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Biodiesel - An Alternative Method for Energy Crisis: A Review

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ABSTRACT: Elevated concern on energy security led to the quest for alternative sources that can replace petroleum dependence to some extent because of their depleting supplies and the significant contribution they make for environmental deterioration. Biofuels are one such alternative that has been emphasized by various countries as a fuel for the future. They are like any other fuel that is derived from biological sources. Wood, charcoal, dung and plant based oils (used as fuel) are some examples of conventional sources for biofuel synthesis. Recent emphasis has been on plant-based oils that can be used to supplement petroleum fuels. In transesterification reaction for knowledge about the nature of raw material, chemical composition of substrate, and the mode of transesterification is of significant importance. This review covers some of the aspects of current scenario of world for energy consumption and dependency followed by alternatives to address this issue. Besides this, the review also includes preliminary perquisites prior to transesterification reaction and mode of analysis of products. Special attention has been given to biocatalytic mode using enzyme such as lipase for biodiesel production. Different benefits which biodiesel offers and its impact on environment is also discussed. In overall the review present significant information related to biodiesel status as an alternative fuel.

Keywords: Biodiesel; Biofuels; Oils; Energy Crisis; Bioethanol; Transesterification.

INTRODUCTION: Current world economy runs on fossil fuels. This is especially true for the transport sector that ineluctably accounts for about 20% of the total world delivered energy consumption and is dependent on petroleum fuels for 98% of its energy requirement. With the emergence of China and India as new growth centres in the world, scrambling for fossil fuels has become intense. The problem arises due to depletion of fossil fuels, environmental damage due to pollution and energy security and has significantly intensified research on biofuels[1]. Biodiesel is the mono alkyl esters of long chain fatty acids that serve as an alternative diesel engines fuel in the coming years due to surging energy crisis since 1970's onwards[2, 3]. Biofuels can be categorized into two types, bioethanol and biodiesel, depending on whether it is a supplement of petrol or diesel respectively. Based on the feedstock biofuels are categorized as first generation (if the feedstock is edible in nature), second generation (if the feedstock is non-edible) and third generation (if the source of oil is whole plant material or microorganism like algae). Biofuels in the conventional sense have always been an important source of energy. Even in the present times almost half of the world's population, especially in the rural areas depend on biofuels to provide cooking energy. However, the new biofuels (bioethanol and biodiesel) have lately gained importance due to various economic, geopolitical and environmental reasons. Biodiesel is regarded as suitable energy source for future because of its biodegradable nature, non toxic properties and lower emission of particulate matter into the environment in contrast to conventional fossil fuels[2, 4].

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All these properties provide an edge for biodiesel to become a successful energy efficient fuel for future[5]. Biodiesel over conventional petroleum based fuel provide higher lubrication for engine which in turn increase the life and also reduced the maintenance cost that ultimately leads to fuel economy[6].

A. BIODIESEL:

The depletion of fossil fuels from their limited resources and the associated pollution problems urge human race to develop renewable energy alternatives that have smaller environmental impact than the traditional ones[7]. In this currently biodiesel shows great promise as a fuel and with respect to sustainability[8]. Biodiesel is defined by the American Society for Testing and Materials (ASTM) as monoalkyl esters of long chain fatty acids derived from renewable feedstocks like vegetable oils and animal fats[9]. It is still controversial if the definition should include other products, such as fatty acid ethyl esters (FAEE) [10]. For biodiesel producers it is very important for their end products to meet the biodiesel standards and specifications mentioned in USA and Europe (ASTMD 6751 and EN 14214) standard[11]. Table 1 shows the EN 14214 specifications for major component and purities in biodiesel.

Table 1: Major components of biodiesel (EN 14214 wt %).

Parameters	FAEE	MAG	DAG	TAG	FFA
EN14214	Min 96.5	Max 0.8	Max 0.2	Max 0.2	Max 0.25

Biodiesel produced from vegetable oils or their blends have different physical and chemical properties to conventional diesel fuel. It is the only alternative fuel to be used in existing diesel engines without modification. In fact, biodiesel has many advantages over conventional diesel or petroleum such as renewable, biodegradable, oxygenated, less toxic, and produces less smoke and particulates and lower CO_2 and less SOx emission. It also has higher cetane number which is used as an indicator for quality of diesel fuel ignition therefore measures the readiness of the fuel to ignite when injected into the engine[12]. Furthermore, biodiesel is less volatile and safer to transport or handle due to its high flash point (150°C)[3, 13, 14]. In addition, biodiesel can enhance engine yield and life because of its better lubricant properties[15].

