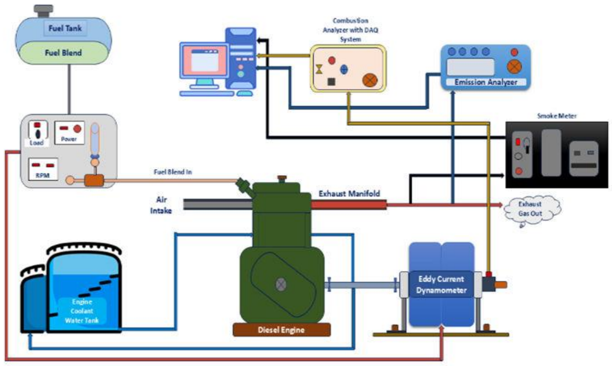


Figure 19. Experimental setup for fueling (HV20D80) Blend

4. Results and Discussion

4.1. Oil extract from mechanical pressing process.

Figure 20 shows this sample added in mechanical pressing machine and pressed continuously, after 60 min time, raw aquatic plant hydrilla oil is obtained which was 130 ml with minimum amount of dust and water. This oil may be used in the next step after removal of unwanted matter.

Figure 20. Obtained Oil from mechanical Pressing method

4.2. The effects of aquatic plant powder size and dryness level for steam distillation process.

The AP oil from100% desiccated hydrilla powder is maximum. What is more, with the known results about hydrilla powder size and degree of dryness, we have concluded that: The smaller the grain size of hydrilla powder and higher degree of dryness, contributes better to oil production %. The correlation between AP powder size and dryness amount is shown in **Figure 24**. 28.5%) compared to the other powder/particles ratio, and the oil yield efficiency was better at 100% dry AP power with 0.5~10 microns with ordinary distilled water, when yield with ordinary distilled water (34.2%). Due to the reduced particle size, water can more easily contact with small particles, thus improving oil yield. Next to **Figure 21 and 22** shows the oil extracted from raw stem and leaves used for steam distillation process.

Figure 21. Oil got from 50% Dried Raw AP Stems and leaves

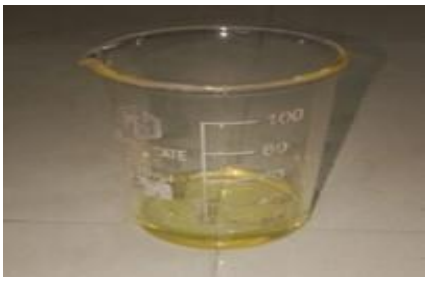


Figure 22. Oil got from 100% Dried Raw AP Stems and leaves

Table 5. Results of oil get from Raw Aquatic plant & Distilled Water

S. No	(%)of Dryness	Raw Aquatic plant Leaves & Stems	Response period (minutes)	Aquatic plant & Distilled Water Ratio(g:ml)	Maintained Temp (˚C)	Obtained Oil Yield (g)	% of Oil Yield
1	50	Raw (AP)	120	1:5	70	6.3	6.3
2	100	Raw (AP)	120	1:5	70	13.7	13.7

Table 5. The influence of aquatic plant powder dryness and particle size on yield with distilled water.

Figure's 23 and 24 is shown as the extracted oil from 50% dried condition of (10-80) micron powder mixture of hydrilla plant.

Figure 23. Oil Got from 50% Dried (10-80) Micron AP Powder Mixture

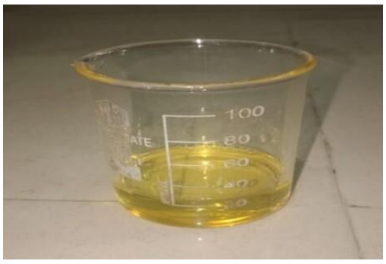


Figure 24. Oil Got from 100% Dried (10-80) Micron AP Powder Mixture

Table 6: The influence of aquatic plant powder dryness and particle size (**10-80)** microns on yield with distilled water.

Table 6. Results of oil get from Aquatic plant (10-80) Micron Powder Mixture & Distilled Water

S. No	(%)of Dryness	Raw Aquatic plant Leaves & Stems	Response period (minutes)	Aquatic plant & Distilled Water Ratio(g:ml)	Maintained Temp (˚C)	Obtained Oil Yield (g)	% of Oil Yield
1	50	Raw (AP)	120	1:5	70	6.3	6.3
2	100	Raw (AP)	120	1:5	70	13.7	13.7

Figures 25 and 26 shows the maximum yield obtained from (0.5-10) mixture powder of hydrilla powder with help of steam distillation process.

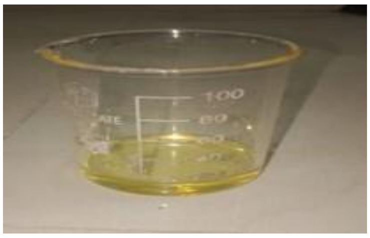


Figure 25. Oil Got from 50% Dried (0.5-10) Micron AP Powder Mixture

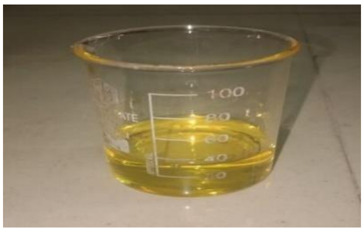


Figure 26. Oil Got from 100% Dried (0.5-10) Micron AP Powder Mixture

Table 7. The influence of aquatic plant powder dryness and particle size (**0-10)** microns on yield with distilled water

Table 7. Results of oil get from Aquatic plant (0.5-10) Micron Powder Mixture & Distilled Water

S. No	(%) of Dryness	Particle size (Micron)	Response period (minutes)	Aquatic plant & Distilled Water Ratio(g:ml)	Maintained Temp (˚C)	Obtained Oil Yield(g)	% of Oil Yield
1	50	0.5-10	120	1:5	70	16.3	16.3
2	100	0.5-10	120	1:5	70	34.2	34.2

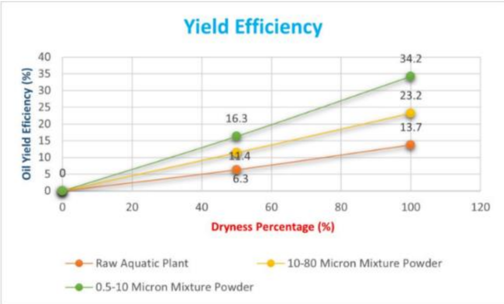


Figure 27. Overall Oil Yield Efficiency Comparison with steam distillation process

It can be seen from **Figure 27**, that efficiency of oil yield increases provided dryness percent and size of powder particles are in inverse correlation, for steam distillation extractor. Therefore, the yellow line in fig 27 shows that it oozes a mixture of (0.5-10) micron aquatic plant particle size with 100% dry condition where oil yield efficiency is better for other powder particle sizes since aquatic plants have high lipid content relative to low density and viscosity; you only have more percentage on oil yield. We found out that the micron size ranges of (0.5-10) microns gave a higher yield % because by reducing the particle sizes of the powder we improved the efficiency in oil extraction yields. We then applied this (0.5-10 micron) micron hydrilla powder combination to the following solvent extraction step to increase yield output.

