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Optimized Biodiesel Production from *Prosopis juliflora* Seeds using NaOH-CZO Green Nano-bio-catalyst – An RSM Approach

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ABSTRACT

This experimental investigation attempts a methodology to produce *Prosopis juliflora* biodiesel through base catalyzed transesterification process with NaOH-CZO (Copper-doped Zinc Oxide) green nano catalyst. The bio-oil from *Prosopis juliflora* seed were derived through Soxhlet extraction process in the presence of *n*-hexane solvent. NaOH-CZO green nano catalyst was prepared by co-precipitation technique using cupric sulphate, zinc sulphate and sodium bi-carbonate with calcination at 600°C. Box-Behnken design in RSM technique developed 29 experimental runs to optimize the transesterification process with methanol to oil molar ratio, catalyst concentration, reaction temperature and agitation speed as the operating influential parameters wherein 1:10, 1.75%, 90°C and 450 rpm, respectively, were found optimal yielding 97.8% of *Prosopis juliflora* biodiesel. The derived biodiesel was standardized by evaluating their physio-chemical properties like kinematic viscosity, density, cetane number, calorific value, flash and fire points for its usage in compression ignition engine along with commercial diesel.

Keywords: *Prosopis juliflora*, Transesterification, Box-Behnken design, Optimization, Physio-chemical properties.

1. INTRODUCTION

Continuous consumption of petro-diesel and proportionate depletion of fossil fuel has created a huge energy scarcity in the automotive and industrial sector. Additionally, the harmful emissions have worsened the greenhouse effect in the earth's atmosphere leading to global warming. Petro-diesel is widely used in internal combustion engine employed in the industrial and transportation sectors, mainly due to their durability and relatively higher thermal efficiency (Hariram and Kumar, 2012). As a result of combustion, increased engine emissions are seen in the environment due to burning of fuel. This could be overcome by infusing fuel additives like oxygenates and supplementing gaseous fuel. Alcohols are one such oxygenates which can contribute to superior engine performance with reduced emission thereby with a proportionate effect on the dependency of petro-diesel. Butanol, methanol, diethyl ether, ethanol etc., are few of the oxygenated fuel additives in the alcohol category which could be used along with diesel fuel in compression ignition (CI) engine. On the other hand, vegetable oil-based biodiesel showed a promising scenario in reducing the dependency on diesel fuel. Viscosity reduced vegetable oil through transesterification or thermal cracking could be blended with diesel fuel upto 20-25% in volumetric ratio for fuelling the CI engine with minimal or no modification in the existing CI engine (John *et al.* 2017). Transesterification is a process of converting the triglycerides in the vegetable oil into its mono alkyl esters in the presence of a catalyst and alcohol at elevated temperature. The reaction temperature and the agitation speed plays a prominent role in producing the vegetable biodiesel. Neem, karanja, cotton seed, pongamia etc., are a few feedstocks that could be used to produce biodiesel. In the present study, bio-oil from *Prosopis juliflora* was used as a feedstock to produce vegetable oil through single stage transesterification process in the presence of Copper-doped Zinc Oxide (CZO) green nano-catalyst along with methanol.

Hundie *et al.* (2022) developed an optimized biodiesel production technique from *Prosopis juliflora* seeds using definitive screening design. They have used magnesium oxide as nano-catalyst and ethanol as solvent for the production of biodiesel with an optimized biodiesel yield of 32.5%. The acidic nature of bio-oil was reduced using acid pre-treatment process with sulphuric acid followed by transesterification process. In the transesterification process using nano magnesium oxide, an initial catalyst concentration equivalent to 1.52 mg KOH/g (H_2SO_4 basis) was applied, followed by 0.88 mg KOH/g in the subsequent step. Molar ratio, nano-catalyst concentration, reaction time and reaction temperature of 10:1, 5%, 80 minutes and 45°C, respectively, produced an optimal biodiesel conversion of 94.83%. Debella *et al.* (2023) derived bio-oil from *Prosopis juliflora* seed

employing solvent extraction process using *n*-hexane solvent in batch process. Transesterification process with NaOH and methanol produced *Prosopis juliflora* biodiesel. Response Surface Methodology (RSM) was used to analyze the effect of operating variables including methanol to oil molar ratio, catalyst concentration and reaction temperature on the biodiesel yield. The optimal condition was identified as 0.5% weight of catalyst concentration, 6:1 methanol to oil molar ratio, 60 minutes of reaction duration and 55°C of reaction temperature which yielded 87.8% of *Prosopis juliflora* biodiesel. Further the FTIR, GCMS and NMR studies revealed the presence of Fatty Acid Methyl Esters (FAME) in biodiesel with erucic acid in higher proportions.

