

A Review on Methods of Transesterification of Oils and Fats in Bio-diesel Formation

T.C.Venkateswarulu^{1*}, Cherukuwada.V.Raviteja¹, Kodali.V.Prabhaker², D.John Babu¹,
A.RanganadhaReddy¹, M.Indira¹, A.Venkatanarayana¹

¹School of Biotechnology, Vignan University, Vadlamudi-522213,India.

²Department of Biotechnology, Vikramashimahapuri University, Nellore-524001,India.

*¹Corres. Author: venki_biotech327@yahoo.com

Abstract: Bio-diesel is an alternative to petroleum-based fuels resultant from animal fats, vegetable oils, and used waste cooking oil including triglycerides. Transesterification is the most familiar method and leads to monoalkyl esters of vegetable oils and fats, called bio-diesel when used for fuel purposes. The used cooking oils are used as raw material, adaption of continuous transesterification process and recovery of high quality glycerol from biodiesel by-product (glycerol) are most important options to be considered to lower the cost of biodiesel. The transesterification reaction is affected by molar ratio of glycosides to alcohol, catalysts, reaction temperature, reaction time and free fatty acids and water content of oils or fats. The mechanism of the transesterification show how the reaction occurs and progresses. The processes of transesterification with supercritical catalysts are also addressed.

Key-words: Bio-diesel, Transesterification, Supercritical catalysts, Vegetable oils.

1. Introduction

Biodiesel has become more attractive recently because of its environmental benefits and the fact that it is made from renewable resources. Biofuels can be produced across the world using resources that are available locally, thereby reducing foreign exchange for buying crude oil and increasing the country's economy. Fuel is any material that stores energy that can later be extracted to perform mechanical work in a controlled manner. This may include combustion, exothermic chemical reactions and nuclear reactions^[1]. Fossil fuels are hydrocarbons, primarily coal and petroleum formed from fossilized remains of ancient plants and animals by exposure to high heat and pressure in the absence of oxygen in the earth's crust over millions of years. Petroleum fuels include diesel, petrol, natural gas, kerosene, gasoline etc.,^[2, 3]. Petroleum diesel, also called petrodiesel or fossil diesel produced from fractional distillation of crude oil at 200-350°C at atmospheric pressure. It contains a mixture of carbon chains that typically contain 8 to 21 carbon atoms per molecule that is like 86% of fuel mass is carbon, density is 0.832kg/L and it has a heating value of 43.1MJ/kg. CO₂ emissions from diesel are 73.25g/MJ with 75% of saturated hydrocarbon composition with a range of C₁₀H₂₀ to C₁₅H₂₅ with an average of C₁₂H₂₃^[4]. Fuel contains hydrocarbons. When hydrocarbons in fuel are subjected to combustion carbon dioxide and water are liberated producing energy. Since fossil fuels are present within the surface of earth, their usage adds carbon residues into the atmosphere resulting in global warming. However, biofuels are also hydrocarbons, their assimilation involves carbon fixation that means hydrocarbons within biofuels are synthesized from carbon dioxide that is already present in the atmosphere. So, combustion of biofuels will not add any foreign carbon derivatives to the atmosphere. Hence environment friendly biofuels are

preferred over hazardous fossil fuels ^[4]. Biofuels are renewable fuels whose energy is derived from carbon fixation. Biofuels include fuels derived from biomass conversion as well as solid biomass, liquid fuels and various biogases. Biofuels are gaining increased public and scientific attention, driven by factors such as oil price hikes, need for energy security, and concern over emission of green house gases from fossil fuels ^[5].

2. Sources for bio-diesel production

Biofuels may be produced from biosources like micro organisms - methane bymethanogens, ethanol by yeast *Saccharomyces cerevisiae* fermentation, transesterification oils of plants and fats of animals to fatty acid methyl esters ^[6]. Biodiesel is produced by transesterification of edible oils and non-edible oils - Jatropha, palm oil ^[7], soybean oil ^[8], rapeseed oil ^[9], palm kernel oil, coconut oil ^[10], Honge ^[11] and even used cooking oil ^[12].

