

# Waste Cooking oil: A Resourceful Waste for Lipase Catalysed Biodiesel Production

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**Abstract-** The growing concerns for environmental sustainability, economic and energy security had made it obligatory to source energy alternatives that harmonize environmental friendliness with biodegradability, renewability, and less reliance on petroleum products dependence. One of such energy sources is called biodiesel. Wide range of substrate; vegetable oils e.g. palm oil, soybean oil, sunflower oil, coconut oil, rapeseed oil, micro algal oils, animal fats, waste products of vegetable oil refinery, used frying/cooking oils was used in its production. To produce biodiesel with these substrates conventionally is complex, as the method is faced with limitations in the downstream processing costs, byproducts recovery and environmental issues. Enzymatic method, involving enzymes particularly lipases in a process of alcoholysis with waste cooking oil emerged to be efficient in curtailing the problems. The potentiality of the waste cooking oil for this purpose among other applications qualified it as a resourceful waste in view of the fact that no concrete waste definitions limit it to only waste. This review focuses distinctively on how biodiesel fuel is produced by lipase catalysis of waste cooking oil, waste concept and definitions and how waste oil can be a viable recourse. Sources of different lipases and substrates were reviewed to include specific substrate modifications to describe the current substrate used in this article. Thus, the economic feasibility of biodiesel production from waste cooking oil is conclusively viable.

**Index Terms-** Immobilized Lipase; Biodiesel production; Waste cooking oil, Waste.

## I. INTRODUCTION

There is an increasingly spurring necessity for an environmentally sustainable alternative energy sources that completely harmonize its environmental friendliness with biodegradability, renewability, low toxicity and less reliance on petroleum product dependence. One of such energy sources is called biodiesel. The current issue of energy security is an additional goal for alternative sources of fuels as prices hikes in recent times; around \$65–67/barrel, approaching \$70/barrel [1]. Shortage of fossil fuels, economic politics and global warming are among other reasons. Biodiesel referred to as fatty acid methyl/alkyl ester can be produced from a wide range of substrate; vegetable oils e.g. palm oil, soybean oil, sunflower oil, coconut oil, rapeseed oil, micro algal oils, animal fats, waste

products of vegetable oil refinery, used frying/cooking oils. Chemically, they are known as monoalkyl esters of fatty acids. The conventional production of biodiesel is complex, as the techniques employed is faced with a number of problems. This conventional method involves the normal acid and base catalysts to form fatty acid alkyl esters. Downstream processing costs, by-product recovery and environmental problems associated with biodiesel production have made it a requisite for the search of alternative production methods and unconventional substrates. The marginal substitute for viable biodiesel synthesis is to supplant the conventional processes with enzymatic methods, involving a wide range of enzymes, particularly lipases in a process of alcoholysis; a form of transesterification reaction. With this enzymatic technique, the aforementioned problems associated with the conventional process is practically solved and the downstream processing costs, a significant limitation of the conventional methods is overcome but replaced with the enzymes cost. Since the outstanding concern now focuses on costs, the application of enzymatic and whole cell immobilization can cut down these costs and enable its multiple and successive usage in an environmental-friendly manner. In further pursuance to reduce these costs, waste cooking oil, a readily discarded by-product which serves as nuisance to many individuals is a potential resourceful waste for enzymatic biodiesel production. The concept of using waste cooking oil as previously highlighted also satisfies the European Waste Act (though emphasis more on prevention) and promotes efficient waste recycling, primarily in the form of biomaterial transformations and secondarily, as a sustainable energy source [2].

Therefore, the aim of this paper is to discuss the potentiality of waste cooking oil as a resourceful waste in biodiesel production and to bring out its cost effective prospect of biodiesel production. Other cost effective improvement means includes the production of quantum lipase using recombinant DNA technology, immobilized lipases and immobilized whole cell technology have tendencies to lower the overall cost, with less downstream processing problems.

## II. CONCEPT OF WASTE RECYCLING

Having understood the importance of waste cooking oil in diverse application particularly its role in biodiesel production become necessary to have a clearer look at its functional

meaning. European Council 1991 Directive recognizes the need to ensure a common terminology and definition of waste and also emphasizes the desirability of heartening the recycling and re-use of waste as raw materials [3]. The waste Act, which emphasis more recovery of waste in primary form as materials and, secondarily, as energy has received a criticism by The European Chemical Industry Council (CEFIC) which argues that it is not the nature of the material that determines if it is a waste, but only the holder's action and intention [4]. Waste definition becomes a serious issue among EU member state and other countries, mainly due to its legal dispute, the implication is that when a substance is defined as waste, is regularly restricted in its transport, sale and re-use, or has a lot of administrative protocols in its import export processing as such become cost incurred. In the 90s, this led to agreeing Waste Catalogue, but individual, state laws still have different definitions and interpretation [5] as a result that it lacks precision individual member has various interpretation as illustrated in Table 1. McKinney [6] describe waste as the unnecessary (meaning preventable) costs that result from inefficient practices, systems or controls.

The notion of waste definition remained the same owing to the fact that a clear and concrete waste characterization is yet to be established since opposing views are now seen by various researchers. The waste notion is relative in different respects. A waste becomes a waste when it loses its primary function for the user, thus someone's waste output is often someone else's raw material input. Secondly, the notion of waste is also relative to the technological state of the art and to the location of its generation. Here going by this definition in this case, the waste cooking oil has a vivid understanding role in energy production as biofuel.