Raja and Premjeyakumar, (2021) blended isopropanol with *Prosopis juliflora* biodiesel to examine the effect on the CI engine in the presence of Exhaust Gas Recirculation (EGR) setup. The blends of fuel were made using ultrasonication technique in volumetric basis. The results notified that the EGR reduced NO_x emission, notably with a marginal reduction in brake thermal efficiency. Increasing the EGR beyond 20% increased the HC and CO emission considerably. John *et al.* (2025) produced hybrid oil blends from *Mimusops elengi, Ricinus communis, Jatropha curcas, Delonix regia* and *Prosopis juliflora* feedstocks due to their abundant availability and high lipid content through solvent extraction method and then filtered. Sodium hydroxide and methanol were used to derive biodiesel through transesterification process in the presence of Cu-Zn bimetallic nano-catalyst. The RSM technique with 30 experimental trials identified the optimal parameters to yield the higher quantity of hybrid biodiesel. The obtained biodiesel was characterized using FTIR, SFM, EDAX, XRD and AFM techniques. Further, the GCMS analysis revealed the existence of saturated, unsaturated and poly-saturated fatty acids in the hybrid biodiesel blends. Sarkar *et al.* (2024) used guava leaves as catalyst in the production of biodiesel from soybean oil. Calcinized catalyst at 850°C produced 95.8% soybean biodiesel at room temperature.

Naveenkumar and Baskar, (2020) showcased the employment of homogeneous and heterogeneous catalyst in the production of vegetable-based biodiesel. They found that heterogeneous catalyst is more suitable and adaptable than homogeneous catalyst which can be later recovered and reused in the subsequent process. Further, nano catalyst in heterogeneous form was found to be more effective in transforming the vegetable oil greatly. Parandi *et al.* (2023) produced biodiesel from sesame waste cake oil through magnetically immobilized lipase nano-catalyst through optimized micro-reactor intensified transesterification reaction process. They used Box-Behnken model in RSM to formulate the experimental trials. *Candida rugosa* lipase (CRL) on TiO_2 nano-catalyst produced 92.23% of biodiesel which was later recovered. The derived biodiesel was subjected to FE-SEM, EDX, FTIR and XRD analysis towards its characterization and standardization. The physio-chemical properties were found to be within ASTM D6751 standards. The transesterification process was enhanced by employing a nano-biocatalyst, whereas the enzyme-catalyzed transesterification process is time-consuming.

Considering the above literatures, it was noticed that meagre number of investigations were carried out with *Prosopis juliflora* seed as a feedstock for biodiesel production and its optimization. Further, usage of statistical tool like RSM in optimizing the input independent variable like molar ratio, catalyst concentration and reaction duration was not noticed. In the present experimental investigation, bio-oil was extracted from processed seeds of *Prosopis juliflora* in a Soxhlet apparatus, followed by base catalysed transesterification using NaOH-CZO green nano-catalyst to produce *Prosopis juliflora* biodiesel. Response Surface Methodology approach optimized the experimental design with Box-Behnken tool up to 29 trial runs. The derived biodiesel was subjected to analysis of its physio-chemical properties towards its standardization and fused as a fuel in compression ignition engine.

2. MATERIALS AND METHODS

Prosopis juliflora seeds were obtained from the rural regions near Kanchipuram, Tamil Nadu, India. Copper-doped zinc oxide nano-catalyst for the transesterification reaction was prepared in the laboratory. The chemical reagents like zinc sulphate, cupric sulphate and sodium bi-carbonate were procured from Rashik Chemical, Chennai, Tamil Nadu. Laboratory grade methanol and *n*-hexane solvents were purchased from Marvic, India.

2.1 Synthesis of Green CZO Nano-bio-catalyst

Co-precipitation methods with zinc sulphate, cupric sulphate and sodium bi-carbonate solution yielded the homogeneous green CZO nano-bio-catalyst (Prajapati *et al.* 2023). First, solution 1 was prepared by adding 0.52% of cupric sulphate and 12.32% of zinc sulphate with 60 mL of double distilled water under constant stirring of 250 rpm at room temperature. Then, solution 2 was prepared by dissolving 2.14% of sodium bi-carbonate in 60 mL of double distilled water. Solution 1 was added in drops to the secondary solution at constant stirring of 300 rpm using a magnetic stirrer and the mixture was maintained at 70°C for 20 minutes. After cooling, filtration process with Whatman filter paper removed the precipitate sedimentation and dying process was initiated at 90°C for 10 minutes for the removal of moisture. The resultant powder was calcinized at 600°C in a microwave oven for 3 hours to make their particle surface active. The synthesized green CZO nano-bio-catalyst was characterized to evaluate its morphological,

