

Investigation of chemical composition and determination physicochemical properties for biodiesel produced from non-edible *Jatropha Curcas* seed oil

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Abstract

Biodiesel is a renewable, biodegradable, environmentally benign, energy efficient and diesel substituent fuel used in diesel engine. It provides a feasible solution to the twin crises of fossil fuel depletion and environmental pollution. It is produced from renewable sources such as vegetable oils or animal fats by transesterification. Although this fuel has gained worldwide recognition for many years, it is not being widely commercialized like petroleum diesel in the world and not widely produced especially in our country Ethiopia. Analysis of fatty acid methyl ester composition in biodiesel prepared from jatropha seed oil (*Jatropha curcas*) was done with the help of GC-MS and 4 major fatty acid methyl esters were identified. These were methyl palmitate, methyl stearate, methyl oleate and methyl linoleate. Major objective of this study was to produce and characterize the biodiesel produced from un-edible jatropha seed oil using NaOH a homogeneous catalyst and to study the variables that affect the amount of biodiesel such as methanol/oil molar ratio, mass weight of catalyst and temperature. In addition to this the chemical composition and the physicochemical properties of the biodiesel were determined and compared with a set of parameter according to European Standard, EN 14214 and American standard test and material, ASTM6751. Results of entire study indicated that chemical composition of the biodiesel product was examined by help of GC-MS and four major fatty acid methyl esters such as methyl palmitate (14.2%), methyl stearate (7.3%), methyl oleate (45.1%) and methyl linoleate (33.4%) were identified. In addition to this the physicochemical properties of the biodiesel such as (density, kinematic viscosity, iodine value, high heating value, flash point, acidic value, saponification value, carbon residue, peroxide value and ester content) were determined and its corresponding values were 886 Kg/m³, 5.94 Mm²/s, 105.42 g I/100g oil, 39.47 MJ/Kg, 135 °C, 0.45 mg KOH/g, 199.76 mg KOH/g, 0.048 %wt, 2.37 meq/kg and 95.34 wt% respectively. The results of the present study showed that all physicochemical properties lie within the ASTM and EN biodiesel standards. Therefore, jatropha seed oil methyl ester could be used as an alternative to diesel engine. Further researches are needed to be conducted to determine the amount of biodiesel produced with heterogeneous catalysts and lipase enzymes.

Key words: Jatropha Seed Oil, Biodiesel, Transesterification, Homogeneous Catalyst, n-hexane, FAME and GC-MS

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1. Introduction

Increasing energy demands, depletion of fossil fuels and environmental pollution make the world under crises now a day's. This is because of many countries worldwide are still heavily dependent on petroleum as their main source of electricity and transportation fuel. The only possible solution to solve this crisis is to find a sustainable (renewable), economically feasible and environmentally friendly source of alternative energy is necessary. There are many alternative energy sources such as hydropower, wind, solar, geothermal and biomass. Biodiesel fuel is a renewable

energy resource which is made from vegetable oils available around us [1-3].

Jatropha curcas is a drought resistant tropical tree and the oil from its seeds is as a fuel substitute [4]. Augustus et al., [5] have reported that *Jatropha curcas* seeds contain around 20-40% oil. Its oil fraction consists of both saturated (14.1% palmitic acid and 6.7% stearic acid) and unsaturated fatty acids (47% oleic acid and 31.6 of linoleic acid).

In addition to this Martinez-Herrera et al., [6] reported that the major fatty acids found in the oil samples

were oleic (41.5-48.8%), linoleic (34.6-44.4%), palmitic (10.5-13.0%) and stearic (2.3-2.8%) acids. This is due to the difference in agro climatic condition such as the nature of the soil grown, amount of rainfall and so on.

The waste or residue of jatropha seed left after oil extraction contains nitrogen, hence it is directly used as fertilizer or as soil improver (or compost) [7].