B. INGREDIENTS REQUIRED FOR BIODIESEL SYNTHESIS:

Lipid substrate and availability: Plant-derived oils such as soybean, sunflower, cottonseed, rapeseed, palm oil, jatropha oil are the main source for producing biodiesel[16]. Technically oil is defined as a liquid form of lipid fraction at ambient temperature[17]. The fatty acid compositions of some of the vegetable oils are shown in Table 2. Among all the available vegetable oils, high oleic acid (C18:1) chain containing oils are favorable due to the increased stability on storage and improved fuel properties[13].

Table 2: Composition of fatty acids of possible oils for biodiesel[13, 18].

Oils	Fatty Acid bound to Glycerol backbone (%)							
	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	Other
Cotton seed oil	28.3	-	0.9	13.3	57.5	-	-	-
Palm oil	42.6	0.3	4.4	40.5	10.1	0.2	-	1.1
Sun-flower oil	7.1	-	4.7	25.5	62.4	-	0.3	-
Soybean oil	11.4	-	4.4	20.8	53.8	9.3	0.3	-
Rape-seed oil	4.0	-	1.5	17.0	13.0	9.0	-	-
Jatropha oil	16.4	1.0	6.2	37.0	39.2	-	0.2	-

In this sense rapeseed oil is outstanding among others. The availability of vegetable oils for biodiesel production varies regionally dependent on the climate, soil conditions and farming traditions. Generally rapeseed oil, having the highest oil yield per acre land and is therefore, serves as the dominating source for biodiesel production in Europe and so is soybean oil in the United States and South America respectively. Owing to the low efficiency of catalysts (alkaline or lipases) working with crude oil, refined vegetable oils are the most suitable and widely used feedstock[19, 20]. However, these high quality agricultural feedstocks are also used for food purposes. However, a new addition to this has been made. Some reports highlight the importance of microbial culture to be used as a potential source for biodiesel[21, 22].

Ironically the large areas of natural land, such as Amazon rainforest in Brazil which plays an important role in adjusting atmosphere, controlling soil erosion and pollution, has been explored to grow soybean or sugar cane, which raises the doubt about the net impact of biofuel on environment[14]. Nevertheless, these controversial discussions about biodiesel bring attentions to the search of more sustainable approaches, for instance, the discovery of alternative non-food feedstock, like algae oil, waste cooking oil, non-edible oils and other low-grade feedstocks.

At present, the cost of feedstock is the main hurdle of commercialization of biodiesel because it takes up 70-95% of the total cost of biodiesel production[23, 24]. Waste cooking oil, a representative of low cost oil is very competitive from economic point of view as well as the ethical considerations about 'food vs. fuel' because it offers the opportunity of making use of surplus or waste biomass. Although the levels of FFA and water in waste oils (typically 2000 ppm water and 10-15% FFA) are manageable by enzyme mediated biocatalytic process[8, 25], however, the source availability appears to be more critical to the potential of utilizing waste oil for industrial biodiesel application. The establishment of channels for collecting the wide spread sources needs the assistance and policy support from local governments.

To this problem genetic engineering of crop plant seems to be a plausible answer where the yield of plant in terms of oil could be increased[13]. But still this seems to be in its early stage. Solutions to the hurdle of feedstock are seeking not only with the existing oil stocks but also with the discovery of potential feedstocks. Algae oil is such a promising potential source for biodiesel production meeting the global demand for renewable fuels. It has high oil productivity with low cost because of its extremely fast growth and more efficient oil production than crop plants[26]. Although high content of polyunsaturated fatty acids may present stability problem due to the susceptibility to the oxidation during storage, but they also entitle algal biodiesel with better cold weather property when compared to other oils and fats because of the lower melting point[4]. Today the technologies of algae cultivation, extracting and converting algae oil to biodiesel are not developing in accordance with each other. There is still a long way to go for the implementation of algae oil in biodiesel industry. In summary, no practical solution has been found to solve the high cost of feedstock so far. Thus, it is more realistic to focus on developing the technology to improve the biodiesel yield out of feedstock and utilizing low quality feedstock.