structural and catalytic properties for biodiesel production. Scanning electron microscopy was used to examine surface morphology, particle size distribution, and agglomeration behaviour. The determination of average crystalline size was made using XRD analysis and further its crystalline phase was confirmed. The metal-O bonding and its functional groups were identified using FTIR, which subsequently confirmed the formation of CZO nano particle. The pore size / volume and the surface area of the CZO nano particle was evaluated using Brunauer-Emmett-Teller surface area analyser. The above methods confirmed that green CZO nano-bio-catalyst possessed uniform surface morphology and higher surface area making it more suitable for its usage as a bio-catalyst in deriving the biodiesel of *Prosopis juliflora* bio-oil.

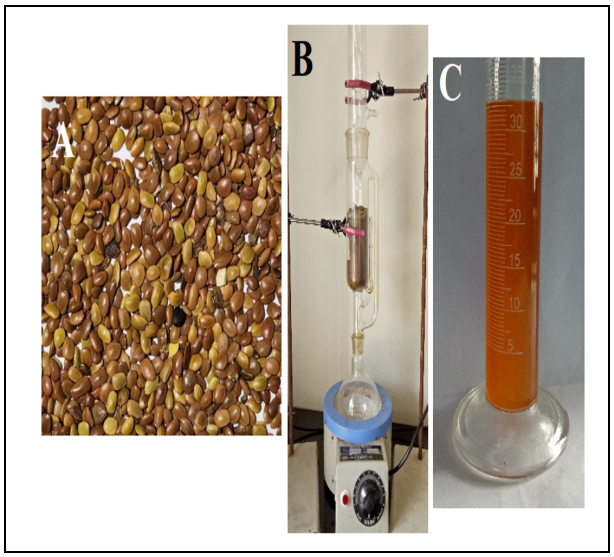


Fig. 1: *Prosopis juliflora* – seeds (A), bio-oil extraction (B) and bio-oil (C)

2.2 *Prosopis juliflora* Bio-oil Extraction

The seeds of *Prosopis juliflora* along with its shell cover were collected from the nearby region of rural Kanchipuram, Tamil Nadu, India. The acquired seeds were then removed from the shell cover, washed thoroughly in distilled water and followed by sun drying for 48 hours and further drying in hot air oven at 120°C for 2 hours. The pre-processed seeds were crushed in a motorized mortar-pestle arrangement running at 350 rpm.

Soxhlet extraction process was utilized to remove the bio-oil from the processed seeds of *Prosopis juliflora*. About 50 g of crushed *Prosopis juliflora* seeds was filled in the thimble of the extractor and placed in the central compartment. The round bottomed flask containing the extractor arrangement was filled with 150 mL of *n*-hexane solvent. Upon heating upto 100°C, the solvent was evaporated and reached the upper layer of the Soxhlet extractor and condensation took place at elevated temperature. The solvent reacted with the crushed *Prosopis juliflora* seeds thereby removing the bio-oil from the ruptured wall membrane, mixing with the solvent and occupying the bottom layer of the chamber. Further heating of the mixture allowed the solvent alone to vaporize and the process was repeated in multiple cycles. This batch cyclic process extracted 42 mL of *Prosopis juliflora* bio-oil in a single batch. Repeating this batch process 24 times in the laboratory condition yielded 1008 mL of *Prosopis juliflora* bio-oil. The detailed bio-oil extraction process is shown in Fig. 1.

2.3 *Prosopis juliflora*– Transesterification of Bio-oil

As the free fatty acid content of *Prosopis juliflora* bio-oil was estimated to be 1.92% through titration method, single stage base catalyzed esterification process was chosen to esterify the bio-oil into its biodiesel. The process was carried out with a 200 mL beaker, round-bottomed conical flask, magnetic stirrer, heating arrangement with a thermometer in a laboratory environment. The operating influential parameters of the transesterification process was found to be methanol to oil molar ratio, catalyst concentration, reaction temperature and reaction speed. Based on the Central Rotatable Composite Design (CCRD) of RSM, an experiment design comprising of 29 experimental runs was developed to identify the optimal range of operating influential parameters on the yield of *Prosopis juliflora* biodiesel. A laboratory scale environment with 200 mL beaker on a heating arrangement equipped with a magnetic stirrer and thermometer was placed on the work table. About 100 mL of *Prosopis juliflora* bio-oil was poured in to the beaker followed by adding the desired quantity of green CZO nano-bio-catalyst at pre-set agitation speed and temperature. The experiments were conducted as per the experimental design (Table 1). The completion of the transesterification process was noted by the formation of ring-like structure separating the *Prosopis juliflora* biodiesel in the upper layer and glycerol in the lower layer. The green CZO nano-bio-catalyst was recovered by separation and dried. Excess methanol recovered was then utilized in the subsequent reactions. The obtained biodiesel was heated upto 100°C for the removal of moisture. The yield of *Prosopis juliflora* biodiesel was estimated using Equation 1.