As scientists aim for progressive research, waste cooking oil as so called, has joined other waste as therefore a very dynamic concept since a concise definition of waste is yet to be provided for regional or global consumptions. To be fair not to go extreme, we viewed it as a resourceful waste or otherwise a raw material.

**Table 1: EU and EU members Definition of waste in Directive 91/156/EEC (Bontoux & Leone 1997 In [2].**

Countries	Waste definition	Legislation
EU	Any substance or object in the categories set out in Annex I which the holder discards or intends or is required to discard	91/156/EEC
	Any substance or object, which the holder disposes or is required to dispose of pursuant to the provisions of national law in force.	75/442/EEC
Austria	Objects which an owner or holder wishes to dispose or disposed	Austrian Waste Management Act 1990 last

	of, or where their collection and treatment as waste is required by the public interest. EWC is not mentioned	revised by GBI. Nr. 434/1996
Belgium	As 91/156/EEC, but without mentioning EWC	Region Wallone: Decree 27 June 1996; Flamish Region: Decree 2 July 1982, amended 20 April 1994
Denmark	As 91/156/EEC with EWC	Statutory Order no. 299 of 30 April 1997
France	Material originating from a production or transformation process, or use, which the holder discards or intends to discard. The distinction between waste and ultimate waste is made	French Act 75-633 1975 revised 13 July 1992
Finland	As 91/156/EEC with EWC.	Waste Act 1072/1993
Greece	As 75/442/EEC not updated to 91/156.	n.a.
Germany	As 91/156/EEC with EWC	Waste Management Act (RWMA) 1994
Italy	As 91/156/EEC with EWC.	Decree 22/97
Ireland	As 91/156/EEC with EWC	Environmental Protection Act July 1996
Luxembourg	Any substance or object which the holder abandons or is required to discard, Also considers waste any product or substance that sent for recovery until it enters the commercial chain again	N.A.
Portugal	As 75/442/EEC not updated to 91/156.	N.A.
Spain	As 75/442/EEC not updated to 91/156	N.A.
The Netherlands	As 91/156/EEC, but without mentioning EWC	Wet Milieubeheer 1993

United Kingdom	As 91/156/EEC, but without mentioning EWC	Environmental Protection Regulations (1991)
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### Sources of Lipase for Biodiesel

Lipase are classified broadly from intracellular and extracellular origins, they are solemnly obtained from life organisms. The sources from which they originates also forms the basis of their classification where there are plants, animals and microbial or microorganism lipases. Plant lipase include those obtained from; castor seed lipase, canola seed lipase, elm seeds, oat seed lipase papaya latex and pinus seed lipases [7, 8]. Animal sources of digestive lipases are pancreatic lipases, gastric and hepatic lipases.

The optimum yield of these enzymes is obtained from microorganisms particularly fungi and bacteria as they are produced at higher concentrations with less restrictions which makes it prominent for its products to possess the properties that ease their commercialization [9, 10, 11].

These lipases are commonly used industrially to obtain a diverse range of products which include biodiesel and other agricultural yields. Lipid modification for industrial application is based on the nature of the modification in question, which dictate the lipase to be selected. These modifications may also include fatty acids- specific modification, position-specific modification of triacylglycerol, modification by synthesis i.e. direct synthesis and transesterification and modification by hydrolysis [1]. The analysis of literature revealed the following microbial lipases, to start with fungi then bacteria; *Aspergillus niger*, *Candida cylindracea*, *Candida rugosa*, *Geotrichum candidum*, *Fusarium heterosporum*, *Fusarium oxysporum*, *Humicola lanuginose*, *Mucor miehei*, *Oospora lactis*, *Rhodotorula rubra*, *Penicillium cyclopium*, *Penicillium roqueforti*, *Rhizopus arrhizus*, *Rhizopus boreas*, *Rhizopus thermosus*, *Rhizopus usarii*, *Rhizopus stolonifer*, *Rhizopus fusiformis*, *Rhizopus circinans*, *Rhizopus delemar*, *Rhizopus chinensis*, *Rhizopus japonicus* NR400, *Rhizopus microsporus*, *Rhizomucor miehei*, *Rhizopus nigricans*, *Rhizopus niVeus*, *Rhizopus oryzae*, *Rhizopus rhizopodiformis*, *Rhizopus stolonifer* NRRL 1478, *Thermomyces lanuginosus*, *Chromobacterium Viscosum*, *Bacillus thermoleovorans*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Pseudomonascepacia*, *Pseudomonasfluorescens*, *Pseudomonas putida* and *Staphylococcus hyicus*, [7, 1, 12]. Several researchers [13, 14, 15] have published the application of lipases for biodiesel production using vegetable oil substrate. The production can also be achieved by waste cooking oil with a considerable productivity. Maceiras *et al.* [16] reported 89.1 methyl ester conversion by *Candida antarctica* (CALB) while Wu *et al.*, [14] reported ethyl ester yield of 85.4%, with Lipase PS-30 (specific activity 34 IU/mg solid) from *P. cepacia* at an alcohol concentration of 95%.

### III. LIPASE MICROENVIRONMENT

The microenvironment of an enzyme molecule depends on its electrostatic charges and the rate at which reaction occurs in non-aqueous media depends on these environmental charges. When the amino acid polar group of protein dissolves in aqueous medium, the pH of the medium determines the charges. In this case, the charge becomes uniform as the protein is transferred to another medium like oil. At this point, the pH memory of protein comes [17]. pH memory has been attributed to a fixation of protein catalytic group ionizations after the biocatalyst preparation. The standard model of the enzymatic fixation process is the maintenance of all of the ionization states present, i.e. buffers species and protein before the freezing. The various effects of additives on the ionization state of low-water proteins has been studied [18].