Biodiesel is mono alkyl esters of fatty acids derived from vegetable oils or animal fats. It is a clean, renewable, biodegradable, environmentally benign, energy efficient and diesel substituent fuel used in diesel engine. It is a carbon neutral fuel because there is no overall increase in CO₂ in the atmosphere due to recycling by the growing plants used to feed the biodiesel industry [7-8]. Emissions of SO₂, SO₃, CO, unburnt hydrocarbons and particulate matter are lower than that of petroleum diesel [8-9]. The most common process used to produce biodiesel is through transesterification, a reaction between triglycerides and an alcohol with a low molecular weight (ethanol or methanol) in the presence of a basic catalyst (NaOH or KOH), to obtain esters and glycerol [10-12]. Transesterification is a three-step reversible reaction of vegetable oils or animal fats with a methanol to form fatty acid methyl esters (FAMES) and glycerol as a final product [13] as shown in figure 1 below.

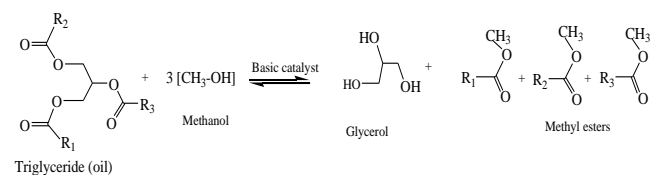


Figure 1: Base catalyzed transesterification processes

The reaction mechanism for the formation of fatty acid methyl esters (FAME) is described as follows (figure 2).

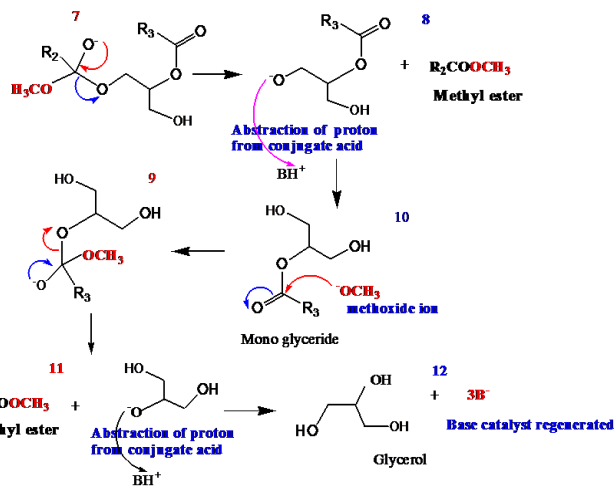
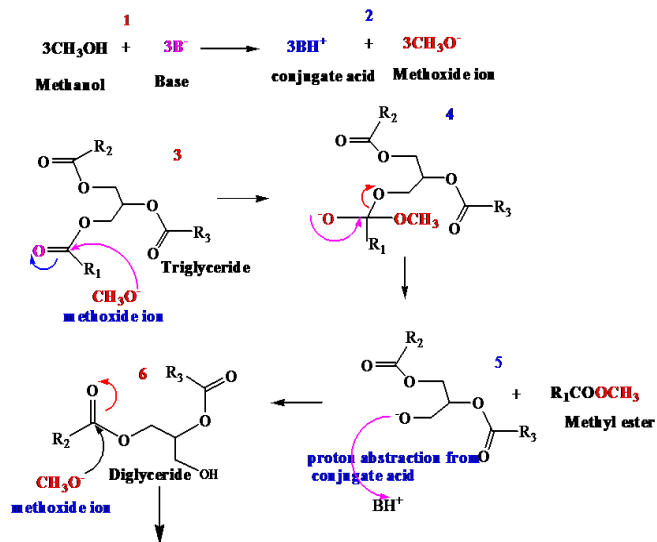


Figure 2: Reaction mechanism for base catalyzed transesterification

2. Materials and Methods

2.1 Chemicals and Reagents Used

Methanol (99%), sodium hydroxide (NaOH), n-hexane, sodium thiosulfate, phenolphthalein, ethanol (96%), anhydrous sodium sulphate and jatropha seed oil.