$$Prosopis\ julifera \text{ biodiesel yield} = \frac{P_{Jb} \times \rho_{pj} \times M_{pj}}{P_{Jo} \times \rho P_o \times M_{PJo}} \times 100 \quad \ldots (1),$$

where P_{Jb} and P_{Jo} are the volume of *Prosopis juliflora* biodiesel and bio-oil respectively, ρ_{pj} and ρ_{Po} are the densities of *Prosopis juliflora* biodiesel and bio-oil and M_{pj} and MP_{Jo} are the molecular mass of *Prosopis juliflora* biodiesel and bio-oil, respectively.

Table 1. Operating influential parameters in the base catalyzed transesterification process

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding
A	Molar ratio	10.00	8.00	12.00	0.0000	Actual
B	Catalyst Concentration	1.25	0.5000	2.00	0.0000	Actual
C	Reaction temperature	90.00	60.00	120.00	0.0000	Actual
D	Reaction Speed	450.00	300.00	600.00	0.0000	Actual

Table 1 enlists the four key process variables molar ratio, catalyst concentration, reaction temperature, and reaction speed used for optimizing biodiesel production. Each factor is assigned actual (non-coded) levels defining its experimental range. The molar ratio (8:1 - 12:1) influences the chemical equilibrium, where higher methanol ratios enhance conversion up to an optimum. Catalyst concentration (0.5 - 2.0 wt%) affects reaction rate; excess leads to soap formation and lower yield (Rajeshwaran *et al.* 2018; Sánchez-Arreola *et al.* 2019). Reaction temperature (60ºC - 120°C) impacts reaction kinetics and methanol vaporization, with around 90°C typically yielding the best conversion. Reaction speed (300 - 600 rpm) improves mass transfer between immiscible reactants, reducing diffusion limitations. The central levels of each factor represent the midpoints used in RSM optimization. These parameters were chosen to study their combined effect on biodiesel yield and to determine the optimum conditions for maximum conversion efficiency.

2.4 Experimental Data and Predicted *Prosopis juliflora* Biodiesel Yield

Box-Behnken design in RSM was employed in predicting the optimal *Prosopis juliflora* biodiesel yield. The model developed with RSM was a quadratic model with 29 experimental runs derived from four operating influential parameters such as methanol to oil molar ratio, catalyst concentration, reaction duration and reaction temperature to identify the *Prosopis juliflora* biodiesel yield. Table 2 represents the outcome of both the experimental trials and predicted value in which the 19th run exhibited 97.8% and 97.53% of *Prosopis juliflora* biodiesel yield in experimental procedure and RSM prediction with 1:10, 1.75%, 90ºC and 450 rpm of methanol to oil molar ratio, green CZO nano-bio-catalyst concentration, reaction temperature and agitation speed, respectively.

Table 2. Experimental design - RSM prediction of *Prosopis juliflora* biodiesel yield

Std	Run	A:Molar ratio	B: Catalyst Concentration	C: Reaction temperature	D: Reaction Speed	*Prosopis juliflora* Biodiesel Yield	*Predicted Biodiesel yield*
		v/v	w/w	Centigrade	rpm	%	%
11	1	8	1.25	90	600	74.9	73.45
25	2	10	1.25	90	450	84.7	91.42
14	3	10	2	60	450	83.78	85.52
29	4	10	1.25	90	600	85.48	85.82
26	5	10	1.5	90	450	86.7	89.97
7	6	10	1.25	60	600	87.48	88.52
6	7	10	1.25	120	300	84.26	87.38
3	8	8	2	90	450	76.7	76.11
22	9	10	2	90	300	81.46	81.22
4	10	12	2	90	450	76.25	77.49
8	11	10	1.25	120	600	80.12	82.31
5	12	10	1.25	60	300	79.7	81.66
2	13	12	0.5	90	450	82.78	87.66
16	14	10	2	120	450	78.9	81.83
20	15	12	1.25	120	450	88.9	86.05

