Introduction

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In the last few years interest and activity has grown up around the globe to find a substitute of fossil fuel. According to Indian scenario the demand of petroleum product like diesel is increasing day by day hence there is a need to find a solution. The use of edible oil to produce biodiesel in India is not feasible in view of big gap in demand and supply of such oil. Under Indian condition only non-edible oil can be used as biodiesel which are produced in appreciable quantity and can be grown in large scale on non-cropped marginal lands and waste lands. Non-edible oils like *Jatropha*, Karanja and mahua contain 30% or more oil in their seed, fruit or nut. India has more than 300 species of trees, which produce oil bearing seeds (Subramanian *et al., 2005*). Around 75 plant species have 30% or more oil in their seeds/kernel, have been identified and listed (Azam *et al.,* 2005). Traditionally the collection and selling of tree based oil seeds were generally carried out by poor people for use as fuel for lightning. Biodiesel has become more attractive because of its environmental benefits and fact that it is made up of renewable resources (Saucedo 2001). Although short term test using vegetable oil showed promising results, longer tests led to injector coking, more engine deposits, ring sticking and thickening of the engine lubricant (Tiwari *et al.,* 2003)

The need for alternative energy sources that combine environmental friendliness with biodegradability, low toxicity, renewability, and less dependence on petroleum products has never been greater. One such energy source is referred to as biodiesel. Biodiesel can be used in its pure form or when mixed with diesel fuel in certain proportions. Most common biodiesel blends are B2 (2% biodiesel, 98% diesel), B5 (5% biodiesel, 95% diesel), B20 (20% biodiesel, 80% diesel).This can be produced from vegetable oils, animal fats, micro algal oils, waste products of vegetable oil refinery or animal rendering, and used frying oils. Chemically, they are known as mono alkyl esters of fatty acids. The conventional method for producing biodiesel involves acid and base catalysts to form fatty acid alkyl esters. The transesterification reaction can be influenced by several factors including molar ratio of alcohol, catalyst, presence of water, free fatty acid in oil samples, temperature, time and agitation speed. In this context, an understanding of the factors affecting the process is very important to make economically and environmentally biodiesel production.

Downstream processing costs and environmental problems associated with biodiesel production and byproducts recovery have led to the search for alternative production methods and alternative substrates. Enzymatic reactions involving lipases can be an excellent alternative to produce biodiesel through a process commonly referred to alcoholysis, a form of transesterification reaction, or through an interesterification (ester interchange) reaction. Protein engineering can be useful in improving the catalytic efficiency of lipases as biocatalysts for biodiesel production. The use of recombinant DNA technology to produce large quantities of lipases, and the use of immobilized lipases and immobilized whole cells, may lower the overall cost, while presenting less downstream processing problems, to biodiesel production. In addition, the enzymatic approach is environmentally friendly, considered a “green reaction”, and needs to be explored for industrial production of biodiesel.

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are the important biocatalysts because of their excellent biochemical and physiological properties. Lipases are the hydrolytic enzymes that can be used in various industrial applications for alcoholysis, acidolysis, amynolysis and hydrolysis reactions. Biodiesel production is one of the stunning applications of lipase. Lipase catalyzed biodiesel production was reported first by Mittelbach in1999. Lipase-catalyzed transesterification takes place in two steps, which involves hydrolysis of the ester bond and esterification with the second substrate (Fan, 2012).

Lipases can also be isolated from many species of plants (papaya latex, oat seed lipase, and castor seed lipase), and animals (pig's and human pancreatic lipases). For industrial enzyme production generally microorganisms are preferred because of their shortest generation time. The other advantages of microorganisms can be listed as high yield of conversion of substrate into product, great versatility to environmental conditions and, simplicity in genetic manipulation and in cultivation conditions. Although lipases from different sources are able to catalyze the same reaction, bacterial and fungal lipases are mostly used in biodiesel production such as *Aspergillus niger, Candida antarctica, Candida rugosa, Chromobacterium Viscosum, Mucor miehei, Pseudomonas cepacia, Pseudomonas fluorescens, Photobacterium lipolyticum, Rhizopus oryzae, Streptomyces sp.,* and *Thermomyces lanuginose* (Yahya *et al.,* 1998). *Candida rugosa* obtained from yeast, is the most used microorganism for lipase production (Freire *et al.,* 2008). Recently, *Streptomyces sp*. was investigated as a potent lipase producing microbe for biodiesel production and found applicable in the field of biodiesel (Cho *et al.,* 2012).