Alcohols: Alcohols used in the production of generally defined biodiesel are of primary and secondary monohydric aliphatic nature in having 1-8 carbon atoms[27]. Methanol is most widely used to produce fatty acid methyl esters (FAME) in conventional alkaline biodiesel production because it is reactive, cheap and easy to recover[13, 19]. Methanol derived FAME is universally recognized and accepted as biodiesel. However, since methanol is still made from natural gas and it is toxic, polar, with a low solubility in oil. Ethanol is gaining attention as an alternative to methanol, since it is more environmental friendly and renewable than methanol because it can be obtained from agricultural products by fermentation process and it is less toxic[25]. Although ethanol derived FAEE has not been well accepted as biodiesel but the fuel properties are similar to FAME and some of them are even better than FAME. For instance, the extra carbon brought by the ethanol molecule slightly increases the heat content, the cetane number and reduces the cloud and pour points[28, 29]. The mass yield of FAEE can also be increased by approximately 5% of the biodiesel weight[8]. Although the first generation bioethanol is currently most available, produced from the sugar and starch, the use of ethanol in biodiesel production can be more sustainable with the success of the second generation bioethanol (cellulosic ethanol).

C. PRODUCTION OF BIODIESEL:

Biodiesel can be produced by transesterification catalyzed by chemical catalyst (alkaline, acid) or enzyme, or non-catalytic transesterification under supercritical conditions[25]. Studies for carrying out transformation reaction using supercritical methanol and carbon dioxide as co-solvent in the presence of heterogeneous catalysts mainly solid acids were reported[30].
Depending on the feedstock quality and the choice of catalyst, the catalytic reaction routes differ in some processing modules.

Conventional chemical method for biodiesel production:

a. Oil degumming: Degumming is the first refining step of vegetable oils, removing phospholipids, mucilaginous gums and metal contaminants that are bound to phosphatidic acid[31, 32]. Van Gerpen and Dvorak recommended the phosphorous content to be less than 50 ppm to avoid noticeable yield loss in the conventional chemical biodiesel production[33]. In this sense refined vegetable oils are qualified for industrial refining standards because the phosphorous content in these is lower than 10ppm[33, 34]. Additionally oil degumming is also a production process for lecithin, which serves as emulsifier in the food and pharmaceutical industries[35].

b. Pre-treatment of FFA content of high acidic feedstocks: Removal of free fatty acids is also crucial at this stage which otherwise can reduce the quality and yield of biodiesel production. FFA content in feedstocks varies with sources and processing procedures. Refined rapeseed and soybean oils usually contain less than 2 % (w/w) free fatty acids, whereas around 10% and 15 % (w/w) are found in animal fats and waste cooking oil respectively[8]. Due to this reason the FFA contents in these non-refined oils and fats have to be reduced to below 2.5 % (w/w) for the conventional biodiesel production process[36]. The FFA level can be lowered by distillation of FFA or converting FFA in the oil to biodiesel in a pre-treatment step, which is an acid catalysis before the alkali transesterification[24]. The former solution will cause yield loss and both solutions need to deal with the whole amount of raw material (oil and the available FFA). Therefore, an integrated solution is proposed where FFA is distillated from high acidic feedstock and later on converted to biodiesel before it rejoins the major oil stream[37].

c. Alkali- and acid catalyzed transesterification: The primary commercial process for biodiesel production is alkaline-catalyzed transesterification of refined vegetable oils with methanol[38]. The product from this process is fatty acid methyl ester (FAME) which is universally accepted biodiesel. The alkali catalysts such as sodium hydroxide and potassium hydroxide etc are soluble with reactants but need their neutralization from the reaction mixture at the end[25, 39]. However, acid serves as suitable catalyst if the amount of free fatty acids and the amount of moisture level is higher in the starting substrate. Generally the maximum amount of FFA acceptable in an alkali-catalyzed system is below 3 wt %. Therefore, and additional pretreatment step is required if the oil or fat feedstock has a FFA content over 3 wt %, before the transesterification process[40]. Along with this the moisture level in raw material should also need to be within defined limits to avoid soap formation during the alkali mediated transesterification reaction. Several reports demonstrate the significant impact of water which it have on transesterification reaction when compared to FFA. Generally presence of water speeds up hydrolysis of triglycerides and increases the FFA content in vegetable oils[41].

Ma et al. (1999) noted that during transesterification reaction, presence of water causes larger negative effect than FFAs[27]. Similarly, Demirbas in 2009, reported that water content is an important factor in the conventional catalytic transesterification of vegetable oils[4]. Canakci and Van Gerpen were of the opinion that presence of water as little as 0.1 wt. % during transesterification reaction will reduce the formation of fatty acid alkyl esters[42]. In the same way, Kusdiana and Saka, stated that conversion of triglycerides to methyl esters can be reduced to 6% when only 5% of water is added. The authors also noted that addition of water as little as 0.1 wt. % might lead to the reduction in the yield of methyl esters[43].