3. Bio-diesel production from vegetable oils

3.1. Literature review

The use of vegetable oils as alternative fuels has been around for one hundred years when the inventor of the diesel engine redolph Diesel first tested peanut oil, in his compression-ignition

Engine. Biodiesel is better for the environment because it is made from renewable resources and has lower emissions compared to petroleum diesel. It is less toxic than table salt and biodegradable as fast as sugar. Its use decreases dependence on foreign oil and contributes to one's own country's economy. Biodiesel is made through a chemical process called transesterification where glycerin is separated from the fat or vegetable oil ^[13].

Oils and fats typically contain monoglycerides, diglycerides, triglycerides, unsaponified lipids and free fatty acids. Since viscosity of vegetable oils is higher, engines designed to burn diesel fuel may not operate efficiently with high viscous and poor volatile vegetable oils. Vegetable oils get solidified at lower temperatures and do not support fuel injection system of engine ^[14]. So, transesterification is adapted to convert triglycerides to fatty acid methyl esters. Free fatty acids react to form glycerin (soap) which ultimately reduces the yield of biodiesel ^[15]. There are four basic routes to biodiesel production from oils and fats.

1. Base catalyzed transesterification
2. Direct acid catalyzed transesterification
3. Conversion of oil into its fatty acids and then into biodiesel
4. Non - catalytic transesterification of oils and fats ^[16].

Transesterification consists of a number of consecutive, reversible reactions. Triglycerides are converted step wise to diglycerides, monoglycerides and finally glycerol liberating a mole of ester in each step. The reactions are reversible, the equilibrium lies towards the production of fatty acid ester and glycerol (fig-1).

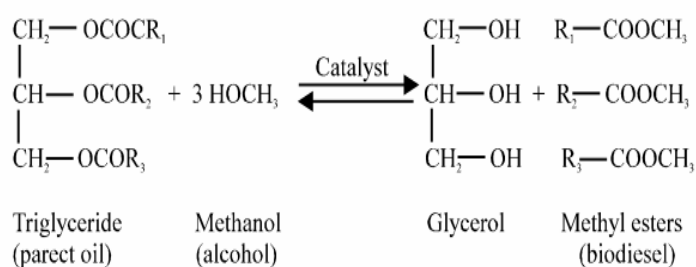
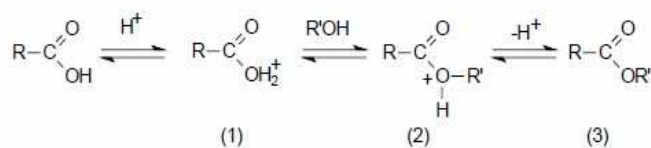


Fig. 1. : The mechanism decomposition of triglycerides (source: Ref. [16]).

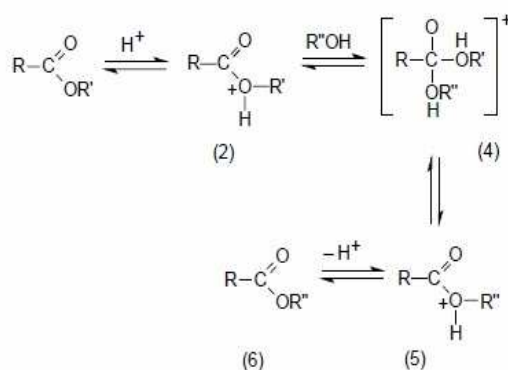
Acid catalyzed transesterification

Carboxylic acids can be esterified by alcohols in the presence of suitable acidic catalyst in water free conditions. The most frequently cited reagent for the preparation of methyl esters is 5% anhydrous hydrogen

chloride in methanol. In a typical esterification procedure, using methanolic hydrogen chloride, the lipid sample is dissolved in at least a 100-fold excess of the reagent and the solution is refluxed for about two hours or is held at 50° C overnight (30 minutes at 50°C will suffice for free acids alone)(fig-2).



Scheme 1. Acid-catalysed esterification of fatty acids.



Scheme 2. Acid-catalysed transesterification of lipids.



Scheme 3. Preparation of methanolic hydrogen chloride via acetyl chloride.

Fig.2. : Mechanism of Acid catalyzed transesterification (source: Ref. [17]).

Free fatty acids in the oil were reduced by using acid catalyst. Free fatty acids reduce the yield of biodiesel by saponification, hence acid catalyzed transesterification has an advantage of more yield. However, the reaction is slow that one run can take more than a day ^[17].

