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Studies on application of fish waste for synthesis of high quality biodiesel

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Studies on application of fish waste for synthesis of high quality biodiesel

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Abstract

A low cost raw material obtained from the discarded parts of fish (*Cirrhinus mrigala*, *Cirrhinus cirrhosa*, *Cirrhinus reba*) was utilized as feedstock oil and catalyst for the synthesis of biodiesel. Esterification followed by transesterification was carried out for the synthesis of biodiesel from waste fish oil. The discarded parts of fish after extraction of oil that included fins, tails and bones were used as a low cost heterogeneous catalyst for the synthesis of biodiesel. The catalyst characterized by thermo-gravimetric analysis and X-ray diffraction analysis showed the calcination temperature required and the phase of the catalyst respectively. The HAP (hydroxyapatite) present in the waste parts of fish got converted into β-tri-calcium phosphate when calcined at 900 °C for 2 h. The catalyst, β-tri-calcium phosphate was studied for its morphology and porous structure by Scanning Electron Microscopy. A moderate experimental condition (1:6.5 molar ratio of oil:methanol, 1.5 wt % of heterogeneous catalyst with respect to oil) was taken for synthesis of biodiesel from waste fish oil. Biodiesel characterized by proton NMR showed a high conversion of waste fish oil to biodiesel (i.e. > 96%). The yield of biodiesel determined by gravimetric method was > 95%. The heterogeneous catalyst, β-tri-calcium phosphate was reused up to five times without significant loss in its activity.

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Keywords: waste fish oil, transesterification, HAP (hydroxyapatite), β-Tricalcium phosphate, biodiesel

1. Introduction

Petrochemical sources, coal and natural gases are finite and at current usage rates will be consumed shortly[1]. The indiscriminate use of fossil resources, coupled with their high cost, unsustainability and their impact on global warming and pollution has led to exploration of renewable sources of energy[2]. The fossil fuels are being discovered at various locations worldwide that enhance its reserves. Among the prominent ones is shale gas reserve in USA. Owing to shale gas discoveries, the estimated reserves of natural gas in the United States in 2008 was 35% higher than in 2006[3]. However, the shale gas potential in other countries is limited. For example, Britain also tried to emulate the USA by exploration of shale gas but was largely unsuccessful and a study reported that there was insufficient potential in fracking (hydraulic fracturing of shale rocks) of the gas[4]. Although, hydraulic fracking produces a significant amount of natural gas, it comes at the cost of numerous environmental effects, mankind safety and health hazards. The waste fluid originating from the exploration of shale gas emits harmful volatile organic compounds that contaminate the air, make the rainwater acidic, and enhance the ground level ozone[3].

The usage of fossil fuels has negative environmental effects such as localized air pollution and climate change on a global scale. In addition, generation and distribution of fossil fuels is greatly influenced by political and economic issues because of the limited locations of reserves and the finite quantity of oil. These drawbacks have driven the need to develop alternative energy resources. Biodiesel has many advantages over conventional petroleum

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derived diesel fuel. Hence, utilizing the renewable sources of energy and to find the alternative sources of energy has become necessary to fulfill the growing global energy demands. Among many sources of renewable energy that play vital role in partially replacing conventional fossil fuel, biodiesel has become increasingly important in catering the global fuel market. Biodiesel being an alternative to diesel fuel has increased worldwide public interest in a number of countries. The feedstock for biodiesel includes the edible and non-edible oils. Biodiesel is non-toxic and biodegradable and produces less harmful substances[5-7]. Biodiesel emits less CO, SO_x, particulate matter and unburned hydrocarbons as compared to mineral diesel[8]. Biodiesel has a relatively high flash point near to 150 ^oC, thus making it less volatile, and therefore safer to transport and handle as compared with petroleum diesel. It can be used directly in diesel engines or as a blend with fossil diesel fuel (called "B7" if this blend contains 7% biodiesel) and provides additional lubrication that can extend engine life.

Biofuel synthesized from biomass requires large land area for the feedstock cultivation; and harvesting. Transportation and pretreatment of biomass are energy consuming, contributing to significant emissions. Also, because of the large land areas required for their feedstock cultivation, especially as one envisions scaling up, there is concern about the impact of biofuels on the food supply chain, the biodiversity loss, and the carbon stock losses from soil because of land-use changes[9]. The low-cost feedstock for biodiesel have increasingly drawn interest, such as waste frying oils and animal fat obtained from by-products of the meat and fish processing industries that cannot be used for human food purposes[10-12]. It is estimated that fish production in India is set to cross 13 million tons (MT) mark by 2016 from the current level of over nine million tons, according to a study by the an industry body Associated Chambers of Commerce and Industry of India (ASSOCHAM). The overfishing of the oceans and the impact of