The chemical route has the advantage of high conversion rates within short reaction time, cost effective catalyst and modest operation conditions[36]. The differences in transesterification catalyzed by enzymatic and conventional processes are depicted in Table 3. However, alkali-catalyzed process can have side reactions like saponification (reaction between alkaline and free fatty acids) which can greatly affect the yield and quality of biodiesel product[44].

Table 3: Comparison of alkaline and enzymatic transesterification methods for the production of biodiesel[8, 19, 45, 46].

Factor	Conventional method	Enzymatic catalysis
Feedstock requirement	Low FFA and water	Flexible
Temperature	Medium and high	Low
Alcohol choice	Methanol	Methanol/ ethanol
Batch reaction time	Short (1 hour)	Long (6-24 hours)
Product yield	Normal	High
Glycerol purity	Low	High
Catalyst cost	Low	High
Catalyst reuse	No	Yes
Commercialization	Yes	No

Therefore, it strictly requires anhydrous conditions specially a problem with wet ethanol and feedstocks of low FFA content. In order to tackle the problem of alkali catalyzed transesterification reaction, an additional prior step of acid treatment step is practised using hydrochloric acid or sulphuric acid[47, 48].

Yusuf and Sirajo, discussed the alkali catalyzed biodiesel synthesis from Groundnut oil under optimum reactions conditions of 1% catalyst at 70°C in only 1 hour for biodiesel synthesis. Biodiesel so formed was evaluated in terms of viscosity, density, flash point and Gross Calorific Value (GCV) and found in accordance with international standards[44]. However, in this particular study the methyl esters transformed were not detailed by author. Patil and Dang in 2009 carried out comparative study for biodiesel synthesis using acid and alkali catalyst for transformation of jatropha oil, pongamia oil, corn oil and canola oil in one-step and two steps, respectively. The results showed that both one-step and two-step transesterification methods are best suitable for both kinds of oil used for biodiesel synthesis[49]. Experimental analysis showed that biodiesel from jatropha oil and corn oil has fuel value quite similar to that of petroleum diesel in contrast to that from karanja and canola methyl esters. Methyl esters formed were evaluated in term of specific gravity, viscosity, calorific value, acid value cetane number and pour point for all four transformed product.

In two steps transesterification reaction acid reaction step is followed by base catalyzed step. Sujan et al. (2009) studied the biodiesel synthesis using jatropha oil using two steps process. Free fatty acid level was achieved to less than 1% in 1 hour at 50°C containing 1% of H_2SO_4 as catalyst in 0.4 w/w methanol to oil ratio. This step was followed by alkali catalyst (0.5% w/w) in 0.20% w/w methanol to oil ratio. They reported biodiesel yield of 95 and 84 % respectively within 1 hour[39].

D. PURIFICATION STRATEGIES FOR BIODIESEL:

After transesterification in the traditional process, the biodiesel products are rarely within the biodiesel specifications due to the short reaction time and thermodynamic limitations (Including driving of reaction towards hydrolysis due to the presence of water content), which means that a product purification process is required. The conventional purification process is complicated and energy intensive, including neutralization of alkali catalyst, water washing of biodiesel followed by FAME distillation and glycerol distillation. The resulting salts from neutralization of alkaline reduce the quality and value of by-product glycerol[19, 50]. Product monitoring is imperative due to the fact, during transesterification process, beside intermediates like MAG and DAG other components like partial glycerols, unreacted TAG as well as unseparated glycerol, FFA, residual alcohol, and catalyst remains can contaminate the final product. These contaminants in biodiesel may lead to severe operational problems such as engine deposits, filter clogging, or fuel quality deterioration with respect to time. Therefore, standards such as those in Europe (EN 14214; EN 14213) and United States (ASTM D 6751) limit the amount of contaminants in biodiesel fuel[11].

E. BIOCATALYSIS FOR BIODIESEL PRODUCTION:

The major differences of the enzymatic vs chemical method for biodiesel production are shown in Table 3. Enzymes are the choice of process development from the inception. Today among other classes of enzymes hydrolases processes approximately 75% of enzyme market[51] as shown in figure 1.