Base catalyzed transesterification

Esters in the presence of base such as an alcoholate anion form an anionic intermediate which can dissolve back to the original ester or form the new ester. The most useful basic transesterifying agents are sodium or potassium methoxide in anhydrous methanol ^[18]. The main advantage of base catalyzed transesterification over acid one is that it is fast and can be conducted at low temperatures(303-308K) and pressure (0.1 MPa) (fig-3).

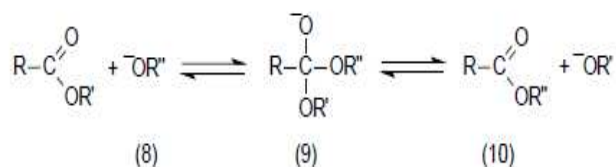


Fig.3.: Base catalyzed transesterification of lipids (source: Ref. [18]).

Acid - base transesterification

In the homogenous acid and base catalyzed transesterification processes, the most commonly used base catalysts are NaOH and KOH while acid catalysts are sulphuric acid and sulphonic acid. The major drawback of base transesterification is saponification which may be due to the presence of free fatty acids in the oil that is subjected for transesterification. Esterification of free fatty acids is possible by acid catalysis which reduces the

soap (glycerine, byproduct) formation, ultimately leads to more yield of alkyl ester (biodiesel). In transesterification process, biodiesel is produced from a reaction between vegetable oil, alcohol in the presence of base or acid catalyst. Methanol is most preferred alcohol because of its low cost and its physical and chemical properties. The highest biodiesel yield was at 3% weight of catalyst, 60° C and reaction time of 120 minutes. The results of acid -base catalyzed synthesis showed that biodiesel yield was improved when compared to base catalyzed synthesis alone. The kinematic viscosity of acid -base catalyzed biodiesel satisfied the standard test method for kinematic viscosity (ASTM D445).

Lipase mediated transesterification

Chemical production of biodiesel by transesterification is cost effective and highly efficient but its problem is with downstream processing which includes separation of catalyst, unreacted methanol, glycerol recovery, removal of inorganic salts. There may be a risk of free acid or water contamination along with soap formation makes separation process difficult^[19]. Enzymatic approach seems to be a promising alternative production pathway. Because it not only decreases the operation cost but also over comes problems associated with chemical production of biodiesel^[20-21]. Enzymatic approach produces high purity product and enables easy separation from byproduct, glycerol. Lipases (triacyl glycerol acyl hydrolase EC 3.1.1.3) are hydrolytic enzymes that catalyze the hydrolysis and synthesis of variety of acylgcerols at interface of lipid and water. Lipases are produced by *Pseudomanas aeruginosa*, *P. flurescens*, *Candida antarctica*, *Rhizopus oryzae*, *Thermomyces lanuginosa*. Transesterification reaction mixture consisted of oil, lipase and methanol. 86.2% conversion of fatty acids to fatty acid methyl ester (FAME) within 36 hours at 45°C by using 7.5 % (w/v) lipases, molar ration of oil to methanol is 1:4.

Microwave assisted transesterification

In conventional transesterification process heating of material takes place by conduction. Whereas microwaves heat by means of thermal radiation and it is very efficient mode of heat transfer. In microwave assisted continuous transesterification, vegetable oil and methanolic NaOH solution were fed separately via 2 pumps and mixed at the tee connector at the inlet of modified house hold microwave oven (800W). Reaction time and molar ratio of the oil to alcohol were controlled via a combination of flow control valves of the pumps. Instantaneous heating of oil mixture by microwave is more advantageous than conventional prolonged heating of 2 hours. The reaction temperature attained during the transesterification in microwave oven (800 W) is around 65 to 800°C. Microwave energy is delivered directly to the reacting molecules which undergo chemical reaction. Reaction time was reduced to 60 seconds with palm oil, 5 to 10 minutes in case of non-edible oils of Honge and Jatropha where their respective conversion in conventional method was 1 hour and 1 to 2 hours. Biodiesel produced by microwave assisted transesterification has reduced performance due to their comparatively high viscosity. Also smoke opacity, HC and CO emissions increased with such oils^[23].