2.2 Instrumentations

Soxhlet apparatus, rotary evaporator, GC-MS, bomb calorimeter, viscometer, hot plate, thermometer, round bottom flask, separatory funnel and grinder were used.

2.3 Experimental Design

A basic catalysts (NaOH) was used to study at which methanol/oil molar ratio, mass weight of catalyst and temperature an optimum biodiesel is produced from the oils of jatropha seed oil. Finally the physicochemical properties of the biodiesel were determined and compared with a set of parameter according to European Standard, EN 14214 and American standard test and material, ASTM6751.

Table1: Experimental Treatments

Methanol to oil molar ratio	Temperature in °C	%w/w of NaOH to Oil
(T ₁)	(T ₂)	(T ₃)
3:1	45, 55, 60 and 65	0.5
6:1		1
9:1		1.5

Where: T = treatment

2.4 Seed Material Preparation

Undamaged jatropha seeds were collected from adigudem agricultural office, cleaned, de-shelled and oven dried at 105°C for 1 hour to remove the water content. After that the dried jatropha seeds were grounded using grinder

and stored in sealed plastic bag for further use.

2.5 Oil Extraction

Oven dried of jatropha seeds were grounded into powder by using grinder. After that 100g of the sample were loaded in to thimble in Soxhlet apparatus. Next to that extraction was carried out using 500 ml normal hexane at 68°C (boiling temperature of hexane) for 12 hours in an electrical heater. The mixture of the extracted oil and the hexane was separated by vacuum rotary evaporator (RE-52A, 220 V/50Hz) machine and the percentage of the oil was calculated by using the formula:

$$\% \text{ Oil} = \frac{\text{Mass of Oil}}{\text{Sample}} \times 100$$

3. Results and Discussions

Table 2: Physiochemical properties of biodiesel prepared from jatropha seed oil, petroleum diesel and biodiesel standards (ASTM D6751 and EN 14214)

Fuel Properties	Biodiesel	Standards (USA and EU)		Petroleum Diesel	Unit
		ASTM D6751	EN 14214		
Specific Gravity	0.886	0.88	-----	0.845	-----
Density at 15°C	886	-----	860 – 900	845	Kg/m ³
Kinematic Viscosity at 40°C	5.94	1.9 -6.0	3.5 - 5.0	2.86	Mm ² /s
Acid Value	0.45	≤ 0.8	≤ 0.5	-----	mg KOH/g
Saponification Value	199.76	-----	-----	-----	mg KOH/g
Flash Point	135	≥ 120	≥ 130	67.5	°C
Higher Heating Value	39.47	-----	-----	45.5	MJ/Kg
Peroxide Value	2.37	-----	-----	-----	meq/kg
Iodine Value	105.42	-----	≤ 120	-----	g I/100g oil
Carbon Residue	0.048	0.05 max	-----	0.15Max	%wt
Ester Content	95.34	-----	≥ 96.5	-----	-----

The biodiesel characterization shows similarities to that of fossil diesel. The physiochemical properties of the biodiesel were determined. Several tests of the properties of biodiesel such as specific gravity, density, kinematic viscosity, iodine value, high heating value, flash point, acidic value, saponification value, carbon residue, peroxide value and ester content profile were determined and all the result were lie within the ASTM and EN standards except the percentage amount of ester produced (95.37%) which is less by 1.13% from its minimum value (96.5%) in EN standard as shown in Table 2 above.

Kinematic viscosity is a measure of resistance of fluid to flow under the influence of gravity [14]. The result from this work shows a kinematic viscosity of 5.94 which is

quite in agreement with the ASTM biodiesel standard but it is slightly higher than the European Standard and Petroleum diesel. Viscosity of a fuel is related to the fuel lubricity. Low viscosity fuels are unlikely to provide satisfactory lubrication in fuel injection pumps; these often lead to seepage and increase in wear [15]. High viscosity in fuel are responsible for atomization of fuel, incomplete combustion and increased exhaust emissions, choking of the injections thereby forming larger droplets on injector, ring carbonization and accumulation of the fuel in the engine [16].