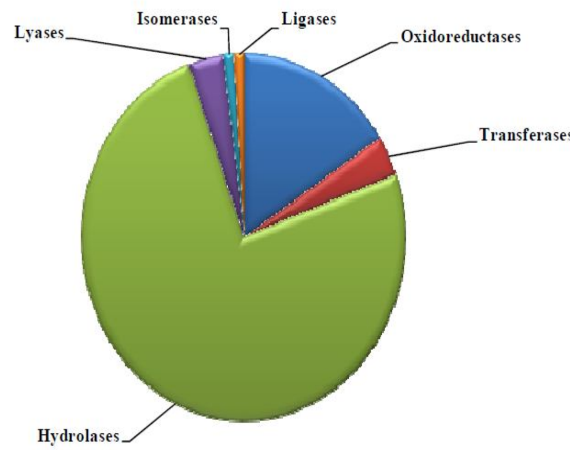

Figure 1: Classes of enzyme and their respective share in market (Faber, 2004)[51]

Transesterification of vegetable oil using enzymatic mode is preferred because of their low cost and simple method requirement for the synthesis of biodiesel[52]. Apart from that, they have similar process modules when using refined vegetable oil including oil degumming, transesterification and product purification. However, when using high acid feedstock e.g. waste oil, the pre-treatment is not necessary since free fatty acid (FFA) can be converted by lipases to biodiesel. Furthermore, the immobilization of lipase and its further application for transesterification reaction requires fewer unit operations for product purification than those required in chemical process. The enzymatic process is more adaptable to feedstock of different qualities, highly selective with fewer side products and more energy efficient. It produces less waste water and high-purity by-product glycerol. Apart from this biocatalytic process for transesterification is considered as environmental friendly. These advantages make the lipase catalyzed biodiesel process promising with great potential to substitute the conventional biodiesel process after overcoming cost limitations.

Lipase for methyl esters synthesis: Among hydrolytic classes of enzymes, lipases are the highly exploited industrial enzyme because of their wide spectrum of applicability in carrying out the reactions in non-aqueous medium[53, 54]. Transesterification is the reaction of a lipid substrate with an alcohol to form esters and a by-product that is glycerol as depicted in figure 2.

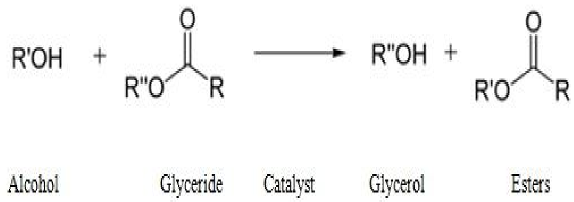

Figure 2: Transesterification of triacylglyceride with alcohol.

It is in principle the action of one alcohol displacing another from an ester. The reaction is reversible where alcohol can be witnessed as the major driving force which pushes the reaction equilibrium to the product formation side. In due course of research, the stability and the re-usability of the enzyme are significantly improves in water limiting reaction and hence the cost of the process can be reduced. Therefore, the synthesis of methyl esters catalyzed by lipases is an alternative to overcome the problem arises due to depletion of available resources.

Different edible oils such as soybean oil in USA, rapeseed oil in Europe and palm oil in Malaysia are being used for the production of methyl ester. However in India, edible oil demand is much higher than its domestic production and the cost is higher compared to diesel fuel. Therefore, non-edible oils are highly welcomed and currently are used for biotransformation under water limiting conditions for methyl esters synthesis.

In Indian scenario, there are several non-edible oil seed species such as *Pogamia pinnata* (Karanja or Honge), *Jatropha curcas* (Jatropha or Ratanjyote), *Azadirachta indica* (Neem) and *Madhuca indica* (Mahua) etc., which could be utilized as a source for production of oil[55]. However, *J. curcas* and *P. pinnata* has been found most suitable for the purpose of production of renewable fuel as biodiesel. It can be planted on under-stocked forest lands, farmer's field boundaries to provide protective hedge, fallow lands, public lands along railway tracks, highways, canals and community and government lands in villages. It can also be planted under the poverty alleviation programmes that deal with land improvement.

Among others, *J. curcus* is probably the most highly promoted oil seed crop at present in the world. *J. curcas* plant belongs to Euphorbiaceae which showed promise for use as an oil crop for biodiesel production. The Jatropha plant is Latin American in origin and is closely related to the castor plant. It is a large shrub/small tree able to thrive in a number of climactic zones in arid and semi-arid tropical regions of the world. An easy to establish perennial, it can grow in areas of low rainfall (250 mm per year minimum, 900-1,200 mm optimal) and is drought resistant. In addition, it is valued for crop protection, prevents wind/water erosion, is not browsed by animals, will reach maximum productivity by year five, and has a 50 year life-span[56]. Also the fuel properties of biodiesel from Jatropha plant are comparable with to those of fossil fuel diesel and confirm to the American and European standards[46]. Table 4 highlights the biodiesel specifications from European standard and properties that jatropha oil biodiesel offers.