Ultra sonically assisted transesterification

Transesterification is a chemical reaction between triglycerides (oil /fats) and methanol, which form immiscible phases when they are in a reaction vessel, the reaction takes place at contact surface between oil and methanol^[24-25]. Ultrasonic reactor drastically reduces the amount of catalyst, molar ratio of oil to methanol 1:6, reaction time, reaction temperature, mass transfer resistance elimination and energy input for production of biodiesel. Low frequency ultra sound (28 and 40 kHz) were found to be more efficient, at higher frequencies the collapse of cavitations bubbles are not strong enough to impinge one liquid to the other^[26]. Transesterification reaction takes place at interfacial region of two immiscible phases (oil and methanol), which may be achieved by vigorous mixing using ultra sonic waves and produces pure fuel with highest possible conversion percentage. Previous reports shown the smaller reaction time (20-40 minutes) with 2-3times lower amounts of catalyst requirements in case of ultrasonic assisted transesterification when compared to mechanical agitation^[27-31]. The reaction rate constants were evaluated and found that rate constants are 3-5 times higher than that of mechanical agitation^[32]. Acoustic and hydrodynamic cavitation found effective tool for intensification of esterification of fatty acids with acid catalyst (H₂SO₄) 99.4 % conversion soybean oil to FAME (fatty acid methyl ester) was achieved in about 15 minutes at 40 °C and molar ratio of 6:1 methanol to oil with 20kHz ultrasonic frequency^[33-34]. Lipase catalyzed transesterification activity enhanced with ultrasonic irradiation. With 8% enzyme (Novozyme 435), 3:1 molar ratio of propanol to oil, power of ultrasonic assistant 100 W, frequency of 28 kHz, temperature 40 to 45 °C for 50 minutes yielded 94.86% of propyl oleate, which is 10.43% higher than mechanical agitation mediated transesterification,also ultrasonic assisted transesterification reduced amounts of lipase and reaction time. Yields of biodiesel were always higher when ultrasound was used^[35].

Transesterification with different catalysts

Basic heterogeneous catalysts

Heterogeneous catalysts are environmentally benign, non corrosive, easily separable from the liquid products, higher activity and longer catalyst life time. Even though homogenous catalysts NaOH, KOH and sulphuric acid, phosphoric acid, sulphonic acid hydrochloric acid are highly reproducible, with a definite stoichiometry and structure. The removal of conventional homogenous catalyst after reaction is technically difficult and large volumes of waste water are produced to separate and clean the catalyst and the products. Therefore, conventional homogenous catalysts are expected to be replaced with environment friendly heterogeneous catalysts^[36-37]. Heterogeneous catalysts can easily be separated from reaction mixtures and reduces environmental pollutants. Most important application is easier working up procedure. Also heterogeneous catalysts are known for their activity, stability, economic benefits, ease of recovery and resistance to poisoning. They can be removed from reaction mixture by coarse filtration technique^[38-39].

Biodiesel production using super critical methanol

Catalyst mediated transesterification process involves separation of catalyst after the reaction. Super critical methanol transesterification for biodiesel production is carried out without catalyst and main variable that affecting yield of biodiesel is methanol-to-oil molar ratio and reaction temperature. In super critical method, acid value and water content do not have a negative effect on reaction. Purification of the reaction products is simpler than in the catalyzed process and non polluting effluents are generated. Super critical methanol method of biodiesel production was carried in a batch stirred tank reactor (83 mL capacity) given 72% yield of biodiesel with methanol to oil ratio of 45.8, temperature of 350°C for 20 minutes. Super critical methanol method has a drawback- expensive apparatus which uses high temperature and pressure limits its application in industrial production^[40]. This has led to many researchers to look for ways to decrease the severity of reaction conditions. For example co-solvents such as carbon dioxide^[41], propane^[42], and heterogeneous catalysts^[43] added into reaction mixture can decrease the operating temperature, working pressure and the amount of alcohol.

Calcium oxide as transesterification catalyst

CaO generates more methoxide anions in the presence of little water (2.03 % water by mass) and they act as catalyst in conversion of soybean oil to biodiesel. Calcium methoxide catalyzed transesterification, represents a result yield of 61% to 97% from 3:1 to 12:1 methanol to oil molar ratios and it has given 95 % conversion within a short reaction time^[44]. CaO was also used for the production of biodiesel from *Jatropha Curcas* oil. CaO becomes a solid super base when treated with ammonium carbonate solution and calcinated at higher temperature to give it a high catalytic activity. 93% conversion was obtained when catalyst dosage was 1.5%, methanol to oil molar ratio 9:1, reaction temperature 70°C for 2.5 hours. Decalcination can be performed by citric acid.