18	16	12	1.25	60	450	89.78	87.29
21	17	10	0.5	90	300	81.7	79.63
9	18	8	1.25	90	300	78.9	76.83
28	19	10	1.75	90	450	97.8	97.53
1	20	8	0.5	90	450	74.2	77.25
27	21	10	1.25	120	450	94.87	93.89
10	22	12	1.25	90	300	79.15	78.45
17	23	8	1.25	60	450	79.7	80.41
15	24	10	0.5	120	450	92.7	90.94
19	25	8	1.25	120	450	80.79	81.14
13	26	10	0.5	60	450	90.7	87.74
23	27	10	0.5	90	600	89.7	87.77
12	28	12	1.25	90	600	83.7	83.62
24	29	10	2	90	600	74.97	74.87

Fig. 2 and 3 expresses the variation between normal probability (%) with external Studentized residual. It could be seen that major scatter falls around the normal expression in the plot which evidenced a successive prediction of *Prosopis juliflora* biodiesel yield of the developed quadratic model. The variation between the external studentized residue and the experimental run number also depicts a closer variation of the *Prosopis juliflora* biodiesel yield around the normal curve with minimal prediction variation.

2.5 Analysis of Variance

The analysis of variance (ANOVA) approach was employed to identify the effectiveness of the developed quadratic model. ANOVA utilizes the necessity of the variables contributing parameters like molar ratio, catalyst concentration, reaction temperature and reaction speed in predicting the *Prosopis juliflora* biodiesel yield at various degree. The "F" and "p" values of the operating influential parameters play a major role in making the developed model significant. Table 3 details the various responses of the influential parameters like molar ratio, catalyst concentration, reaction temperature and reaction duration. The inter-relationship between the influential operating parameters are also given in Table 3. The Model F-value of 2.71 implies the model is significant. There is only a 3.62% chance that an F-value this large could occur due to noise. p-values less than 0.0500 indicate model terms are significant. In this case A, B, A^2, B^2, C^2 and D^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

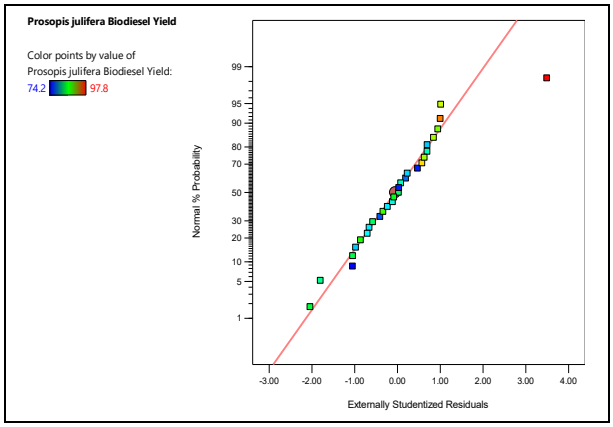


Fig. 2: Normal plot of residuals

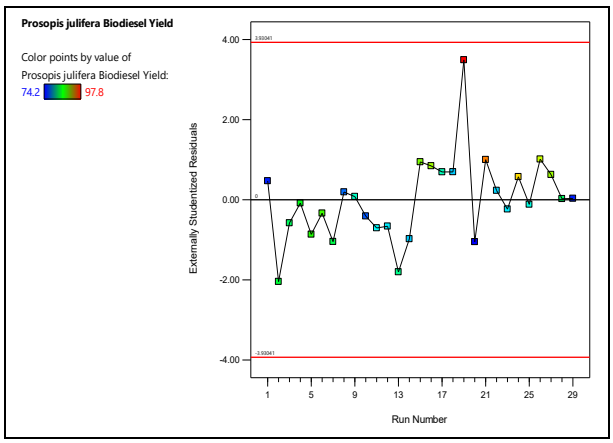


Fig. 3: Variation between externally studentized residuals and experimental run number

Table 3. ANOVA table for quadratic model

Source	Sum of Squares	df	Mean Square	F-Value	p-Value	
Model	773.03	14	55.22	2.71	0.0362	significant
A-Molar ratio	104.25	1	104.25	5.12	0.0401	
B-NaOH-CZO Catalyst Concentration	98.63	1	98.63	4.84	0.0451	
C-Reaction temperature	0.1969	1	0.1969	0.0097	0.9231	
D-Reaction Speed	2.56	1	2.56	0.1256	0.7283	
AB	20.39	1	20.39	1.00	0.3341	
AC	0.9702	1	0.9702	0.0476	0.8304	
AD	18.28	1	18.28	0.8971	0.3596	
BC	11.83	1	11.83	0.5809	0.4586	
BD	52.49	1	52.49	2.58	0.1308	
CD	35.52	1	35.52	1.74	0.2078	
A^2	292.80	1	292.80	14.37	0.0020	
B^2	102.43	1	102.43	5.03	0.0416	
C^2	0.8645	1	0.8645	0.0424	0.8398	
D^2	190.52	1	190.52	9.35	0.0085	
Residual	285.20	14	20.37			
Cor Total	1058.23	28				

