catalysts

Article

Response Surface Methodology Approach for Optimized Biodiesel Production from Waste Chicken Fat Oil

Fatima Shafiq [1], Muhammad Waseem Mumtaz [1,*], Hamid Mukhtar [2], Tooba Touqeer [1], Syed Ali Raza [3], Umer Rashid [4,*], Imededdine Arbi Nehdi [5,6] and Thomas Shean Yaw Choong [7]

[1] Department of Chemistry, University of Gujrat, Gujrat 50700, Pakistan; fatimashafiq369@gmail.com (F.S.); tuba.toqir@gmail.com (T.T.)
[2] Institute of Industrial Biotechnology, Government College University, Lahore 54000, Pakistan; hamidwaseer@yahoo.com
[3] Department of Chemistry, Government College University, Lahore 54000, Pakistan; chemstone@yahoo.com
[4] Institute of Advanced Technology, Universiti Putra Malaysia, UPM Serdang, Selangor 43400, Malaysia
[5] Chemistry Department, College of Science, King Saud University, Riyadh 1145, Saudi Arabia; inahdi@ksu.edu.sa
[6] Laboratoire de Recherche LR18ES08, Chemistry Department, Science College, Tunis El Manar University, Tunis 2092, Tunisia
[7] Department of Chemical and Environmental Engineering, Universiti Putra Malaysia, UPM Serdang, Selangor 43400, Malaysia; csthomas@upm.edu.my
* Correspondence: muhammad.waseem@uog.edu.pk (M.W.M.); umer.rashid@upm.edu.my (U.R.); Tel.: +60-3-9769-7393 (U.R.)

Received: 1 February 2020; Accepted: 26 February 2020; Published: 6 June 2020

Abstract: Biodiesel is gaining acceptance as an alternative fuel in a scenario where fossil fuel reserves are being depleted rapidly. Therefore, it is considered as the fuel of the future due to its sustainability, renewable nature and environment friendly attributes. The optimal yield of biodiesel from cheap feed stock oils is a challenge to add cost effectiveness without compromising the fuel quality. In the current experiment, waste chicken fat oil was taken as the feedstock oil to produce biodiesel through the chemical and enzymatic route of transesterification. The process of chemical transesterification was performed using KOH and sodium methoxide, while enzymatic transesterification was done by using free *Aspergillus terreus* lipase and *Aspergillus terreus* lipase immobilized on functionalized Fe_3O_4 nanoparticles (Fe_3O_4_PDA_Lipase) as biocatalysts. The physico-chemical properties of the understudy feedstock oil were analyzed to check the feasibility as a feedstock for the biodiesel synthesis. The feedstock oil was found suitable for biodiesel production based upon quality assessment. Optimization of various reaction parameters (the temperature and time of reaction, catalyst concentration and methanol-to-oil mole ratio) was performed based on the response surface methodology (RSM). The maximum yield of biodiesel (90.6%) was obtained from waste chicken fat oil by using Fe_3O_4_PDA_Lipase as an immobilized nano-biocatalyst. Moreover, the above said optimum yield was obtained when transesterification was done using 6% Fe_3O_4_PDA_Lipase with a methanol-to-oil ratio of 6:1 at 42 °C for 36 h. Biodiesel production was monitored by FTIR spectroscopic analysis, whereas compositional profiling was done by GC–MS. The measured fuel properties—cloud point, pour point, flash point, fire point and kinematic viscosity—met the biodiesel specifications by American Society for Testing and Materials (ASTM).

Keywords: biodiesel; transesterification; immobilized lipase; RSM; fuel properties

1. Introduction

The rapid industrial growth and population explosion have built an immense pressure on natural resources, including fossil fuels. The whole world is determined to find suitable solutions in context with the forthcoming energy crisis. The world is in search of alternate sources of fuel to reduce its dependency on conventional fuels. Biodiesel has emerged as a promising alternative fuel in recent years due to its renewable nature and environment friendly attributes. Biodiesel may be characterized as alkyl esters of fatty acids and may be utilized easily in diesel engines without major alterations [1]. The emissions of CO and NOx from diesel burning are issues of keen interest as both are greenhouse gases and responsible for tropospheric ozone formation. It is an established fact based on the work of many researchers that, comparative to conventional diesel, combustion of biodiesel produces less CO and unburnt hydrocarbons but higher NOx emissions, probably due to a higher oxygen content in biodiesel [2,3].

Initially, synthesis of biodiesel was extensively carried out using vegetable oils and seed oils of non-edible origin. Usually, the production of biodiesel from edible oils is not cost-effective and these vegetable oils are used in food, hence are valuable. To avoid the problems associated with cost, edibility and food shortage, biodiesel production from non-edible fractions of food and related wastes is gaining sound gravity. Similarly, the biodiesel preparation from non-edible seeds like *Jatropha* is not completely feasible as the cultivation of non-edible seed oil plants may create competition with edible crops on shrinking fertile agriculture land [4]. Recently many studies were carried out for biodiesel synthesis from low cost vegetal and animal-based feed stocks like waste cooking oils and animal fats by reducing their viscosity through the transesterification process [5]. The chemical and enzymatic transesterification processes are adopted to convert fatty acids of feedstock into their alkyl esters. Both transesterification modes have their own modalities and advantages but may be optimized for high quality biodiesel [6].

Chicken fat is a poultry waste that can be used to produce biodiesel. The fat content in chicken is about 10% by weight, which is very high, and its cost is low. Commercial broiler chicken meat was reported to have relatively high contents of polyunsaturated lipids as compared with organic chicken [7]. Researchers have reported that chicken fat constitute about 25% to 35% saturated and 40% to 75% unsaturated fatty acids. Palmitic acid, along with stearic acid, linoleic acid and oleic acid, are major fatty acids in chicken fat [8,9]. The fats can be converted into alkyl esters by the process of transesterification. In an alkali-catalyzed transesterification reaction, both the glyceride and alcohol should be extensively free of water contents as the water compels the reaction to partly change into a saponification reaction, resulting in soap formation [6]. Sodium hydroxide and potassium hydroxide are commonly used as alkali catalysts, but they result in water formation during transesterification, that is why sodium and potassium methoxides are preferred for biodiesel production. Alkali catalysts are good especially for those feed stocks that contain minimal acid value. However, if the acid value of the feed stock is high, then it is recommended to perform pre-treatment acid esterification to reduce the free fatty acid contents before performing base-catalyzed transesterification of the feedstock [10].