4.3. Soxhlet (solvent) Extraction Process:

When 100% of one hundred percent dried AP powder fully ground with a particle size of 0.5 to 10 µm was mixed with isopropyl alcohol solvents for 120 minutes, the yield was determined as being 54.2% by the same process used in the conventional solvent extraction method. The hydrilla verticillata plants oil extruded to 63.8% when the one hundred percentage dried AP powder with a particle size in the range of 0.5–10 micron was mixed with n-hexane solvents for 120 minutes, and to 58.5% when it was mixed with chloroform solvent for 120 minutes. **Table 8** Effect of dryness and particle size (0.5-10 µ) of aquatic plant powder upon yield by isopropyl alcohol as a solvent.

Figure's 28 and 29 depicts the maximum yield of obtained from (0.5-10) mixture powder of hydrilla powder using solvent extraction process with isopropyl alcohol as a solvent.

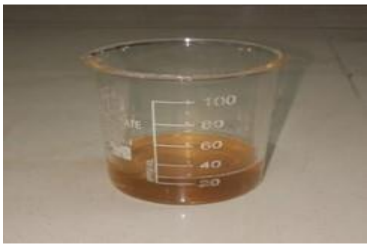


Figure 28. Oil Got from 50% Dried (0.5-10) Micron AP Powder Mixture

Figure 29. Oil Got from 100% Dried (0.5-10) Micron AP Powder Mixture

Table 8. Results of oil get from Aquatic plant (0.5-10) Micron Powder Mixture & isopropyl alcohol

S. No	(%) of Dryness	Particle size (Micron)	Response period (minutes)	Aquatic plant & isopropyl solvents Ratio(g:ml)	Maintained Temp (˚C)	Obtained Oil Yield (g)	% of Oil Yield
1	50	0.5-10	120	1:5	70	22.7	22.7
2	100	0.5-10	120	1:5	70	54.2	54.2

Table 9. Effect of dryness and particle size (0.5-10) microns of aquatic plant powder on yield with N-hexane as solvent. then (0.5-10) mixture powder of hydrilla powder with the help of solvent extraction shows highest yield as **Figure's 30 and 31** of n-hexane.

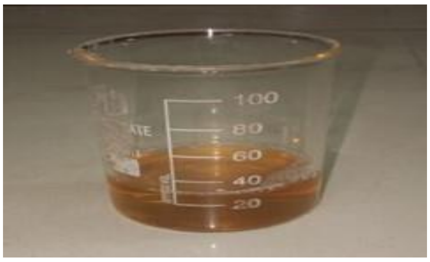


Figure 30. Oil Got from 50% Dried (0.5-10) Micron AP Powder Mixture

Figure 31. Oil Got from 100% Dried (0.5-10) Micron AP Powder Mixture

Table 9. Results of oil get from Aquatic plant (0.5-10) Micron Powder Mixture & n-hexane solvent

S. No	(%)of Dryness	Particle size (Micron)	Response period (minutes)	Aquatic plant & n-hexane solvents Ratio(g:ml)	Maintained Temp (˚C)	Obtained Oil Yield (g)	% of Oil Yield
1	50	0.5-10	120	1:5	70	28.7	35.2
2	100	0.5-10	120	1:5	70	63.8	63.8

Table 10: the effect of the dryness and particle size (0.5-10) micron of the aquatic plant powder on yield by Chloroform solvents. **Figure's 32 and 33** shows the maximum yield of pure compound (0.5-10) who's having a mixture powder hydrilla powder for solvent extraction process with use of chloroform solvent.

Table 10. Results of oil get from Aquatic plant (0.5-10) Micron Powder Mixture & Chloroform solvent

S. No	(%) of Dryness	Particle size (Micron)	Response period (minutes)	Aquatic plant & chloroform solvents Ratio(g:ml)	Maintained Temp (˚C)	Obtained Oil Yield (g)	% of Oil Yield
1	50	0.5-10	120	1:5	70	26.2	26.2
2	100	0.5-10	120	1:5	70	58.5	58.5

Figure 32. Oil Got from 50% Dried (0.5-10) Micron AP Powder Mixture

Figure 33. Oil Got from 100% Dried (0.5-10) Micron AP Powder Mixture

It is shown in **Figure 34** that the higher the dryness percentage, a smaller particle size is obtained by using steam distillation extraction plant and this condition Favors for the oil production efficiency. Since an aquatic plant has high lipid content, low density and little viscosity, a green line on the graph above shows that (0.5-10) micron of the aquatic plant is mixed with 100% dried condition to maximize oil yield than powder having other particle sizes. The maximum oil yield efficiency was obtained with the finest particle size and we have found that the microns interval (0.5-10) was superior than other sizes ranges used. We have therefore taken advantage of this with n-hexane solvents. A (0.5-10) micron comminated hydrilla powder is employed for a solvent extraction process which has had better yield efficiency (63.8%) than alternative chloroform and isopropyl solvents

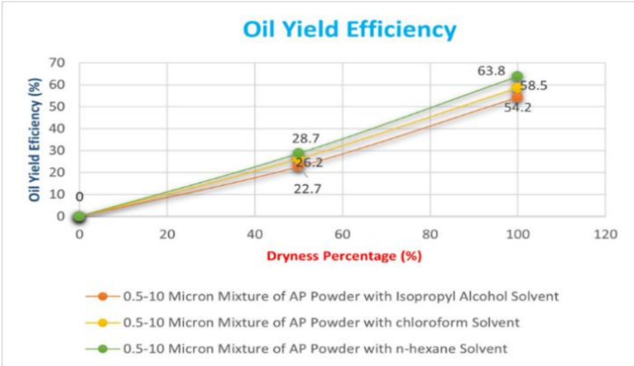


Figure 34. Overall Oil Yield Efficiency Comparison with Solvent Extraction process

Table 11 Properties of hydrilla oil compared with diesel and standard bio fuel.

Table 11. Properties of Hydrilla verticillate Aquatic Plant Oil

Physiochemical Properties	ASTM Standards	Diesel	Biodiesel	Mechanical pressing	Steam distillation method	Solvent Extraction Method
Color	-	Light green	Amber	Dark brown	Pale yellow	Dark pale yellow
Odor	-	Aromatic	Sweet	Sweet Earthy Aroma	Earthy Aroma	Earthy Aroma
Density Kg/m^3	4052-91	830	860	865	838	842
Kinematic Viscosity @40̊ cSt	D445	2.5	4.5	5.1	3.18	3.47
Calorific Value MJ/kg	D240	45	40	33	36	38
Cetane Index	D613	45-50	40-50	37-41	40-45	43-50
Flash Point °C	D92	55	130	97	73	66
Fire Point °C	D93	60	136	103	79	72
Latent Heat of Evaporation kJ/kg	D975	260	310	350	330	320
Boiling point °C		200-350	300-380	295	278	265
Ref. No		(Aravind *et al.* 2021; Yuvarani *et al.* 2017)	(Aravind *et al.* 2021; Yuvarani *et al.* 2017)			

4.4. Chemical properties of present aquatic plant oil by solvent extraction method with high yield efficiency oil get from n-hexane solvent.