Table 4: Depicting the characteristics of biodiesel from Jatropha oil which is within range on EU standard.

Properties	Units	EU standard for bio-diesel	Jatropha oil bio-diesel
Density (at 20°C)	g/ml	0.86-0.90	0.879
Flash point	°C	>101	191
Cetane Number	-	>51	57-62
Kinematic Value (40°C)	cSt	3.5-5	4.20
Calorific value	MJ/Kg	Undefined	39.5
Iodine Number	-	<120	95-106
Sulphated ash	%	<0.02	0.014
Carbon residue	%	<0.3	0.025

Numerous patents have been filled at national and international level regarding the transesterification reaction catalyzed by lipase enzyme. Basheer (2010) claims the enzymatic synthesis of fatty acids alkyl esters in a solvent free microaqueous system, from fatty acids and an alcohol employing lipase single or jointly immobilized on support with the objective to prevent denaturation of enzyme from excess of methanol, glycerol and water formed at the end of the reaction[57]. This biocatalyst (Novozyme® 435 *Candida antarctica* Lipase B) possesses different specificity for glycerol backbone of the fatty acid source. Schorken et al. (2010) was granted a patent for process development involving the lipase from *Thermomyces lanugenosus*. The main objective was to provide a biofuel which could meet the guidelines of European Parliament where process would be ecofriendly and less polluting. Second objective in the investigation was to find the appropriate enzymatic preparation that would increase the yield of monoglyceride and diglycerides from polyol ester. The invention primarily directed for the composition, comprising C1-C8 alkyl esters and partial glycerides, with a free glycerol content produced by enzyme and which are useful as biofuel[58]. Du et al. (2009) described the production of biodiesel catalyzed by lipase from Lipozyme® TL, Lipozyme® RM and Novozyme® 435 and a mixture thereof[59]. Inventor claims that the process is beneficial in terms of improvement of lipase reactivity, prolongation of life and enhanced yield of biodiesel. Process employing the short chain alcohol ROH as an acyl acceptor, hydrophilic organic solvent and renewable oil as starting raw material for the synthesis of biodiesel catalyzed by lipase.

In addition to this, biodiesel synthesis has been reported using lipases in free or immobilized form. Mendes et al. (2011) reported use of immobilized lipase of *Thermomyces langinosus* (TLL) and *Pseudomonas fluorescens* (PEL) through multipoint covalent attachment for biodiesel synthesis using babassu and palm oil with ethanol in solvent-free media[60]. However, multipoint covalent attachment works only for lipase from TLL on Toyopearl AF-amino-650M resin which was activated with glycidol, epichorohydrin and gluteraldehyde. For biodiesel synthesis the optimum ratio reported was 9:1 for ethanol and babassu whereas it was 18:1 for ethanol and palm oil respectively. Products of reaction i.e. fatty acids ethyl ester (FAEE) were determined by gas chromatography (GC) using FID (varian CP 3800).

F. WORLD SCENARIOS FOR BIODIESEL QUALITY:

Biodiesel is used as fuel commercially in many countries for last couple of decades either in blended form with conventional fuel or in pure form. However, due to the strict vigilance of European and American Standard for Biodiesel (EN14214 and ASTM D 6751) only chemically and physically characterized biodiesel which meets the set standards of these agencies could be allowed as biofuel[61]. Romdhane et al. (2011) reported the esterification of 1-butyl oleate as a biodiesel using *Talaromyces thermophilus* lipase in immobilized form[62]. Immobilization was achieved by different methods like adsorption, ionic binding and covalent coupling using chitosan as support material. TLL in immobilized biocatalyst was reproducible for at least ten cycles without much loss in its hydrolytic activity. A high performance synthesis of 1-butyl oleate with 95% yield was observed at 60°C with a molar ratio of 1:1 for oleic acids to butanol using 100 U (0.2 g) of immobilized lipase. The authentication of product was done using GC-MS to confirm the conversion percentage calculated by titration. Shah and Gupta in 2007, reported the synthesis of biodiesel using immobilized *Pseudomonas cepacia* lipase on celite for the transformation of jatropha oil[63]. For the synthesis of biodiesel they optimize the process by screening various commercial lipase preparation, pH tuning, their immobilization followed by varying the temperature and water content in the reaction. The best yield of 98% (w/w) at 50°C in the presence of 4-5% (w/w) water in 8 hours was observed. Moreover, they find that the biocatalyst is reproducible at least for four times without any activity loss.