Specificity of lipases has a great importance in the selection of the usage area of lipases.

Lipases can be divided into three groups due to their specificity as 1,3-specific lipases, fatty acid-specific lipases and nonspecific lipases. Especially, 1,3-specific lipases which release fatty acids from positions 1 and 3 of a glyceride and hydrolyze ester bonds in these positions such as *Aspergillus niger*, *Rhizopus oryzae* and *Mucor miehei* catalyze transesterification reactions efficiently (Ribeiro *et al.,* 2011, Antczak*et al.,* 2005). The study of Du *et al* (2005), showed that higher yield (90%) was achieved for biodiesel production by using a sn-1,3-specific lipase, *Thermomyces lanuginosa* immobilized on silica gel (Lipozyme TL IM). Thus, the use of sn-1,3- specific lipases can give rise to biodiesel yield of above 90% under appropriate conditions (Antczak *et al.,* 2005). Substrate specificity of lipases is also a crucial factor towards the biodiesel production which acts on the choice of the proper enzyme based on the composition of raw materials by consisting in the capability of distinguishing structural features of acyl chains. Lipases from *Pseudomonas fluorescens, Pseudomonas cepacia, Candida rugosa, Candida antarctica* and *Candida cylindracea* are suitable for transesterification reaction by displaying both wide substrate specificity and region. However, the limited reserve of fossil fuel has drawn the attention of many researchers to look for alternative fuels which can be produced from renewable feedstock.

Vegetable oils are candidates as alternative fuels for diesel engines with their high heat content (Hartmeier *et al.,* 1998). But, direct use of vegetable oils is not possible because of the high kinematics viscosity of them which are varies in the range of 30–40 cSt at 38 °C and are about 10 times higher than of diesel fuel that leads to many problems (Hartmeier *et al.,* 1998, Koh *et al.*, 2011).

The edible vegetable oils such as soybean, sunflower, palm, corn cottonseed, canola and olive oils have been widely used in enzymatic transesterification. In developed countries, edible oils constitute more than 95% of biodiesel production feedstock because the produced biodiesel from these oils have properties very similar to petroleum-based diesel. Also, the country and its climate, the oil percentage and the yield per hectare are effective parameters in selecting the potential renewable feedstock of fuel. For example, while rapeseed oil prevailing the EU production, soybean oil prevailing the US and Latin American production, and palm oil mainly being used in Asia.

Inedible oils do not find a place in human consumption due to including toxic components. Therefore, inedible oils do not compete with food crops. Thus, inedible vegetable oils are an alternative feedstock for biodiesel production. *Babassu (Orbinya martiana), Jatropha curcas (Linnaeus),* neem *(Azadiracta indica), polanga (Calophyllum inophyllum),* Karanja *(Pongamia pinnata),* rubber seed tree *(Hevea brasiliensis),* mahua *(Madhuca indica* and *Madhuca longifolia),* tobacco *(Nicotina tabacum),* silk cotton tree, etc. are promising inedible vegetable oil sources. *Jatropha curcas* is an attractive feedstock between various oil bearing seeds as it has been developed scientifically and found to give better biodiesel yield and productivity. Crude *Jatropha* oil contains about 14% of free fatty acid that is too high for alkaline catalyzed biodiesel production (Hartmeier *et al.,* 1998). However, high free acid content is not a problem in the production process of biodiesel via using enzyme catalysts. Besides *Jatropha curcas*, 26 species of fatty acid methyl ester of oils of including *Azadirachta indica, Calophyllum inophyllum, and Pongamia pinnata* were found most suitable for use as biodiesel, which adjust to the major specification of biodiesel standards of European Standard Organization, Germany, and USA (Mohibbe *et al.,* 2005). Modi *et al*. reported conversion of crude oils of *Pongamia pinnata (*karanj*), Jatropha curcas (Jatropha)* via immobilized Novozym 435 to biodiesel fuel with yield 90, and 92.7%, respectively (Modi *et al.,* 2007).