On the other hand, enzyme-catalyzed transesterification is gaining acceptance and is considered technically comparable to alkali transesterification. This method normally employs lipase as a catalyst. Lipase-catalyzed transesterification of feedstock oils with a relatively higher free fatty acid content can be carried out without performing any pre-treatment acid esterification step that is normally required in case of alkaline transesterification [11]. However enzymatic transesterification is a high-cost process, because enzymes can be denatured easily in the presence of short-chain alcohols and it is difficult to recover [12]. To cope with these problems, enzymes are immobilized on various supports to enhance their durability. Immobilized enzymes are adoptable to harsh conditions as compared to the free enzymes and are easy to recover. Immobilization of enzymes on the matrix and beads may reduce the enzyme activity by blocking its active site and lowering the mass transfer. However, due to very small size and Brownian movement of nanoparticles, these are a potent choice for enzyme immobilization [13]. There are few reports on enzymatic transesterification of chicken fat oil [14,15].

In the present work the transesterification process was optimized to synthesize biodiesel from a cheaper source in the form of waste chicken fat. The relative effects of various catalysts and their concentration were studied and optimized for improved yields of biodiesel by involving the methanol-to-oil ratio along with reaction time and temperature. The synthesized biodiesel was also analyzed for fuel properties to check its feasibility for use in a compression ignition (CI) engine.

2. Results and Discussion

2.1. Physico-Chemical Characterization of Waste Chicken Fat Oil (WCFO)

The pre-analysis tests of WCFO revealed that the acid value of the oil was 6.56 ± 0.05 mg KOH/g, saponification value 200 ± 7.50 mg KOH/g, refractive index 1.46 ± 0.01, density 0.85 ± 0.07 g/cm^3, iodine number 75 ± 10.70 g iodine/100 g and the peroxide value was computed as (5.5 ± 0.50 meqO$_2$/kg). These values were depicted comparable with that reported by previous studies [16]. Chicken fat oil has a high acid value, which is why the acid esterification of the feedstock was done prior to the alkaline transesterification to reduce the free fatty acid content and avoid saponification.

2.2. Optimization of Biodiesel Production Process

The experimental results obtained after performing reactions as per CCRD were statistically analyzed to select the most appropriate model from the linear, 2F1, cubical and quadratic models. The model that was best suited was chosen by considering the *p*-values, R^2 values, lack-of-fit tests and adjusted R^2 values. It was observed that the quadratic model was most suited for both the chemical and enzymatic routes of biodiesel production (Table 1).

Table 1. Summary of selected quadratic models.

Feedstock	**Catalysts/Biocatalysts**		**Selected Models**	**Sequential *p*-Value**	**Lack-of-Fit *p*-Value**	**Adjusted R-Squared**
WCFO	Enzymes	Fe_3O_4_PDA_Lipase	Quadratic	<0.0001	0.0701	0.9713
WCFO		*Aspergillus terreus* lipase	Quadratic	<0.0001	0.1276	0.9679
WCFO	Chemicals	CH_3ONa	Quadratic	<0.0001	0.4021	0.9519
WCFO		KOH	Quadratic	<0.0001	0.0916	0.9518

The summary statistics clearly determined the fitness of quadratic models for chemical as well as enzymatic biodiesel production process for WCFO.

2.3. Graphs of Predicted vs. Actual Values

The predicted vs. actual value graphs for biodiesel yield depicts the fitness of the selected quadratic model. The graphs of predicted vs. actual values are shown in Figure 1, where Figure 1a–d describes the predicted vs. actual graphs based on experimental data about yield of biodiesel obtained through the transesterification of waste chicken fat oil by Fe_3O_4_PDA_Lipase (Figure 1a), *Aspergillus terreus* lipase (Figure 1b), sodium methoxide (Figure 1c) and KOH (Figure 1d). The distribution of the data along the straight line and the small difference between the predicted and actual value reveals the fitness of the quadratic model for all four experimental designs.