Generally, the reaction product in transesterification reaction can also be ester with short chain fatty acids which find their application in food and flavor industry. Liaquat in 2011 reported the synthesis of (Z)-3-hexen-1-yl caproate using rapeseed lipase[64]. Enzyme was found to catalyze ester formation more efficiently with non-polar solvent then their counterpart polar organic solvents in spite of the same stability in both. Also the specificity of lipase is more for straight and branched chain alcohol whereas for secondary alcohol low or no reactivity was reported. Maximum ester yield was (95%) after 48 hours and at 25°C when 0.125 M (Z)-3–hexen-1-ol and caproic acid were used as reactant. The product transformed was analyzed by GC-MS and determined quantitatively using calibration graph of peak area versus concentration. Ahmed et al. (2010) reported the esterification of ethyl caprylate which is used as a constituent in fruity flavour and also as flavour enhancer in wine and whisky[65]. The partially purified lipase from *Bacillus subtilis* 37 was used in both free and immobilized form and the esterification efficiency of the reaction calculated was found to be 55.8% and 75% respectively. Quantification of ethyl caprylate formed was done using gas chromatography equipped with FID and Rtx-R-20 capillary column. Nitrogen serves as carrier gas at the flow rate of 90ml/min. Table 5 highlighting the methods used for the analysis of biodiesel transformed using lipase from different oils under different reaction conditions.

Table 5: Method used for analysis of transformed product.

Source/s	Catalyst	Reaction conditions	Method for analysis	Yield	Reference
Crude jatropha oil, Karanj oil, sunflower oil and Propan-2-ol	Immobilized lipase (Novazym-435) 200mg	50°C, 8 hours, 4:1 ratio of alcohol to oil	GC	92.8, 91.7 and 93.4% respectively	66
Salad oil and methanol Plant oil and waste oil	Immobilized lipase from *Candida* sp 99-125 (2g)	40°C, 180rpm, 1:3 ratio of alcohol to oil, petroleum ether in 1:1 ratio with oil as solvent media, 10% water	GC	90 and 92% respectively	2
Lard + methanol (1:3 Molar)	Immobilized lipase *Candida sp* 99-125 (0.2g)	n-hexane, 20% water, 40^{o}C for 30 hours	GC	87.4%	67
Jatropha oil and ethanol	Immobilized lipase from *Pseudomonas cepacia* (0.05g)	50°C, 4-5% water in reaction media, 8 hours	GC	80%	63
Sunflower oil: ethanol (2:1 v/v)	Pig Pancreatic lipase (PPL) (00.1-0.1% W/W) of the total substrate	60°C, 8-12 hours	GC	-	6
Canola oil and Methanol	Immobilized *Candida antarctica* lipase	40°C, 250 rpm, Iso-octane and tert-butanol	GC	-	68
Soybean oil and methanol	Immobilized and free lipase from *P. aeruginosa* LX1	Water (0.27g), 30°C, 180rpm, tert-butanol as reaction media	GC	80 and71.5% respectively	69
Palm oil, crude palm oil and free fatty acid and ethanol (1:4 Molar)	*Thermomyces langinosus* lipase expressed in *Aspergillus* Strain	45°C, tert-butanol (1:1 molar ratio) as reaction media	GC	89,82 and 75.5% respectively	70
Oleic acid and 1-butanol	Immobilized lipase from *Talaromyces thermophilus*	60°C, hexane as reaction media	GCMS	95%	62
Pistacia chinensis bge seed oil and methanol (1:5 Molar)	Immobilized lipase from *Rhizopus oryzae*	Enzyme dosage 25 IUAI-ROL/g PCO or 7 IUMI-ROL/g PCO, water content 20% by weight of oil, 37°C, 60 hours.	TLC and GC FID	92, 94%	71