Specific gravity of the fuel is very important in diesel engine because fuel injection system operates on a volume metering basis. The values of specific gravity obtained for mango seed oil methyl ester was 0.886 with a corresponding density value of 886 kg/m³. This value lies within the ASTM standards and also in close proximity to the findings of other studies. The carbon residue is an indicator of residual carbon after combustion. The carbon residue for this study is measured to be 0.048 %wt which is less than the maximum value of ASTM standard (0.05% wt). Gerpen et al., [14] reported that the major cause of surplus carbon residue in biodiesel is due excessive total glycerin present in it. The low value obtained in this study is due the complete separation and effective removal of glycerol after the transesterification process.

Flash point is the minimum temperature at which a fuel must be heated for it to ignite air-vapor mixture. The U.S. Department of Transportation specified 90°C as the flash point for nonhazardous fuel [17]. The flash point for this work is 135°C. This result shows appreciable consistency with both ASTM, EN standard for biodiesel and works of other researchers. The high value obtained in this study clearly signifies that the biodiesel produced is basically free from methanol; this is because even small quantity of methanol can reduce the flash point reasonably and also negatively affects diesel engine parts such as fuel pumps, seals and elastomers.

The acid value obtained in this work is very small (0.45) to compare with the ASTM and EN standard (≤ 0.8 and ≤ 0.5) respectively and reported work of [18]. Acid value measures directly the free fatty acids content of the methyl ester. It clearly helps to state the corrosive nature of the fuel, its filter clogging tendency and the amount of water that may be likely present in the biodiesel. This parameter can also be used to measure the freshness of the biodiesel. The higher the acid value the lower the quality of the fuel.

Generally from the physiochemical properties of the biodiesel produced from mango seed oil Table 2 above it can be used as diesel substituent fuel.

3.1 Effect of Different Variables in the Amount of Biodiesel Produced

Several variables which affect the yield of FAMES were studied. These are mass weight of catalyst, methanol to oil molar ratio and temperature. Their effect of each variable was shown in Tables 3, 4 and 5 below.

3.2 Effect of Variation of Methanol/Oil Molar Ratio in the Amount of Biodiesel Produced

The amount of methyl ester in transesterification process activity depends on the molar concentrations of methanol to oil (Table 3). Large excess methanol is required to shift the equilibrium favorably during transesterification for better yields of biodiesel [19]. When the methanol/oil molar ratio was increased from 3:1 to 6:1, an increase in methyl ester content was observed but further increasing methanol/oil molar ratio from 6:1 to 9:1 a decrease in methyl esters content was observed. This is because the higher alcohol molar ratio interferes with the separation of glycerol from the biodiesel due to an increase solubility of glycerol in the alcohol (Table 3).

Table 3 Methyl ester content, as function of mass weight of catalyst at methanol/oil molar ratio 3:1, 6:1 and 9:1; reaction temperature 60 °C with reaction time of 3 hours

Methanol/Oil Molar Ratio	Mass Weight of Catalyst, wt.%	Temperature in °C	Methyl Ester Content, w/w%
			Adigudem Agricultural Office
3:1	1%wt NaOH	60	86.85
6:1		60	95.34
9:1		60	87.74

3.3 Effect of Variation Temperature in the Amount of Biodiesel Produced

Temperature plays an important role during biodiesel production; this is because the rate of reaction is strongly influenced by the reaction temperature [20-21]. Table 4 below shows the amount of biodiesel varies with temperature variation from 45°C to 65°C at a catalyst concentration of 1% wt. As the temperature increase from 45°C-60°C, the conversion yields of biodiesel also increases considerably. Further increase in temperature results in decrease in the yield of biodiesel.

Literatures have reported that alkaline transesterification are conducted close to the boiling point of the alcohol used and that temperature higher than this burns the alcohol resulting into lower yield. Patil and Deng [22] reported that alkaline transesterification at temperature above 60°C cause excessive methanol loss due to evaporation and significantly reduce overall biodiesel yield. This is due to the saponification of glycerides by alkali catalyst is much faster than the transesterification reaction above 60°C.

