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Optimized Biodiesel Production from *Dunaliella Salina*, A Unicellular Green Algae through Artificial Neural Network

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Abstract

Renewable energy is a sure bet for energy needs in the future and algae biofuels for instance can go a long way to provide the energy needed. In this work, the process of obtaining the bio-oil from *Dunaliella Salina*, a hypersaline, unicellular microalga, having greenish-orange color, is described. The microalgae were cultured in f/2 nutrition medium supplemented with carbon dioxide and vitamins and trace metals. 650 mL of bio-oil were obtained, where ultrasonic extraction frequency of 60 Hz was carried out on the sample for a period of 90 minutes to isolate the bio-oil. The bio-oil was then processed to biodiesel through a single stage base-catalyzed transesterification using methanol and sodium hydroxide as the catalyst. This procedure produced pure *Dunaliella Salina* biodiesel with an extraction efficiency of 93%. The prediction tool ANN was utilized with trainlm algorithm which predicted the yield which was similar to experimental yield with error percentage of 0.09016. Nuclear Magnetic Resonance (NMR) spectral analysis, Gas Chromatography-Mass Spectrometry (GCMS), and Fourier Transform Infrared Spectroscopy (FTIR) are the three analysis performed on the produced biodiesel. This work paves the path for more research and development in the field of algae biofuels by demonstrating the effectiveness of *Dunaliella Salina* as a sustainable feedstock for biofuel production and offering a thorough explanation of the mechanisms involved in its conversion to biodiesel.

1. Introduction

Increasing urbanization, population growth, and energy consumption along with depletion of conventional petroleum-based oil reserves and deteriorating air quality are all driving forces behind these trends. Researchers are moving to identify greener and more sustainable energy sources. Because of the special advantages over first- and second-generation biodiesels, algae biodiesel has garnered a lot of attention. Compared to conventional feedstock's, algae can produce more biodiesel per unit of growth area because of their high lipid content (up to 70%). Algae grow quickly, which makes harvesting more convenient and increases yield overall. The capacity to grow algae in a variety of settings, such as non-arable land and wastewater, lessens the need for it to compete with food crops for resources. The ability of algae to absorb carbon dioxide is approximately ten times greater than that of land-based plants, which contributes to the algae's increased environmental sustainability. Algal biodiesel's appeal is increased by its capacity to produce useful by-products, maintain biodiversity, and need less resources [1, 2]. Because of its beneficial qualities, Chlorella vulgaris is a widely preferred strain for the production of biodiesel. Because they develop quickly and contain 50% lipids. Microalgae are essential for the production of biodiesel because they can be harvested more frequently and produce more biomass. Its adaptability to different environments—such as freshwater and wastewater—makes it more alluring since it reduces competition with food crops for resources [3].

Animal fat, leftover cooking oil, and both edible and non-edible vegetable oil are the sources of biodiesel, a monoalkyl ester of long-chain fatty acids. The process of making biodiesel involves interacting oils and alcohol stoichiometrically to produce glycerol and biodiesel (methyl ester). Because it can run diesel engines more reliably than petroleum, regardless of environmental concerns, biodiesel is growing in importance [4]. Among which, the production of biodiesel has grown in popularity as a resource for energy due to a number of factors, including its high biodegradability, renewable nature, lack of toxicity, increased energy security, ability to save foreign exchange, and social benefits for the rural economy [5], Its lower combustion emission profile allows it to be used in current diesel engines with little to no modification and good engine performance, makes it superior than petroleum-based diesel [6], Blending it in any ratio with ordinary diesel fuel can lead to better combustion compared to petroleum-based diesel due to its high cetane number, flash point, and oxygen concentration [7], To reduce the cost of producing biodiesel and food competition, feedstock's utilized in the process must come from non-edible oil containing biomass. More than 300 oil-bearing crops have been identified as possible global sources for the production of biodiesel [8]. Nonetheless, as the cost of raw materials makes up roughly 75% of the entire cost of producing biodiesel, selecting a suitable feedstock is crucial to guaranteeing the production cost of biodiesel [9], When selecting feedstock, factors to take into account include land availability, agricultural methods, greenhouse gas emissions, pesticide injections, soil erosion and fertility, contribution to biodiversity value losses, direct economic value of feedstock's accounting for co-products, water requirement and availability, and feedstock effects on air quality.

The authors studied the different crude extracts of S. longata which is a common filamentous green alga through the GC-MS technique. With the turn of the spectrometer with the carrier gas as helium, the electron impact ionization mode was used. It is also explained there that concerning the molecular ion concentration, the depicted fatty acid sums up in the area of the GC graph; the percentages of the various fatty acids briefly revealed in the sample. Antimicrobial, hydrocarbons, fatty acids, fatty alcohols, phenolic, ketones, terpenes, esters, and esters desirable materials were proved to be existing. Also, they have quantified the phytochemical contents of the filamentous algae through the GC-MS analysis and extraction solvents [10]. The authors have described how to isolate long chain aliphatic alcohol from beeswax using supercritical carbon dioxide in the Methanolysis process. They have also discovered that the beeswax samples have 40% of long chain esters which when put through the transesterification process, which uses also the supercritical carbon dioxide, the beeswax can be converted to fatty acid methyl esters (FAME). He also demonstrated how to fractionate triglycerides and waxes from the corn bran wax [11]. The researchers have discussed on how various insects can be utilised to make biodiesel depending on the amount of fat that the insects possess. He observed that 25 to 30 percent of an insect's dry biomass was lipids; Arophalus sp. containing the highest lipid content at 56.8%. He has analysed the lipid content in the development periods of larvae, perpupa, pupae, nymph and adults of insects belonging to the orders Coleoptera, Hymenoptera and Orthoptera [12]. FTIR, NMR (^1H and ^{13}C), and GCMS were used to describe the biodiesel after conducting chromatographic experiments on the biodiesel derived from sunflower oil. The mass spectrum has been utilized to analyse the chain length and the amount of double bonds present in the different FAMEs that they discussed, including seven saturated, three mono unsaturated, and one poly unsaturated FAME. The NMR results revealed them to be true. Additionally, they have offered the procedure for sunflower oil methanolysis that is alkali catalyzed [13]. The authors adopted both one and two step transesterification to convert rubber seed oil with high FFA to biodiesel. In the first stage, a molar ratio of 6:1 concentration of KOH as a catalyst at a reaction temperature of about 45 °C and a reaction time of 30 minutes gave the highest biodiesel yield. The result obtained depicted that the maximum reduction of Free Fatty Acid content was obtained with 5% H_2SO_4 catalyst. It is therefore clear that biodiesel had relatively high viscosity, slightly lesser calorific value and a rather higher flash point than the diesel [14].