Olive oil and methanol (1:6 Molar)	Immobilized lipase from *Staphylococcus haemolyticus* L62	30°C, 10 hours, 230 rpm	TLC and GC with FID	90% within 6 hours	72
Alkali and Acid catalyzed transesterification					
Karanja oil and Methanol (12:1Molar)	KOH 1% Conc.	65°C/3hours, 360rpm	>97%	Physical properties like viscosity, flash point, cloud point and pour point	73
Soybean oil and Ethanol (10.0 Mol/Mol)	.035% in KOH	30min, 29°C	91.8%	TLC, GCMS with (TCD)	74
Rapeseed oil and Methanol	KOH 0.6% Conc.	50°C, 90min	-------	GC	75
Karanja oil and Methanol Jatropha oil and Methanol	KOH 1% Conc.	65°C, 3hours	89 and 91% respectively	H^1NMR	76
Wastewater sludge as lipid feedstock (Primary and Secondary and Methanol (1:12)	H_2SO_4 (5% v/v)	75°C	14.2 and 2.5% respectively	GC	47
Mucor circinelloides fungus as lipidic feedstock and methanol (60:1)	BF3, H2SO4 and HCl (8%)	65°C, 8 hours	99% in direct transesterifcation, 91.4 and 98.0% in two step process for all three catalyst	Thin Layer Chromatography	48

G. POTENTIAL OF BIODIESEL IN INDIA:

Continuous increase in the price of crude oil, together with the uncertainty of the future price trends and the limited crude oil production, has forced India to start considering biofuels as one alternative path. For example in order to meet the supply and production gap of oil, the country starts importing approximately 75% of crude oil since 2004, and still this fraction is increasing yearly. Statically, India produces only 33 million tonnes per year of its annual crude oil requirement of about 120 million tonnes where it is speculated that about 50% of oil use is consumed by the transport sector alone. For the remaining 75% India relies on costly imports (Table 6).

Table 6: Projected use of gasoline and diesel in India.

Years	Diesel (High Speed Diesel)	Gasoline (Motor Spirit)
2001-2002	39.8	7.07
2006-2007	52.3	10.1
2011-2012	66.9	12.8
2016-2017	83.6	16.4

Apart from the economics related to this percentage, political and national security issues continuously pushing for the need for the development of biofuels.
In Europe, the United States, Australia, etc., biodiesel is produced from rapeseed, soybean, etc. In India, these products are highly valuable since most of the people need these products for fulfilling other priorities such as nourishment. On the other hand, India has enormous potential for production of non-edible seeds from which oil can be derived to develop biodiesel, depending upon the potential of specific seeds in the locality.

Since the Indian government does not consider the use of edible oils owing to "feed and fuel" debate thus the focus is only on non-edible seeds for biodiesel production. Due to this, Planning Commission has proposed a National Mission on Biodiesel and *J. curcus*. This include mass scale plantation, seed collection and setting up plants for biodiesel production.

H. ENVIRONMENTAL BENEFITS USING BIODIESEL:

According to US EPA, the use of biodiesel in a conventional diesel engine results in substantial reduction of unburned hydrocarbon, carbon monoxide and particulate matter. Also, emission of nitrogen oxides are either slightly reduces or increased depending on the duty cycle of the engine and testing methods used. Biodiesel is non-toxic therefore, has no health risk to humans. Vehicles that run on biodiesel emit less sulphur dioxide (SO_2), particulate matter (soot), carbon dioxide (CO_2), with fewer heavy hydrocarbons (HC) and polycyclic aromatic hydrocarbons (PAH) as shown in Table 7.

Table 7: Reduction in gaseous emission using biodiesel.

Emission	B10	B100
Regulated Emission total unburned	30%	-93%
Hydrocarbon	-20%	-50%
Particulate Matter	-22%	-30%
NO_X	+2%	+13%
CO_2	-	-80%
SO_2	-	-100%

CONCLUSION: Meanwhile, biofuel production is booming around the globe and gradually gaining acceptance in the market as an environmentally friendly alternative fuel. As Brazil, the United States, and Europe account for the lion's share of today's biofuel production and consumption, Indian is also making effort to devise alternatives for energy crisis. This means that the countries can produce their own fuel, and reduce their dependence on foreign sources for energy[77]. However, for biodiesel to establish and continue to mature in the market, various aspects must be examined routinely. Some of the key issues such as improving efficiency of the production processes by using low cost feedstock, method of production either through chemically or enzymatically need careful attention and monitoring. Considering the prowess of enzymatic process for transesterification over chemical process, different strategies needed to be put into practice where the overall efficiency of the process could be enhanced. Finally with any new technology or products, biodiesel will require continuous improvement especially in producing cleaner emissions and having less impact on the environment.

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