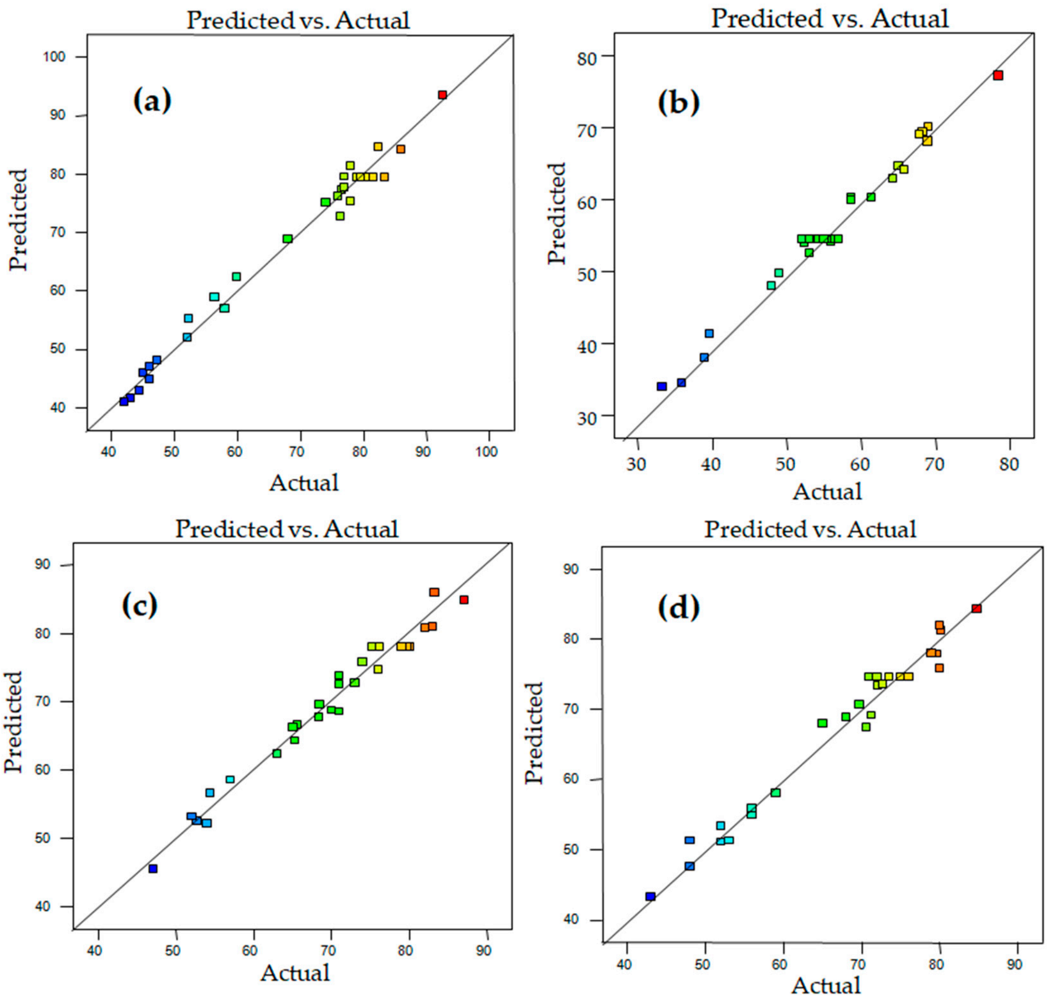

Figure 1. Graphs of predicted vs. actual yield for waste chicken fat oil-based biodiesel by Fe_3O_4_PDA_Lipase (**a**), *Aspergillus terreus* lipase (**b**), Sodium methoxide (**c**) and KOH (**d**).

2.4. Optimization of Reaction Parameters for Manufacturing of Biodiesel Using Chicken Fat Oils

The enzymatic transesterification of waste chicken oil using Fe_3O_4_PDA_Lipase as a bio-catalyst resulted in optimal biodiesel yield when transesterification reactions were performed by employing 6% Fe_3O_4_PDA_Lipaseconcentration with a 6:1 molar ratio of methanol to oil, at 42 °C for 36 h. While in case of enzymatic transesterification by *Aspergillus terreus* lipase, a 1% enzyme concentration, methanol-to-oil ratio of 6:1 and reaction temperature of 35 °C for 36 h were the optimal process conditions. However, when the sodium methoxide-catalyzed transesterification of WCFO was conducted, the optimum conditions for the reaction were a 1% catalyst level and a 6:1 methanol:oil mole ratio at 60 °C for a 1.25 h reaction time (Table 2). The optimum biodiesel yield in case of a potassium hydroxide (KOH)-catalyzed reaction was obtained at a 1% catalyst concentration, 1 h of reaction time, a methanol-to-oil ratio of 6:1 and 60 °C. Highest biodiesel yield was obtained for the nano-biocatalyst (Fe_3O_4_PDA_Lipase), which might be due to the high stability and activity of the immobilized enzyme at an elevated temperature and adoptability towards harsh conditions [17]. Moreover, the lipase can also convert the free fatty acids present in the feedstock to FAMEs. Lower yield obtained by the free lipase can be explained by the reduction of enzyme activity due to denaturation of the free enzymes at a higher temperature, which is required for biodiesel production from chicken fat oil, and the presence of short-chain alcohol [18]. For chemical transesterification, sodium methoxide was proven to be better than KOH, because sodium methoxide did not produce water, which might be responsible for saponification, and the separation of glycerol from biodiesel could be difficult, thus reducing process efficiency. Comparable results for enzymatic and chemical transesterification of waste chicken fat oil have been reported in the published literature. Coppini et al. has reported a 90.61% biodiesel yield from chicken fat by using a 0.3 wt % NS-40116 enzyme, 1.5 of methanol:oil and 1.5 wt % water at 45 °C for 24 h [11]. Da Silva et al. has reported a 77% esterification yield by using 0.3 wt % lipase, 1:4.5 methanol:oil and 2 wt % water at 30 °C in 24 h [15]. Alptekin et al. has reported an 87.4% biodiesel yield from waste chicken fat using a 1% concentration of a KOH catalyst and a 6:1 methanol-to-oil ratio at 60 °C [19]. Mata et al. has reported a 76.8% biodiesel yield by transesterification of chicken fat using a 0.8% KOH catalyst, 6:1 methanol:oil at 60 °C for 2 h [20]. The few variations in the results are probably due to the different fatty acid profiles of chicken fats and different enzyme sources.

Table 2. Optimized factors for biodiesel synthesis via enzymatic and chemical modes of transesterification of chicken fat oil.

Feedstock Oil	**Catalysts/Biocatalysts**	**Reaction Time (Hours)**	**Reaction Temperature °C**	**CH_3OH:Oil Molar Ratio**	**Catalyst's Concentration (%)**	**Biodiesel Yield (%)**
WCFO	Fe_3O_4_PDA_Lipase	36	42	6:1	6	90.6
WCFO	*Aspergillus terreus* Lipase	36	35	6:1	1	78.4
WCFO	CH_3ONa	1.25	60	6:1	1	87.1
WCFO	KOH	1	60	6:1	1	84.8