Basic alkaline earth metal compounds as catalysts

Barium hydroxide, calcium methoxide, CaO and NaOH were studied as catalysts. Powdered catalysts were dispersed in methanol and heated to boiling point of methanol and stirred intensively for 30 minutes. Then oil was poured in a thin stream. Barium hydroxide was slightly active, calcium methoxide was medially active. Tetra hydrofuran was used as additive to accelerate the process. Maximum conversion was obtained around 0.72 hours with Barium hydroxide, calcium methoxide and sodium hydroxide. CaO yielded 75% in 2.5 hours reaction time with rapeseed oil. Calcium methoxide yielded 98% with soybean oil when 4% Calcium methoxide was used at 65°C, methanol to oil molar ratio of 1:1 and 2 hours reaction time^[33].

Alkaline earth metal - doped ZnO catalysts were prepared by impregnation with aqueous solution of an alkaline earth metal nitrate. Best activity was achieved with Sr(NO₃)₂/ZnO. 95% conversion of soybean oil was achieved with molar ratio of oil to methanol 1:12^[45]. Thirteen different kinds of metal oxides containing calcium, barium, magnesium or lanthanum were prepared as catalysts. The calcium series catalysts showed a high yield of methyl ester. CaMnO₃, Ca₂Fe₂O₅, CaZrO₃, CaCeO₃ and CaTiO₃ whose catalytic basicity were highest showed 92%, 92%, 88%, 89% and 79% of ester yields respectively. BaTiO₃, BaZrO₃, BaCeO₃, MgTiO₃, MgZrO₃, MgCeO₃, LaZrO₃ and LaCeO₃ ester yields were 1% or less. A high durability of catalytic activity was found for the catalyst samples of CaZrO₃ and CaO-CeO₃ which were able to provide methyl ester yields greater than 80%^[46].

Alumina supported heterogeneous catalysts

Potassium nitrate was loaded onto alumina by impregnation from aqueous solution and calcined at 773K prior to each reaction. The chemical groups K_2O and $Al-O-K$ on surface were probably main active sites, 87.43% conversion of soybean oil was achieved with 6.5% catalyst and molar ratio of 15:1, however further increase in ratio yielded little improvement at high stirring speeds^[47]. Alumina loaded with KI/Al_2O_3 demonstrated superior catalytic activity with 87.4% conversion, reaction time of 8hours with 35 % KI . 96% conversion was obtained with KI/Al_2O_3 and calcined for 3 hours, methanol to oil molar ratio 15:1, 8 hours of reaction time and catalyst amount 2.5%^[48].

$Na/NaOH/\gamma-Al_2O_3$. $\gamma-Al_2O_3$ was pretreated which was heated up to 320°C and stirred to form a white solid in the presence of sodium hydroxide and metal sodium. Dessicated catalyst along with co-solvent n-hexane was used to convert soybean oil in the presence of 9:1 oil to methanol at 600°C. 5:1 molar ratio of oil to n-hexane was most effective in conversion^[49]. Heterogeneous catalyst $Na/NaOH/\gamma-Al_2O_3$ showed same activity that of homogenous $NaOH$, 12:1 molar ratio, reaction time of 1.5 hours^[50]. Calcined $Mg-Al$ hydrotalcites used as catalysts for methanolysis of soybean oil with oil to methanol ratio of 15:1, reaction time 9hours, 7.5% catalyst and conversion was 67%^[40].

Zeolites as effective heterogenous catalysts

NaX Zeolites loaded with 10:1 molar ratio of methanol to oil, 3wt % of catalyst, reaction time of 8hours yielded 85.6%. The used catalyst was regenerated by impregnating in an aqueous solution of 5% KOH showed 84.3% conversion^[51]. Several acidic and basic solids like ZrO_2 , ZnO , SO_4^{2-}/ZrO_2 , KNO_3/KL Zeolites and KNO_3/ZrO_2 were used as catalysts for palm kernel oil and coconut oil among their ZnO and SO_4^{2-}/ZrO_2 exhibited highest activity. 1wt% of SO_4^{2-}/ZrO_2 resulted in yield more than 90% from crude palm kernel oil 86.3% from crude coconut oil and reaction time of 1 hour. Catalyst can easily be regenerated by filtration^[50]. Nickle was most catalytic among several identified effective metals and zeolites, more than 90% conversion was achieved at temperature of 1200 and 1500°C^[51].