Asokan et al. did analyse the effect of Jatropha methyl ester before and after oxidation. They looked at the analysis based on the activation energy. The results showed that there was a considerable decrease in the size of the carbon smoke particles and a steady decrease in CO emission following oxidation [15]. Mattana et al. analysed the biodiesel blends in the engine considering the emission and performance parameters. The benchmark was verified using both experimental and numerical analysis. Based on calculations and performance, it was determined that the biodiesel blend is perfect for replacing diesel fuel in a conventional engine without modification because the added oxygen content improved combustion and reduced emissions while also increasing brake thermal efficiency at each blend percentage [16]. Venkatesan et al. studied the palm oil biodiesel properties and how they influenced the performance of the diesel engine. Both of the fuels that were studied—palm oil methyl and ethyl ester—performed better in the diesel engine. It was observed that the ethyl ester of palm oil revealed reduced emissions of both nitric oxide and carbon monoxide in comparison. Based on the blend study, POEE10 was recommended as the optimal substitute for diesel engine usage [17]. Hariram et al. studied the parameters of engine such as emission and performance of palm oil biodiesel while adding bio-butanol through carburetion. Notable decrease in CO_2 (5%), NO_x (6%) and particulate matter (24%) emissions were noticed [18]. Paneerselvam et al. analysed the biodiesel produced from Parinari polyandra oil usage in diesel engine. It has been found that the defined fuel's qualities are a lot more similar to diesel fuel. The power and thermal efficiency data indicated that the B10 mix was the best option. In a similar vein, B30 was discovered to be a helpful diesel fuel substitute with steady performance [19]. Thus, it can be said that the biodiesel produced from this resource is a sustainable biofuel. In recent days, modeling and optimization of the production process of biodiesel played a crucial role. To overcome these issues, researchers are implementing various modelling techniques to define the biodiesel production process. The various techniques include artificial neural network (ANN), adaptive neuro-fuzzy logic inference systems (ANFIS), Taguchi grey method, and response surface methodology (RSM) [20–22]. It is relevant to consider various factors such as time, temperature, methanol-to-oil ratio, catalyst concentration, and agitation speed for the higher biodiesel yield. These operating parameters were selected based on the type of reactor utilized for the production of biodiesel.

A machine learning approach such as artificial neural network (ANN) is a technique used to model the transesterification of biodiesel. ANN is used in modelling various transesterification techniques [23,24].

ANN deals the nonlinearity, uncertainty, complexity, and multivariate nature of biodiesel production [25]. The multiple training algorithms developed by using ANN are the benefits over the other mathematical techniques [26]. The convergence of the neural network was enhanced by training algorithm and improved the prediction of the output response.

Dunaliella Salina's high lipid content and capacity to flourish in hypersaline conditions have made it a viable option for the manufacture of biodiesel. Because it can withstand harsh environments like high salinity and nutritional stress, which cause lipid accumulation—a necessary building block for biodiesel—this microalga is especially prized. In contrast to conventional biofuel crops, *Dunaliella Salina* uses saline water to grow in non-arable terrain, protecting freshwater supplies and preventing competition with food crops [27]. *Dunaliella Salina*'s high lipid content—which, under times of stress, can surpass 50% of its dry biomass—makes it a desirable microalga for the generation of biodiesel. Because of its ability to flourish in hypersaline conditions, this plant can be grown in non-arable terrain with salty water, minimizing its negative effects on the environment and lowering competition for freshwater resources. Because of its excellent flexibility and quick growth, it may be produced on a huge scale [28]. Godwin et al. examined the fatty acid composition of Scenedesmus obliquus and Chlorella pyrenoidosa microalgae at different CO_2 concentrations as well as the bio-fixation of CO_2. The study showed that both species changed their lipid profiles in response to CO_2 levels and successfully used CO_2 to increase biomass [29]. A rise in CO_2 concentrations boosted the fatty acid synthesis that is good for biodiesel. The study emphasizes these algae's potential for producing biofuel and sequestering carbon [30]. The synthesis of biodiesel from Nannochloropsis sp., a microalga recognized for its high lipid content, was investigated by Halim et al. [31]. Their study demonstrated that nannochloropsis could be converted into biodiesel with efficiency, and they also outlined ways to maximize the yield of biodiesel by optimizing lipid extraction and transesterification [32]. The high hydrocarbon content of Botryococcus braunii was the subject of Halim et al. investigation into the plant's potential for producing biodiesel. Botryococcus braunii may produce a sizable amount of biodiesel, as the study showed, but it also highlighted difficulties in refining extraction and processing methods [33]. Ekkachai et al. noted the high protein content and lipid output of Spirulina platensis used in the generation of biodiesel. In addition to offering insights into enhancing lipid extraction and biodiesel production efficiency, the study determined that spirulina is a feasible source of biodiesel with significant economic potential [34].

Based on the literature study it can be determined that algal biodiesel will be more environmentally friendly and also biodiesel conversion from algae will be eco-friendly. Hence in this study it was determined to work with algae source for biodiesel conversion. Later the experimental biodiesel conversion was verified with ANN prediction tool and produced fuel shall be characterized.

2. Materials and Methods

The unicellular greenish-orange halophilic microalga *Dunaliella Salina* is frequently found in saline conditions. This species is a member of the Dunaliellaceae family, the Order of Chlamydomonas, the Class Chlorophyceae, and the Division Chlorophyta. *Dunaliella Salina* is an organism of great interest for its possible applications in biotechnology and nutrition because of its remarkable biomass rich in carotenoids and lipids, as well as its well-known capacity to flourish in high salinity environments. The above mentioned algae is the one which has been selected for this study as shown in Figure 1 from which the biodiesel will be extracted, which was procured from National Institute of Ocean Technology, Chennai. When compared to conventional feedstocks through literature study, *Dunaliella Salina* offers a more ecologically benign, economically feasible, and sustainable choice for biodiesel production. It is a better option for biodiesel production in terms of the environment and economy due to its high lipid content, capacity to grow in harsh conditions, minimal resource requirements, and potential for co-products [35].