4.4.1. Heating Value

Hydrilla oil behaves as a fuel for IC engines and has very high calorific value due to its high energy density structure, good combustion characteristics with ease of handlings. The recovered oil from Hydrilla largely contains triglycerides and long-chain fatty acids that release abundant energy when burned. Its heat value is enhanced by the fact that it has a relatively low oxygen and moisture content, allowing for better combustion. Hydrilla oil content also creates better mixing and atomization of air in IC engines (and hence more complete combustion and higher thermal efficiency). And with small modifications it could serve as a blend stock or an alternative to IC engines because its heating value is close to that of the conventional diesel.

Rate of heat release from fuel burning in air or oxygen, which is proportional to the heating value of fuel (**Figure 35**). The calorific value of the oil obtained from Hydrilla verticillata ranged from 37 to 38 MJ/kg. **Table 11** shows the oils that we have extracted for different biomasses by employing distilled water and via steam distillation technique. It is clear that just about every oil must have a higher HHV.

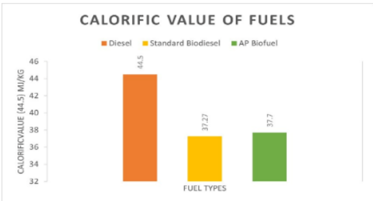


Figure 35. Calorific value of AP Bio-Fuel.

4.4.2. Flash point and Fire Point

Hydrilla oil is a better substitute for IC engines than the other due to higher flash point and fire point properties. This temperature is not so high – it is called the flash point of hydrilla oil and very few oils have such a low value. This lowers the risk of accidental ignition during storage and handling making it safer than traditional petroleum fuels. Likewise, an engine that has a higher fire point—the temperature at which the oil's burning actually will be significant—is guaranteed to display consistent burning properties. Such property contributes towards safe storage and efficient ignition in operation, making it an acceptable fuel for IC engines.

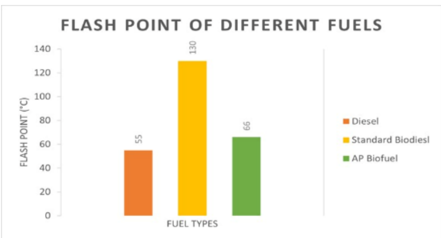


Figure 36. Flash Point Value of AP Bio-Fuel.

The flashing point temperature of benzene is determined by its flashpoint, which can be seen in **Figure 36**. Traditional diesel fuel has a flash point in the range of 55-65 ˚C, while the algae-based oil shown to be available have the flash points that vary in the range of 65-130 ˚C (Aravind *et al.* 2021). The crude products of algal oil extracted from hydrilla verticillata's flash point of 74˚C were shown in **Figure 37** reported that the AP fuel fire point is 79˚C (Ellappan and Rajendran 2021), suggesting that such an oil perhaps could be used in blended fuels to replace diesel.

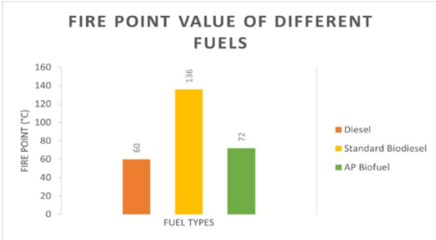


Figure 37. Fire Point Value of AP Bio-Fuel

4.4.3. Density and Kinematic Viscosity

The dens it and the kinematic viscosity of hydrilla oil determine its suitability as a fuel for internal combustion (IC) engines. The water hyacinth oil generally has slightly more density than common diesel, but this does not affect the efficiency of combustion or injection. Even more mass per volume can be obtained with a density from which even a higher energy content per litter obtainable without requiring fuel injection parameter changes for optimal performance.

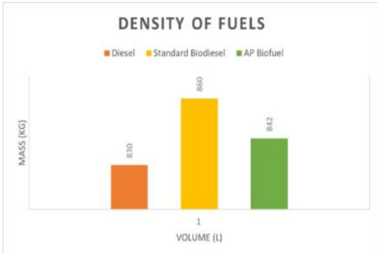


Figure 38. Density of AP Bio-Fuel.

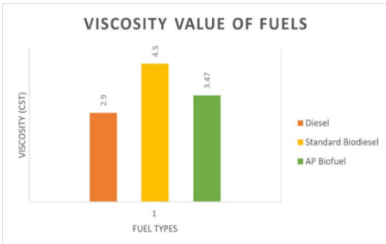


Figure 39. Viscosity Value of AP Bio-fuel

Hydrilla oil's kinematic viscosity is also only slightly higher than that of diesel which will have no impact on the fuel flow, atomization or mixing with air in the combustion chamber. Unregulated, the lower viscosity could lead to carbon on the piston and incomplete combustion. But you don't have to boil the oil or mix it with simpler fuels of lower viscosity then pour them into the engine to see how it performs.

The density of AP oil obtained by Stea distillation method was approximately 842 kg/m³, compared to regular biodiesel (**Figure 38**). It suggests that the fuel we obtained by such method can be used as a bio-substitute fuel for internal combustion engines. The kinematic viscosity of the oil was 3.47 cSt that is much lower than typical biodiesel value as shown in **Figure39**.

4.4.4. Cetane number

A fuel's cetane number indicates its (Ignition Quality) quality and performance when ignited. The higher the cetane number, the better a fuel will perform. Biodiesel fuel made from algae oil has the cetane number is as high, or higher, than the Petro diesel. Petroleum diesel has a cetane value between 40 and 55 higher numbers correlating to better ignition quality. Biodiesel derived from algae oil may match or even surpass the range of this price, depending upon specific feedstock and processing methods utilized. **Figure 40** Show" Hydrilla verticillata value of the cetane index of oil extracted "(43) is in line with the current reporting range and It costs less than conventional biodiesel or diesel, but not significantly so. Thus, the oil could be used as alternative energy when mixed other biodiesels

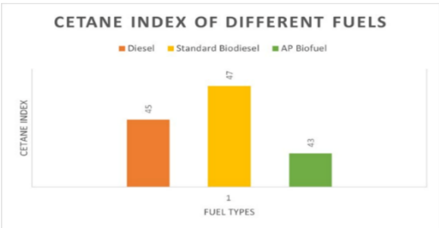


Figure 40. Cetane Index of AP Bio-Fuel

The physiochemical properties of the oils are presented in **Table 11** This table also compares the properties of algal oil used in our study with those of conventional diesel fuel and biodiesel. On the other hand, dry H. verticillata aquatic plant fat may be considered as a good potential feedstock for alternative fuel.

4.5. FT-IR Analysis:

Therefore, **Figure 41**. The presence of characteristic hydrocarbon and oxygenated functional groups normally associated with lipids were expected to appear in the FTIR spectra of Hydrilla verticillate oil. The oil obtained from Hydrilla verticillate would contain principally triglycerides (the fats and oils), fatty acids, and perhaps a very few other oxygenated compounds. If the oil contains hydroxyl groups, O-H stretching vibrations can be observed in the range of 3325.64–3647.7 cm^{-1}. There would be a wide, striking peak in this area for oils containing phenolic compounds or fatty acids. This may indicate the presence of minor levels of oxidation contaminants or free hydroxyl groups fatty acids (i.e., from hydroxy fatty acids). Due to the hydrogen bonding, broad and intense peaks are also observed H-O-H stretching mode.