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aquaculture in marine ecology may become an issue that need to be addressed. The indiscriminate fishing may not only pose danger to the sustainability of aquatic ecosystem, it will also generate colossal waste of the discarded inedible parts of fish. This part of fish waste may be diverted for the generation of oil that could be utilized for several purposes. These include higher value creating applications viz. poly unsaturated fatty acid rich oils that has health benefits. Apart from this, the surplus fats obtained from the waste parts of the edible fish could be utilized for synthesis of biodiesel. The most broadly established industrial technologies for biodiesel manufacturing are continuous alkali catalyzed transesterification at low pressure (2-4 bar, 60 - 90°C calling for refined oils with low FFA) or at high pressure (90 bar, 240 °C) using crude triacylglycerides (TAGs)[13-14]. Oils with high FFA such as soap stock, used frying oils or recycled greases, can be esterified prior to transesterification by an "integrated" process[15]. A broad number of heterogeneous catalysts have been investigated by researchers that includes zeolites, clays, heterogenized guanidines, aluminum orthophosphate, ion-exchange resins and pure or mixed oxides; among others[16]. Chakraborty et al., (2011)[17] utilized the scale of Rohu fish (*Labeo rohita*) for preparation of a heterogeneous catalyst. The fish scale that contains hydroxyapatite was converted to β-tri-calcium phosphate upon calcination at temperature above 900° C for 2 h. In the present work, fat was extracted from the discarded portion of fish (*Cirrhinus mrigala*, *Cirrhinus cirrhosa*, *Cirrhinus reba*) that included all the residues (viscera, eyes, fins, tails and maw) left after separating the edible part of the fish. After extraction of oil, the same residual portion of fish along with bones upon calcination was utilized to synthesize heterogeneous catalyst[18].

2. Materials

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2.1. Waste fish oil extraction

The discarded parts (viscera, eyes, fins, tails and maw) of fish species *Cirrhinus mrigala*, *Cirrhinus cirrhosa*, *Cirrhinus reba* were collected from the local fish market from Varanasi, India. At first, pre-treatment processes were done by absorption of the fish residue by active clay. The waste parts of fish were then washed with hot distilled water to remove blood and other solid impurities viz. gelatin matter. Thereafter, the waste fish parts were dried at 102 ^{0}C for 40 min to remove water content. The dried matter was crushed to smaller pieces. Waste fish oil was expelled from the dried matter using a mechanical expeller followed by solvent extraction using n-hexane. After extraction of oil, the dried matter still contained some residual oil that was removed by adding petroleum ether as a solvent. The dried matter after treatment with petroleum ether was used to synthesize heterogeneous catalyst. The insoluble impurities present in the waste fish oil were determined by weighing the solid residue after it got settled at bottom of the container.

All the chemicals used were of analytical reagent (AR) grade. Methanol (>99% purity) was purchased from Merck (India).

3.2. Catalyst Preparation

After extraction of oil from the discarded parts of fish, the dry residual matter included fins, tails and bones. This dry matter was washed thoroughly with hot distilled water several times to remove gelatinous matter, after which it was subjected to drying in hot air oven at 100 ^{0}C for about 3 h. The dried matter was calcined in a muffle furnace at varying temperature ranging from 400 ^{0}C to 1000 ^{0}C for 2 h and ground to fine powder. Characterization of dry matter was carried out through thermo-gravimetric analysis (TGA) over temperature range from

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27 ^{0}C (room temperature) to 1000 ^{0}C and by X-ray diffraction (XRD) pattern. The surface morphology of the catalyst was observed using scanning electron microscope (SEM).

3. Experimental Method

3.1. Acid-catalyzed esterification

The acid value of the waste fish oil was 11.89 mg KOH/g oil which was higher than the maximum acid value of 4 mg KOH/g oil for transesterification as specified in literature (Sharma et al., 2008). Due to high acid value of waste fish oil, acid esterification of waste fish oil was performed to make the feedstock suitable for base transesterification. Conversion of free fatty acids into corresponding fatty acid methyl esters (biodiesel) with methanol was investigated in presence of sulfuric acid as a catalyst. The esterification reaction were carried out using a 3-necked 500 mL round bottom flask fitted with a stirrer, a thermometer and a reflux condenser with the round bottom flask immersed in a constant-temperature water bath. The accuracy of the temperature measurement was ± 0.5 ^{0}C. 100 ml of the waste fish oil was taken for esterification followed by addition of methanol and H_2SO_4 as catalyst. The reaction mixture in the reactor contained methanol to oil molar ratio from 3:1 to 9:1 evaluated in this study. Optimum methanol to oil molar ratio was 6:1 and 1.0 wt % H_2SO_4 in 120 min reaction time at 55 ^{0}C.

3.2. Base-catalyzed transesterification

After esterification, the acid value of waste fish oil got lowered to 2.89 mg KOH/g. Thereafter, transesterification reaction was carried out for synthesis of biodiesel. All the transesterification reactions were carried out for 2 h. The variables that affect the conversion efficiency of transesterification reactions are reaction time, molar ratio of alcohol: oil and the amount of catalyst. Moderate experimental condition {1:6.5 molar ratio of oil: methanol, 1.5 wt

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% of catalyst (β-Tri-calcium Phosphate) with respect to oil} was taken for synthesis of biodiesel from waste fish oil. 1.5 wt % of catalyst (β-Tri-calcium Phosphate) was added to this mixture to start the reaction. The mixture was stirred at a speed of 900 rpm and at 55 ^{0}C for 2 h duration. At the completion of reaction, the products of the reaction were allowed to settle overnight in a separating funnel that resulted in formation of three distinct phases (methyl ester on top, glycerol in the middle layer, and catalyst phase at the bottom). Glycerol was removed by decantation. Catalyst was collected (and reused for the further experiments) by filtration and methanol was evaporated in vacuum rotavapor.