Continuous heterogeneous catalysed transesterification

The catalyst section includes two fixed bed reactors made of mixed oxide of Zinc and Aluminum and is fed with vegetable oil and methanol at a given ratio. Esters and glycerol are separated in a settler. Glycerol byproduct is of high quality i.e., >98% glycerin^[52].

Cell catalyzed transesterification of biodiesel production

Transesterification was catalyzed by Lipase in the production of fatty acid alkyl esters / biodiesel from oils or fats. Lipase mediated transesterification has more advantages in downstream processing over chemical conversion. However its high cost restricts its use in industrial production. Utilising whole cell biocatalyst is a potential approach to reduce the cost of catalyst in lipase-catalyzed biodiesel production^[48]. Filamentous fungus *Rhizopus oryzae* (R. oryzae PTCC5174) whole cells immobilised within biomass support particles (BSP) by aseptic inoculation with spores by incubating at 30°C for 90 hours on a reciprocal shaker in basal medium for growth of *R. oryzae*. After cultivation, BSP-immobilized cells were separated by filtration, washed repeatedly and dried and then treated with 0.1 % (v/v) glutaraldehyde solution at 25°C for 1 hour, then used as whole-cell biocatalyst for methanolysis reaction^[53]. Used cooking oil (UCO) pretreated by filtration with Whatman 42 and the filtrate was heated for 15 minutes with magnetic stirring apparatus that has an influence on transesterification reaction^[53]. Pretreated UCO, 3:1 molar ratio of methanol to oil in three step additions at 35°C along with immobilised whole cell bio catalyst yielded 88% conversion of free fatty acids to methyl esters was obtained^[54].

Conclusion

Biofuels are renewable fuels which are produced by living organisms by carbon fixation. These fuels do not influence climatic adverses like global warming that are caused by conventional, non-renewable fossil fuels. Transesterification of oils and fats results in the formation of biodiesel (fatty acid methyl ester). Economics of biodiesel is influenced by cost of oil, transesterification procedure and downstream processing. Economical conversion of oil to biodiesel depends on transesterification and it involves in production of soap and addition of catalyst. Soap and catalyst are to be separated to obtain pure biodiesel. Fuel quality may be influenced by

catalyst residues, methanol and soap left in biodiesel. So, it is necessary to choose a process which is economic, ecofriendly, minimal downstream processing requirements and maximum conversion of oils to biodiesel. A combination of microwave assisted transesterification; ultrasonically assisted transesterification and heterogeneous catalyst mediated transesterification would give optimal in biodiesel production. Heterogeneous catalysts can be regenerated and recycled and separated from reaction mixture by coarse filtration. Maximum energy can be transferred using microwave assisted transesterification, whereas ultrasonically assisted transesterification gives maximum contact between two immiscible phases. Hence yields are always higher with ultrasonically assisted transesterification. Minimizing costs involved in transesterification finally produce cheaper biodiesel.

