CHAPTER 3

3.1 Materials

Measuring cylinders, conical flasks, burettes, volumetric flasks, test-tubes, mortar-pestle, double distilled water, micropipettes, pipettes, corning, borosil separating funnels, etc.

3.2 Reagents

Hexane, toluene, tetrahydrofuran (THF), acetone and diethyl ether were of analytical grade. Chemicals such as, methanol, sodium sulphate, and deuterated chloroform were of analytical reagent (AR) grade and are purchased from Merck Ltd, Mumbai, India. Waste fish oil was derived from discorded parts fish, calcium oxide derived from waste crab shell, β-tricalcium phosphate derived from solid matter, *Pongamia pinnata* oil from its seeds. The oil was extracted from the kernel by mechanical expeller and thereafter by Soxhlet extraction. The oil was then filtered with Whatman No. 42 filter paper of pore size 20-25 µm. Potassium hydroxide (KOH), sodium hydroxide (NaOH), and sodium methoxide ($NaOCH_3$) were purchased from Qualigens Fine Chemicals, Mumbai, India. Synthesis grade methanol of ≥ 99 % assay, ortho-phosphoric acid (85 % pure), sodium sulfate dry purified, and sulphuric acid (H_2SO_4) 99 % GR grade were procured from Merck Limited, Mumbai, India. Distilled water was prepared in the laboratory in quartz apparatus.

3.3 Instruments

The instruments were used in the process of synthesis of catalysts, and production And characterization of biodiesel. X-Ray Diffractometer, TG/DTA/DTG, Magnetic

Stirrer, Scanning Electron Microscope, Fourier Transform Infrared Spectroscopy, Tubular furnace (IKON), Raman Spectrometer (Reni Shaw in Via Raman spectrometer), X-Ray Photoelectron Spectroscopy (Kratos Analytical Instrument, Shimadzu group company, Amicus XPS UK), Surface area analyzer (Smart instruments, SMART SORB 92/93, Mumbai, India), Serological water bath with stirrer (Narang Scientific Works, New Delhi, India), Analytical balance (VIBRA), pH meter (IKON, India) and Nuclear Magnetic Resonance Spectroscopy (Bruker, Germany) Avance III HD, Ascend^{TM} 500) were used in the present study.

3.4 Preparation of catalysts

3.4.1 Calcium oxide preparation

Crab shells were collected from the Jas Dry Fish Merchant – India MART Thoothukudi, Tamil Nadu, India. The collected crab shells were washed thoroughly with distilled water to eliminate dust particles associated with the crab shells. After washing, crab shells were kept in a hot air oven at 100 ℃ for 2 hour to remove water content present in the crab shells. Thereafter, crab shells was converted into powder with the help of ball mill apparatus. This powder was calcined for 5 h ranging from 450 ℃ to 850 ℃ with the heating rate of 10/min. Calcium carbonate ($CaCO_3$) present in the crab shells was converted into calcium oxide (CaO) when the temperature reached 850 ℃ in a tubular furnace for 4 h. The calcined material was powdered in agate mortar and kept in desiccators to avoid moisture and was used as efficient solid base catalyst in transesterification reactions for the synthesis of biodiesel from fish oil derived from waste parts of fish as well as *Pongamia pinnata* oil extracted from *Pongamia pinnata* seeds

since calcium oxide used as an efficient catalyst in the transesterification reactions [Boey et al., 2011a].

3.4.2 β - tricalcium phosphate preparation

Waste fish oil was extracted from the discarded parts of fish, after extraction of waste fish oil the dry residual matter was left which included tails, bones and fins. Dry matter was washed with hot distilled water to remove gelatinous matter and flesh associated with dry matter and kept in a hot air oven for 5 h at 102 °C to remove water content. The dried matter was ground into powder with the help of agate mortar, thereafter powder was calcined at different temperatures ranging from 400 °C to 1000 °C with constant heating rate 10 °C /min in muffle furnace for 4 h. After calcination, powder was ground into fine powder with the help of ball mill followed by agate mortar. This fine powder was characterized and used as solid base catalyst in the transesterification reactions using waste fish oil as well as *Pongamia pinnata* oil as feedstock.

3.5 Esterification

By titration with KOH, the acid value of both oils (waste fish oil as well as *Pongamia pinnata* oil), was measured as per ASTM test method. In spite of these worthiness, the major drawback of fish oil is its free fatty acid content. For the transestrification process, researchers have recommended to reduce acid value of oil and it should be less than 4 mg KOH/g. [Sahoo et al., 2007; Veljković et al., 2006]. Before conducting transesterification reaction, esterification process was carried out. Esterification was carried out with both the oils (waste fish oil as well as *Pongamia pinnata* oil) using sulphuric acid as

homogeneous acid catalyst (1 %) and methanol to oil molar ratio was 8:1 at 60 °C temperature for two hours. The reaction took place in a 3 necked round bottom flask kept in a serological water bath. After completion of reaction, reaction mixture was poured out in a separating funnel to separate water from the FAME (Fatty Acid Methyl Ester) by the gravitational force. The removal of unreacted methanol was done by rotavapour. Acid values of both oils (waste fish oil as well as *Pongamia pinnata* oil) were reduced and further transestrification process has been carried out.