4. Results and Discussion

Characterization of catalyst

Table 1 depicts the properties of the waste fish oil. The waste fish oil was brown in color with typical smell. The kinematic viscosity of the fish oil was quite high (25.51 mm^2/s at 40 ^{o}C). The waste fish oil also possessed insoluble impurities amounting to 0.0305 wt%.

4.1. DGA/DTA analysis of dry matter

Fig.1 depicts the thermo-gravimetric analysis (TGA) plot for the annealing of the dried matter which is left after extraction of waste fish oil. Thermo-gravimetric analysis (TGA) was run at 20 ^{0}C per min over a temperature range from 27 ^{0}C (room temperature) to 1000 ^{0}C. TGA curve showed a total weight loss of about 43.48 %. To observe each step in the process, the first derivative of this curve has been examined (Fig.1, curve TGA), first weight loss occurred at $T < 200$ ^{0}C, corresponding to the release of the adsorbed water. Higher weight losses can be seen for 200 ^{0}C < T < 500 ^{0}C; a large peak with a smaller shoulder is present, the first being related to the release of organic matter while the smaller shoulder corresponds to the release of more

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residual organics and of water present in the lattice structure. Comparing this curve with the DTA spectrum (Fig.1, curve DTA) it can be seen that two exothermic peaks correspond to these two weight losses. A smaller endothermic heat exchange is observed for $T > 800\ ^0C$, which is probably associated with lattice rearrangements and apatite crystallization

4.2. X-ray diffraction patterns (XRD)

Fig. 2 depicts the powder X-ray diffraction patterns of calcined powder over a temperature range from 400^0C-1000^0C. These diffraction patterns show a gradual increase in the degree of sharpness of peaks with increasing heat treatment temperature. The calcined powder patterns were collected in the angular range 10^o-80^o, with scanning rate of 0.4 θ/s. Presence of β-tri-calcium phosphate and hydroxyapatite (naturally occurring mineral form of calcium apatite with the formula $Ca_5(PO_4)_3$, usually written $Ca_{10}\ (PO_4)_6(OH)_2$ to denote that the crystal unit cell comprises two entities) of calcined powder were understood by indexing of the diffraction peaks using a standard JCPDS files for both β-tri-calcium phosphate and hydroxyapatite are (09-0169), (09-0432) and intense peaks indicates the small portion of HAP present. Narrow and highly intense peaks of the calcined powder attributed to the highly crystalline structure of the developed catalyst (β-tri-calcium phosphate). Intensification of the XRD peaks upon calcinations at above 800 ^0C shows reflections of prominent peaks of β-Tri-calcium phosphate and the prominent peaks values according to JCPDF file (09-0169) are c.a. 31.02, c.a.39.8, c.a. 34.3, c.a. 46.9, c.a. 50.3, c.a.51.4. While, for hydroxyapatite, prominent peak values according to JCPDF file (09-0432) are c.a. 28.9, c.a.32.9, c.a. 51.3. Equation 1[19] shows the conversion of hydroxyapatite to β-tri-calcium phosphate at temperature above 800 ^0C.

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$$\mathbf{Ca_{10-X}(HPO_4)_X(OH)_{2-X} \longrightarrow (1-X)Ca_{10}(PO)_6(OH)_2 + 3X\beta\text{-}Ca_3(PO4)_2 + XH_2O} \quad (1)$$

The retention of active β-$Ca_3(PO_4)_2$ crystalline phase over a calcined range from 800^{0}C to1000^{0}C is indicative of thermal stability of the developed catalyst hence, the XRD results supplement with the TGA in confirming the bulk structural stability i.e., β-Tri-calcium Phosphate.

4.3. SEM analysis of catalyst

Fig.3 shows the images of scanning electron microscope (SEM). All SEM images were taken after calcinations of developed catalyst at 900^{0}C. Effect of calcination from 800^{0}C to 1000^{0}C was required not only to evaporate protein and fats (that caused catalyst to be whitened) present in the catalyst but also a small increase in porous structure of developed catalyst.

The catalyst (β-Tri-calcium Phosphate) particles are porous and distributed with hydroxyapatite particles (small portion present in the developed catalyst). The possible mechanism of β-tri-calcium phosphate in transesterification has been demonstrated by scientific workers[17]. Due to basic nature of β-tri-calcium phosphate, it will abstract a proton from methanol and asr reaction proceeds, after formation of biodiesel, catalyst can be recovered for further use. The mechanism is similar to that of a conventional base catalyst. In the process, the surface O^{2-} extracts H^+ from methanol to form methoxide anion. The methoxide anion attacks the carbonyl carbon of the triglyceride molecule to form a terrahedral intermediate. A rearrangement of tetrahedral intermediate results in the formation of diglyceride and a methyl ester. Subsequently, diglyceride upon further reaction forms monoglyceride and methyl ester. Lastly, monoglyceride forms methyl ester and glycerol. And in the process the catalyst is regenerated[17].