References

1. Meher LC, Sagar DV, Naik SN. Technical aspects of biodiesel production by transesterification – a review. *Renew Sustain Energy Rev* 2006; 10:248–68.
2. Paul Mann, Lisa Gahagan and Mark B. Gordon, "Tectonic setting of world's giant oil and gas fields", in Michel T. Halbouty(ed.) *giant oil and gas fields of the decade, 1990-1999*, Tulsa, Okla: American Association of Petroleum geologists, p. 50.
3. <http://www.sciencedirect.com/articles/f/fossil-fuel.htm>
4. www.biofuelswatch.com/why-are-biofuels-better-than-fossil-fuels
5. V. Manienyan, R.Senthil kumar and Dr. S. Sivaprakasam – Experimental investigation on optimised biodiesel (based on various catalysts) used in a twin cylinder diesel engine, *JAOCS*. 2006; 830:836.
6. Lertsathaporn Suj V. Ruangying P, Pairintra R and Krishnangkura K., continuous transmethylation of vegetable oils by microwave irradiation. *School of energy and materials King Mongkut's University of technology Thonburi, Bangkok*, 1989 ; 2(3):97
7. Huaping Zhu, Zongbin WU, Yuanxiong C, Ping Z, Shijie duan, Xiaohua liu and Zong qiang Mao *Chin journal of catalysis*, Preparation of biodiesel catalyst by solid super base of Calcium oxide and its refining process 2006:27(5):391-396.
8. Yang Z and Xie W, Soybean oil transesterification over ZnO modified with alkaline earth metals, *Fuel processing technology*. 2007; 88:631-638.
9. Suppes GJ, Dasari MA, Doskosi EJ, Mankidy PJ and Goff MJ. Transesterification of soybean oil with zeolite and metal catalyst, *Applied catalysis A : general* 200; 257: 213-223.
10. Liu X, Piao X, Wang Y, Zhu S and He H. Calcium methoxide as a solid base catalyst for the transesterification of soybean oil to biodiesel with methanol. *Fuel*. 2008;87:1076-1082.
11. Stavarche C, Vinatoru M, Yim B, Maeda Y. *chem. Lett*. 2003; 32(8) : 716-717.
12. www.biodiesel.org
13. A.B.Bhake, S.L.Bankar, S.S.Shartri and N.M.Limaye, Aspects of ultrasonically assisted transesterification of vegetable oils, *biosource technology*, 1989; 70:1-15.
14. <http://www.osti.gov/bridge>
15. Fangrui M and Hanna MA., Biodiesel production: A review : *biosource technology*, 1979;70:1-15.
16. Acid-Base transesterification of oil with high free fatty acid content - Anil kumar, Redeem valoyi, Aoyi ochieng and Maurice onayango, *Journal of Biosciences Bioengineering*.2002;67:77-82.
17. Canakci M and Gerpen J. Van A pilot plant to produce biodiesel from high free fatty acid feed stocks, *American society of agricultural engineers*, 2003;46(4):945-954.
18. Ramdas AS, Jayaray S and Muraleedharan C biodiesel production from high free fatty acid rubber seed oil. *Fuel* 2005;84(4) : 335-340.
19. Enzymatic conversion of waste oil to biodiesel in a solvent – free system. *Prepr.pap.-Am.Chem. Soc.,Div. Fuel chemistry*. 2003;48(2) : 533-534.
20. Shimada Y, Watanabe Y, Samukaroa T, Sugihara A, Noda H, Fukuda H and Tominga Y conversion of vegetable oil to biodiesel using immobilised *Candida antarctica* lipase. *Journal of the American oil Chemists' society*. 1999;76(7):789-793.
21. Joaquin Quesada and Pilaq Olivarer : super critical biodiesel production from raw soybean oil, *American oil Chemists' society*. 2009;76(7):709-713.
22. Fukuda H, Kondo A and Noda H - biodiesel production by transesterification of oils. *Journal of Biosciences Bioengineering*.2001;92(5) : 405-16.
23. Stavarche C, Vinatoru M and Maeda Y. *ultrasonic sonochemistry*. 2004; 12:367-372.