Table 4: Methyl ester content, as function of mass weight of catalyst at methanol/oil molar ratio, 6:1; reaction temperature, 45°C, 55°C, 60°C and 65°C; reaction time of 3 hours

Methanol/Oil Molar Ratio	Mass Weight of Catalyst, wt.%	Temperature in °C	Methyl Ester Conte nt, w/w%
			Adigudem Agricultural Office
6:1	1%wt NaOH	45	90.64
6:1		55	92.78
6:1		60	95.34
6:1		65	87.76

3.4 Effect of Variation of Mass Weight of Catalyst in the Amount of Biodiesel Produced

From the result obtained as shown in table 5 below, as the mass weight of catalyst increase from 0.5 to 1 %wt a progressive increase in percentage conversion in the reaction was achieved and thereafter experienced a decrease in the yield above this concentration (1%wt of NaOH). It was obvious that increase in catalyst concentration beyond 1% wt of NaOH results in a decrease in biodiesel yield. Hence, the yield at 1.5% wt (88.57%) was lower than the yield obtained at 0.5%wt (90.23%). This can be clearly explained by the reversible nature of transesterification reaction. The findings from this studies is very consistent with opinion of Darnoko and Munnir [23], who reported that catalyst concentration greater than 1% wt may have favored the backward reaction, thereby shifting the equilibrium from the right to the left, hence the formation of glycerol. In comparing results of this findings with that of previous researchers such as [24] for *Jatropha Curcas* oil and [14] for Tiger nut oil as biodiesel resource, the result is in accordance with their findings.

Table 5: Methyl ester content, as function of mass weight of catalyst at methanol/oil molar ratio, 6:1; reaction temperature, 60 °C; reaction time of 3 hours

Methanol/Oil Molar Ratio	Mass Weight of Catalyst, wt.%	Temperature in °C	Methyl Ester Content, w/w%
			Adigudem Agricultural Office
6:1	0.5	60	90.23
6:1	1	60	95.34
6:1	1.5	60	88.57

3.5 GC-MS analysis of biodiesel prepared from jatropha seed oil

Based on GC-MS analysis, the FAME of biodiesel prepared from jatropha seed oils collected from adigudem agricultural office, four major compounds were identified as described in detail in Table 3 below.

Table 3. Chemical Composition of FAMES of jatropha seed oil by GC-MS analysis

Fatty Acid Methyl Ester	Position of Double Bond in FAMES	Molecular Mass	Amount (%)
Methyl Palmitate	----	270	14.2
Methyl Linoleate	18:2(n-6), Δ 9,12	294	45.10
Methyl Oleate	18:1(n-6), Δ 9	296	33.4
Methyl Stearate	-----	298	7.3
Saturated Fatty Acid Methyl Esters	-----	----	21.5
Unsaturated Fatty Acid Methyl Esters	-----	----	78.5

3.6 Analysis of Mass Fragmentation of Fatty Acid Methyl Esters Prepared from Jatropha Seed Oil

Based on the GC-MS analysis on chemical composition of the biodiesel produced from jatropha seed oil collected from adigudem agricultural office, four major fatty acid methyl esters such as methyl palmitate, methyl stearate, methyl oleate and methyl linoleate methyl were identified. The molecular ion (parent) peaks of methyl palmitate, methyl linoleate, methyl oleate and methyl stearate were observed at 270, 294, 296 and 298 respectively. The saturated FAMES detected in the biodiesel from jatropha seed oil (methyl palmitate and methyl stearate) show $\text{CH}_3\text{OC}(=\text{OH}^+)\text{CH}_2$ fragment and appears at $m/z = 74$ as the base peak (100%) which is the result of McLafferty rearrangement due to the formation of a six member ring structure of an intermediate.