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Discarded parts of fish utilization for energy production purpose could be important for economical and also for environmental aspects. This study was initiated to evaluate and optimize the conversion of waste fish oil into methyl ester known as biodiesel. The physical and chemical characteristics of these esters were much closer to those of diesel fuel than those of fresh vegetable oil or fat, which makes them a good substitute for diesel fuel. Experiments have been performed to determine the optimum conditions for this conversion process using a three factor factorial design for producing biodiesel. The major variables in the transesterification process are determined from the pre-experiments as: reaction temperature, molar ratio of alcohol/oil, alcohol type utilized and catalyst type.

4.4. Characterization of biodiesel

Proton NMR analysis

The conversion of oil to fatty acid methyl ester (biodiesel) was quantified according to the Proton NMR signal of methoxy group because proton NMR is a strong evidence to quantify the content of biodiesel since ^1H (Proton) is the most naturally abundant and most sensitive NMR active isotope. Fig.4 shows the Proton NMR spectrum of biodiesel synthesized from waste fish oil. The conversion of the waste fish oil to methyl esters (biodiesel) was calculated by the ratio of integrated signals at 3.6 ppm (methoxy groups of the methyl esters written as A_{ME}) and 2.30 ppm (methylene groups of all fatty acid derivatives written as A_{CH2}).

$$C=100^* (2\ A_{ME})/\ 3A_{CH2} \qquad (2)$$

The conversion of biodiesel was equated using equation 2 and was found to be 96.47%.

Table 2 depicts the properties of the biodiesel. Biodiesel was found to fulfill the specification of ASTM D6751 for water content, kinematic viscosity and flash point. However, the density of

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biodiesel obtained was lower than the minimum limit specified by ASTM D6751 limits. The cloud point of the fuel was observed to be 1 ^{o}C which indicates that it will be suitable even in moderate cold climatic conditions.

4.5. Optimization of transesterification reaction

Effect of calcination temperature on the yield of biodiesel

The optimization of reaction was done at varying conditions (molar ratio and catalyst amount) using catalyst calcined at different temperature (600, 800, 900 ^{o}C). The reaction temperature of was kept constant at 55 ^{0}C as a higher temperature decreases the time required to reach maximum conversion. The reaction was run for 2 h for the conversion of waste fish oil into biodiesel. At calcination temperature of 600 ^{o}C, the maximum conversion obtained was 30%. The low conversion could be attributed to presence of both HAP and β-Tri-calcium hydroxyapatite in the catalyst. The constituent in the catalyst calcined at this temperature has HAP in slightly higher concentration than β-Tri-calcium hydroxyapatite. The catalyst calcined at a higher temperature (800 ^{o}C) showed an increase in the conversion of waste fish oil to a large extent. The conversion obtained at various molar ratio and catalyst amount ranged from 73.63 % to 88.89 %. The lowest obtained conversion i.e. 73.63 % was obtained using 1.0 wt % of catalyst and 5.5:1 methanol to oil molar ratio. The reaction condition at highest obtained conversion (88.89 %) was 1.5 wt % catalyst amount and 6.5:1 methanol to oil molar ratio. The reaction conditions have been depicted in Table S_1 and Table S_2 (Supplementary Material).

Using catalyst calcined at 900 ^{o}C, a further significant enhancement in the conversion of waste fish oil to biodiesel was observed. With the same reaction conditions that resulted in highest conversion with catalyst calcined at 800 ^{o}C (i.e. 1.5 wt % catalyst amount, and 6.5:1 methanol to

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oil molar ratio at 55 °C in 2 h reaction time), highest conversion (96.47%) of waste fish oil to biodiesel was observed. The optimized reaction condition is shown in Table S_3 (Supplementary Material). The European Norms (EN 14214) states that the product after transesterification of triglycerides will be called as biodiesel only if the fatty acid alkyl ester content is at least 96.5%[20] . The high conversion (96.47 %) which is almost near to the International Specification of EN confirms the biodiesel to meet specification to be utilized as a fuel. Transesterification reaction using a homogeneous catalyst (viz. NaOH/KOH) requires just a small amount of catalyst (0.5 − 1% with respect to oil weight)[21]. However, when heterogeneous catalysts are used for synthesis of biodiesel, a comparatively higher catalyst amount has been reported to be needed for transesterification[20]. The catalyst amount most frequently reported by most of the researchers using CaO or MgO as heterogeneous catalyst in synthesis of biodiesel ranges from 2.5 to 3.0 wt% (with respect to oil weight). Few researchers have reported even a higher dose of catalyst (up to 10 wt% of CaO with respect to oil) amount required for synthesis of biodiesel. Using hydrotalcite and hydrotalcite doped compounds as heterogeneous catalyst, researchers have reported catalyst amount to range from 1 wt% to 7.0 wt% with respect to oil weight[20]. The molar ratio (methanol to oil) reported by researchers using heterogeneous catalyst (CaO) ranges from 6:1 to 15:1. Using supercritical condition, the molar ratio requirement reported is much higher (41:1). In the present work, the reaction conditions are quite moderate (methanol to oil molar ratio, 6.5:1; catalyst amount 1.5 wt% with respect to oil, reaction temperature, 55 °C; and reaction time 2 h) and comparable to that reported by researchers using heterogeneous catalysts. The advantage of heterogeneous catalyst over the conventional homogeneous catalyst is the reusability of the former. The yield of biodiesel was also determined by gravimetric method that showed a high value of greater than 95%.