C–H stretching band due to alkyl groups (saturated hydrocarbons) 2806.92–2979.48 cm^{-1}: They alone can account for the C–H stretching bands. These bands are wide and intense in oils, particularly for the methyl (CH_3) and methylene (CH_2) groups located in triglycerides or fatty acid chains. This is the most pronounced feature in the FTIR spectrum of the oils. 1460.81–1635.34 cm^{-1}: The stretching vibration of carbonyl groups (C=O) suggests the presence of an ester bond, common in triglycerides (fatty acid esterified glycerol). This peak, which occurs frequently and is easy to see signifies fatty acids or triglycerides in the oil. If oxidised compounds, like aldehydes or ketones in the oil, are present, extra peaks can be found in the 1460.81–1635.34 cm^{-1} region for carbonyl derivatives.

1251.58-1382.71 cm^{-1}: C=C stretching vibration (which represents the fact that if oil contained unsaturated fatty acids (e.g., oleic acid, linoleic acid, or others polyunsaturated fatty acids), presence of double bonds in the chains was observed). This peak may give an idea of how unsaturated the oil is, as it typically weaker compared to that of alkanes. The polyethylene has the C-H bending (methylene and methyl) vibrations, which appear in the 1073.19–1152.26 cm^{-1} region. As the long carbon chains in these triglycerides are saturated, this often appears as a large peak in many oils and fats.

The C–O stretch vibrations of 419.442–953.627 cm^{-1} are associated with the ester bonds present in fatty acids or triglycerides (Zhong *et al.* 2025). Also, in this region may be observed peaks of ether or alcohol.

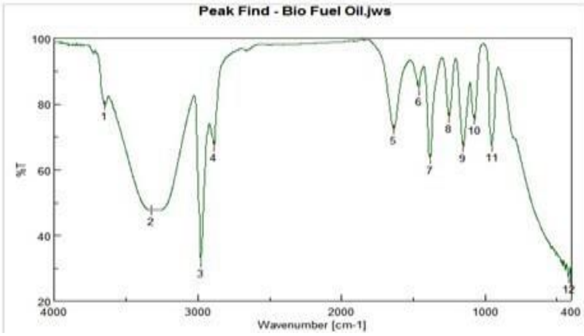


Figure 41. FT-IR Analysis of Hydrilla Oil.

Figure 41 shows the values got from FTIR analysis process.

No.	Position	Intensity	No.	Position	Intensity
1	3647.7	79.527	2	3325.64	47.6975
3	2979.48	31.7191	4	2886.92	67.0087
5	1635.34	72.4312	6	1460.81	84.0607
7	1382.71	63.1061	8	1251.58	75.8034
9	1152.26	66.8094	10	1073.19	75.2231
11	953.627	67.1077	12	419.442	27.0183

Figure 42. Position of Chemical Components

4.6. SEM Analysis

Figure 43. Demonstrates the importance of SEM study for interpretation of the morphological features and physical structure of a new discovery. The SEM analysis of Hydrilla verticillata oil content has often been applied to an understanding of the oil microstructural, morphological and droplet detail. The objective of this analytical method is to locate and identify the functional group and chemical composition in Hydrilla oil. Scanning electron microscopy (SEM) analysis of candlenut oil also presents some useful properties as a biofuel for internal combustion (IC) engines, and an interesting phenomenon is that information contact angle of this oil on glass at 30 °C using is low. The study indicates that a uniform microstructure with minimum impurities is exhibited, which leads to higher purity and good combustibility. The result of carbon deposits obtained from the surface morphology of Hydrilla oil residue in combustion process indicates low

carbon problem, and reduces engine wear and maintenance. Also, the uniform fatty acid molecules of a structure of oil improve atomization and air mixing and enhance combustion efficiency. No large agglomerates or impurities exist, assuring smooth fuel flow and preventing clog of an injector as confirmed by the SEM analysis. These ultrasmall features confirm the clean-burning nature and assurance of Hydrilla oil as a substitute for diesel engine.

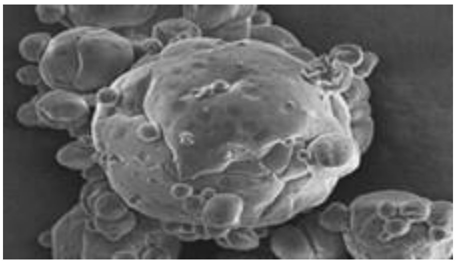


Figure 43. SEM Image of Hydrilla Oil

Refer **Figure 44** The physicochemical characterisation of hydrilla oil only indicates that it has a good potential as an alternate biofuel. Major fuel parameters (density 842 kg/m³, kinematic viscosity at 40 °C 3.47 mm²/s, lower heating value of gross calorific value 38 MJ/kg, cetane index 43-50 as well as flash point >66 °C) meet or exceed typical ASTM D6751 standard requirements for diesel type biodiesel. Based on these results, Hydrilla oil possesses the fuel quality properties which are needed for safe handling and engine performance.

Properties	Hydrilla Oil (Measured)	ASTM Standards Range	Meets Standards
Density at 15°C (kg/m³)	842	820-900	Yes
Kinematic Viscosity @40° (mm²/s)	3.47	1.9-6.0	Yes
Calorific Value (MJ/kg)	38	>35	Yes
Cetane Index	43-50	>47 (Typical Minimum)	Yes
Flash Pont (°C)	66	>52 (for biodiesel)	Yes

Figure 44. Physicochemical Properties Discussions

The cetane index is in the acceptable level, although biased slightly below the minimum ASTM limit (≥47). In contrast to a low flash point, the storage and transport safety is enhanced due to the much higher flash point. The suitability of Hydrilla oil as an alternative renewable fuel is discussed based on these preliminary results, however rigorous research including statistical validation and field engine testing is required.

4.7. The measure of performance.

4.7.1. Brake Thermal Efficiency (BTE) values for the combination of diesel and hydrilla fuel (HV20D80).

In this research study, the influence of filling Hydrilla fuel in a B20 form has been studied on brake thermal efficiency (BTE) of internal combustion engine under variations in loads. The objective of this work is to investigate the performance and energy conversion efficiency achievable by Hydrilla biofuel for replacing traditional diesel fuel. The BTE vs load plots for this experimental data are plotted in for and discuss chapter.

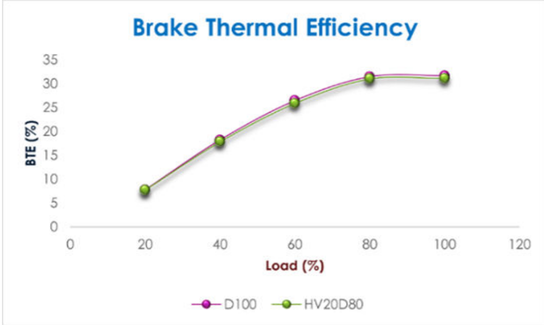


Figure 45. BTE values for the diesel vs. hydrilla fuel blend

For clarity, **Figure 45** data are presented alongside the B20 Hydrilla blend and standard diesel. The key findings from BTE investigation using the Hydrilla B20 blend reveals that it complies up to 31.13% of normal diesel fuel and a small drop of 1.70%. Hydrilla fuel, which has more oxygen than diesel, runs better and may increase the combustion efficiency of diesel when mixed with it. Improved energy conversion due to this improved combustion can increase BTE.