Methyl linoleate shows $[\text{CH}_2=\text{CHCH}=\text{CHCH}_2]^+$ fragment which appears at $m/z = 67$ as the base peak (100%). Methyl oleate shows $[\text{CH}_2=\text{CHCH}_2\text{CH}_2]^+$ fragment which appears at $m/z = 55$ as the base peak (100%).

The methyl palmitate base peak ion at $m/z = 74$ undergoes McLafferty rearrangement losing the methyl ester which is fragmented between α and β substituted carbons while the ion at $m/z = 87$ is fragmented between C_4 - C_5 also losing methyl ester and a hydrogen atom. An ion with $m/z = 57$ is fragmented between C_3 and C_4 losing a methylene diol and three hydrogen atoms via McLafferty rearrangement. Methyl linoleate's molecular ion occurs at 294 m/z . Both ions at $m/z = 67$ and 81 represent hydrocarbon fragments with general formula $[\text{C}_n\text{H}_{2n-3}]$ losing dialkenes and a hydrogen atom. Methyl oleate's parent peak is observed at $m/z = 296$. The peak at $m/z = 74$ represents the rearranged McLafferty methyl ester fragment while the peak at $m/z = 87$ represents fragmented hydrocarbon ions with general formula $[\text{CH}_3\text{OCO}(\text{CH}_2)_n]$. Methyl stearate's molecular ion occurs at $m/z = 298$. The ion present at $m/z = 74$ corresponds to the McLafferty rearranged methyl ester fragmented between the α and β carbons while the ion at $m/z = 87$ represents loss of methyl ester and a hydrogen atom fragmented between C_3 - C_4 respectively. Molecular ion at $m/z = 67$ and 81 represent hydrocarbon fragments with general formula $[\text{C}_n\text{H}_{2n-3}]$ due to loss of alkenes and a hydrogen atom. Based on environmental pollution issues

[25-37] and energy needs, there is need to use renewable energy sources and mango seed oil found to be efficient for the production of biodiesel.

4. Conclusions

Biodiesel from jatropha seed oil is obtained by transesterification process using sodium hydroxide as catalyst. Optimum amount of methyl ester content (95.34%) was obtained at 60°C with methanol to oil molar ratio 6:1 and at 1%wt of NaOH. The physicochemical properties of the biodiesel were determined and compared ASTM and EN biodiesel standards and fulfill both properties. Therefore, jatropha seed oil methyl ester could be used as an alternative energy resource to diesel engine. In addition to this further researches are needed to be conducted to determine the amount of biodiesel produced with heterogeneous catalysts and lipase enzymes.

References

- [1] N. Kondamudi, S.K. Mohapatra, M. Misra. (2008). Spent coffee grounds as a versatile source of green energy. *Journal of agricultural and food chemistry*. 56(24): 11757-11760.
- [2] S. Lebedevas, A. Vaicekaskas, G. Lebedeva, V. Makareviciene, P. Janulis, K. Kazancev. (2006). Use of waste fats of animal and vegetable origin for the production of biodiesel fuel: quality, motor properties, and emissions of harmful components. *Energy & Fuels*. 20(5): 2274-2280.
- [3] B.A. Nebel, M. Mittelbach. (2006). Biodiesel from extracted fat out of meat and bone meal. *European Journal of Lipid Science and Technology*. 108(5): 398-403.
- [4] G.M. Gübitz, M. Mittelbach, M. Trabi. (1999). Exploitation of the tropical oil seed plant *Jatropha curcas* L. *Bioresource technology*. 67(1): 73-82.
- [5] G. Augustus, M. Jayabalan, G. Seiler. (2002). Evaluation and bioinduction of energy components of *Jatropha curcas*. *Biomass and Bioenergy*. 23(3): 161-164.
- [6] J. Martinez-Herrera, P. Siddhuraju, G. Francis, G. Davila-Ortiz, K. Becker. (2006). Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico. *Food Chemistry*. 96(1): 80-89.
- [7] S. Lebedevas, G. Lebedeva, V. Makareviciene, P. Janulis, E. Sendzikiene. (2008). Usage of fuel mixtures containing ethanol and rapeseed oil methyl esters in a diesel engine. *Energy & Fuels*. 23(1): 217-223.
- [8] F. Ma, M.A. Hanna. (1999). Biodiesel production: a review. *Bioresource technology*. 70(1): 1-15.
- [9] C.R. Coronado, J.A. de Carvalho Jr, J.T. Yoshioka, J.L. Silveira. (2009). Determination of ecological efficiency in internal combustion engines: The use of biodiesel. *Applied Thermal Engineering*. 29(10): 1887-1892.