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4.6. Catalyst reusability

Reusability of the developed catalyst (β-Tri-calcium Phosphate calcined at 900^0C) was tested by carrying out transesterification reaction with the used catalyst. Retention of catalytic activity was found to be chemically stable up to five times in transesterification reaction. The catalytic activity of a catalyst can be reduced after five times by the adsorption of water. Equation 3 shows adsorption of water molecules on β-Tri-calcium Phosphate

$$4Ca_3(PO_4)_2 + 2H_2O \longrightarrow Ca_{10}(PO4)_6(OH)_2 + 2\,CaHPO_4 \qquad (3)$$

4.7. Cost of biodiesel

The feedstock contributes to the majority of the cost of biodiesel which is reported to be up to 80 % of the total cost of biodiesel[22-23]. The present study deals with the usage of waste fish oil that has the potential to significantly lower the production cost of biodiesel. The pretreatment of the discarded fish residue via water washing will not add significantly to the cost of biodiesel production. The catalyst too is obtained from the same residual part of the fish. The synthesis of the catalyst by calcination will add to the cost of production of biodiesel. However, as the catalyst being heterogeneous, it will compensate for the cost incurred during calcination of the catalyst. The detailed cost aspect of biodiesel so obtained is in progress and will be reported in the next coming manuscript.

5. Conclusion

An increasing amount of waste has become a second generation energy resource. Fish waste derived oil was successfully converted into biodiesel. Synthesis of biodiesel using waste fish oil

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and developed catalyst (β-Tri-calcium Phosphate) were potential to generate relatively inexpensive biodiesel because both oil and catalyst were taken from the waste i.e., discarded parts of fish. Esterification followed by transesterification was carried out that resulted in high yield and purity of biodiesel. Developed catalyst was thermally stable at 900 ^{0}C. The heterogeneous catalyst (β-Tri-calcium Phosphate) was reused up to five times without loss of its efficiency. When used for more than five times, a small decrease in yield and conversion of oil to biodiesel was observed because of adsorption of water molecule on the surface of catalyst. Proton NMR was chosen as the primary analytical method for conversion of waste fish oil into biodiesel. The peak observed in Proton NMR spectra showed no traces of triglycerides that was confirmation for the formation of biodiesel of high quality.

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References

1. Hansen, A.C., Zhang, Q., Lyne, P.W.L., 2005. Ethanol-diesel fuel blends - a review. Bioresour. Technol. 96, 277–285.
2. Janaun, J., Ellish, N., 2010. Perspectives on biodiesel as a sustainable fuel, Renew. Sustain. Energy Rev. 14, 1312–1320.
3. Knothe, G., Van Gerpen, J.H., 2009. The Biodiesel Handbook, 2nd ed.; AOCS Publishing: Urbana.
4. Sharma, Y.C., Singh, B., Korstad, J., 2011. Latest developments on application of heterogeneous basic catalysts for an efficient and eco friendly synthesis of biodiesel: A review. Fuel. 90, 1309-1324.
5. Srivastava, A., Prasad, R., 2000. Triglycerides-based diesel fuels. Renew. Sustain. Energy Rev. 4, 111–33.
6. Sharma, Y.C., Singh, B., Upadhyay, S.N., 2009. Response to the comments on ‘‘Advancements in development and characterization of biodiesel: A review”. Sharma, Y.C., Singh, B., Upadhyay S.N. Fuel 2008;87:2355–73 by Clifford Jones. Fuel. 88, 768-769.
7. Macalister, T., 2014. No shale gas potential in weald basin, concludes British geological survey. The Guardian. 23 May 2014.