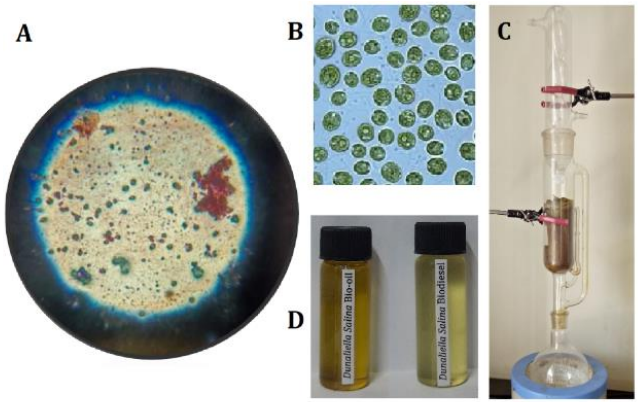

Figure 1. Microscopic View and Bio-oil extraction from *Dunaliella Salina*

2.1. Bio Oil Extraction

Utilizing the ultrasonic bio-oil extraction technique on harvested *Dunaliella Salina* biomass, algal oil was extracted. To increase the extraction efficiency of the ultra-sonication process, 100 g of *Dunaliella Salina* biomass was combined with 30 mL of a mixture consisting of methanol, chloroform, and double distilled water in the ratio of 1:2:4. The membranes of the *Dunaliella Salina* cell wall broke and the algal oil came out as the ultrasonicator was functioning at 60 Hz for 90 minutes. Subsequently, sediments and extracted oil were transferred and placed in a beaker and 10ml of methanol added in order to get them mixed together. Finally, to filter out the algal oil from the unwanted particles, Whatman filter paper was used. To extract out the final traces of H2O in the obtained algal oil, 6 mL of acetone was added and heated. Finally, *Dunaliella Salina* bio-oil was extracted which had 650 mL of oil.

2.2. Biodiesel Conversion

Triglycerides were converted into their mono alkyl methyl esters by a single-stage transesterification process using sodium hydroxide methanol, since the free fatty acid content of the *Dunaliella Salina* bio-oil was determined to be less than 2 percentiles. The transesterification parameters were adjusted to the optimistic levels suggested by the literature, which included a molar ratio of 1:8, a catalyst of 0.6% by weight of NaOH, a reaction period of 180 minutes, and a temperature of 50°C. To create sodium methoxide solution, 1.2 g of sodium hydroxide pellets were first dissolved in 100 mL of methanol solution in a beaker and stirred at 450 rpm for 20 minutes. As previously mentioned, in a single batch base catalyse transesterification process, 40 mL of sodium methoxide solution was mixed with 120 mL of algal oil to keep the molar ratio between algal bio-oil and methanol at 1:8. The entire mixture was put into a conical flask with a flat bottom, and the transesterification reaction was started by keeping it at 50°C for 180 minutes.

After the entire contents was transferred into a separating funnel, a 180-minute cooling interval was allowed. When the bottom and upper layers of the glycerol and algal biodiesel conformed to the transesterification process conclusion, a ring formation was observed. To remove the glycerol, the separating funnel's turning knob was turned on. Algal oil was thus separated. After adding 5 mL of acetone, the resulting algal oil was heated to 60°C for 2 minutes in order to eliminate water molecules and trace. After six iterations of this batch procedure, *Dunaliella Salina* biodiesel with an 93% transesterification efficiency was extracted.

2.3. Physio-chemical properties

The fuels used in the diesel engine was checked for its properties in order to verify with the standard operating values and it detailed closer values as shown in Table 1. In the properties of biodiesel, density was noted as 864 kg/m^3 which was closer to diesel fuel, similarly the viscosity of the fuel was found to be 5.2 mm^2/s which is little higher but was closer to the ASTM standard universally accepted. Calorific value of the biodiesel is 41 MJ/kg, flash point is 115^{o}C.

Table 1. This is the example of table formatting

Properties	*Dunaliella Salina* Biodiesel	Neat diesel	ASTM Biodiesel standards
Density (Kg/m3)	864	838	ASTM D240
Viscosity (at 40° C) (mm^2/s)	5.2	1.9-4.1	ASTM D445
Calorific value (MJ/kg)	41.0	43.8	ASTM D240
Cloud Point (°C)	7	-15 to 5	ASTM D2500
Flash Point (°C)	115	75	ASTM D93

2.4. ANN yield prediction in comparison with Experimental yield

The ability of response surface methodology (RSM) is limited to control the un-controllable factors. Machine learning based artificial neural network (ANN) is a computational method, which is used to predict the results from the available experimental dataset. Total 29 experimental datasets are available for the analysis of ANN and theses dataset are collected by using RSM based Box-Behnken design (BBD) [35]. BBD is used to develop the experimental design for biodiesel yield from *Dunaliella Salina* bio-oil and considered the input parameters such as catalyst concentration, methanol-oil ratio, temperature and reaction time. These input parameters—catalyst concentration, methanol to oil ratio, temperature, and time—have a major effect on the production, quality, and conversion efficiency of biodiesel that is produced by the transesterification process, according to Lam et al. (2010). Achieving high-quality biodiesel production on an industrial scale requires optimizing these characteristics [36]. ANN consists of three layers, i.e., input layer, hidden layer, and output layer. The sigmoidal transfer function (Tansig) is used in the hidden layer and the linear transfer function is utilized in the output layer. MATLAB R2019a was used to develop the ANN model. Serpil et al. [37] state that because artificial neural networks (ANN) are more flexible, accurate predictors, and better at modeling complex nonlinear interactions than traditional statistical techniques, they have been successfully used in biodiesel yield optimization.

The architecture of the developed ANN model is illustrated in Figure 2. The developed architecture of ANN model consists of 4 input parameters (Catalyst concentration, Methanol to oil ratio, Temperature, Time) in the input layer and one output response in the output layer [38, 39]. The developed model describes the number of neurons presented in the both input and output layer depends on the number of input parameters, output responses and number of neurons in the hidden layers respectively (Eq. (1)).