Therefore, production by optimization of enzyme activity in the non-aqueous medium requires the stabilization of the pH of the microenvironment, as previously observed in our study [19] (Deba, 2013) with *Candida rugosa* lipase with phosphate buffer, at both free and immobilized form in preparation for transesterification to attain higher catalytic activity. Thus, the process of enzyme pre-treatment and solution buffering ensure the maintenance of its pH ionization power is essential for all biocatalysis and contributes to higher catalytic activity of enzymes as well as its stability [10].

### IV. ENZYMATIC ALCOHOLYSIS OF TRIGLYCERIDES

Enzymes are biological molecules (catalysts) which promote numerous chemical reactions to take place within the homeostasis limit of a living system. Enzymes have enormous potential for reducing environmental crisis and energy claim in the chemicals and pharmaceutical industries [11]. Mass scale enzyme applications have been reported for the production of drinks and textiles, leather, detergents, baking, rare sugars, starch hydrolysis and fructose production, pulp, antibiotics (semisynthetic penicillins) and genetic engineering [20, 11].

Transesterification or alcoholysis can be carried out or without enzymes, and numerous examples are overflowing in the literature on the application for biodiesel [12, 11, 21]. Recently, interest is ongoing in using lipases as the biocatalyst, which is basically to commercially convert vegetable oils/ fats to FAME as biodiesel fuel, as a result of the benefits it has over the acid and alkali catalysts;

- Free of soap formation
- Washing step is avoided to esterify both FFA's and triglycerides in one step mechanism
- Capitate a better quality glycerol
- Ability in handling large variation in feedstock quality
- A second generation raw materials like waste cooking oils, animal fat and similar waste fractions, with high FFA and water content, can be catalyzed with complete conversion to alkyl esters with considerably reduced quantity of wastewater and
- Works under friendly conditions leading to less energy consumption
- Highly selective

- Can be genetically engineered to improve their efficiency
- The reactions catalyzed by enzymes are considered "green" reactions

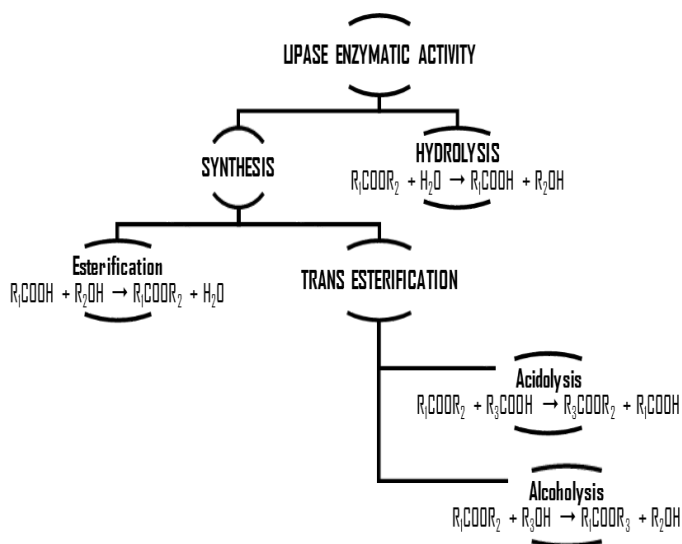
[1, 22, 21]

A comparison of conventional (alkali catalyst) transesterification versus biocatalyst transesterification is presented in Table 2.

**Table 2: Distinctive differences between Conventional and Biocatalyst Transesterifications [11]**

Major factors	Biocatalyst Transesterification	Conventional Transesterification (Alkali)
Biodiesel production yield	Relatively lower than alkali catalyst, around 90%	High, nearly 99%
Downstream processing	None	Multi-step purification of end products
Production cost of biodiesel	Really expensive as biocatalyst are expensive	Cheap, as catalysts are comparatively cost less
Commercialization	Not exactly	100% commercialized
Waste water generation	No waste water generation	Saline and alkaline effluents need treatment No waste water generation before discharge
Temperature	20-60°C	60-80°C
Presence of FFA's in feed stock	Completely conversion into the methyl ester	Soap formation
Presence of water	No effect on final product	Towards for more soap formation as hydrolysis No effect on the final product of the oil may take place

The reactions catalysed by lipase can be classified into two (2) stages; Hydrolysis and Synthesis reaction: the later is further subdivided as represented in Figure 1.



**Figure 1: Reaction classifications of lipase enzymatic activity**

The vast majority of enzyme researches for biofuel synthesis have been concerned in determining the most reliable enzyme source and optimizing the reaction conditions; substrate molar ratio, solvent, temperature, acyl migration, water content [1, 22, 23, 24], for increase in the production yield to enable industrial scale application. The problem associated with the enzyme reaction, particularly lipase is methanol inactivation. This problem has been examined and most probably solved by Shimada *et al.* [13], the authors reported stepwise addition of methanol in alleviating methanol inactivation of *Candida antarctica* lipase and the results was 90% yield of FAME from waste edible oil. Moreover, they reported enzyme stability in recycling for >50 times without considerable lost in activity. Few studies have considered the nature of the alcohol used in the transesterification reaction. In the presence or absence of solvents enzymes catalyzed alcoholysis can occur, this requires even less energy and practically no purification in obtaining FAME compared to base-catalyzed alcoholysis, in which soap formation presents downstream processing drawbacks. Table 3 presents the enzymatic production of biodiesel from waste cooking under various reaction conditions with various lipases.