4.7.2. The HV20D80 Blend's Brake Specific Fuel Consumption (BSFC)

Brake specific fuel consumption (BSFC) is a key metric for the fuel economy of internal combustion engines. It is the amount of fuel consumed for each unit of power generated over time. This value of the latent heat of evaporation for the hydrilla fuel is slightly more than that of HV20 and a little larger than that for diesel. Superior atomization and smaller droplets at injection time are the main causes why we cannot achieve a common diesel fuel; as can be observed also from **Figure 45** Diesel over which was just a % increase less than 5 (4.5%).

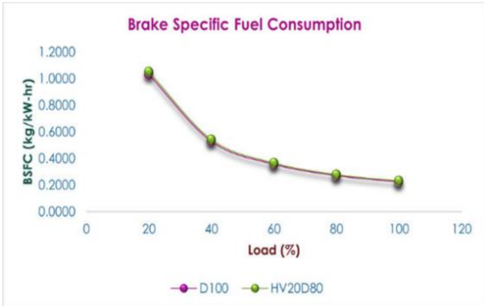


Figure 46. Blend HV20D80's BSFC

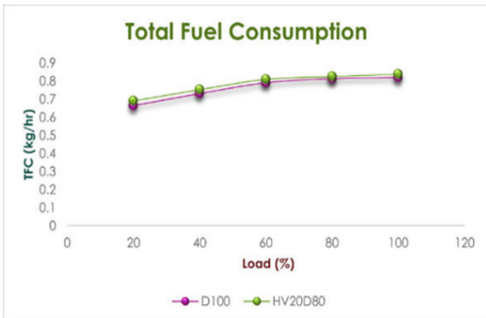


Figure 47. TFC for the HV20D80 Blend

4.7.3. Blend HV20D80's Total Fuel Consumption (TFC).

Total Fuel Consumption (TFC) of a diesel engine using an admixture of 20% Hydrilla oil and 80% diesel is more as compared to that with neat diesel. This increase in TFC could be as a result of the low calorific value and high viscosity of Hydrilla oil, which may influence engine atomization fuel combustion. It's actually not much more expensive than diesel. TFC is less than three percent in **Figure 47** when compared with pure diesel. The blend contains less energy per unit of fuel, so the engine must take in more of it to generate the same amount of braking power that could be obtained with typical diesel.

4.8. Combustion Parameters

4.8.1. HV20D80 Crank Shaft Pmax Value vs. Crank Angle Degree

In **Figure 48**, the peak cylinder pressure of a diesel engine with Hydrilla oil (20%) mixed with diesel (80%) was considerably lower (63.79 bar) compared to that of an engine using pure diesel (63.09 bar). The higher viscosity and lower cetane number of hydrilla oil, which have a slight negative impact on combustion characteristic are the main factors that cause this drop in peak cylinder pressure. A retarded ignition delays due to a lower cetane number results in poorer combustion and less average pressure rise in the cylinder. In addition, the flow of such viscous fuel-water blend might fail to atomize the fuel into small enough droplets avoiding air mixing sufficiently, resulting in lower combustion efficiency. The maximum cylinder pressure drops the energy released during power stroke is 0.87 percent lower.

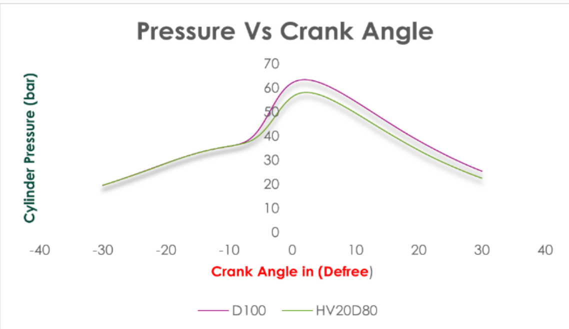


Figure 48. Peak Cylinder Pressure for the HV20D80 Blend vs. Crank Angle Degree

4.8.2. Heat Release Rate (HRR) vs Crank Shaft Crank Angle Degree of HV20D80 Blend.

Diesel 20% Hydrilla oil & 80% diesel **Figure 49**, when a CI engine operates on an organic blend of 20% hydrilla and80% pure diesel, HRR is less than that of the neat fuel. (63.79 J/Deg against 65.11 J/Deg). These contribute significantly to the rise in HRR loss (2.02%) of hydrilla oil: higher viscosity, lower cetane number and lower vapor pressure all are against combustion **Figure 48**. This is followed by a longer ignition delay, due to lower cetane number, and hence slower and less efficient combustion. In addition, the insufficient air mixing and poor fuel atomization caused by the increased viscosity also result in a decrease in rate of fuel energy delivered to combustion. The general thermal efficiency of the motor is affected because higher-peak heat release (as well as the more sluggish burn) is reduced. Variations in the combustion parameters, which lead to performance and emission alterations, are reproduced by this decrease of HRR.

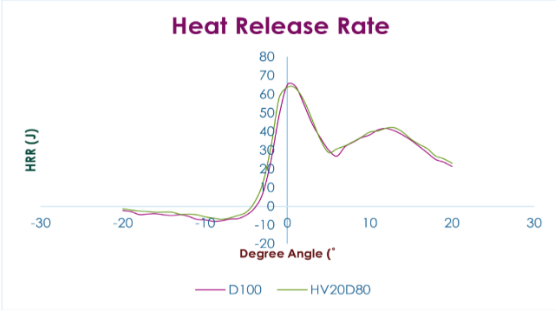


Figure 49. HRR vs Crank Angle Degree for the HV20D80 Blend.

4.9. Parameters for emissions

4.9.1. Emission of Carbon Monoxide from the HV20D80 Blend.

Carbon Monoxide (CO) emission from a diesel engine operating with 20% Hydrilla oil blended with 80% Diesel indicates an increase of only 0.134%, since hydrilla has more C and H than the pure diesel. Hydrilla oil has high viscosity and low volatility, resulting in poor atomization of the fuel mix, leading to the main factor of increased CO emissions. These combinations are the cause of poor air/fuel mixing, and inadequate atomization. A slightly lower cetane number of hydrilla oil also results in slight ignition delays, which would decrease the combustion efficiency. Additional partially oxidized carbon molecules, such as CO, are formed in the process. The small 1.51 % increase in CO emissions found in the investigation may imply that during combustion (when using biodiesel) blends of Hydrilla oil adjustments must be made to the combustion parameters e.g injecting pump and timing can be **Figure 50**.