Table 2. Experimental design with various input parameters and output responses

Run	Catalyst conc.	Methanol to oil ratio	Temp (°C)	Time	Exp. Yield (%)	Pred. Yield (%)	% Error
1	1.5	8	50	2	93.82	93.61	0.22884
2	1	8	50	3	93.99	93.91	0.09016
3	1	8	40	2	86.95	86.85	0.11943
4	1	7	40	1	85.62	85.59	0.03244
5	1	7	50	2	92.09	91.99	0.10502
6	1	8	60	2	90.51	89.95	0.62099
7	1	7	50	2	92.09	91.99	0.10502
8	1	8	50	1	90.84	90.03	0.88991
9	0.5	7	60	2	83.41	83.11	0.36465
10	1	7	40	3	86.09	86.03	0.06796
11	0.5	7	50	3	89.03	89.02	0.00971
12	1	7	50	2	92.09	91.99	0.10502
13	1.5	6	50	2	91.89	89.88	2.18937
14	1.5	7	50	1	92.01	92.01	0.00435
15	1	7	50	2	92.09	91.99	0.10502
16	1	7	50	2	92.09	91.99	0.10502
17	1	7	60	1	89.37	88.88	0.54371
18	1	6	50	1	88.92	88.58	0.38698
19	1.5	7	60	2	92.95	92.51	0.47346
20	1	7	60	3	82.5	82.50	0.00000
21	1.5	7	50	3	92.74	91.37	1.47577
22	0.5	7	40	2	84.07	84.06	0.01570
23	0.5	8	50	2	89.18	88.14	1.16894
24	1	6	40	2	83.33	83.18	0.18336
25	0.5	7	50	1	88.28	87.34	1.06661
26	1	6	60	2	87	86.71	0.33661
27	1.5	7	40	2	87.92	87.45	0.53094
28	0.5	6	50	2	87.16	86.96	0.22479
29	1	6	50	3	89.57	89.31	0.28604

The number of neurons in the hidden layer were estimated based on their performance. The outperformed number of neurons has been selected and based on the selection of neuron numbers the developed network was applied. The error values are estimated by utilizing Eqs. (2-4) and considering these values for the prediction of the output responses. The experimental data set were trained by using trainlm algorithm [40].

$$2\left(n_{\frac{i}{p}} + n_{\frac{o}{p}}\right) \leq n_h \leq \left(n_a\left(n_{\frac{i}{p}} + n_{\frac{o}{p}}\right) - n_{\frac{o}{p}}\right)/\left(n_{\frac{i}{p}} + n_{\frac{o}{p}} + 1\right) \quad (1)$$

$$\text{Mean Absolute Error} = \sum_{i=1}^{n_3}[[X_i - \overline{X_i}] / n_a \quad (2)$$

$$\text{Mean Absolute Percentage Error} = \frac{100}{n_a}\sum_{i=1}^{n_a}\{(X_i - \overline{X_i} / X_i)\} \quad (3)$$

$$\text{Root Mean Square Error} = \sqrt{\sum_{i=1}^{n_a}\{(X_i - X_i)^2\} / n_a} \quad (4)$$

where, ni/p – number of neurons in the input layer, no/p – number of neurons in the output layer, nh – number of neurons in the hidden layer, na – total experimental data.

The mean absolute error (MAE), mean absolute percentage error (MAPE) and root mean square error (RMSE) were calculated based on the equations obtained from Rocabruno et al. [41] and Rauf et al. [42]. The total 29 experimental data set were obtained from BBD, at which 21 data set were utilized for training (73 %), 4 data set (13 %) for testing and remaining 4 data set for validation purpose. The testing and validation data set was provided by the performance of the algorithm based on the independent evaluation and data set not affected during or after the training.

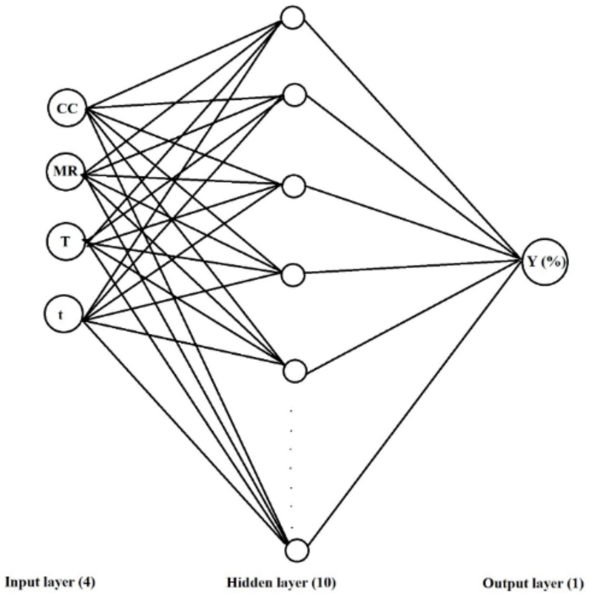

Figure 2. Architecture of the developed ANN model

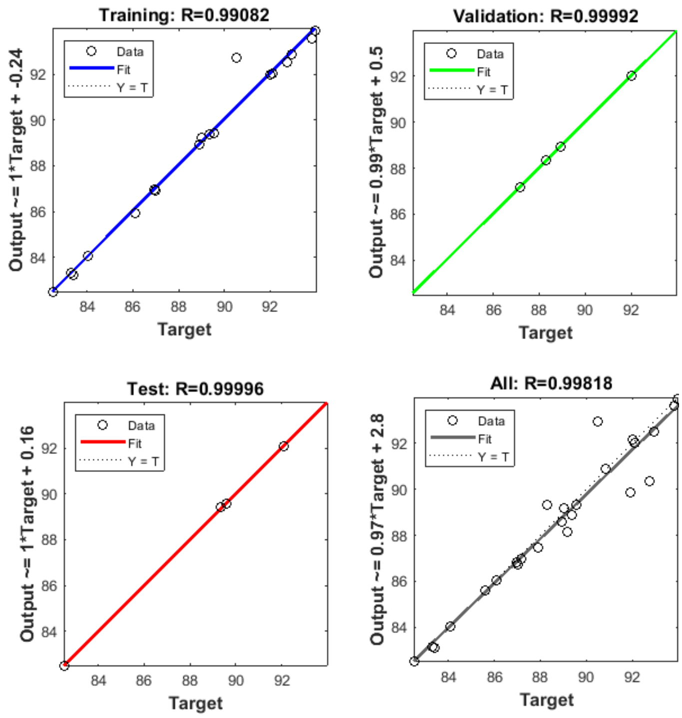

Figure 3. ANN modelling for Biodiesel Yield from *Dunaliella Salina*

2.5. Modelling by ANN

Figure 3 shows the training data, test data, validation data, and all data for the developed ANN model. The trainlm algorithm was utilized to develop the ANN model and developed model was used to predict the biodiesel yield. The predicted results of trainlm algorithm was compared with the experimental dataset and indicated the comparison between weight and transfer function of developed model. The coefficient of correlation (R^2) was measured by the input variables and output response. The optimal ANN model was computed by the minimal error value [43]. The training, validation, and testing of R2 was utilized to estimate the performance of the protocol of developed ANN model. Initially, 10 neurons were selected in the hidden layer to perform the optimization of the trainlm algorithm. The dataset utilized in the training (70 %) exposed that R2 value of trainlm which detailed better result.