Nevertheless, enzymatic transesterification have been characterized with limitations such as:

- Longer reaction time
- Higher catalyst concentration is needed to complete reaction,
- High cost in enzyme production i.e. ~ \$1000 US/ kg, whereas sodium hydroxide is only \$0.62 US/ kg
- Although reuse of the lipase becomes possible after immobilization, it loses its activity in 100 days of use [25, 21].



**Table 3: Enzymatic biodiesel production from waste cooking oil under various reaction conditions with various lipases**

Oil/fat	Lipase/form	Alcohol/ Acyl-acceptor	Reaction Conditions	Yield realized	Reference
Waste frying oil	<i>Candida antarctica</i> (CALB)/ Imm on acrylic resin (Novozym 435) with an activity of 10,000 PLU/g	Methanol	2 g of WFO, 0.2 g of lipase, methanol to oil molar ratio of 25:1, 10% of Novozym 435, period of 4 h at 50 C incubation, & constant stirring at 150 rpm.	89.1%	Maceiras <i>et al.</i> , [16]
Waste cooking oil	<i>Pseudomonas cepacia</i> /Imm. on ceramic beads	Methanol	Methanol:oil molar ratio 1.00, 50 g of WCO and different methanol conc ranging 4.3 to 8.3 ml. 1 ml of enzyme solution containing 0.2% g immobilized lipase per g oil	> 40 %	Al-Zuhair, [31]
Waste cooking oil	<i>Candida</i> sp. 99-125,	Methanol	Batch reactor, temperature controlled at 40 °C, mixture was stirred with an electric stirrer at 220 rpm, methanol step-wise addition into 150 g WCO, 20 min.	2.5 mmol/g after 30 h	Liu <i>et al.</i> , [38]
Recycled restaurant grease	<i>Pseudomonas cepacia</i> (PS-30, specific activity 34 IU/mg solid)	95 % Ethanol	Mole ratio of grease to ethanol of 1:6.6 , 13.7 wt% lipase (PS-30), at 38.4°C for 2.47 h.	85.4%	Wu <i>et al.</i> , [14]
Waste vegetable oil (acidic oil)	<i>Candida antarctica</i> (Novozymes)/ Imm. on macroporous acrylic resin	Methanol	30-g mixture of dehydrated first-step product and 5.5 wt% MeOH was stieed at 30°C using 6 wt% imm. lipase. Reaction repeated 5 times by enzyme transfered to a fresh substrate mixture every 48 hrs.	>98.5 wt% after a24hrs	Watanabe <i>et al.</i> , [39]
Waste vegetable oil (In waste bleaching earth)	<i>Rhizopus oryzae</i> / Free form	Methanol	Oil/methanol molar ratio of 1:4, 75% water content (by weight of substrate), an, and 67 IU/g of substrate, agitation of 175 rpm for 96 hrs. at 35 °C.	55% (w/w) after 96 hrs.	Lara Pizarro and Park, [69]
Sunflower, soybean, and waste cooking oils	<i>Thermomyces lanuginosus</i> /Imm. covalently on Microporous polymeric matrix (MPPM)	Methanol	Operation conditions were: Alcohol:oil ratio 6:1, water 15%, substrate flow 21 ml/min, temperature 65 °C, , total reaction time 5 hrs & 24 hrs, temperature 25 °C	Sunflower oil (63.8%), Soybean oil (55.5%) & Waste cooking oil (50.9%). In 24 hrs reaction 90.2%, 93.9%, & 97.0%	Dizge <i>et al.</i> , [70]
Waste edible oil	<i>Candida antarctica</i> (Novozym 435)	Methanol	At 30°C with shaking (130 oscillations/min) in a 30 g mixture of oil, 1/3 molar equivalent of MeOH for the stoichiometric amount, and 4 wt.% immobilized lipase	>90%, >100 days without loss in activity.	Shimada <i>et al.</i> , [13]
Waste cooking oil	<i>Candida</i> sp. 99–125/ imm. on textile cloth.	Methanol	Lipase/hexane/water/WCO weight ratio of 25:15:10:100, temperature of 45 °C and reactant flow of 1.2 ml/min	91.08%	Chen <i>et al.</i> , [71]
Waste cooking oil	<i>Penicillium expansum</i> / Imm. on resin D4020	Methanol	2 g waste oil with, 0.4 g t-amyl alcohol, 168 U immobilized PEL	92.8%, 68.4%	Li <i>et al.</i> , [72]

(high acid value)			and different amounts of blue silica gel. 1 molar equivalent of methanol was added at 35°C and 200 rpm for 24 hrs.	original activity retained after used for 10 batches	
Waste cooking oil	Lipase enzymes (Lipozyme-TL IM)/Imm. on hydrotalcite and zeolites, & free form	Methanol	9.65 g of waste cooking oil mixed with lipase (4% by weight of grease), 1.4 g methanol. Alcohol to oil molar ratio as 4:1 mixture kept at 24 °C, 200 rpm.	92.8% (imm.) & 95% (free)	Yagiz <i>et al.</i> , [40]

## V. MECHANISM OF LIPASE REACTIONS AND ITS KINETICS

Lipases are hydrolases as a result of their natural tendency in hydrolysing triacylglycerols. Lipase like other enzymes have a three dimensional structure in an aqueous environment with their non polar group retained inert and the polar region is always exposed. In contrast to other enzymes, lipase lipolytic reaction is somewhat complex, as its lipid substrate are insoluble in water [7]. It has become necessary in order to have active lipase catalysis, lipid immiscibility of water to form a liquid-liquid interface. Thus the interface is the point of accessibility of lipase to the substrate to bring about the reaction [1]. Therefore the nature of the interface and interfacial area as well as interfacial properties influence the activity.