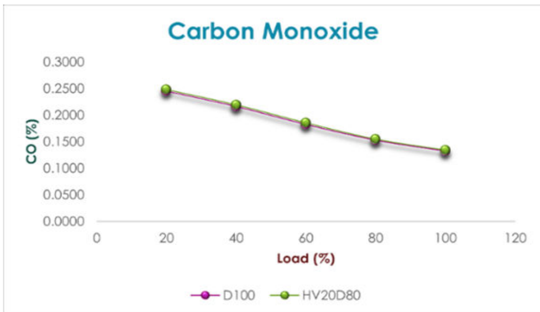


Figure 50. CO Emissions against Various Loads for the HV20D80 Blend

4.9.2. Emission of Hydrocarbons from the HV20D80 Blend

As a result of its chemical composition and the combustion characteristics, hydrilla oil slightly produces more hydrocarbons (HC) over diesel (12.5%) for low load and 9.4% high load case while under high load condition 1.38 percent compared to that on **Figure 51**. higher oxygen content and how it burns as a biodiesel, or bio-based fuel. Due to its higher molecular weight and viscosity, however, inefficient combustion leads to greater HC emissions. In addition, because the unsaturated fatty acids in Hydrilla oil adversely affect atomization and retarded oxidation in the engine, they contribute to a elevate unburned hydrocarbons. Diesel, on the other hand, produces less HC due to its higher volatility, more complicated chemical connection and better combustion performance.

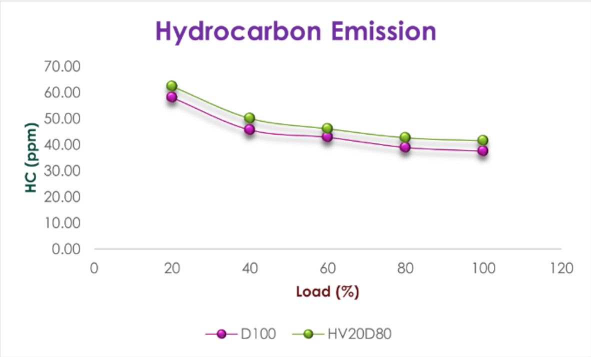


Figure 51. HC Emission Vs Different Load for the Blend of HV20D80

4.9.3. HV20D80 Blend Emissions of Nitrogen Oxide.

As compared to conventional diesel, it has been determined that use of a hydrilla diesel blend at an amount of 20% hydrilla decreases NOx (Nitrogen Oxides) by 1.97 ppm. The aquatic plant hydrilla may contain a lot of fertile material for making biofuel There's an awful lot of organic matter in the plant hydrilla, and it could be processed into ethanol. When mixed with diesel, it alters how the fuel burns. The oxygenated molecules within hydrilla are mainly responsible for the decline of NOx emission. By further promoting more complete burning of fuels at lower temperatures, they increase combustion efficiency and thus reduce NOx emissions. The physical properties of the mix, with a higher boiling point and lower cetane number with respect to pure diesel, are also favourable to a more delayed and regulated combustion. Accordingly, less thermal NOx is produced at high combustion temperatures. Therefore, a blend of 20% hydrilla in diesel as possible choice to reduce NOx emissions and improve overall environmental desirability of engines can be shown from **Figure 52.**

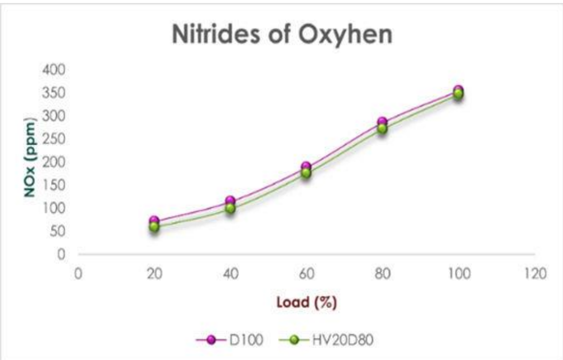


Figure 52. NOx Emission against Various HV20D80 Blend Loads

4.9.4. Emissions of Carbon Dioxide from the HV20D80 Blend

From **Figure 53**, comparing against the normal diesel, using a 20% hydrilla fuel blend with diesel also contributes to CO2 reduction of about 1.8%. This decline could be related to hydrilla being renewable as a biofuel feedstock. Hydrilla is a carbon sink because it is derived from plant material, and during its growth phase, utilizes photosynthesis to extract CO2. Because the carbon released from burning hydrilla in a fuel blend came from the atmosphere, not fossil fuels, it is a part of the carbon cycle. So, when you use the hydrilla biofuel, you cause release of less net carbon than if you were using straight diesel, because that is made from fossil fuel and releases lots of carbon that has been buried for millions of years, so in total: less CO2 => overall. Consequently, the hydrilla blend helps reduce CO2 emissions by reducing the overall carbon footprint of the fuel.

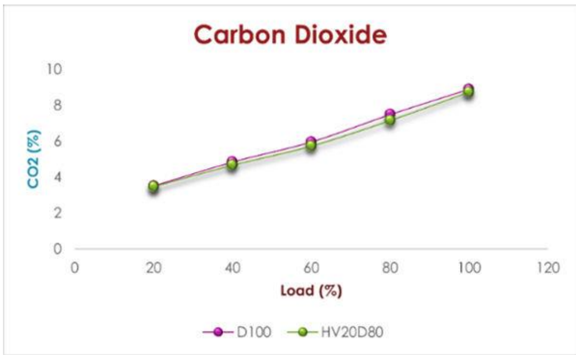


Figure 53. CO_2 Emission against Various HV20D80 Blend Loads

4.9.5. The blend of HV20D80 emits smoke.

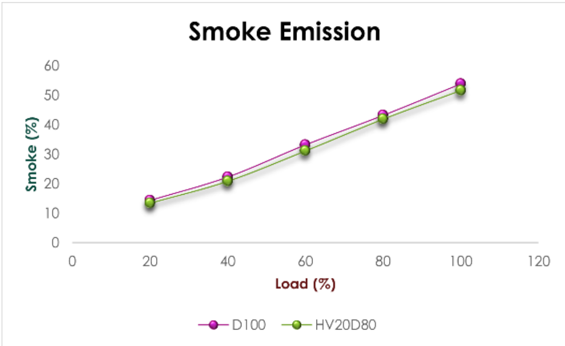


Figure 54. Smoke Emission against HV20D80 Blend Loads

At higher load situations, the hydrilla 20% fuel mixture with diesel also reduces smoke by 4.06%. This is predominantly due to the improved burning characteristics of hydrilla. The oxygenates of the hydrilla fuel mix enhance fuel combustion by promoting a more efficient and complete burning of the fuel. As a result, there has been the added benefit of a reduction in particulate emissions and unburned hydrocarbons associated with smoke from combustion. Unburned residues and smoke are often created by what is called incomplete combustion in diesel engines, especially when they are operated under high load or badly managed. The hydrilla biofuel mix also results in lower particulate emissions, since it encourages a cleaner and more controlled burn.

Regarding the air pollution and environmental impact, it is a cleaner fuel from the brake specific smoke, which visually shows very little or no visible smoke is emitted out of the exhaust while this reduction in soot formation. The CO2 and NOx emissions are lowered with the use of Hydrilla, a cleaner exhaust also led to a better engine output and environmental protection perspective.