2.5. ANOVA for Transesterification Data of WCFO

The influence of various reaction parameters such as linear factors, 1st order interactions and quadratic expressions on percentage biodiesel yield are described in the ANOVA table (Table 3). The terms (a)–(d) represents the quadratic models based on findings of Fe_3O_4_PDA_Lipase, *Aspergillus terreus* lipase, sodium methoxide and KOH-catalyzed transesterification of WCFO, respectively. The statistical analysis depicted that the linear term, A—reaction time, had a significant impact for models a, b and c on biodiesel yield ($p < 0.0001$, 0.0003 and 0.0003, respectively), which were <0.05, while for model d it was not significant. The linear term B–reaction temperature, showed p values of 0.1743, <0.0001, 0.0004 and <0.0001 for models a, b, c and d, respectively. The Fe_3O_4_PDA_Lipase catalyzed transesterification was not affected significantly by temperature change in the selected range. Reaction temperature significantly affected the biodiesel yield for *Aspergillus terreus* lipase, which was temperature sensitive. The p values for the linear term C—CH_3OH:Oil, was <0.05 for model (a) and (c) but it was 0.1524 for model (b) and 0.7970 for model (d), which is >0.05. D—catalysts/biocatalysts concentration, was proven to have a significant effect on biodiesel yield for all the four models. A previous report on *Jatropha curcas* seed oil transesterification showed the significant impact of catalyst concentration, methanol-to-oil molar ratio, reaction temperature and reaction time on biodiesel yields [21] In case of Model (a), the 1st order interaction terms AC, AD and CD were found to be significant having p-values of 0.0007, 0.0001 and 0.0018, respectively, which were less than 0.05; however, for Model (b) only BD and CD were found significant. In case of Model (c), the 1st order interaction variables, i.e., AD and CD, were significant with p-values of 0.0065 and 0.0017 being less than 0.05; for Model (d), only AC 1st order interactions were imparting a significant impact on biodiesel yield with p-values lower than 0.05. Where the quadratic terms C^2 and D^2 were significant for Models (a) having a $p < 0.05$, for Model (b) the statistical significance was noted among the quadratic terms B^2, C^2 and D^2. In Model (c), B^2 and C^2 were significant, while in the case of Model (d), A^2 and D^2 were significantly affecting the biodiesel yield with $p < 0.05$.

Table 3. RSM-based ANOVA for transesterification of waste chicken fat oil (WCFO).

Source	Df	SS (MS) [a]	F-Value (*p* Value) [a]	SS (MS) [b]	F Value (*p* Value) [b]	SS (MS) [c]	F Value (*p* Value) [c]	SS (MS) [d]	F Value (*p* Value) [d]
Model	14	7213.87 (515.28)	70.99 (< 0.0001)	3018.69 (215.62)	63.48 (< 0.0001)	3159.24 (225.66)	41.95 (< 0.0001)	3806.96 (271.93)	41.87 (< 0.0001)
A—Reaction Time	1	310.24 (310.24)	42.74 (< 0.0001)	76.08 (76.08)	22.40 (0.0003)	114.01 (114.01)	21.19 (0.0003)	4.84 (4.84)	0.75 (0.4014)
B—Reaction Temperature	1	14.76 (14.76)	2.03 (0.1743)	1656.84 (1656.84)	487.79 (< 0.0001)	107.62 (107.62)	20.01 (0.0004)	268.40 (268.40)	41.33 (< 0.0001)
C—CH_3OH:Oil	1	188.55 (188.55)	25.97 (0.0001)	7.72 (7.72)	2.27 (0.1524)	376.29 (376.29)	69.95 (< 0.0001)	0.45 (0.45)	0.069 (0.7970)
D—Catalyst/Biocatalyst Concentration	1	3164.57 (3164.57)	435.97 (< 0.0001)	277.26 (277.26)	81.63 (< 0.0001)	460.40 (460.40)	85.59 (< 0.0001)	2557.47 (2557.47)	393.81 (< 0.0001)
AB	1	9.79 (9.79)	1.35 (0.2637)	15.05 (15.05)	4.43 (0.0525)	0.46 (0.46)	0.085 (0.7750)	0.45 (0.45)	0.070 (0.7952)
AC	1	129.39 (129.39)	17.83 (0.0007)	8.70 (8.70)	2.56 (0.1303)	16.61 (16.61)	3.09 (0.0993)	105.50 (105.50)	16.25 (0.0011)
AD	1	104.67 (104.67)	14.42 (0.0018)	0.83 (0.83)	0.25 (0.6277)	53.66 (53.66)	9.97 (0.0065)	0.13 (0.13)	0.021 (0.8880)
BC	1	3.05 (3.05)	0.42 (0.5264)	2.16 (2.16)	0.64 (0.4376)	1.27 (1.27)	0.24 (0.6346)	1.56 (1.56)	0.24 (0.6313)
BD	1	0.81 (0.81)	0.11 (0.7424)	222.82 (222.82)	65.60 (< 0.0001)	17.21 (17.21)	3.20 (0.0939)	1.17 (1.17)	0.18 (0.6774)
CD	1	193.91 (193.91)	26.71 (0.0001)	47.01 (47.01)	13.84 (0.0021)	77.88 (77.88)	14.48 (0.0017)	12.23 (12.23)	1.88 (0.1901)
A^2	1	2.49 (2.49)	0.34 (0.5670)	11.18 (11.18)	3.29 (0.0897)	5.19 (5.19)	0.096 (0.3416)	115.85 (115.85)	17.84 (0.0007)
B^2	1	1.19 (1.19)	0.16 (0.6913)	22.09 (22.09)	6.50 (0.0222)	79.02 (79.02)	14.69 (0.0016)	0.15 (0.15)	0.023 (0.8811)
C^2	1	524.04 (524.04)	72.19 (< 0.0001)	112.00 (112.00)	32.97 (< 0.0001)	1011.94 (1011.94)	188.12 (< 0.0001)	1.20 (1.20)	0.19 (0.6731)
D^2	1	96.56 (96.56)	13.30 (0.0024)	112.82 (112.82)	33.21 (< 0.0001)	2.48 (2.48)	0.46 (0.5078)	117.67 (117.67)	18.12 (0.0007)
Residual	15	108.88 (7.26)	-	50.95 (3.40)	-	80.69 (5.38)	-	97.41 (6.49)	-
Lack of Fit	10	96.75 (9.67)	3.99 (0.0701)	30.77 (5.13)	2.29 (0.1276)	58.46 (5.85)	1.31 (0.4021)	85.11 (8.51)	3.46 (0.0916)
Pure Error	5	12.13 (2.43)	-	20.18 (2.24)	-	22.24 (4.45)	-	12.30 (2.46)	-
Cor Total	29	7322.75	-	3069.64	-	3239.93	-	3904.37	-

Note: Fe_3O_4_PDA_Lipase (a), *Aspergillus terreus* lipase (b), sodium methoxide (c) and KOH (d). SS stands for sum of squares and MS is mean square.