Table 4. External and internal studentized residuals with cook's distance

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residuals	Externally Studentized Residuals	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
1	74.90	73.45	1.45	0.559	0.484	0.471	0.020	0.530	11
2	84.70	91.42	-6.72	0.349	-1.844	-2.043	0.121	-1.495	25
3	83.78	85.52	-1.74	0.575	-0.592	-0.578	0.032	-0.672	14
4	85.48	85.82	-0.340	0.224	-0.086	-0.082	0.000	-0.044	29
5	86.70	89.97	-3.27	0.305	-0.870	-0.862	0.022	-0.571	26
6	87.48	88.52	-1.04	0.561	-0.347	-0.335	0.010	-0.379	7
7	84.26	87.38	-3.12	0.561	-1.042	-1.045	0.092	-1.181	6
8	76.70	76.11	0.5917	0.573	0.201	0.194	0.004	0.224	3
9	81.46	81.22	0.2421	0.575	0.082	0.079	0.001	0.092	22
10	76.25	77.49	-1.24	0.573	-0.420	-0.407	0.016	-0.471	4
11	80.12	82.31	-2.19	0.541	-0.716	-0.703	0.040	-0.763	8
12	79.70	81.66	-1.96	0.583	-0.674	-0.660	0.042	-0.781	5
13	82.78	87.66	-4.88	0.581	-1.672	-1.801	0.259	-2.122	2
14	78.90	81.83	-2.93	0.558	-0.978	-0.976	0.080	-1.096	16
15	88.90	86.05	2.85	0.559	0.949	0.946	0.076	1.065	20
16	89.78	87.29	2.49	0.582	0.854	0.846	0.068	0.998	18
17	81.70	79.63	2.07	0.582	0.708	0.695	0.047	0.820	21
18	78.90	76.83	2.07	0.582	0.710	0.696	0.047	0.822	9

19	97.80	87.53	10.27	0.236	2.603	3.492	0.139	1.941	28
20	74.20	77.25	-3.05	0.581	-1.046	-1.049	0.101	-1.236	1
21	94.87	90.89	3.98	0.224	1.002	1.002	0.019	0.538	27
22	79.15	78.45	0.7006	0.582	0.240	0.232	0.005	0.274	10
23	79.70	80.41	-0.706	0.582	-0.242	-0.234	0.005	-0.276	17
24	92.70	90.94	1.76	0.558	0.588	0.574	0.029	0.645	15
25	80.79	81.14	-0.353	0.559	-0.118	-0.114	0.001	-0.128	19
26	90.70	87.74	2.96	0.582	1.013	1.014	0.095	1.197	13
27	89.70	87.77	1.93	0.558	0.643	0.629	0.035	0.707	23
28	83.70	83.62	0.0823	0.559	0.027	0.026	0.000	0.030	12
29	74.97	74.87	0.1039	0.558	0.035	0.033	0.000	0.037	24

3. RESULTS AND DISCUSSION

In this present work, the optimization of the biodiesel yield through parameters like molar ratio, catalyst concentration, reaction temperature, and reaction speed were analysed and were run in RSM to study them.

he experimentally run values interpreted with the RSM with external and internal studentized residuals with Cook's distance were based on the below parameters as shown in Table 4, It was noted that most residuals were small (close to 0), e.g., Runs 1 (residual 1.45), 3 (−1.74), 8 (0.592), indicating good agreement between measured and predicted yields for the majority of experiments. It was detailed that Run 19 is a clear outlier: residual 10.27, internally studentized residual - 2.60, externally studentized residual - 3.49, and Cook's distance - 0.139. These values indicate that run 19 has a large deviation from the model prediction and it exerts notable influence on fitted coefficients i.e., studentized residuals > 2 approximately and Cook's D non-negligible.