3. Results and Discussion

3.1. Biodiesel Conversion Optimization with ANN prediction

The oil extracted from the algae *Dunaliella Salina* was converted into biodiesel using single stage transesterification process as detailed in the previous section. The conversion process was verified by optimizing the parameters that were used in the transesterification. The parameters that had the influence in conversion were the catalyst used, methanol to oil ratio, temperature during the process and the time duration of the process. While performing the transesterification in order to optimize the conversion by the influence of these parameters the following changes were experimented. The methanol to oil ratio widely practiced in biodiesel conversion are 1:8, 1:7 and 1:6 which was also taken into consideration for this experimental analysis, the catalyst concentration used in this study in terms of weight are 0.5%, 1% and 1.5%, the temperature used for the study are 40oC, 50oC and 60oC and the time duration in terms of 1 hr, 2 hr and 3hr.

The various operating parameters considered are detailed in Table 2, the methanol to oil ratio 1:8, catalyst concentration 1% wt, temperature 50oC, time duration 3 hr and the best output response of biodiesel yield is 93.99 %. Similarly, the methanol to oil ratio 1:7, catalyst concentration 1.5% wt, temperature 60 0C, time duration 2 hr and the output response is 92.95 %. Considering the next methanol to oil ratio 1:6, catalyst concentration 1.5% wt, temperature 50 0C, time duration 2 hr and the output response is 91.89 %. All the experimental result was taken into the ANN prediction and the predicted yield value is set to be a benchmark for the biodiesel conversion. The error values were observed as mean absolute error 0.735, mean absolute percentage error 0.819, and root mean square error 1.017. The maximum biodiesel production of 93.99% has been estimated by ANN method with the help of operating parameters such as temperature 50 0C, time 3 hr, catalyst content 1% wt, and 1:8 methanol to oil ratio.

3.2. Gas Chromatography - Mass Spectrometer (GCMS)

Bio-oil produced from the marine algae *Dunaliella Salina*. was converted into biodiesel through a one-stage base-catalyzed transesterification method with sodium hydroxide/methanol. A biodiesel sample was esterified in order to ascertain the presence of diverse FAMEs and to highlight the degree of efficiency by the transesterification procedure and hence analyzed by GC-MS. In the biodiesel mass chromatogram nine different FAMEs were identified within RT between 19. 93 to 30. 48 minutes. As a result of the analysis performed to obtain the structure of fatty acid methyl ester, different fragmentation patterns were obtained, all of which had a base peak at m/z 74. The obtained mass chromatogram and the fragmentation patterns of the esterified biodiesel and the algal bio-oil demonstrated the occurrence of the McLafferty rearrangement process. Some common mass fragmentation patterns of the FAME are the carbo-methoxy ion loss resulting from β cleavage as shown in Figure 4. Several of the mass fragmentation patterns exhibited multiple peaks: These multiple peaks may be based on the fact that the methoxy group has been ejected out and the carbon and hydrogen atoms redistributed through the process of transesterification. Consequently, olefins containing, for instance, 14,17-Octadecadienoic acid methyl ester are found at RT 22. 36 minutes due to this hydrogen ion redeployment and organization within the carbonyl group. The different fatty acid methyl ester that presented the biodiesel sample were identified as follows: Retention time 19. 93 for 9-hexadecenoic acid methyl ester, Retention time 20. 27 for hexadecanoic acid methyl ester, retention time 21.79 (heptadecanoic acid methyl ester), RT 22.36 (14, 17-octadecadienoic acid methyl ester), RT 24.79 (eicosanoic acid methyl ester), RT 26.87 (13-docosenoic acid methyl ester), RT 27.19 (docosanoic acid methyl ester), RT 28.49 (tricosanoic acid methyl ester), and RT 30.48 (tetracosanoic acid methyl ester).

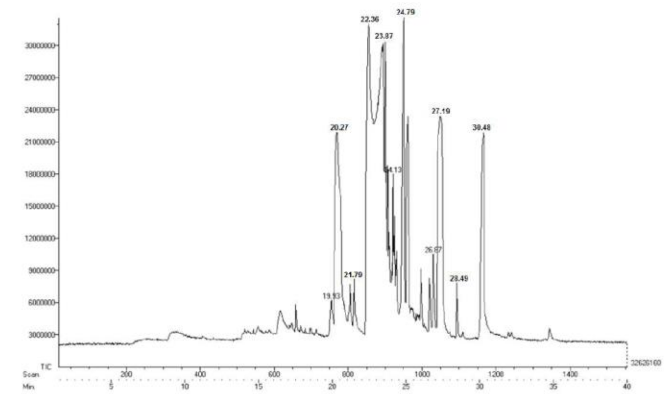

Figure 4. GCMS Chromatogram of *Dunaliella Salina* Biodiesel

3.3. Fourier Transform Infrared Spectrometer (FTIR)

Dunaliella Salina biodiesel details the FTIR spectra vibration between 547 to 3008cm^{-1} as shown in Figure 5. Conversion of oil to biodiesel is briefly signaled in the vibration stretch 1741 cm^{-1}. A long hydrocarbon chain was noted between 1019 cm-1 and 1460 cm-1, which is a group bending-stretching vibration. It was noticed that there was presence of carboxylic group at various stretches like 844 cm^{-1}, 914 cm-1, and 1437 cm^{-1}. From the FTIR spectra result it was confirmed that the complete conversion of the bio-oil and its FAMEs was detailed through the strong stretches at 2853 cm^{-1} and 2923 cm^{-1} and the weak stretch like 3008 cm^{-1} also confirms the same.