The 3D surface plots of the significant 1st order interaction terms are presented in Figure 2. Figure 2a–c presents the significant 1st order interaction terms of Model (a). Figure 2a shows the 3D surface plot between the methanol-to-oil ratio and reaction time; it reveals that the yield increases with an increase in reaction time and methanol:oil, but further increases in the methanol-to-oil ratio resulted in a decreased biodiesel yield. The joint impact of time and concentration to increase the biodiesel yield is given in Figure 2b. Figure 2c presents the 3D plot between bio-catalyst/enzyme concentration and methanol:oil for Model (a). The plot shows that enzyme concentration directly increases the biodiesel yield but the increase of metahonl:oil after a specific limit decreases the biodiesel yield.

Figure 2d,e presents the 3D response surface plots for Model (b). Figure 2d reveals the relation between catalyst concentration and reaction temperature; an increase in temperature decreases the biodiesel yield probably due to the denaturation of free enzyme. Figure 2e presents possible impact of methanol:oil and bio-catalyst/enzyme concentration on biodiesel yield.

Figure 2f,g are the 3D plots of the significant 1st order interaction terms of Model (c). Figure 2f presents the relation between catalyst concentration and reaction time. It is observed that increase in both parameters increases the biodiesel yield. Figure 2g shows the relation between the methanol-to-oil ratio and catalyst concentration. The catalyst concentration increased the biodiesel yield but by further increasing the methanol-to-oil ratio up to certain level, however beyond optimal level, a decrease in biodiesel yield was noted.

Figure 2h presents the response of surface plot on the only significant interaction term of Model (d), which is between the reaction time and methanol-to-oil ratio. It showed that biodiesel yield increased with time but after a specific period any further increase in time was not effective.

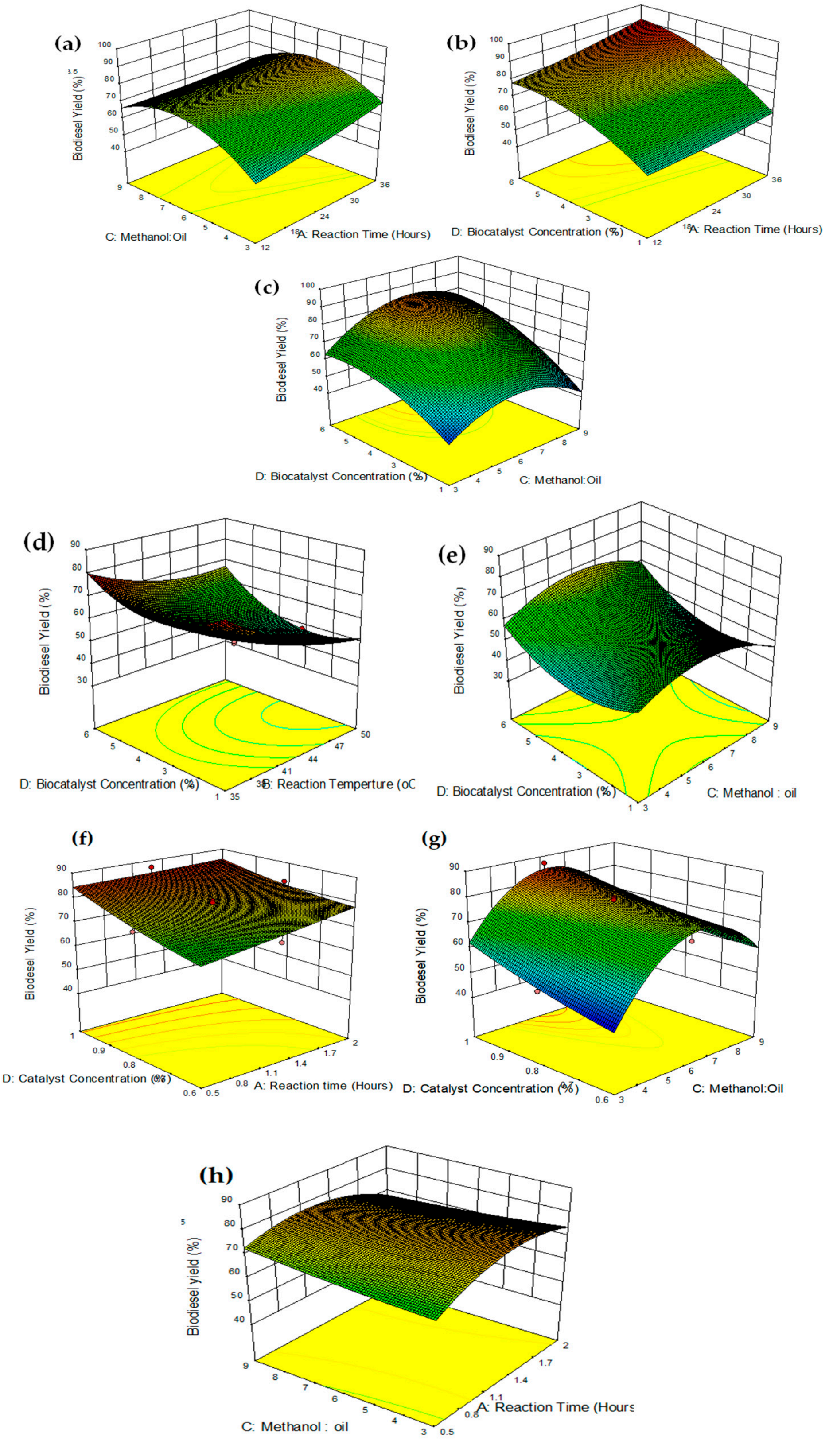

Figure 2. Response surface graphs for the significant 1st order interaction terms of Model (**a**) (A × C), (**b**) (A × D), (**c**) (C × D); Model (b), (**d**) (B × D), (**e**) (C × D); Model (c), (**f**) (A × D), (**g**) (C × D); Model (d), (**h**) (A × C).