5. Conclusion

The novel Biofuel was easily and efficiently extracted from the abundant and rapidly growing aquatic plant Hydrilla verticillata in this work. And the following oil extraction methods mechanical pressure, steam distillation and

Soxhlet solvent were used to determine the best method for maximum oil yield. This study confirms that Hydrilla verticillata-derived oil, particularly extracted using the solvent method with n-hexane, yields up to 63.8% oil, exhibiting fuel properties comparable to diesel. The 20% blend demonstrated promising engine performance with reduced NOx and CO_2 emissions. These findings establish Hydrilla as a viable feedstock for sustainable third-generation biofuel production. The drying of biomass and its particle size reduction to 0.5-10 µm were key components in the oil extraction process.

Good quality characteristics for the oil obtained based on physicochemical analysis was observed to be acceptable for use as an alternate fuel. Blended with diesel, bio-oil produced from hydrilla indicates its potential as an alternative fuel for CI engines. The Blend HV20D80 give very better performances but not exceeding standard diesel fuel, The key findings from BTE investigation using the Hydrilla B20 blend reveals that it complies up to normal diesel fuel and a small drop of 1.70%, in cylinder pressure was reduced due to the lack lacking of combustion properties present in blends, the energy released during power stroke is 0.87 percent lower and heat release rate of the engine was decreased 2.02% when compared to standard diesel. Beside emission part CO 15% and HC emission 1.38% increased due to the slightly improper combustion, NO_X and CO_2 emissions are decreased due to low temperature occurred in combustion chamber. a comparison with some other algal feedstocks like Water Hyacinth, Spirogyra, and Scenedesmus parvus that shows differences in oil yields, extraction efficiency and engine performances. The performance of Hydrilla biofuel in context with these well-developed feedstocks have been discussed and literature references have been provided to support it, highlighting its potential advantages and challenges in comparison to them. Here, concluded the novelly found and extracted biofuel is most suitable alternate biofuel for blend with diesel and reduce greenhouse gaseous emission and carbon foot prints.

6. Future Work

- Increasing blend ratio with help of modification to the diesel engine like (LHR-Low Heat Rejection Engie and Trinary Blend of adding alcohols as a co blend to minimize diesel fuel needs.
- Add some additives like graphene oxide to promote combustion characteristics.
- Increasing injection pressure to improve blend molecule size for good atomization inside the combustion chamber.
- Tested with hybrid vehicles.

Conflict of Interest

The authors have no conflicts of interest to disclose.

Data Availability Statement

Upon reasonable request, the corresponding author will make the datasets created and/or analysed during the current work available.

Nomenclature

S No	Symbol	Abbreviation
1	CIE	Compression Ignition Engine
2	AP	Aquatic Plant
3	HV	Hydrilla Verticillata
4	NAABB	National Alliance for Advanced Biofuels and Bioproducts.
5	GHG	Greenhouse Gas Emission
6	EHMT	Extraction of biodiesel via Hexane following Microwave-assisted Transesterification
7	FAME	Fatty Acid Methyl Ester
8	ASTM	American Society for Testing and Materials
9	KOH	Potassium hydroxide
10	ICUN	International Union for Conservation of Nature
11	CITES	Convention on International Trade in Endangered Species
12	W	Watts
13	rpm	Revolution Per Minute
14	L	Litter
15	Kg	Killo Grame
16	µ	Micron
17	˚C	Degree Celsius
18	%	Percentage
19	m	Meter
20	INR	Indian Rupees
21	g	gram
22	mL	Milli Litter
23	MJ	Megajoule
24	HC	Hydrocarbon
25	CO	Carbon monoxide
26	NO_X	Nitrides of Oxygen
27	CO_2	Carbon dioxide
28	HRR	Heat Release Rate
29	BSFC	Break Specific Fuel Consumption
30	TFC	Total Fuel Consumption

References

Anyanwu, I. N., Okeke, C. S., Nwankwo, S. C., Nwachukwu, M. O., Michael, M. O., Opara, V. C., Anorue, C. O., *et al.* (2022). Aquatic macrophytes (*Spirogyra porticalis* and *Nymphaea L.*) as substrates for biofuel production: Potentials and challenges. *Scientific African, 18*, e01412. https://doi.org/10.1016/j.sciaf.2022.e01412

Aravind, S., Barik, D., Ragupathi, P., & Vignesh, G. (2021). Investigation on algae oil extraction from algae *Spirogyra* by Soxhlet extraction method. *Materials Today: Proceedings, 43*, 308–313. https://doi.org/10.1016/j.matpr.2020.12.527

Arun, J., Raghu, R., Hanif, S. S. M., Thilak, P. G., Sridhar, D., Nirmala, N., Dawn, S. S., Sivaramakrishnan, R., Chi, N. T. L., & Pugazhendhi, A. (2022). A comparative review on photo and mixotrophic mode of algae cultivation: Thermochemical processing of biomass, necessity of bio-oil upgrading, challenges and future roadmaps. *Applied Energy, 325*, 119808. https://doi.org/10.1016/j.apenergy.2022.119808

Baig, R. U., Malik, A., Ali, K., Arif, S., Hussain, S., Mehmood, M., Sami, K., Mengal, A. N., & Khan, M. N. (2018). Extraction of oil from algae for biodiesel production, from Quetta, Pakistan. *IOP Conference Series: Materials Science and Engineering, 414*(1), 012022. https://doi.org/10.1088/1757-899X/414/1/012022

Bangari, N., Singh, V. K., & Sharma, V. K. (2024). Experimental investigation of thin-film solar cells as a wearable power source. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, *46*(1), 9341-9361.

Barry, A., Wolfe, A., English, C., Ruddick, C., & Lambert, D. (2016). *2016 National Algal Biofuels Technology Review.* U.S. Department of Energy.

Bhuiya, M. M. K., Rasul, M., Khan, M., Ashwath, N., & Mofijur, M. (2020). Comparison of oil extraction between screw press and solvent (n-hexane) extraction technique from beauty leaf (*Calophyllum inophyllum* L.) feedstock. *Industrial Crops and Products, 144*, 112024. https://doi.org/10.1016/j.indcrop.2019.112024

Brindhadevi, K., Anto, S., Rene, E. R., Sekar, M., Mathimani, T., Chi, N. T. L., & Pugazhendhi, A. (2021). Effect of reaction temperature on the conversion of algal biomass to bio-oil and biochar through pyrolysis and hydrothermal liquefaction. *Fuel, 285*, 119106. https://doi.org/10.1016/j.fuel.2020.119106

Cheng, J., Huang, R., Li, T., Zhou, J., & Cen, K. (2014). Biodiesel from wet microalgae: Extraction with hexane after the microwave-assisted transesterification of lipids. *Bioresource Technology, 170*, 69–75. https://doi.org/10.1016/j.biortech.2014.07.064

Demirbas, A., & Demirbas, M. F. (2011). Importance of algae oil as a source of biodiesel. *Energy Conversion and Management, 52*(1), 163–170. https://doi.org/10.1016/j.enconman.2010.06.055

Ellappan, S., & Rajendran, S. (2021). A comparative review of performance and emission characteristics of diesel engine using eucalyptus-biodiesel blend. *Fuel*, *284*, 118925.