Lipase catalysed alcoholysis reaction occurs in two-step mechanism, occurring for each bond (ester) of triglyceride molecule. It begins with the hydrolyzation of ester bond releasing the alcohol moiety, then esterification with the second substrate followed [15, 26]. The Ping-Pong Bi-Bi mechanism is followed by alcoholysis of triglycerides, the most commonly used reaction to illustrate reactions catalyzed by lipases [27, 28, 12]. To fit to experimental results, simplification kinetics i.e the Michaelise Menten kinetics could be applied [29], as Michaelise Menten kinetics reaction can possibly explain the enzymatic conversion agreeably with appropriate fitting of models of varying complexity, though further rationalization is needed for the accuracy [21]. Numerous kinetic studied for the alcoholysis of triacylglycerols have been established in the literature [28, 30, 21, 12]. The Al-Zuhair kinetic model that follows the classical Ping-Pong Bi-Bi mechanism with alcohol inhibition but also take in an extra parameter, taking into account the release of fatty acids primarily [31]. Another kinetic model is that of Pilarek and Szweczyk [30] which also consider the Ping-Pong Bi-Bi manner with competitive inhibition by alcohol with the suppositions of an irreversible acyl bond sliced in glycerides, a reversible monoglyceride isomerization and an irreversible enzyme deactivation. To enable one have a full image of the rate of enzymatic alcoholysis of triglycerides, parameters to be considered include; lipase type, presence of organic solvents, amounts of reactants, mass transport limitations, the temperature's influence on enzyme deactivation (the equilibrium limitation for conversion) formation and conversion of intermediates, [21].

To add to the aforementioned parameters, the nature of the enzymes which is either in free form or immobilized form. As all the mentioned parameters can seriously have an effect on it. Therefore, by trying to determine kinetics in such systems all these factors become significant.

## Sources of Renewable Oils and Fats for Biodiesel Production

Non renewable recourses are the major sources of our fuel energy, such resources are the fossil fuels which are characterized as non renewable exhaustible, non biodegradable and highly toxic. These resources are in the form of petroleum, coal, natural gas. etc. The renewable energy sources come primarily from plants, animal and microbe, which are categorised as plant derived oils, waste oils/fats and microbial oils respectively. The microbial oils are basically algal derivatives.

## A. Plant-Derived Oils

These category of fuel source are biodegradable, renewable, nontoxic and inexhaustible, with an energy content analogous to that of the conventional fuel (fossil diesel fuel). Fuel obtained from this source is more expensive than doing so from petroleum-based fuels. This is partly as a result of the the contention between their needs either for food, food industry, feed, oleochemical industries or as a biofuel. Plant derived oils as a feed stock was identified as an eligible environmentally friendly fuel source as a result of the CO<sub>2</sub> fixation during the course of plant light production. Where the fixed carbon dioxide serve as a carbon source to the process. For that reason, combustion of fuel from this source is more or less paying back what was fixed naturally. The whole dwindling process of the enzymatic method lied to the fact that the method is not cost wise. With development in agro-genetic engineering, Non edible types of these oils would surely be produced, with that the competition for use as food, fuel or oleochemical industry surely would fade, cost arising conflict with massive production of such oil would surely fade. Other conflicting issues adding to the cost is the cost of enzyme, though immobilization method significantly tried to cut down, but not to the level of cost balancing. Through advancement in microbial molecular genetics (Genetic engineering), enzymes might be produced at huge number.

The fatty acid content of the source crops of oil or fat is significant in biodiesel because, in the winter, oil rich in saturated fatty acid tend to solidify as such clogging the fuel ways [1]. Low production scale are seen with refined oil which are even more costly. Rapeseed, palm kernel, sunflower, Soybean, peanut/ groundnut, cottonseed, castor bean, safflower oils are the more regularly used oils in biodiesel production, [1]. Upon all the vegetable oil used oleic acid rich oil is most suitable because of their better fuel properties [32] and increased stability of their alkyl ester on storage.

Other plant derived oils which are equally important include *Camelina sativa* oil [33], rubber seed oil [34], Coffee oil [35], peanut (*Arachis hypogea* L.) seed oil [36]. Seed oil like *Camelina sativa* (camelina) is a annual oilseed plant of the genus *Cruciferae* that grows well in temperate conditions, and matures earlier than other oilseed crops [33]. Therefore its early maturity can highly be an advantage in its market flow. Fröhlich and rice [33] also reported that methyl ester from this oil were mainly within specification, though low-temperature behaviour could be a problem in some climate which can of course be overcome by the use of suitable pour-point depressants or by blending with diesel oil. Nowadays research has shown the feasibility of defective crop seed to be employed in bio production which otherwise may result in poor quality oil to the market. Oliveira *et al.*, [35] tested the viability coffee oil for biodiesel production, both from healthy and defective beans, both of which were good conversion to fatty acid methyl and ethyl esters. *Arachis hypogea* L. commonly refered to as Peanut, oil from this plant was solvently extraxted and investigated for biodisel by transesterification, methyl ester conversion was 89%.