2.6. FTIR Spectroscopic Analysis of Feedstock Oil, Biodiesel and Composition of Fatty Acid Methyl Esters

Asymmetric bending of the CH_3 group was observed in the region between 1425 and 1447 cm^{-1} and in the region ranging from 1188 to 1200 cm^{-1} which were basic characteristic peaks of biodiesel. While the C=O stretch vibrations observed in the region between 1700 and 1800 cm^{-1} and CH_2 asymmetric and symmetric stretching vibrations appeared at 2900–3100 cm^{-1} were present in FTIR spectra of both feedstock oil and synthesized biodiesel samples. However, signals in the 1390–1400 cm^{-1} region confirmed the O–CH_2 group and in the 1095–1101 cm^{-1} region defined the asymmetric axial stretching of O–CH_2–C for WCFO in FTIR spectra; however, these bands were absent in their respective biodiesel spectra. The above spectroscopic observations were according with the findings of a previous study [22]. The palmitic acid methyl ester (C16:0) 17.96%, stearic acid methyl ester (C18:0) 20.85%, oleic acid methyl ester (C18:1) 42.92% and linoleic acid methyl ester (C18:2) 16.54%, respectively, were the major FAMEs (Table 4). The current findings were found comparable with those reported by a previous study [16].

Table 4. The fatty acid methyl ester (ME) composition of the synthesized biodiesel.

Biodiesel Type	Palmitic Acid (C16:0) ME %	Stearic Acid (C18:0) ME %	Oleic Acid (C18:1, cis) ME %	Linoleic Acid (C18:2, cis)
WCFAO	17.96	20.85	42.92	16.54

2.7. Fuel Characteristics of WCFO Biodiesel

Fuel analysis plays a vital role in the evaluation of the manufactured biodiesel for its technical compatibility with conventional diesel. Fuel analysis of the understudy biodiesel samples was carried out in accordance with ASTM standard methods and the findings are mentioned below.

Kinematic viscosity is considered as one of the most significant fuel properties, as it is related to the resistance of flow that mainly occurs due to internal friction. If a biofuel contains greater values of kinematic viscosity, it will result in poor fuel atomization or incomplete combustion. Flash point and fuel volatility are inversely related to each other. Similarly, high values of cloud point generally result in problems such as fuel-line clogging. The kinematic viscosity (mm^2/s), flash point (°C), fire point (°C), pour point (°C) and cloud point (°C) values for WCFO biodiesel are given in Table 5. The mentioned fuel-quality parameters were found comparable with those from previous studies [19,20].

Table 5. Fuel characteristics of WCFO biodiesel.

Properties	WCFOB
Kinematic viscosity (mm^2/s) at 40 °C	4.9 ± 0.55
Flash point °C	171 ± 2.51
Fire point °C	187 ± 3.51
Pour point °C	3.0 ± 2.0
Cloud point °C	6.3 ± 2.37

3. Materials and Methods

Chemicals and reagents of analytical grade were utilized during study and were procured from Sigma-Aldrich (Saint Louis, MO, USA) and Merck (Darmstadt, Germany). Lipase from *Aspergillus terreus* was produced through fermentation at the Institute of Industrial Biotechnology, GC University, Lahore, Pakistan. The nano-biocatalyst (Fe_3O_4_PDA_Lipase) prepared and characterized in our previous work has been used as the immobilized lipase for biodiesel production from chicken fat oil [17]. The chicken fat was collected from the local market of Gujrat City, Pakistan.

3.1. Pre-Treatment of Feedstock

The collected waste chicken fat was heated at 100 °C to convert it into liquid. The liquid was then filtered to remove the solid waste. Since the chicken fat oil contains a high free fatty acid (FFA) content, alkaline catalysts are not suitable for un-treated chicken fat oil. In this case, acid esterification was used to reduce the acid value before alkaline transesterification. For this purpose, the chicken fat oil was taken in three neck flasks equipped with a thermometer and a glass condenser. The third neck was used to withdraw the sample. Oil was homogenized by heating and stirring at 600 rpm. Briefly, 50 mg concentrated sulfuric acid and 2.25 g methanol for each gram of FFA present in the oil was mixed in a beaker. The acid value of the sample was checked after specific time intervals by taking small aliquots. The process was carried out till the acid number reduced to the desired value. After completion of the process, the mixture was put in a separating funnel. Three layers were formed after some time. The top layer consisted of unreacted methanol and the lower layer was water while the middle layer was fatty acid methyl esters (FAMEs) and oil. The middle layer was collected for chemical transesterification [23]. The enzymatic transesterification, however, was done without pre-acid esterification. The collected oil was subjected to analysis for some basic parameters, including saponification value, acid value (AV) and peroxide value. The degree of unsaturation of feedstock oil was determined by iodine number. Similarly, the specific gravity was also determined along with density and the refractive index.

3.2. Experimental Design

The Central Composite Response Surface Methodology (CCRD–RSM) was used to evaluate the impact of the different conditions, namely A) reaction time, B) reaction temperature, C) CH_3OH: oil and D) the catalyst/biocatalyst's concentration on percentage biodiesel yield, for different transesterification routs using catalysts (KOH, CH_3ONa, free *Aspergillus terreus* lipase and Fe_3O_4_PDA_Lipase). The ranges of the selected parameters for the four models are presented in Table 6.

Table 6. The ranges of reaction parameters: reaction time, temperature, CH_3OH-to-oil ratio and enzyme concentration used for optimization studies of enzymatic and chemical transesterification.