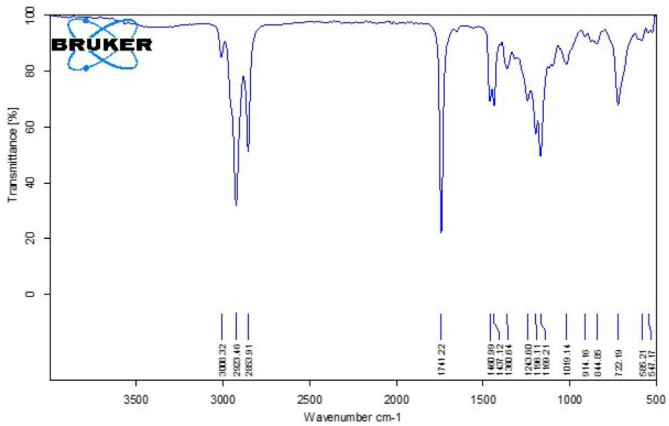

Figure 5. FTIR Transmittance of *Dunaliella Salina* biodiesel

3.3.1. Nuclear Magnetic Resonance (NMR)

NMR investigations offer comprehensive insights into the chemical composition, purity, and efficiency of the transesterification process, they are crucial to the manufacture of algae-based biodiesel. They are essential instruments for maximizing the manufacturing process and guaranteeing that the biodiesel produced is of the highest calibre and complies with legal requirements [41]. *Dunaliella Salina* algal biodiesel was dissolved using 0.8 ml of methanol in order to create a 1H NMR solution. The sample was injected inside the equipment with pulse 90 degrees and relaxation delay of 10 seconds. It was noted that the primary components in the biodiesel were fatty acid esters, it was also noted that there were traces of steroids, alkaloids and alkanes. It can be noted from the Figure 6 that fatty acid ester is the peak observed at 3.669 ppm in the 1H NMR spectrum of *Dunaliella Salina* fuel sample. Carbonyl functional group was noted at 5.387 ppm with a strong signal. The peaks at 3.330 ppm to 2.108 ppm range shows the long unsaturated hydrocarbon chains, OCH (at 5.397 ppm) and OCH_2 ester groups (at 5.408 ppm),due to the conversion of triglyceride to mono-alkyl fatty acid ester. Alkenes were seen as a faint doublet peak at 3.336 ppm. Presence of polyunsaturated fatty acids in the sample was noted due to the triplet peak at 1.377 ppm.

13C NMR analysis was done using deuterated methanol as a solvent, much like the sample injection proton NMR method. The peaks of monoglycerides, diglycerides, triglycerides, and epoxy ester were seen ranging between 0 ppm and 200 ppm as shown in the Figure 7. A singlet peak was seen at 47.967 ppm (ie., mono-alkyl long-chain hydrocarbon). A cluster peak ranging from 127.681 ppm to 129.557 ppm explains the presence of unsaturated esters with carbonyl groups. Transesterification occurrence was confirmed by the presence of a terminal peak at 174.417 ppm. Group cluster of peaks between 26.814 ppm and 33.444 ppm (represented the carboxylic group) and 16. 442 ppm peak includes the existence of monoglycerides.

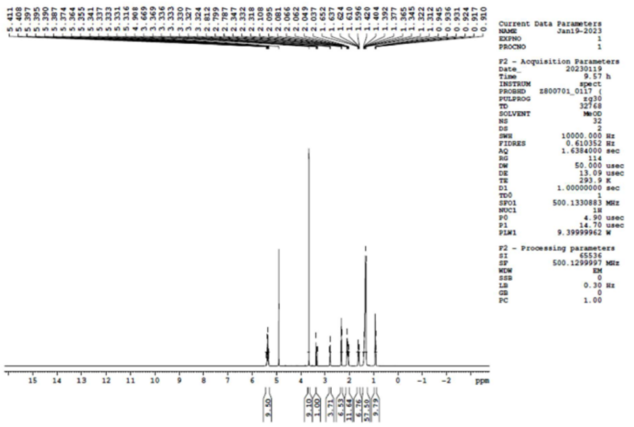

Figure 6. ^{1}H NMR Spectrum of *Dunaliella Salina* biodiesel

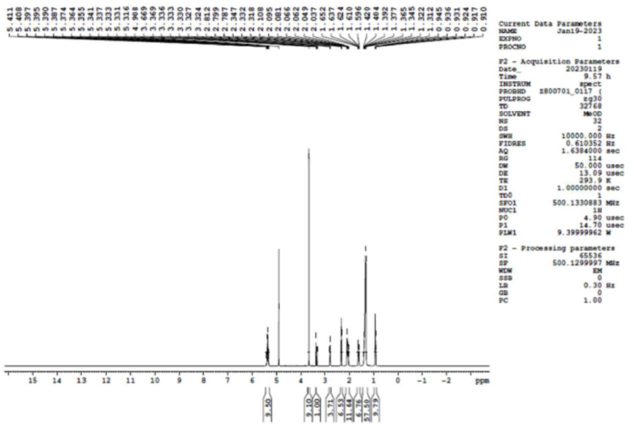

Figure 7. ^{13}C NMR Spectrum *Dunaliella Salina* biodiesel

4. Conclusion

The *Dunaliella Salina* microalgae was used as the resource for making biodiesel. Through the process the following conclusions were made,

- By means of using ultra-sonication assisted bio-oil extracting method 650 mL of *Dunaliella Salina* bio-oil was extracted in the ratios 1:2:0.4 of methanol, chloroform and double distilled water with ultra-sonication at 60 Hz for 90 minutes.
- Among the various influencing parameters considered for optimization for biodiesel conversion, the parameters which gave the optimal biodiesel yield was the methanol to oil ratio 1:8 with the catalyst concentration 1% weight, temperature 50^{o}C and time duration 3 hours. The biodiesel conversion efficiency was noted to be 93.99% using single stage transesterification process which utilized a 1:8 molar ratio, 0.6% by weight of NaOH, 50°C reaction temperature, and 180 min reaction time as the parameters.
- The Experimental Biodiesel yield was verified with ANN prediction tool using trainlm algorithm which predicted the similar yield of 93.91% which had only the error value of up to 0.09016%.
- Fourier Transform Infrared transmittance detailed the conversion of bio-oil to Fatty acid methyl ester through the stretching vibrations between 1741 cm^{-1} and 2853 cm^{-1}.
- NMR results confirms the fatty acid esters presence through peaks 3.669 ppm (^{1}H NMR), and the transesterification reaction through peaks 127.681 ppm and 129.557 ppm (^{13}C NMR)

Based on the aforementioned qualitative results, it can be inferred that *Dunaliella Salina*-derived biodiesel has the potential to be a viable alternative to petro-diesel feedstock.