Elnajjar, E., Purayil, S. T. P., Alnuaimi, F., Al Khawaja, H., Shaikhoun, L., Arnaoud, N., & Almutawa, S. (2023). Mechanistic investigation of efficient cell disruption methods for lipid extraction from various macro and micro species of algae. *Bioresource Technology Reports, 22*, 101482. https://doi.org/10.1016/j.biteb.2023.101482

Gong, J., Mei, Y., Wang, B., Zhang, S., Hou, Y., Hou, J., ... Pang, S. (2025). Study on enhanced torrefaction of elm with Mg(OH)2. Renewable Energy, 254, 123800. doi: https://doi.org/10.1016/j.renene.2025.123800

Ijaz, A., Anwar, Z., & Zafar, M. (2023). Screening of wastewater *Oedogonium oblongum* algae for hyper-oil transformation into biodiesel by response surface methodology. *Kuwait Journal of Science.* https://doi.org/10.48129/kjs.v50i2.11605

Jain, A., Bora, B. J., Kumar, R., Sharma, P., Medhi, B. J., Farooque, A. A., Tirth, V., Senthilkumar, N., & Peyyala, P. K. (2023). Impact of titanium dioxide (TiO_2) nanoparticles addition in *Eichhornia crassipes* biodiesel used to fuel compression ignition engine at variable injection pressure. *Case Studies in Thermal Engineering, 49*, 103295. https://doi.org/10.1016/j.csite.2023.103295

Kale, B. N., Patle, S. D., & Kalambe, S. R. (2021). Microalgae biodiesel and its various diesel blends as promising alternative fuel for diesel engine. *Materials Today: Proceedings, 44*, 2972–2977. https://doi.org/10.1016/j.matpr.2020.11.911

Liu, S., Pu, S., Deng, D., Huang, H., Yan, C., Ma, H.,... Razavi, B. S. (2020). Comparable effects of manure and its biochar on reducing soil Cr bioavailability and narrowing the rhizosphere extent of enzyme activities. Environment International, 134, 105277. doi: https://doi.org/10.1016/j.envint.2019.105277

Qiu, Z., Chen, R., Gan, X., Wu, C., Yang, H, ... Zhao, Y. (2025). An Iterative Approach to Solve the Viscous Damper Temperature and the Torsional Vibration Amplitude of a Diesel Engine. Journal of Vibration Engineering & Technologies, 13(6), 437. doi: 10.1007/s42417-025-01983-7.

Rajendran, S. (2021). A comparative study of performance and emission characteristics of neat biodiesel operated diesel engine: a review. *Journal of Thermal Analysis & Calorimetry*, *146*(3).

Rajendran, S., & Ganesan, P. (2021). Experimental investigations of diesel engine emissions and combustion behaviour using addition of antioxidant additives to jamun biodiesel blend. *Fuel*, *285*, 119157.

Rajendran, S., Ganesan, P., & Anandkumar, G. (2023). A comparative assessment on performance, combustion and emission characteristics of diesel engine fuelled by juliflora biodiesel-diesel blends. *Australian Journal of Mechanical Engineering*, *21*(1), 257-269.

Rajendran, S., Selvanarayanan, R., Pappa, C. K., & Thomas, B. (2025). Wastewater recycling integration with IoT sensor vision for real-time monitoring and transforming polluted ponds into clean ponds using HG-RNN. Global NEST Journal, 27(4). https://doi.org/10.30955/gnj.06758. The additions can be found in page 41, lines 12–28 of the revised manuscript.

Rashd, J. A., Lalung, J., Kassim, M. A., Wijaya, D., Allzrag, A. M. M., & Shaah, M. A. (2024). Kinetics and thermodynamic studies on biodiesel synthesis via Soxhlet extraction of *Scenedesmus parvus* algae oil. *Energy Conversion and Management: X, 23*, 100633. https://doi.org/10.1016/j.ecmx.2024.100633

Sayed, K., Baloo, L., & Sharma, N. K. (2021). Bioremediation of total petroleum hydrocarbons (TPH) by bioaugmentation and biostimulation in water with floating oil spill containment booms as bioreactor basin. *International Journal of Environmental Research and Public Health*, *18*(5), 2226.

Sayed, K., Baloo, L., Kutty, S. R. B., & Makba, F. (2021). Potential biodegradation of Tapis Light Crude Petroleum Oil, using palm oil mill effluent final discharge as biostimulant for isolated halotolerant Bacillus strains. *Marine Pollution Bulletin*, *172*, 112863.

Sayed, K., Baloo, L., Kutty, S. R. B., Al Madhoun, W., Kankia, M. U., Jagaba, A. H., & Singa, P. K. (2022). Optimization of palm oil mill effluent final discharge as biostimulant for biodegradation of tapis light crude petroleum oil in seawater. *Journal of Sea Research*, *188*, 102268.

Sayed, K., Baloo, L., Yekeen, S. T., Kankia, M. U., & Jagaba, A. H. (2021). Determination of total petroleum hydrocarbons concentration in coastal seawater of Teluk Batik Beach, Perak, Malaysia. *Key Engineering Materials*, *888*, 119-128.

Sayed, K., Syakir, M. I., Othman, A. A., Azhar, B., Tohiran, K. A., & Nobilly, F. (2025). Introducing the RICE framework in paddy-duck farming: a novel approach to enhance the adaptive capacity of paddy farmers among asnaf community in managing the risk of climate change. *Archives of Agronomy and Soil Science*, *71*(1), 1-16.

Selvanarayanan, R., Rajendran, S., Pappa, C. K., & Thomas, B. (2024). Wastewater recycling to enhance environmental quality using fuzzy embedded with RNN-IOT for sustainable coffee farming. Global NEST Journal. https://doi.org/10.30955/gnj.006346.

Shi, R., Handler, R. M., & Shonnard, D. R. (2019). Life cycle assessment of novel technologies for algae harvesting and oil extraction in the renewable diesel pathway. *Algal Research, 37*, 248–259. https://doi.org/10.1016/j.algal.2018.12.019

Shrivastava, M., & Srivastava, S. (2021). Application and research progress of *Hydrilla verticillata* in ecological restoration of water contaminated with metals and metalloids. *Environmental Challenges, 4*, 100177. https://doi.org/10.1016/j.envc.2021.100177

Yuvarani, M., Kubendran, D., Aathika, A. R. S., Karthik, P., Premkumar, M. P., Karthikeyan, V., & Sivanesan, S. (2017). Extraction and characterization of oil from macroalgae *Cladophora glomerata. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects, 39*(23), 2133–2139. https://doi.org/10.1080/15567036.2017.1340781

Zhong, R., Lyu, H., Su, X., Yan, X., Teng, Y., Dong, W, ... Shen, X. (2025). Freeze–Thaw–Induced Regulation of Petroleum Hydrocarbon Adsorption in Cold-Region Soils: Role of Organic Matter Dynamics. Water Research, 124495. doi: https://doi.org/10.1016/j.watres.2025.124495.