RSC Advances Accepted Manuscript

8. Semwal, S., Arora, A.K., Badoni, R.P., Tuli, D.K., 2011. Biodiesel production using heterogeneous catalysts. Bioresour. Technol. 102, 2151–2161.
9. Dinjus, E., Arnold, U., Dahmen, N., Höfer, R., Wach, W., 2009. Green Fuels – Sustainable Solutions for Transportation, in R. Höfer, ed., Sustainable Solutions for Modern Economies. RSC Publ., Cambridge, 125-163.
10. Demirbas A., 2009. Biodegradability of biodiesel and petrodiesel fuels Energy Sour, Parts A: Recov Utiliza Environ Effects, 31, 169–174.
11. Yee, K.F., Tan, K.T., Abdullah, A.Z., Lee, K.T., 2009. Life cycle assessment of palm biodiesel: revealing facts and benefits for sustainability Appl Energy. 86, 189–196.
12. Breucker, C., Jordan, V., Nitsche M., Gutsche, B., 1995. Oleochemie - Chemieprodukte auf der Basis nachwachsender Rohstoffe. Chem. Ing. Tech. 67, S. 430-440.
13. Fritsche, U. R., Sims, R.E.H., Monti, A., 2010. Direct and indirect land-use competition issues for energy crops and their sustainable production-An overview. Biofuels, Bioprod. Biorefin. 4, 692-704.
14. Encinar, J.M.; Gonzales, J.F.; Rodriquez-Reinares, A. 2007. Ethanolysis of used frying oil. Biodiesel preparation and characterization Fuel Process. Technol. 88, 513-522.
15. Horel, A., Schiewer, S., 2011. Influence of constant and fluctuating temperature on biodegradation rates of fish biodiesel blends contaminating Alaskan sand. Chemosphere. 83, 652-660.
16. Sabudak, T., Yildig. M., 2010. Biodiesel production from waste frying oils and its quality control. Waste Manage. 30, 799-803.
17. Araujo, B.Q., Nunes, R.C.R., Moura, E.M., Cito, A.M.G.L., Santos Junior, J.R. 2010. Synthesis and characterization of beef tallow biodiesel. Energy Fuel. 24, 4476-4480.
18. Sunarso, Noor, A.F.M., Kasim, S.R., Othman, R., Ana, I.D., Ishikawa, K., 2013. Synthesis of Biphasic Calcium Phosphate by Hydrothermal Route and Conversion to Porous Sintered Scaffold. J Biomaterials and Nanobiotechnology, 4, 273-278.
19. Chakraborty, R., Bepari, S., Banerjee, A., 2011. Application of calcined waste fish (*Labeo rohita*) scale as low-cost heterogeneous catalyst for biodiesel synthesis. Bioresour. Technol. 102, 3610–3618.
20. Demirbas, A., Importance of biodiesel as transportation fuel. 2007. Energy Policy. 35, 4661–4670.
21. Singh, Bhaskar, Korstad, John, Sharma, Yogesh C., 2012. A critical review on corrosion of compression ignition (C.I.) engine parts and its inhibition by biodiesel and biodiesel blends, Renewable and Sustainable Energy Reviews 16, 3401– 3408.
22. Nair, P., Sharma,Y.C., Singh, B., Upadhyay,S.N.,2012. Synthesis of biodiesel from waste frying oil using calcium oxide derived from *Mereterix mereterix* as a heterogeneous catalyst, Journal of Cleaner Production, 29-30,82-90.
23. Sharma, Y.C., Singh, B., Upadhyay, S.N., 2008. Advancements in development and characterization of biodiesel: A review. Fuel. 87, 2355-2373.

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Captions for figures:

Fig.1 Thermo-gravimetric analysis (TGA) of dry matter of fish discarded parts

Fig. 2 XRD Diffractogram of (a) β-Tri-calcium Phosphate (b) hydroxyapatite

Fig. 3 Surface configuration (porosity) of the β-Tri-calcium Phosphate by SEM

Fig.4 Proton NMR spectrum of biodiesel

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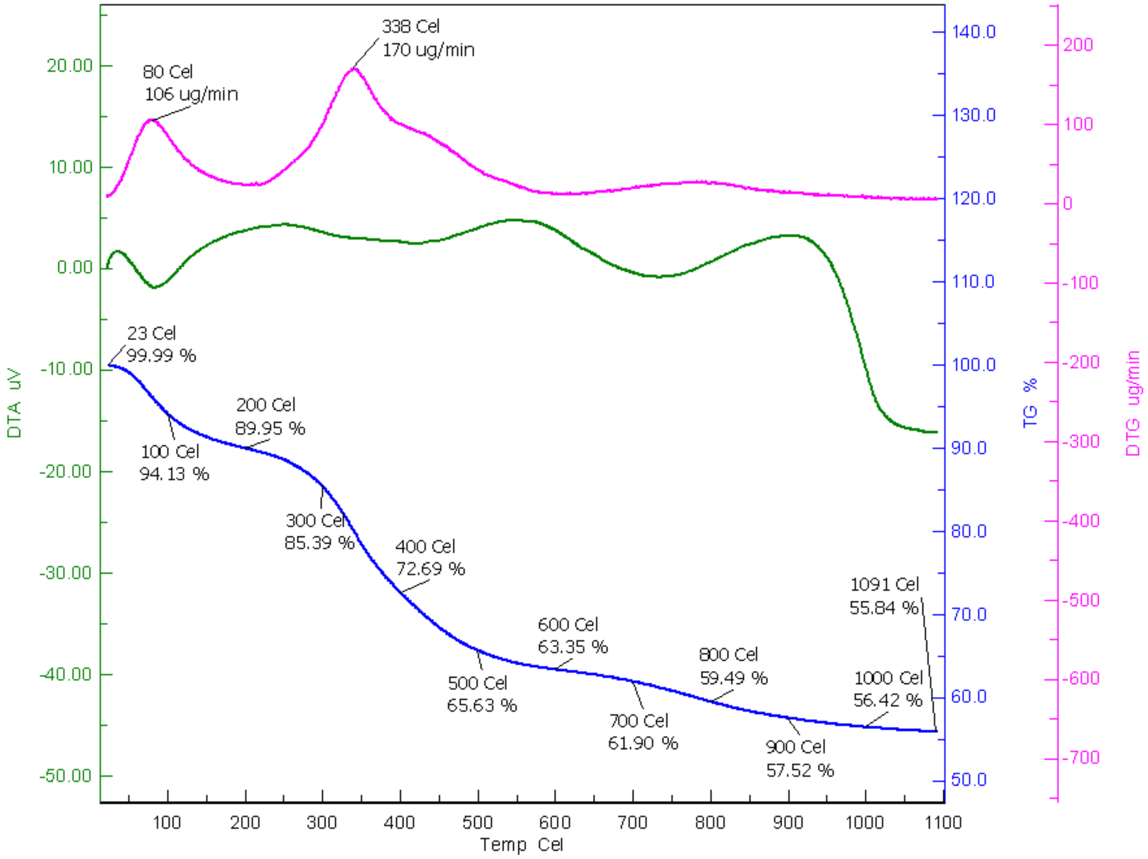