- [10] G. Knothe, C.A. Sharp, T.W. Ryan. (2006). Exhaust emissions of biodiesel, petrodiesel, neat methyl esters, and alkanes in a new technology engine. *Energy & Fuels*. 20(1): 403-408.
- [11] T. Mata, N. Cardoso, M. Ornelas, S. Neves, N. Caetano. (2010). Sustainable production of biodiesel from tallow, lard and poultry fat and its quality evaluation. *CHEMICAL ENGINEERING*. 19: 3.
- [12] T.M. Mata, N. Cardoso, M. Ornelas, S. Neves, N.S. Caetano. (2011). Evaluation of two purification methods of biodiesel from beef tallow, pork lard, and chicken fat. *Energy & Fuels*. 25(10): 4756-4762.
- [13] S. Morais, T.M. Mata, E. Ferreira In *Life cycle assessment of soybean biodiesel and LPG as automotive fuels in Portugal*, Chemical Engineering Transactions, Vol. 19 [4th International Conference on Safety & Environment in Process Industry-CISAP4, SS Buratti, Ed.], 2010; Italian Association of Chemical Engineering (AIDIC): 2010; pp 267-272.
- [14] M. Umaru, I.A. Mohammed, M. Sadiq, A. Aliyu, B. Suleiman, T. Segun In *Production and characterization of biodiesel from Nigerian mango seed oil*, Proceedings of the World Congress on Engineering, 2014; 2014; p 5.
- [15] S.A. Raja. (2011). Biodiesel production from jatropha oil and its characterization. *Res J Chem Sci*. 1: 81-87.
- [16] Y. Wang, T. Al-Shemmeri, P. Eames, J. McMullan, N. Hewitt, Y. Huang, S. Rezvani. (2006). An experimental investigation of the performance and gaseous exhaust emissions of a diesel engine using blends of a vegetable oil. *Applied Thermal Engineering*. 26(14-15): 1684-1691.
- [17] R. Kenneth, B. McCarl. (2010). Market penetration of biodiesel. *International Journal of Energy and Environment*. 1(1): 53-68.
- [18] C. Enweremadu, O. Alamu. (2010). Development and characterization of biodiesel from shea nut butter. *International Agrophysics*. 24(1): 29-34.
- [19] R. Sree, N.S. Babu, P.S. Prasad, N. Lingaiah. (2009). Transesterification of edible and non-edible oils over basic solid Mg/Zr catalysts. *Fuel Processing Technology*. 90(1): 152-157.
- [20] M. Devanesan, T. Viruthagiri, N. Sugumar. (2007). Transesterification of Jatropha oil using immobilized *Pseudomonas fluorescens*. *African Journal of Biotechnology*. 6(21).
- [21] D. Bajpai, V. Tyagi. (2006). Biodiesel: source, production, composition, properties and its benefits. *Journal of OLEO science*. 55(10): 487-502.
- [22] P.D. Patil, S. Deng. (2009). Optimization of biodiesel production from edible and non-edible vegetable oils. *Fuel*. 88(7): 1302-1306.
- [23] D. Darnoko, M. Cheryan. (2000). Kinetics of palm oil transesterification in a batch reactor. *Journal of the American Oil Chemists' Society*. 77(12): 1263-1267.
- [24] T.T. Kywe, M.M. Oo. (2009). Production of biodiesel from Jatropha oil (*Jatropha curcas*) in pilot plant. *World Academy of Science, Engineering and Technology*. 50: 477-483.