Design	Catalysts/Biocatalysts	A—Reaction Time (h)	B—Reaction Temp (°C)	C—CH_3OH:oil	D—Catalyst/Biocatalyst Concentration (%) (with Respect to Substrate)
(a)	Fe_3O_4_PDA_Lipase	12–36	35–50	3:1–9:1	1–6
(b)	*Aspergillus terreus* lipase	12–36	35–50	3:1–9:1	1–6
(c)	CH_3ONa	0.5–2	40–60	3:1–9:1	0.6–1
(d)	KOH	0.5–2	40–60	3:1–9:1	0.6–1

In each case, thirty experiments were carried out as per CCRD factorial design. Chemically, this process was performed in three neck flasks equipped with a temperature regulator. A stirrer and reflux condenser were also attached with the flask. The reactions were accomplished at 500 RPM.

3.2.1. Chemical Transesterification

Briefly, 50 g of pre-treated waste chicken fat oil (WCFO) in a flat-bottom three-neck flask was subjected to pre-heating for moisture removal. The molecular weight of the understudy chicken fat oil (873.72 g/mol) was calculated as per a previously reported method [24]. The next step involved the mixing of known amounts of methanol and catalysts, respectively. The resultant mixture was transferred gently to the flask. The whole mixture present in the flask was kept for heating with stirring. According to the CCRD design, the reaction conditions were maintained. The reaction was allowed to proceed, and on completion the mixture was separated into two layers. The upper layer was taken and further processed to obtain the refined biodiesel.

3.2.2. Enzymatic Transesterification

For enzymatic biodiesel production, firstly waste chicken oil was mixed with methanol. A specific amount of immobilized lipase (Fe_3O_4_PDA_Lipase) for design (a) and free *Aspergillus terreus* lipase for design (b) was introduced to the oil/methanol solution and the reaction mixture was subjected to orbital shaking at 200 rpm with a 0.5% water content (with respect to oil), for a specific time period [25]. The CCRD was followed to set the alcohol to oil molar ratio, enzyme concentration, reaction temperature and time. After completion of the reaction, the glycerol was removed to obtain crude biodiesel, which was purified to get refined biodiesel. Magnetic nano-biocatalyst was separated from biodiesel and glycerol by magnetic decantation.

For the optimization studies, suitable statistical models based on experimental data were employed. Linear, 2FI, cubical and quadratic models were tested. The lack-of-fit test values, model significance (p-value), the R^2 and adjusted R^2 values provided the base to select most appropriate statistical model. Finally, the response surface graphs were utilized to check the influence of the studied reaction conditions on the yield of biodiesel.

3.3. Quantification and Characterization of Synthesized Biodiesel

For the FTIR spectroscopic study, a Carry660 FTIR spectrophotometer (Agilent Technologies, Santa Clara, CA, United States) was used and FTIR spectra were drawn over 400–4000 cm^{-1} scanning range.

The biodiesel from waste chicken oil was subjected to GC–MS analysis in order to evaluate the fatty acid methyl esters content (FAMEs). For this purpose, the GC–MS (QP 2010) instrument with a dB 5 column (Shimadzu, Japan) having diameter of 0.15 mm was used. The sample size (1.0 μL) was taken with a split ratio of 1:100, while a source of carrier gas, helium, was used having a 1.20 ml/min flow rate. The oven temperature was kept at 160.0 to 260.0 °C with a ramp rate of 4 °C per minute. The scanning of mass was done from 40.0 to 560.0 *m/z*. The detection of FAMEs was ascertained by comparing the relative retention time of each discrete FAMEs with reliable standards of FAMEs and by comparison with the NIST mass spectral library.

Fuel characteristics of the biodiesel were evaluated by some test experiments utilizing the ASTM standard procedure, i.e., cloud point (ASTM D 2500), viscosity (ASTM D 455), pour point (ASTM D 97) and flash point (ASTM D 93) [19]. The measurements were made in triplicate and the results were analyzed with the help of statistical tools.

4. Conclusions

The waste chicken fat oil was transformed into biodiesel by alkaline and enzymatic transesterifications. The reaction time, temperature, methanol:oil ratio and catalyst concentration were selected for the process optimization. Among all the catalysts and enzymes used, Fe_3O_4_PDA_Lipase-catalyzed transesterification of the studied feedstock oil was proved to be the most efficient to give maximum biodiesel yield. On the other hand, in case of chemical catalysis, CH_3ONa was proved to be better than KOH when chicken fat oil was used as the feedstock. FTIR spectroscopy and GC/MS characterization further confirmed biodiesel formation. The compositional profiles and fuel characteristics of the synthesized biodiesel showed a promising compatibility of WCFO as a potential candidate for biodiesel production for future fuel regimes.

Author Contributions: The idea and concept for the current research was floated by, F.S., T.T., M.W.M., H.M. and U.R.; the methodology was also designed by F.S., M.W.M. and U.R.; the enzyme production was carried out by H.M.; the writing of the original draft was prepared by F.S. with the help and supervision of M.W.M., H.M., S.A.R., T.S.Y.C.; and I.A.N. reviewed the manuscript for improvement of the final version. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: One of the authors acknowledges his gratitude to King Saud University (Riyadh, Saudi Arabia) for the support/technical assistance of this research through Researchers Supporting Project number (RSP-2019/80).

Conflicts of Interest: The author declares no conflict of interest.