Fig.1

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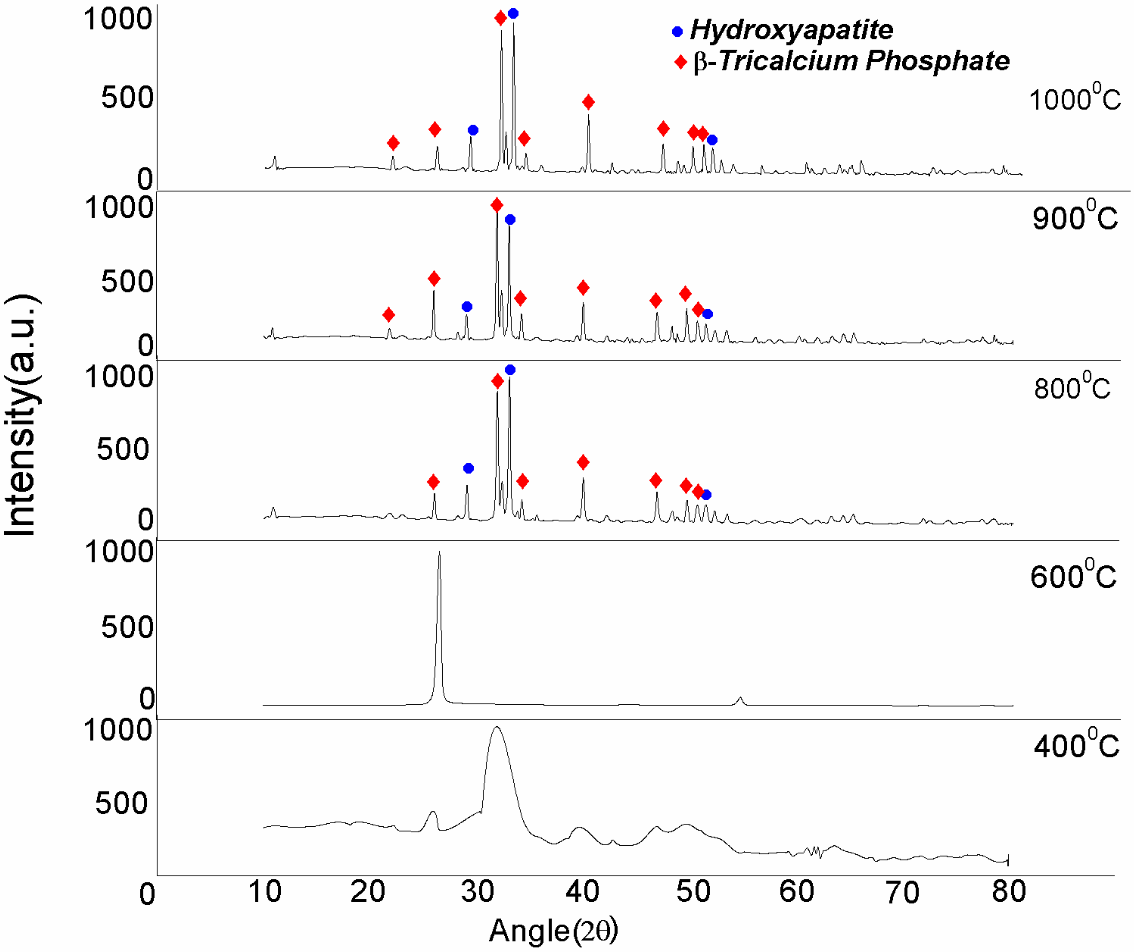

Fig. 2

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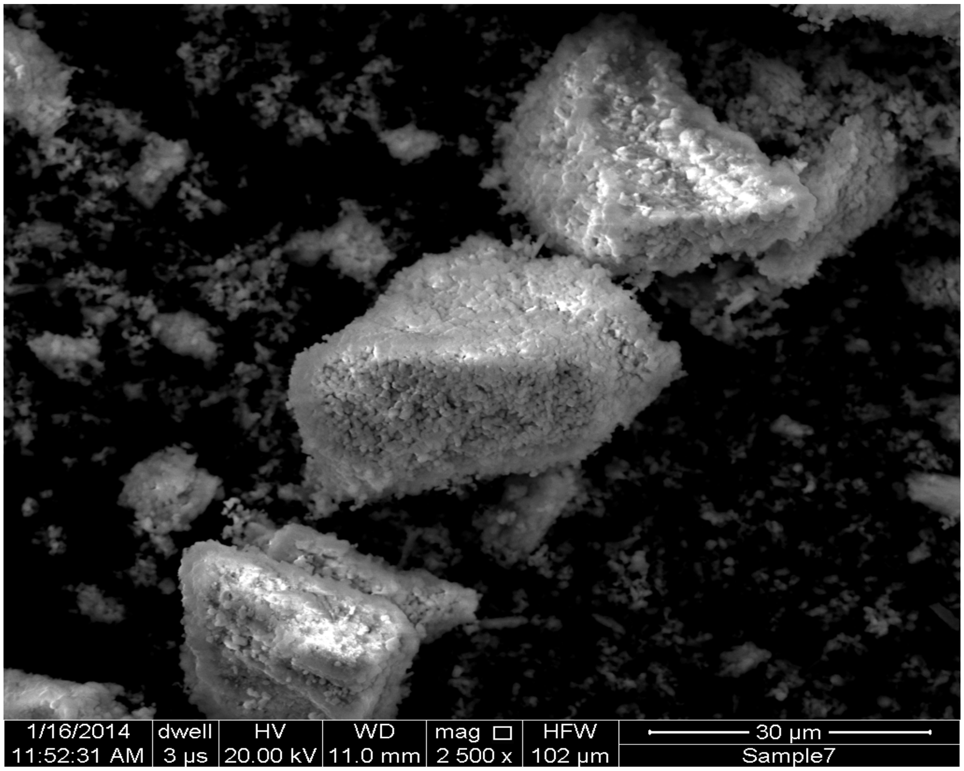