Most of the later seed oils are also relatively available like most of the commonly used oils (Rapeseed, palm kernel,

sunflower, Soybean, peanut/ groundnut, cottonseed, castor bean, safflower oils). Being that almost all the feedstock of biodiesel are regionally and seasonally specific. There is need for more exploration on enzymatic catalysis of these oils.

## B. Waste Oils and Fats

This category of oil are derivatives of plant oil (vegetable oils) and Animal fats. As such they are oil-based substance consisting of vegetable matter that has been used in preparing food and is no longer suitable for human consumption. Waste/used frying oils, beef tallow, lard, yellow grease [14], Waste Edible Oil [37] and other hard stock fats [1] as generally called are good substrate for biodiesel production. These oils are cheap, may have some drawback due to vulnerability to oxidation, high free fatty acid composition, the contents of high polymerization products and high viscosity. As a result, pretreatment of such oils become necessary in order to reduce the mentioned components, in preparation to transesterification if basic catalyst where to be used, a times even enzymes [1].

Researchers established the application of waste cooking oil as an alternative energy in the form of biodiesel. Maceiras *et al.* [16] investigated the used of waste frying oil and reported methyl ester 89.1 % using *Candida antarctica* (CALB). Al-Zuhair [31], reported methyl ester > 40 % with *Pseudomonas cepacia* Immobilized on ceramic beads Recent study by Liu *et al.* [38], reported the applicability of waste cooking oil in batch reactor by *Candida sp.* 99-125 (2.5 mmol/g after 30 h). Acidic oils can also be applied in the quest for the alternative fuel. Watanabe *et al.* [39] established the potential waste vegetable oil (acidic oil from vegetable refining) in biodiesel production, fatty acid methyl ester conversion was >98.5 wt% after a 24-h reaction, catalysed by *Candida antarctica* (Novozymes) , immobilized on macroporous acrylic resin. And also reported reusability of the biocatalyst >100 days without significant loss in activity. Yagiz *et al.* [40] using waste cooking oil obtained 92.8% methyl ester for from Lipozyme-TL IM Immobilized on hydrotalcite and zeolites. Even the free lipase from the same reaction gave 95%.

Other prospective energy supply can be from the biodiesel produced from the waste cooking oil in the form of blend. Blending of fuels such as biodiesel and fossil diesel (for example, B20) 20% B100 and 80% diesel), or pure 100% biodiesel (B100) and 100% vegetable oils and ethanol and gasoline (gasohol), are global effort strive to reduce the 100 % dependency on petroleum diesel fuels. Researchers [41,42] identified the following advantages with the used of blend fuels; less smoke and particulate discharges, a low sulfur content and higher cetane numbers, oxygenated, readily available, little aromatic content and higher heat content of about 88 percent of number 2 diesel fuel, lesser carbon monoxide and hydrocarbon emissions above all they are biodegradable and renewable.

## C. Microbial Oils

Microalgal oils represent another low-priced source of renewable raw materials for biodiesel production that has more or less received attention. Out of the estimated more than 50,000 species known, only a limited number, of around 30,000, have been studied [43], among which *Chlorella* seems that greater option for biodiesel production. However, other species are as efficient and fruitful as this one, but the selection needs to take

into account factors, such as the capability of microalgae developing using the nutrients available or under definite environmental conditions [44].

The use of bioreactor are normally employed for algal oil conversion to biodiesel. This is achieved through substrate feeding and heterotrophic fermentation [1]. Photobioreactors are normally used in cultivating micro algae for biodiesel production. Viewing it pragmatically, the points are; they are simple to handle because they easily cultivated and grow with little or even no attention, using water not fitting for human utilization and easy to acquire nutrients [44]. Biodiesel production was reported by Li *et al.* [45] on large scale *Chlorella protothecoids*, micro alga in bioreactors. Lipid content derived reached up to 44–48% (of the cell dry weight), and then used to obtain 98% conversion to FFAE, catalysed by *Candida sp.* lipase (immobilized), substrate molar ratio of 3:1 and a reaction time of 12 h. Such biodiesel produced was sulphur free and performs as well as petroleum diesel, while reducing discharges of particulate matter, hydrocarbons, and SOX and CO. Nevertheless emissions of NOX could be higher in some engine types [46]. The resulting biomass that accumulates after oil extraction can be further used in methane, ethanol, livestock feed and organic fertilizer [47].

## VI. VIRGIN OIL MODIFICATION TO WASTE COOKING OIL

The term waste cooking oil is a product of frying different foods in vegetable oil. During the course of the frying a number of changes are accompanied the natural structure of the molecules. These changes are in the form of, thermolytic, oxidative and hydrolytic reactions.

At the thermolytic stage, heat has a central role during food preparation, as such different degrees of temperature are exerted in the form of frying depending on the food in question, technique and frying conditions (oxygen concentration, time, temperature, presence or absence metals) and oil/fat (presence of native antioxidants) [48]. Oil are usually heated in the air in the presence of light for a long time at around 160-200 °C [49]. Though depending on the number of frying cycles which varied based on socioeconomic status and also the oil composition, In general the changes would be in these forms [50];

- Alteration in the surface tension,
- Increase in thickness and stickiness
- Colour modification
- Increase in the specific heat
- Increase in the tendency of fat to foam.