Yield increases as the methanol - oil molar ratio increases from 8 to approximately 10 – 11, then plateaus near the maximum region observed yields up to approximately 97.8% in the design as shown in Fig. 4. Catalyst concentration shows an optimum at an intermediate value approximately 1.0 to 1.5% w/w which if it is too low catalyst lowers conversion, if it's too high gives no further increase and can slightly reduce apparent yield and may likely due to soap formation or difficulties in phase separation. Increasing methanol ratio increases FAME yield up to an optimum since excess methanol helps drive equilibrium, while catalyst has an optimum beyond which soap formation or emulsification reduces apparent recovery. Literature study has reported mixed oils report optimal methanol - oil ratios in the 6:1–12:1 range and catalyst around 0.5–2% w/w. The present study reveals that catalyst loading positively influences yield up to 8 wt%, beyond which the yield stagnates which was in-line with the reports of Lee *et al.* (2011) and Hariram *et al.* (2018). This may be attributed to increased viscosity or catalyst saturation, leading to mass transfer limitations. The highest yields in this surface were in the high-molar-ratio and moderate-catalyst zone therefore, for a reaction conducted at 90°C and 450 rpm, methanol - oil ratio approximately around 10–12:1 and catalyst approximating 1.0 to 1.5% w/w to maximize yield was found consistent with the region where the measured maximum value appeared.

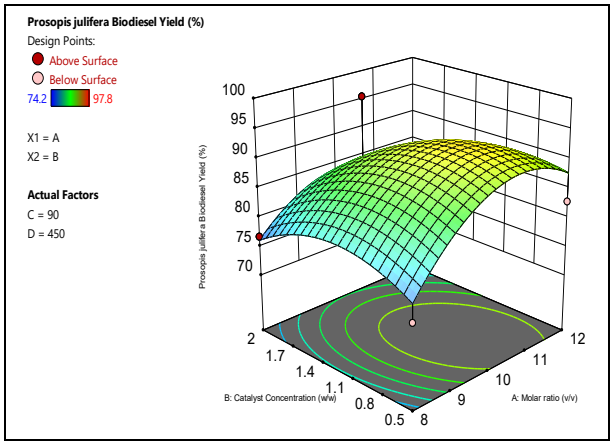


Fig. 4: Catalyst concentration and methanol to oil molar ratio variation at constant reaction temperature

The effect of methanol to oil molar ratio and reaction temperature at constant catalyst concentration is shown in Fig. 5. The yield rises with increasing temperature from 60ºC to approximately 90ºC to 100°C, beyond that point, the surface flattens i.e., marginal gains are there. Highest yields cluster where A is higher (i.e., approximately 10–12) and temperature is moderate to high. Literature studies depicted that the temperature accelerates reaction kinetics and reduces viscosity improving mass transfer; however too high a temperature increases methanol vaporization (i.e., loss of alcohol) and

can promote side reactions. It was reported optimum reaction temperatures often between was between 50°C - 90°C depending on alcohol and reactor setup. At catalyst concentration 1.25% and stirring 450 rpm, raising temperature to 90°C while using high methanol ratio i.e., near 10:1, produces near-maximum conversion and further temperature increase gives little improvement and may not be economical. Similar outcomes were also seen in Lee *et al.* (2011) and Shahedi *et al.* (2019).

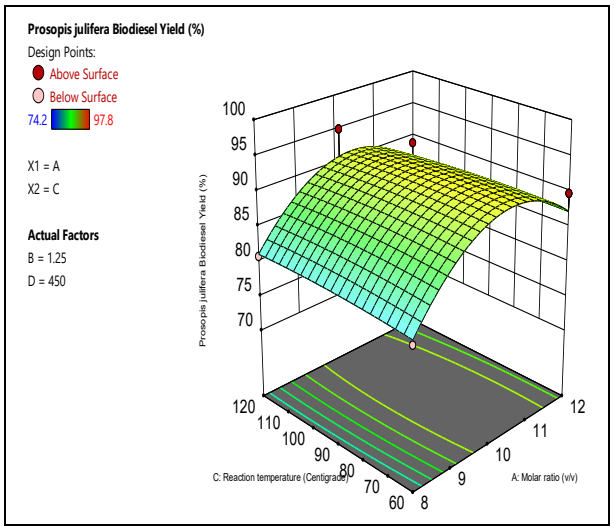


Fig. 5: Methanol to oil molar ratio and reaction temperature variation at constant catalyst concentration

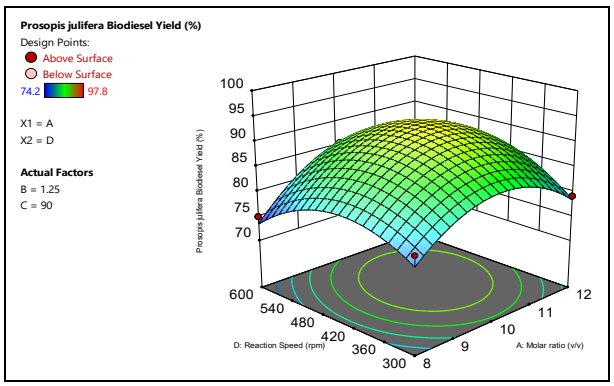


Fig. 6: Reaction speed and methanol to oil molar ratio variation at constant and reaction temperature