Fig. 3

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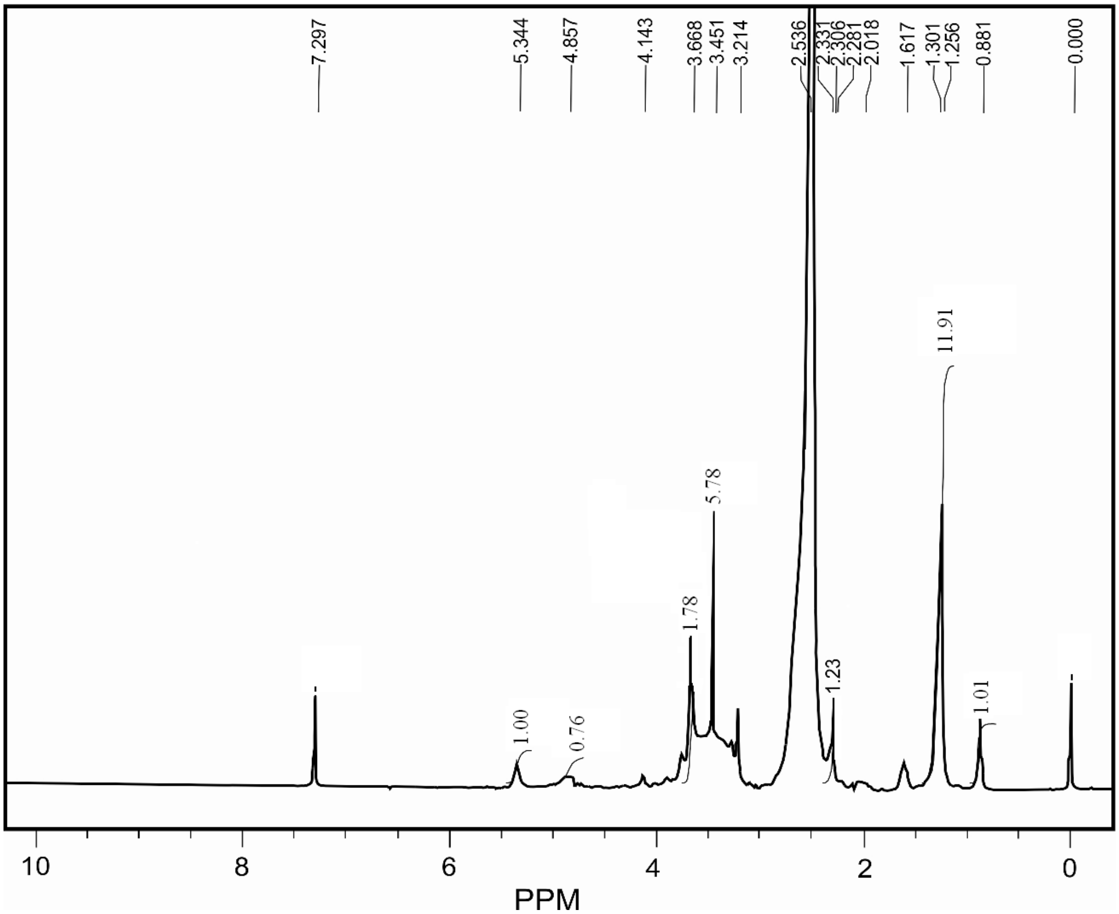

Fig.4

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Table-1 Properties of waste fish oil

Properties	Value
Visual observation	Liquid at 43 ^{0}C, brown color, typical smell
Water content (%)	0.10
Density (kg/m^3)	893
Kinematic Viscosity (mm^2/s at 40°C)	25.51
Moisture and Volatile Matter (wt %)	0.9570
Insoluble Impurities (wt %)	0.0305

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Table-2 Properties of biodiesel

Properties	Value
Water content (%)	0.03%
Density (kg/m^3)	843
Kinematic Viscosity (mm^2/s at 40°C)	4.99
Cloud Point (°C)	1
Flash Point (^{o}C)	150

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