While in the absence of oxygen thermolytic reaction may follow, this is at a very high temperature. If the oil is heated up to 180 °C in the free oxygen state and the triglycerides contain saturated fatty acids, sequence of regular alkanes, alkenes, symmetric ketones, CO and CO<sub>2</sub>, and lower fatty acids. But if it is unsaturated fatty acids mainly dimeric compounds, including saturated dimers, and polycyclic compounds are formed [49].

In two other processes, oxidation and hydrolysis reactions; in the former, Hydroperoxides are formed as a primary product during the process and could form compounds, such as isomeric hydroperoxides (conjugated diene group content). This a result of the complex reactions between unsaturated fatty acid and molecular oxygen through free radical mechanism as illustrated in Figure 2. Hydrolytic reaction occur due to the steam produced



during food making, this steam causes the hydrolysis of triglycerides to monoglycerides and diglycerides, FFA and glycerol [51]. Modification of oil composition can be determined by the content of monoglyceride and diglyceride since FFA are lost in the frying process [52].

products negatively influence properties of UFO for their further utilization as substrate in biofuels production.

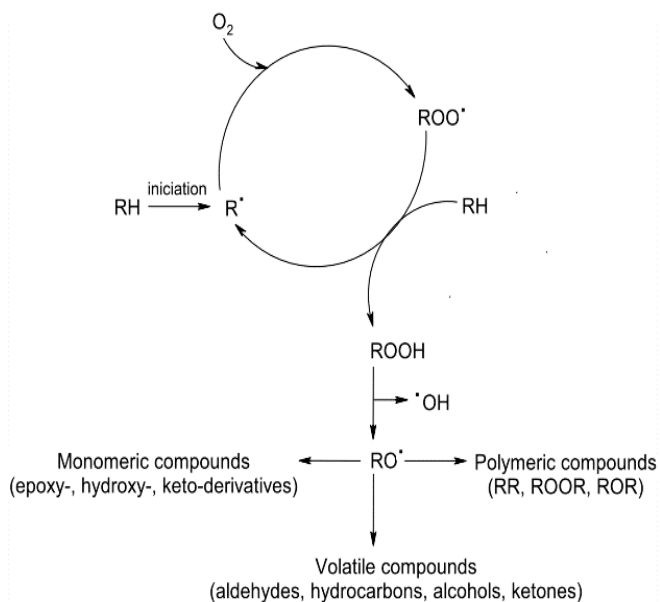
### Economics Feasibility of Biodiesel from Waste Cooking Oil and Future Expression

The economics of biodiesel production have taken a new look with the current flow in crude oil price in the world market. Recent report from BBC [58] reported that the new crude oil price was set at \$109 per barrel. In US diesel price is around \$4 per gallon [59] the value of diesel fuel indicate shoot in price over 2 decade. This value correspond to a 33% raise over 2010, a 60% raise over 2009. As conventional diesel fuel prices continue to amplify, impose allocation should therefore be channelled towards transportation costs, against other sectors.

In the year 2008, Chinese petroleum utilization increased from 164 million tons to 553 million tons, while crude oil imports increased from 2.9 million tons to 178.9 million tons, 1990-2008 [60]. This may be due to high economic growth which make even the number of cars to raise. Data from 1990 to 2008 showed that the market passenger cars grew from 0.51 to 9.38 million. The Chinese car market is the second-largest car market in the world with sales of about 7.28 million, in 2006 followed by Japan [61]. The IEA projection had it that Chinese oil consumption for transportation use would raise by 5.3% per annum from 2006 to 2030. This is an assumption that Chinese oil consumption will get bigger in future time. After the USA, China is the leading CO<sub>2</sub> emission country on earth [62]. The increase in fuel consumption has caused air pollution problems.

With continuous hike in oil prices in the global market and consumption, biodiesel turn out to be a more feasible alternate energy source. Transportation sector presently produced around 25 percent of worldwide energy-related CO<sub>2</sub> emissions and accounts for approximately 50 percent of worldwide oil consumption [62]. Data from clean air task force has shown that Public health expenses as a result of conventional diesel emissions pollution is approximately \$139 billion per annum [63]. Vulnerable groups (aged, children) especially Children, due to their developing respiratory systems, are at a bigger risk to filthy air quality. It is further stressed that approximately 4.5 million US children are suffering from asthma, even though not all of these cases can be attributed to diesel emission exhaust. However, exposure to diesel exhaust can cause swelling and irritation of airways and can aggravate symptoms in children that already have respiratory ailment [63].

An improvement in the biodiesel market is so much more related to the development of agricultural industry ensuring environmental sustainability. In U.S for example, improvement of the U.S. biodiesel industry was buoyant by the efforts of soybean producers who sought to extend markets and demand for their crops. After federal policies to gear up biodiesel production were introduced beginning in 1998 the industry commenced meaningful production. A reflection of the past, in 2004-2005 Production of biodiesel in the U.S. has risen dramatically tripling from 25 million to 75 million gallons, in 2006 this value spirochete to 250 million gallons. By September 2008, reaching an estimated 700 million gallons [64]. It may also be on this light and also to improve on the air pollution situation the Chinese government decided that all biofuel producers receive subsidies to cover operating losses, moreover, VAT of 17% for



**Figure 2: Reaction Pathway for free radical autoxidation**

Quality assessment of oil after frying is based on the polar content, any further heating increase the polarity. Fresh unused oil have around 0.4 and 6.4 mg/100 g [53]. In Europe, most countries have set highest polar content level of 25% in edible oil meaning that the fats and oils must be discarded when its polar fraction is greater than 25% [53]. In Slovak Republic, current legislative give a yardstick of polar components in edible oil set to 25 %. Any limit exceeding necessitate replacement of the oil/fat [48]. But for Polymeric substances, composition of more than 10 % is also a ground for oil/fat replacement. A research conducted on olive oil, sunflower oil, and a mixture of the two oils demonstrated that after 20 fryings cycles, the polar content of olive oil by 480% and that of sunflower oil is raised by 640% and after 40 fryings, all the used oils have a polar fraction of >25% [53].