Fig. 6 depicts the effect of agitation speed and the methanol-to-oil molar ratio at a constant reaction condition. It is observed that the yield increases slightly with stirring speed from 300 to 450 rpm and then plateaus. However, the methanol-to-oil ratio remains the dominant factor, with higher ratios yielding the highest conversion across all stirring speeds.The surface suggests diminishing returns for rpm beyond the speed 450 to 500 rpm. The stirring speed was maintained at 450 rpm, as further increases do not significantly enhance yield when the methanol-to-oil ratio and temperature are near their optimal values. Similar reasons were also determined (Yadav *et al.* 2025; Khan *et al.* 2019 and Lee *et al*. 2011). This study indicates that the optimum methanol-to-oil ratio is 10:1. The biodiesel yield across experimental runs ranged from 74.2% to 97.8%. The optimal catalyst concentration was found to be in the range of 1–1.5%, consistent with values reported in the literature (Asaad *et al.* 2024; Sánchez-Arreola *et al*. 2019).

The derived *Prosopis juliflora* biodiesel was assessed based on the physio-chemical properties as detailed in Table 5. The base catalysed transesterification process using NaOH-CZO green nano-catalyst reduced the kinematic viscosity from 7.789 mm^2/s to 3.9542 mm^2/s at 40°C which was carried out employing D6079 ASTM method. The specific gravity was reduced by 8.75% from 0.9174 to 0.8657 considerably. The calorific value of *Prosopis juliflora* was significantly increased from 31.78 MJ/kg to 43.89 MJ/kg and it was considered to be an added advantage for replacing it as a substitute for diesel fuel. ASTMD240 method was adopted to estimate the calorific value (Hariram *et al.* 2018). The esterification process substantially reduced the flash and fire point of *Prosopis juliflora* biodiesel to make the fuel parcels to combust within their flammability limits. The cetane number was found to be favourable with 51.9 as it encourages better atomization and combustion phenomenon (Hariram *et al*. 2021; Venkatesan *et al.* 2019). The fuel density was found to be lowered from 935 kg/m^3 to 894 kg/m^3 which supported in atomization of fuel particles and enhancing the premixed combustion phase greatly during the burning of fuel.

3.1 Physio-chemical Properties

Table 5. Physio-chemical properties of *Prosopis juliflora* bio-oil and its biodiesel

	Units	ASTM methods	Diesel (D100)	Biodiesel standards	*Prosopis juliflora* bio-oil	*Prosopis juliflora* biodiesel
Kinematic viscosity @ 40ºC	mm^2/s	D6079	3.03	2.5-6	7.789	3.9542
Specific gravity @ 30ºC		D1298	0.836	-	0.9124	0.8657
Calorific value	MJ/kg	D240	42.3	-	31.78	43.89
Acid value	mg/NaOH	D664	0	0.85	3.98	0.27
Flash point	ºC	D93	50	120	148	127
Cetane number		D4737	46	51	39.4	51.9
Iodine value	g I2/100g	--	1.35	120 max	-	97.8
Density @ 15ºC	kg/m^3	D7052	836	860-900	935	894.1
Fire point	ºC	D93	56	-	161	143
FFA	%	D664	0	-	4.15	0.25

4. CONCLUSION

The present experimental investigation aimed to extract bio-oil from processed seeds of *Prosopis juliflora* and convert it into biodiesel via transesterification. Bio-oil was obtained using Soxhlet extraction with *n*-hexane, yielding a total of 1008 mL over 24 batches. A base-catalyzed transesterification process employing a green CZO nano-biocatalyst, synthesized *via* co-precipitation using cupric sulphate, zinc sulphate, and sodium bicarbonate, was used to reduce kinematic viscosity. Process optimization using the Box–Behnken design under Response Surface Methodology achieved a maximum yield of 97.8% at a methanol-to-oil molar ratio of 10:1, catalyst concentration of 1.75%, reaction temperature of 90 °C, and stirring speed of 450 rpm. The physico-chemical properties of the produced *Prosopis juliflora* biodiesel, including kinematic viscosity, density, cetane number, calorific value, and flash and fire points, were within ASTM standards. It can therefore be concluded that the produced biodiesel is suitable for use in compression ignition engines in blended form with conventional diesel, with minimal or no engine modifications.

DATA AVAILABILITY

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

AUTHORS' CONTRIBUTIONS

All authors have equally contributed to the manuscript.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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