- [25] H.N. Bhatti, Q. Zaman, A. Kausar, S. Noreen, M. Iqbal. (2016). Efficient remediation of Zr (IV) using citrus peel waste biomass: kinetic, equilibrium and thermodynamic studies. *Ecological engineering*. 95: 216-228.
- [26] M.J. Iqbal, F. Cecil, K. Ahmad, M. Iqbal, M. Mushtaq, M. Naeem, T. Bokhari. (2013). Kinetic Study of Cr (III) and Cr (VI) Biosorption Using *Rosa damascena* Phytomass: A Rose Waste Biomass. *Asian Journal of Chemistry*. 25(4).
- [27] M. Iqbal. (2016). *Vicia faba* bioassay for environmental toxicity monitoring: a review. *Chemosphere*. 144: 785-802.
- [28] M. Iqbal, M. Abbas, M. Arshad, T. Hussain, A. Ullah Khan, N. Masood, M. Asif Tahir, S. Makhdoom Hussain, T. Hussain Bokhari, R. Ahmad Khera. (2015). Short Communication Gamma Radiation Treatment for Reducing Cytotoxicity and Mutagenicity in Industrial Wastewater. *Polish Journal of Environmental Studies*. 24(6).
- [29] M. Iqbal, I.A. Bhatti. (2015). Gamma radiation/H₂O₂ treatment of a nonylphenol ethoxylates: degradation, cytotoxicity, and mutagenicity evaluation. *Journal of hazardous materials*. 299: 351-360.
- [30] M. Iqbal, N. Iqbal, I.A. Bhatti, N. Ahmad, M. Zahid. (2016). Response surface methodology application in optimization of cadmium adsorption by shoe waste: a good option of waste mitigation by waste. *Ecological engineering*. 88: 265-275.
- [31] M. Iqbal, J. Nisar. (2015). Cytotoxicity and mutagenicity evaluation of gamma radiation and hydrogen peroxide treated textile effluents using bioassays. *Journal of Environmental Chemical Engineering*. 3(3): 1912-1917.
- [32] M. Iqbal, J. Nisar, M. Adil, M. Abbas, M. Riaz, M.A. Tahir, M. Younus, M. Shahid. (2017). Mutagenicity and cytotoxicity evaluation of photocatalytically treated petroleum refinery wastewater using an array of bioassays. *Chemosphere*. 168: 590-598.
- [33] Q. Manzoor, R. Nadeem, M. Iqbal, R. Saeed, T.M. Ansari. (2013). Organic acids pretreatment effect on *Rosa bourbonia* phyto-biomass for removal of Pb (II) and Cu (II) from aqueous media. *Bioresource technology*. 132: 446-452.
- [34] M. Mushtaq, H.N. Bhatti, M. Iqbal, S. Noreen. (2016). *Eriobotrya japonica* seed biocomposite efficiency for copper adsorption: isotherms, kinetics, thermodynamic and desorption studies. *Journal of environmental management*. 176: 21-33.
- [35] R. Nadeem, Q. Manzoor, M. Iqbal, J. Nisar. (2016). Biosorption of Pb (II) onto immobilized and native *Mangifera indica* waste biomass. *Journal of Industrial and Engineering Chemistry*. 35: 185-194.
- [36] J. Nisar, M. Sayed, F.U. Khan, H.M. Khan, M. Iqbal, R.A. Khan, M. Anas. (2016). Gamma-irradiation induced degradation of diclofenac in

aqueous solution: kinetics, role of reactive species and influence of natural water parameters. *Journal of Environmental Chemical Engineering*. 4(2): 2573-2584.

[37] A. Rashid, H.N. Bhatti, M. Iqbal, S. Noreen. (2016). Fungal biomass composite with bentonite efficiency for nickel and zinc adsorption: a mechanistic study. *Ecological engineering*. 91: 459-471.