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Author contributions

Hariram V: Conceptualization, Methodology and Writing. **Godwin John J**: Software. **Saravanan A**: Data curation. **Sangeeth Kumar E**: Writing-Original draft preparation. **Vinoth Kumar M**: Visualization and Editing. **Ramanathan V**: Investigation. **Balachandar M**: Investigation. **Baskar S**: Software, Validation.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Murugesan, P., Hoang, A. T., Venkatesan, E. P., Kumar, D. S., Balasubramanian, D., & Le, A. T. (2022). Role of hydrogen in improving performance and emission characteristics of homogeneous charge compression ignition engine fueled with graphite oxide nanoparticle-added microalgae biodiesel/diesel blends. International Journal of Hydrogen Energy, 47 (88), 37617–34. https://doi.org/10.1016/J.IJHYDENE.2021.08.107
2. Venkatesan, H., Sivamani, S., Bhutoria, K., & Vora, H. H. (2019). Assessment of waste plastic oil blends in performance, combustion and emission parameters in direct injection compression ignition engine. International Journal of Ambient Energy, 40(2), 170–8. https://doi.org/10.1080/01430750.2017.1381155
3. Chitradevi, V., & Balu Pandian. (2024). An experimental study on performance, emissions, and combustion characteristics of a CI engine running on Citrullus Colocynthis biodiesel blends. Journal of Thermal Engineering, 10(4), 954–960.
4. Ogunkunle, O., & Ahmed, N. A. (2019). A review of global current scenario of biodiesel adoption and combustion in vehicular diesel engines, Energy Reports, 5, 1560–1579. https://doi.org/10.1016/J.EGYR.2019.10.028
5. Demirbas, A. (2009). Political, economic and environmental impacts of biofuels: A review. Applied Energy, 86, (1) 108–117. https://doi.org/10.1016/J.APENERGY.2009.04.036

6. Okcu, G. D. (2022). Microalgae biodiesel production: a solution to increasing energy demands in Turkey. Biofuels, 13(1), 77–93. https://doi.org/10.1080/17597269.2019.1637070
7. Balat, M. (2011). Potential alternatives to edible oils for biodiesel production - A review of current work. Energy Conversition Management, 52 (2), 1479–1492. https://doi.org/10.1016/J.ENCONMAN.2010.10.011
8. Atabani, E., Silitonga, A. S., Badruddin, I. A., Mahlia. T. M. I., Masjuki, H. H., & Mekhilef, S. (2012). A comprehensive review on biodiesel as an alternative energy resource and its characteristics, Renewable and Sustainable Energy Reviews, 16 (4), 2070–2093. https://doi.org/10.1016/J.RSER.2012.01.003
9. Ahmad, L., Yasin, N. H. M., Derek, C. J. C., & Lim, J. K. (2011). Microalgae as a sustainable energy source for biodiesel production: A review. Renewable and Sustainable Energy Reviews. 15(1), 584–593, 2011. https://doi.org/10.1016/J.RSER.2010.09.018
10. Abdel-Aal, E. I., Haroon, A. M., & Mofeed, J. (2015). Successive solvent extraction and GC–MS analysis for the evaluation of the phytochemical constituents of the filamentous green alga Spirogyra longata, Egypt. J. Aquatic Res, 41 (3), 233–246. https://doi.org/10.1016/J.EJAR.2015.06.001
11. Jackson, M. A., & Eller, F. J. (2006). Isolation of long-chain aliphatic alcohols from beeswax using lipase-catalyzed methanolysis in supercritical carbon dioxide, Journal of Supercritical Fluids 37 (2), 173–177. https://doi.org/10.1016/J.SUPFLU.2005.08.008
12. Manzano-Agugliaro, F., Sanchez-Muros, M. J., Barroso, F. G., Martı´nez-Sa´nchez, A., Rojo, S., & Pe´rez-Ban˜o´n, C. (2012), Insects for biodiesel production, Renewable and Sustainable Energy Reviews, 16 (6), 3744–3753. https://doi.org/10.1016/J.RSER.2012.03.017
13. Naureen, R., Tariq, M., Yusoff, I., Chowdhury, A. J. K., & Ashraf, M. A. (2015) Synthesis, spectroscopic and chromatographic studies of sunflower oil biodiesel using optimized base catalyzed methanolysis. Saudi Journal of Biological Science, 22 (3), 332–339. https://doi.org/10.1016/J.SJBS.2014.11.017
14. Ramadhas, A. S., Jayaraj, S., & Muraleedharan, C. (2006). Theoretical modeling and experimental studies on biodiesel-fueled engine, Renewable Energy, 31 (11), 1813–1826. https://doi.org/10.1016/J.RENENE.2005.09.011
15. Asokan, M. A., Senthur Prabu, S., Shikhar Kamesh., & Wasiuddin Khan. (2018). Performance, combustion and emission characteristics of diesel engine fueled with papaya and watermelon seed oil bio-diesel/diesel blends. Energy, 145, 238-245. https://doi.org/10.1016/J.ENERGY.2017.12.140
16. Mattana Santasnachok., Charoen Chinwanitcharoen., Wirogana Ruengphrathuengsuka., & Ekkachai Sutheerasak. (2023), Diesel-engine generator tests fueled with ethyl and methyl esters of palm oil as catalyzed by potassium hydroxide, Energy Reports, 9(10), 48-55. https://doi.org/10.1016/J.EGYR.2023.05.061.
17. Venkatesan, H., Sivamani, S., Premkumar, & T., Tharun, P. (2017). Reduction of Exhaust Emissions Using a Nanometallic Enriched Lemongrass Biodiesel Blend. Energy Sources, Part A: Recovery, Utilization and Environmental Effects, 39 (21), 2065–2071. https://doi.org/10.1080/15567036.2017.139549
18. Hariram, V., Saravanan, A., Nadanakumar, V., Vinoth Kumar, M., Balachandar, M., John Godwin, J., Seralathan, S., & Vasudev, K. L. (2022). Optimized Grapeseed Biodiesel Production and its Effect on the CI engines Combustion Characteristics at Variable Compression Ratios. International Journal of Vehicle Structures & Systems, 14(2), 242-256. https://DOI.ORG/10.4273/IJVSS.14.2.19
19. Panneerselvam, N., Murugesan, A., Vijayakumar, C & Subramaniam, D. (2016). Optimization of biodiesel produced from watermelon (*Citrullus vulgaris*) using batch-type production unit. Energy sources, Part A: Recovery, Utilization, and Environmental Effects, 38 (16), 2343–2348. https://doi.org/10.1080/15567036.2015.1048389
20. Betiku, E., Osunleke, A., Odude, V., Bamimore, A., Oladipo, B., Okeleye, A., & Ishola, N. (2021) Performance evaluation of adaptive neuro-fuzzy inference system, artificial neural network and response surface methodology in modeling biodiesel synthesis from palm kernel oil by transesterification, Biofuels, 12 (3), 339–354. https://doi.org/10.1080/17597269.2018.1472980
21. Balaji Dhanapal., Hariram Venkatesan., & Balachandar Moorthy. (2024). Synergetic effect of hydrogen supplementation with waste cooking oil biodiesel – An assessment of the emission, combustion and performance parameters of a compression ignition engine. International Journal of Hydrogen Energy, 49 (B), 1292–1302. https://doi.org/10.1016/j.ijhydene.2023.11.052
22. Betiku, E., Omilakin, O. R., Ajala, S. O., Okeleye, A. A., Taiwo, A. E., & Solomon, B. O. (2014) Mathematical modeling and process parameters optimization studies by artificial neural network and response surface methodology: A case of non-edible neem (Azadirachta indica) seed oil biodiesel synthesis, Energy, 72, 266–273. https://doi.org/10.1016/J.ENERGY.2014.05.033
23. Stamenković, O. S., Rajković, K., Veličković, A. V., Milić, P. S., & Veljković, V. B. (2013), Optimization of base-catalyzed ethanolysis of sunflower oil by regression and artificial neural network models, Fuel Processing Technlology, 114, 101–108. https://doi.org/10.1016/J.FUPROC.2013.03.038
24. Tu, J. V. (1996). Advantages and disadvantages of using artificial neural networks versus logistic regression for predicting medical outcomes, J. Clin. Epidemiol, 49 (11), 1225–1231. https://doi.org/10.1016/S0895-4356(96)00002-9
25. Lam, M. K., & Lee, K. T. (2012). Microalgae biofuels: A critical review of issues, problems and the way forward. Biotechnology Advances, 30(3), 673-690.