Therefore to determine such quality, technique that can be applied include column chromatography [54]. However, this technique is require chemicals input and enormous time. The use of spectrophotometer was reported as one of the simple and fast method [55]. Sample that were examined were measured at an absorbance of 490 nm, in 250 to 650 nm region. Region of 350–2500 nm were used using UV/Vis/NIR [56]. Collected results revealed that NIR is comparatively faster and non destructive technique for UFO quality determination. And during intensive frying FTIR spectroscopy can be used for monitoring of oil/fat quality [57].

In general, as vegetable oils are been fried they possess various polar point and should be discarded upon its attainment. Additionally, UFO requires systematic monitoring in order to uphold the quality as the quantity and the type of unattractive

biofuel plants had been cut down [61]. Lack of feedstock availability can be a limiting factor as the main feedstock for biodiesel is used cooking oil and china a net importer of oilseed and vegetable oil. This kind of intervention was seen by some government, as part of the Energy Policy in US, Fund authorization was disbursed to DERA ranged from \$100 million to \$300 million yearly, to be circulated by the Environmental Protection Agency for Clean Diesel Campaign. The first four years since 2008, appropriated funding has fluctuated between \$50 million to \$60 million per year. It further reduced to 1 half to that of the year 2008 in 2012 (\$30 million). This followed by \$20 million for 2013 [65]. A dramatic reduction was seen in the year 2014, the Administration's proposed 2014 budget further reduces DERA's funding with a 70% cut, bringing it to \$6 million. This decline in fund may be attributed to the economic fluctuations as a result of direst in various part of the world [66]. Kemp [67] reported the division of biodiesel production costs which is attributed to the major cost in the production accounting up to 70 % to that of the Oil feedstock, which can also be followed by the price of petroleum diesel and the cost of transportation to distant areas. As it is a known fact that increase in demand of fuel with limited supply would cause increase in cost of the fuel. For these reason, if the waste vegetable oil is utilized as biodiesel raw material, biodiesel economics would be significantly improved. In addition, reduction in the waste treatment costs since there are no stringent legislations prohibiting the disposal of waste cooking oil into drainage systems [58]. Food store and restaurant as well do not need to spend money conveying the waste oil to dump site. Several decades had passed with restaurant operators had to pay for a service to collect their unwanted waste vegetable oil in compliance with state and local environmental regulations. Producers of biodiesel for their own use had the chance to take for free. However, with the eye opener of biodiesel as lucrative business many collection services began offering token to take a restaurant's oil in order to make their personal biodiesel. Such collectors in some cases began paying restaurants to collect their waste oil. Collector services can pay \$0.20 per gallon or above depending on the oil quality, collection frequency, market force and the quantity collected. Although this new income stream does not provide substantial supply of income, nonetheless can help make up for other business expenses.

Table 4 shows the domestic waste cooking oil generated by some selected countries. As a giant industrial stride, which is also a zero discharge concept, Currently, Fast-food giant McDonald's is developing a biodiesel program in several countries, after its efforts in Austria in 2003. The used oil from the company's restaurants is converted into biodiesel, which is then used to fuel the company's distribution trucks. It has extended its tentacles to Malta and of recent the United Kingdom (UK). Effort is on progress to establish in United States which is under experimentation. The Delta Institute, which is a Chicago-based environmental and economic development profit free organization is exploring the viability of a project that would translate McDonald's used cooking oil into biodiesel in the Chicago area [68].

Biodiesel from waste cooking oil is noteworthy in that it has the potential for considerable cost savings and serve as one of the several emission reducing technologies. Therefore The

establishment of a sustainable biodiesel industry is a feasible way for to turn away the dependency on fossil fuel imports, create new employment opportunities particularly in the agricultural sector and improve the economic.

**Table 4 Used domestic waste oil generation by various countries [37]**

Country	Quantity (million tons/yr)
Europe	0.7-10
United States	10.0
Canada	0.12
Ireland	0.153
China	4.5
Malaysia	0.5
Japan	0.45-0.57
Taiwan	0.07

## VII. CONCLUSION

Biodiesel has become very attractive replacement to petroleum fuel. Literatures mostly covered alcoholysis of biodiesel by edible oils with little insight in the application of waste cooking oil and non edible oils. Lipase catalysing the production of biodiesel from waste cooking oil has been successful as reported in the bulk of this article. The potentiality of waste cooking oil for this purpose among other applications qualified it as a resourceful waste in view of the fact that it can be an economic booster and a supplement for the current dilemma of environmental sustainability and dependence on petroleum recourses. It is a major cost saving raw-materials.

Bearing in mind a notion that waste management is often viewed as the last step of the material chain.,the actuality in this regards is that waste management is part of resources management. Our view to the waste is a huge loss of resources in material form and energy. Therefore reusage effort is a necessity to regain what seemed to be missing.

An assumption is that if a waste vegetable source is available for free, the key recurring capital costs of biodiesel production would be the enzymes necessary to convert the oil into biodiesel, therefore the free or cheap supply of the oil would cover up the expensive cost of the enzyme.

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