References

1. Berchmans, H.J.; Hirata, S. Biodiesel production from crude *Jatropha curcas* L. seed oil with a high content of free fatty acids. *Bioresour. Technol.* **2008**, *99*, 1716–1721. [CrossRef] [PubMed]
2. Niculescu, R.; Clenci, A.; Iorga-Siman, V. Review on the use of diesel–biodiesel–alcohol blends in compression ignition engines. *Energies* **2019**, *12*, 1194. [CrossRef]
3. Rehan, M.; Gardy, J.; Demirbas, A.; Rashid, U.; Budzianowski, W.M.; Pant, D.; Nizami, A.S. Waste to biodiesel: A preliminary assessment for Saudi Arabia. *Bioresour. Technol.* **2018**, *250*, 17–25. [CrossRef]
4. Talebian-Kiakalaieh, A.; Amin, N.A.S.; Mazaheri, H. A review on novel processes of biodiesel production from waste cooking oil. *Appl. Energy* **2013**, *104*, 683–710. [CrossRef]
5. Jamil, F.; Al-Muhtaseb, A.H.; Al-Haj, L.; Al-Hinai, M.A.; Hellier, P.; Rashid, U. Optimization of oil extraction from waste "Date pits" for biodiesel production. *Energ. Conver. Manag.* **2016**, *117*, 264–272. [CrossRef]
6. Lotero, E.; Goodwin, J.G., Jr.; Bruce, D.A.; Suwannakarn, K.; Liu, Y.; Lopez, D.E. The catalysis of biodiesel synthesis. *Catalysis* **2006**, *19*, 41–83.
7. Gürü, M.; Koca, A.; Can, Ö.; Çınar, C.; Şahin, F. Biodiesel production from waste chicken fat based sources and evaluation with Mg based additive in a diesel engine. *Renew. Energy* **2010**, *35*, 637–643. [CrossRef]
8. Chiu, M.C.; Gioielli, L.A. Solid fat content of abdominal chicken fat, its stearins and its binary mixtures with bacon. *Food Sci. Technol.* **2002**, *22*, 151–157.
9. Gugule, S.; Fatimah, F.; Rampoh, Y. The utilization of chicken fat as alternative raw material for biodiesel synthesis. *Anim. Prod.* **2011**, *13*, 115–121.
10. Liu, K.S. Preparation of fatty acid methyl esters for gas-chromatographic analysis of lipids in biological materials. *J. Am. Oil Chem. Soc.* **1994**, *71*, 1179–1187. [CrossRef]
11. Coppini, M.; Magro, J.D.; Martello, R.; Valério, A.; Zenevicz, M.C.; Oliveira, D.D.; Oliveira, J.V. Production of methyl esters by enzymatic hydroesterification of chicken fat industrial residue. *Braz. J. Chem. Eng.* **2019**, *36*, 923–928. [CrossRef]
12. Christopher, L.P.; Kumar, H.; Zambare, V.P. Enzymatic biodiesel: Challenges and opportunities. *Appl. Energy* **2014**, *119*, 497–520. [CrossRef]
13. Dumri, K.; Hung Anh, D. Immobilization of lipase on silver nanoparticles via adhesive polydopamine for biodiesel production. *Enzym. Res.* **2014**, *2014*, 389739. [CrossRef]
14. Aryee, A.N.; Simpson, B.K.; Cue, R.I.; Phillip, L.E. Enzymatic transesterification of fats and oils from animal discards to fatty acid ethyl esters for potential fuel use. *Biomass Bioenergy* **2011**, *35*, 4149–4157. [CrossRef]
15. da Silva, J.R.P.; Nürnberg, A.J.; da Costa, F.P.; Zenevicz, M.C.; Lerin, L.A.; Zanetti, M.; Valério, A.; de Oliveira, J.V.; Ninow, J.L.; de Oliveira, D. Lipase NS40116 as catalyst for enzymatic transesterification of abdominal chicken fat as substrate. *Bioresour. Technol. Rep.* **2018**, *4*, 214–217. [CrossRef]
16. Feddern, V.; Kupski, L.; Cipolatti, E.P.; Giacobbo, G.; Mendes, G.L.; Badiale, F.E.; de Souza-Soares, L.A. Physico-chemical composition, fractionated glycerides and fatty acid profile of chicken skin fat. *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 1277–1284. [CrossRef]
17. Touqeer, T.; Mumtaz, M.W.; Mukhtar, H.; Irfan, A.; Akram, S.; Shabbir, A.; Rashid, U.; Nehdi, I.A.; Choong, T.S.Y. Fe_3O_4-PDA-Lipase as surface functionalized nano biocatalyst for the production of biodiesel using waste cooking oil as feedstock: Characterization and process optimization. *Energies* **2019**, *13*, 177. [CrossRef]
18. Andrade, M.F.; Parussulo, A.L.; Netto, C.G.C.M.; Andrade, L.H.; Toma, H.E. Lipase immobilized on polydopamine-coated magnetite nanoparticles for biodiesel production from soybean oil. *Biofuel Res. J.* **2016**, *3*, 403–409. [CrossRef]
19. Alptekin, E.; Canakci, M. Optimization of pretreatment reaction for methyl ester production from chicken fat. *Fuel* **2010**, *89*, 4035–4039. [CrossRef]
20. Mata, T.M.; Cardoso, N.; Ornelas, M.; Neves, S.; Caetano, N.S. Evaluation of two purification methods of biodiesel from beef tallow, pork lard, and chicken fat. *Energy Fuels* **2011**, *25*, 4756–4762. [CrossRef]
21. Kamel, D.A.; Farag, H.A.; Amin, N.K.; Zatout, A.A.; Ali, R.M. Smart utilization of jatropha (*Jatropha curcas* Linnaeus) seeds for biodiesel production: Optimization and mechanism. *Ind. Crops. Prod.* **2018**, *111*, 407–413. [CrossRef]

22. Mumtaz, M.W.; Mukhtar, H.; Anwar, F.; Saari, N. RSM based optimization of chemical and enzymatic transesterification of palm oil: Biodiesel production and assessment of exhaust emission levels. *Sci. World J.* **2014**, *2014*. [CrossRef] [PubMed]
23. Chai, M.; Tu, Q.; Lu, M.; Yang, Y.J. Esterification pretreatment of free fatty acid in biodiesel production, from laboratory to industry. *Fuel Process. Technol.* **2014**, *125*, 106–113. [CrossRef]
24. Marulanda, V.F.; Anitescu, G.; Tavlarides, L.L. Investigations on supercritical transesterification of chicken fat for biodiesel production from low-cost lipid feedstocks. *J. Supercrit. Fluids* **2010**, *54*, 53–60. [CrossRef]
25. Katiyar, M.; Ali, A. Onepot lipase entrapment within silica particles to prepare a stable and reusable biocatalyst for transesterification. *J. Am.OilChem. Soc.* **2015**, *92*, 623–632. [CrossRef]