Acid value calculated using following equation (Eq. 3.1):

$$\text{Acid Value (mg KOH/g)} = V_{KOH} * 56.1 * C_{KOH} / m_{sample} \quad (3.1)$$

FAME conversion was calculated using following equation (Eq. 3.2):

$$\text{Free Fatty Acid Conversion (\%)} = (AV_1 - AV_2)/AV_1 * 100 \quad (3.2)$$

Where AV_1 (mg KOH g^{-1}) is the acid value of original oil sample, and AV_2 (mg KOH g^{-1}) is the acid value of catalysed product.

3.6 Transesterification

After esterification, acid values of both oils (waste fish oil as well as *Pongamia pinnata* oil) were reduced to 0.44 mg KOH/g (*Pongamia pinnata* oil) and 0.42 mg KOH/g (waste fish oil) respectively. Thereafter transesterification reactions were performed using calcium oxide and β-tricalcium phosphate were as base catalysts. Transesterification reaction parameters such as methanol: oil was varied from 1:6 to 1:14 molar ratio, stirrer speed was varied from 350 rpm to 850 rpm, reaction time varied from 30 min to 190 min, catalyst concentration was varied from 1 % to 5 % (with respect oil) and temperature of

the reaction mixture was varied from 35 °C to 85 °C. Effect of each reaction parameter on biodiesel yield was observed. In each experiment, the effect of co-solvent (methanol: co-solvent 1:1) on biodiesel yield was studied. After completion of the transestrification reaction, in each and every experiment, reaction mixture was poured out in separating funnel to separate the by product (glycerol). Further purification of biodiesel was done which included removal of unreacted and removal of water content. Purified biodiesel was characterized with Proton NMR, FT-IR and yield was calculated using following equation (Eq. 3.3):

Biodiesel yield (%) = Wt. of fatty acid methyl ester/Wt. of fish oil used*100 % (3.3)

3.7 Characterization of biodiesel

3.7.1 Proton NMR analysis of biodiesel

Purified biodiesel was analyzed with proton NMR spectroscopy since proton NMR spectroscopy is more sensitive and accurate to analyze the biodiesel. Proton NMR analysis was done by using Bruker SFO1 500MHz. Each and every ^{1}H NMR spectra was observed when 180 µl biodiesel was dissolved in 0.8 ml of deuterated $CDCl_3$ chloroform. Horst et al., 2009 studies showed that the methyl esters consist of methoxy proton provide a singlet at around 3.66 ppm and methylene group of methyl esters provide a triplet at around 2.3 ppm. Effect of biodiesel parameters on biodiesel yield was observed by calculating the conversion of biodiesel using following well known equation (Eq. 3.4):

$$C= 100(2*A_{CH3})/(3*A_{CH2}) \quad (3.4)$$

3.7.2 Characterization of biodiesel by FT-IR

FT-IR spectroscopy was performed on a BRUKER ALPHA Eco ATR in the wavelength range from 4000–500 cm^{-1}. Biodiesel synthesized from both oils (waste fish oil as well as *Pongamia pinnata* oil) was characterized by using Fourier Transform Infrared Spectroscopy (FT-IR). Fourier transform infrared spectroscopy was employed to identify the functional groups present in the synthesized biodiesel.

3.7.3 Fatty acid composition of feedstocks through Gas Chromatograph Mass Spectrometry (GC-MS) analysis

The extracted raw feedstocks, viz. such as waste fish oil and *Pongamia pinnata* oil were converted to respected methyl esters and were analyzed with Gas Chromatograph Mass Spectrometry (GC-MS) to determine the fatty acid composition present in the respected raw feedstocks. Fatty acid composition was determined using Gas-Chromatograph Mass Spectrometry (GC-MS) Perkin Elmer, Mass range: 20 to 620 Daltons (amu). Samples were prepared to analyze when 0.03 mL of each oil methyl ester was mixed with 2.1 mL of hexane. Temperature was increased from 30 °C to 530 °C with the heating rate 4 °C /min. Parentage of relative abundance was calculated according to respected retention time. Using the known compound spectrum from the NIST 2011 library, unknown compound spectrum was predicted with the help of Turbo Mass software.

3.8 Characterization of catalysts

3.8.1 XRD analysis

The XRD analysis was carried out with a Shimadzu diffractometer model XRD 6000. The

diffractometer employed Cu Kα radiation to generate diffraction patterns from the powder crystalline samples at ambient temperature. The Cu Kα radiation was generated by a Philips glass diffraction X-ray tube (broad focus 2.7 kW type). The peaks were matched with Joint Committee on Powder Diffraction Standards (JCPDS) files.

3.8.2 Characterization of catalysts by DTA/TGA

DTA/TGA experiments were performed under nitrogen flow with a structured text analyzer (DTA/TGA), model STA 409, NetzschGer¨ atebau GmbH (Germany). Ten milligrams of sample is taken and kept in a 1 mL aluminum crucible with a heating rate of 10 °C/min at a 50 mL/min air flow rate. Pure aluminium powder was taken as reference material. Weight loss of prepared samples were observed with respect to temperature range.

3.8.3 Characterization of catalysts by SEM

All images were taken from Scanning Electron Microscope (SEM) with a model JSM-7600F is a versatile high resolution scanning electron microscope with three modes of operation, namely, the high vacuum (HV) mode for metallic sample (electrically conducting), low vacuum (LV) and environment scanning electron microscope (ESEM). The resolution is 1.0 nm at 15 kv and 1.5 at 1 kv,in GB mode. The magnification of instrument is 25x to 10,000x at low and 100x to 1,000,000x at high. The probe range is varied from 1 pA to ≥ 200 nA.