https://doi.org/10.1016/J.BIOTECHADV.2011.11.008

26. Lam, M. K., Lee, K. T., & Mohamed, A. R. (2010). Homogeneous, heterogeneous and enzymatic catalysis for transesterification of high free fatty acid oil (waste cooking oil) to biodiesel: A review. Biotechnology Advances, 28(4), 500-518. https://doi.org/10.1016/J.BIOTECHADV.2010.03.002
27. Tang, D., Han, W., Li, P., Miao, X., & Zhong, J. (2011). CO2 biofixation and fatty acid composition of Scenedesmus obliquus and Chlorella pyrenoidosa in response to different CO_2 levels. Bioresource Technology, 102 (3), 3071-3076. DOI: 10.1016/J.BIORTECH.2010.10.047. https://doi.org/10.1016/j.biortech.2010.10.047
28. Talebian-Kiakalaieh, A., Amin, N. A. S., & Mazaheri, H. (2013). A review on novel processes of biodiesel production from waste cooking oil. Applied Energy, 104, 683-710. https://doi.org/10.1016/J.APENERGY.2012.11.061
29. Godwin John, J., Hariram, V., & Seralathan, S. (2018). Emission reduction using improved fuel properties of algal oil biodiesel and its blends. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects, 40 (1)1, 45-53. https://doi.org/10.1080/15567036.2017.1405108
30. Lee, J. H., Lee, T. H., & Park, J. M. (2010). Biodiesel production from Nannochloropsis microalgae: A review. Biotechnology Advances, 28(5), 526-535. https://doi.org/10.1016/J.BIOMBIOE.2024.107240
31. Halim, R., Danquah, M. K., & Webley, P. A. (2012). Oil extraction from microalgae for biodiesel production. Bioresource Technology, 102(1), 178-185. https://doi.org/10.1016/J.BIORTECH.2010.06.136
32. Amin, N. A. S., & Lee, K. T. (2009). Biodiesel production from microalgae as renewable energy: A review. Renewable and Sustainable Energy Reviews, 13(3), 683-693. https://doi.org/10.1016/J.FUEL.2024.131547
33. Turan., Mesci, N. G., & Ozgonenel, B. (2011). Artificial neural network (ANN) approach for modeling Zn(II) adsorption from leachate using a new biosorbent. Chemical Engineering Journal, 173, 98–105. https://doi.org/10.1016/J.CEJ.2011.07.042
34. Ekkachai Sutheerasak., Worachest Pirompugd., & Sathaporn Chuepeng. (2023). The performance and emission of a generator-diesel engine fueled with palm oil methyl ester combined with carbureting biobutanol, Energy Reports, 9(3), 210-218. https://doi.org/10.1016/J.EGYR.2022.12.109
35. Oyetola Ogunkunle., & Noor A, Ahmed. (2020), Exhaust emissions and engine performance analysis of a marine diesel engine fuelledwith Parinari polyandra biodiesel–diesel blends, Energy Reports, 6: 2999-3007. https://doi.org/10.1016/j.egyr.2020.10.070
36. Latchubugata, C. S., Kondapaneni, R. V., Patluri, K. K., Virendra, U., & Vedantam, S. (2018). Kinetics and optimization studies using Response Surface Methodology in biodiesel production using heterogeneous catalyst, Chemical Engineering Research and Design, 135, 129–139. https://doi.org/10.1016/J.CHERD.2018.05.022
37. Serpil Savcı. (2017), Treatment of Biodiesel Wastewater Using Yellow Mustard Seeds, Turkish Journal of Engineering, 1(1), 11 - 17, https://doi.org/10.31127/TUJE.315927
38. Rocabruno-Vald´es, C.I., Ramírez-Verduzco, L.F., & Hern´andez, J.A. (2015). Artificial neural network models to predict density, dynamic viscosity, and cetane number of biodiesel. Fuel, 147, 9–17. https://doi.org/10.1016/J.FUEL.2015.01.024
39. Gul Muhammad., Ange Douglas Potchamyou Ngatcha., Yongkun Lv., Wenlong Xiong., Yaser A. El-Badry., Eylem Asmatulu., Jingliang Xu., & Md Asraful Alam. (2021). Enhanced biodiesel production from wet microalgae biomass optimized via response surface methodology and artificial neural network, Renewable Energy, 184, 753-764. https://doi.org/10.1016/J.RENENE.2021.11.091
40. Mehmet Çağatay., & Suleyman Karacan. (2022), Multivariable generalized predictive control of reactive distillation column process for biodiesel production, Turkish Journal of Engineering, 6 (1), 40 – 53. https://doi.org/10.31127/TUJE.801441
41. Venkatesan, H., Sivamani, S., Bhutoria, K., & Vora, H. H. (2019). Assessment of Waste Plastic Oil Blends in Performance, Combustion and Emission Parameters in Direct Injection Compression Ignition Engine. International Journal of Ambient Energy, 40 (2): 170–178. https://doi.org/10.1080/01430750.2017.1381155
42. Alam, F., Mobin, S., & Chowdhury, H. (2015). Third generation biofuel from algae. Procedia Engineering, 105, 763-768. https://doi.org/10.1016/j.proeng.2015.05.068
43. Gouveia, L., & Oliveira, A. C. (2009). Microalgae as a raw material for biofuels production. Journal of Industrial Microbiology and Biotechnology, 36(2), 269-274. https://doi.org/10.1007/s10295-008-0495-6