24. Aspects of ultrasonically assisted transesterification of vegetable oils—A.B. Bhake, S.L. Bankar, S.S.Shastri and N.M. Limaye, . *Renewable energy*. 2009; 34(3):756-762.
25. Stavarche C, Vinatoru M, Nishimura & Maeda Y. *ultrason.sonochem*.2005;12:367.
26. Stavarche C, Vinatoru M & Maeda Y.*ultrason.sonochem*.2007; 14:380.
27. Stavarche C, Vinatoru M & Maeda Y. *ultrason.sonochem*.2006;13:401.
28. Stavarche C, Vintoru M, Maeda Y and Bandow H. *Ultrason.sonochem*.2007;14:413
29. Stavarche C, Vintoru M, Maeda Y and Nishimua R. *Chem.Lett*.2003;32(8):716.
30. Colucci JA, Ernesto BE, Fabio A and JAm. *Oil chem. Soc.* 2005;82:525.
31. Kelkar M, Gogate P and Pandit P. *Ultrason.Sonochem*. 2008;15:188
32. Jose A, Colucci JA, Ernesto BE & Fabio A. *JAOCS*. 2005;82:525.
33. Hoang D. H and Nguyen TD. *Renewable energy*. 2009; 34(3):766-768.
34. B.K. Barnwal and M.P. Sharma:Renewable and sustainable energy reviews, Prospects of biodiesel production from vegetable oils in India : 2005 ; 9 : 363-378.
35. Saka S and Dadan K. Biodiesel fuel from rapeseed oil as prepared in super critical methanol *Fuel* 2001;80:225.
36. Butterworth AJ, Clark JH, Walton PH and Barlow SJ. *Journal of chemistry society, Chemical communication*. 1996;1859.
37. Xuejun L, Huayang H, Wang Y, Zhu S and Piao P. Transesterification of soybean oil to biodiesel using CaO as a solid base catalyst. *Fuel*. 2008; 87:216-221.
38. Han H, Cao W and Zhang J. - preparation of biodiesel from soybean oil using supercritical methanol and CO₂ as co-solvent. *Process biochemistry* 2005; 40:3148-3151.
Han H, Cao W and Zhang J. - preparation of biodiesel from soybean oil using supercritical methanol and co-solvent. *Fuel* 2005; 84:347-351.
39. Demirbas A. Biodiesel from sunflower oil in supercritical methanol with calcium oxide, *Energy conversion and management* 2007; 48:937-941.
40. N.R. Banapurmath, R.S Hosmath, Satish kambali math and P.G. Tewari - Microwave assisted continuous biodiesel production of vegetable oils and their feasibility studies in diesel engines,*Journal of American oil Chemists' society (JAOCS)* 1959 ; 25(3):97.
41. Huaping Zhu, Zongbin WU, Yuanxiong C, Ping Z, Shijie duan, Xiaohua liu and Zong qiang Mao *Chin journal of catalysis*, Preparation of biodiesel catalyst by solid super base of Calcium oxide and its refining process 2006;27(5):391-396.
42. Kawashima A, Matsubara K and Honda K. Development of heterogenous base catalyst biodiesel production, *Bio resource technology*. 2008; 99:3439-3443.
43. Xie W and Peng H and Chen L. Transesterification of soybean oil catalysed by potassium loaded on alumina as solid base catalyst, *applied catalysis A: General* 2006; 300:67-74.
44. Xie W and Li H. Alumina -supported potassium iodide as a heterogenous catalyst for biodiesel production from soybean oil. *Journal of molecular catalysis A: Chemical* 206; 255:1-9.
45. B.K. Barnwal and M.P. Sharma:Renewable and sustainable energy reviews, Prospects of biodiesel production from vegetable oils in India : 2005 ; 9 : 363-378.
46. Kim HJ, Kang BS, Kim MJ, Park YM, Kim DK, Lee JS and Lee KY. Transesterification of vegetable oil to biodiesel using heterogenous base catalyst, *catalysis Today*. 2004;93-9:315-320.
47. Arzamendi G, Campol, Arguinaena E, Sanchez M, and Gandia LM. Synthesis of heterogenous NaOH/Alumina catalysts: comparision with homogenous catalyst, *chemical engineering journal*. 2007; 134:123-130.
48. Xie W, Huang X and Li H. Soybean oil methylesters preparation using NaX zeolites loaded with KOH as a heterogenous catalyst. *Bioresource technology*. 2007; 98:936-939.
49. Bournay L, Casanave D, Delfort B, Hillion G and Chodorge JA. New heterogenous process for biodiesel production : A way to impove the quality and the value of crude glycerin produced by biodiesel plants, *catalysis today*. 2005 ; 106 : 190-192.
50. Poonam Gera, S.K. Puri and M.K. Jha – Use of basic heterogenous catalyst for biodiesel production, *Journal of molecular catalysis B : enzymatic* 2004 ;17 : 154-161.
51. Ban K, Hama S, Nishizuka K, Kaieda M, Matsumoto T, Kondo A, Nada H and Fakuda H. Repeated use of whole cell biocatalysts immobilized within biomass support particles for biofuel production. *Journal of molecular catalysis B : enzymatic* 2002 ;17 : 157-165.

52. Hama S, Yamaji H, Fukumiju T, Tamalpudi S, Kondo A, Nada H and Fakuda H. Biodiesel-fuel production in a packed bed reactor using lipase producing *Rhizopusoryzae* cells immobilized with biomass support particles. Journal of biochemical engineering journal. 2007;34:273-278
53. Mohammad Pazouki, FarzaneZamani, Amir Hosseinzamzadian, Samira lotfi and Ghasem Najafpour. Studying the effect of used cooking oil pretreatment on *Rhizopusoryzae* cell-catalysed transesterification of biodiesel production, chem. Lett. 2004; 32(8) : 714-717
54. Stavarche C, Vinatoru M, Yim B, Maeda Y. chem. Lett. 2003; 32(8) : 716-717.