Jatropha curcas is a species of flowering plant in the spurge family, Euphorbiaceae, that is native to the American tropics, most likely Mexico and Central America. It is cultivated in tropical and subtropical regions around the world, becoming naturalized in some areas. The specific epithet, "*curcas*", was first used by Portuguese doctor Garcia de Orta more than 400 years ago and is of uncertain origin. Common names include Barbados nut, purging nut, physic nut, or JCL (abbreviation of *Jatropha curcas Linnaeus*). *J. curcas* is a poisonous, semi-evergreen shrub or small tree, reaching a height of 6 m (20 ft). It is resistant to a high degree of aridity, allowing it to be grown in deserts.

Biofuel development in India centres mainly around the cultivation and processing of *Jatropha* plant seeds which are very rich in oil (40%). The drivers for this are historic, functional, economic, environmental, moral and political. *Jatropha* oil has been used in India for several decades as biodiesel for the diesel fuel requirements of remote rural and forest communities; *Jatropha* oil can be used directly after extraction (i.e. without refining) in diesel generators and engines. *Jatropha* has the potential to provide economic benefits at the local level since under suitable management it has the potential to grow in dry marginal non-agricultural lands, thereby allowing villagers and farmers to leverage non-farm land for income generation. As well, increased *Jatropha* oil production delivers economic benefits to India on the macroeconomic or national level as it reduces the nation's fossil fuel import bill for diesel production (the main transportation fuel used in the country); minimizing the expenditure of India's foreign-currency reserves for fuel allowing India to increase its growing foreign currency reserves (which can be better spent on capital expenditures for industrial inputs and production). Since *Jatropha* oil is carbon-neutral, large-scale production will improve the country's carbon emissions profile. Finally, since no food producing farmland is required for producing this biofuel (unlike corn or sugar cane ethanol, or palm oil diesel), it is considered the most politically and morally acceptable choice among India's current biofuel options; it has no known negative impact on the production of the massive amounts grains and other vital agriculture goods India produces to meet the food requirements of its massive population (circa 1.1 Billion people as of 2008). Other biofuels which displace food crops from viable agricultural land such as corn ethanol or palm biodiesel have caused serious price increases for basic food grains and edible oils in other countries.

Karanja is a medium sized tree found almost throughout India. Karanja tree is wonderful tree almost like neem tree. The common name of the oil is Karanja Seed Oil and the botanical name is *Pongamia glabra* of Leguminosae family. *Pongamia* is widely distributed in tropical Asia and it is non edible oil of Indian origin. It is found mainly in the Western Ghats in India, northern Australia, Fiji and in some regions of

Eastern Asia. The plant is also said to be highly tolerant to salinity and can be grown in various soil textures viz. stony, sandy and clayey. Karanja can grow in humid as well as subtropical environments with annual rainfall ranging between 500 and 2500 mm. This is one of the reasons for wide availability of this plant species. The tree bears green pods which after some 10 months change to a tan colour. The pods are flat to elliptic, 5-7 cm long and contain 1 or 2 kidney shaped brownish red kernels. The yield of kernels per tree is reported between 8 and 24 kg. The kernels are white and covered by a thin reddish skin. The composition of typical air dried kernels is: Moisture 19%, Oil 27.5%, and Protein 17.4%. The present production of Karanja oil approximately is 200 million tons per annum. The time needed by the tree to mature ranges from 4 to 7 years and depending on the size of the tree the yield of kernels per tree is between 8 and 24 kg.

India is a tropical country and offers most suitable climate for the growth of Karanja tree. It is found in abundance in rural areas and forests of entire India, especially in eastern India and Western Ghats .The seeds are crushed in expeller to get the oil. As the tree of Karanja is naturally found in forests, there are so far no reports on adverse effects of Karanja on fauna, flora, and humans or even on environment but that is a different area of research. Karanja oil has been reported to contain furanoflavones, furanoflavonols, chromenoflavones, flavones and furanodiketones which make the oil non-edible and hence further encourages its application for biodiesel production.

With this background the present study was undertaken with the following objectives

1. To isolate and screen fungi for production of the enzyme-lipase.
2. To optimize the cultural conditions of few fungi for best lipase production.
3. To produce and evaluate the yield of biodiesel from *Jatropha* and (*Pongamia)* Karanja oil by using the following as catalysts.
    a. Extracellular enzyme (Culture filtrate/ partially purified enzyme by dialysis)
    b. Intracellular enzyme (Whole cell lipases)
    c. Commercially available lipase enzyme/lyophilized/immobilized enzymes of the isolated fungi.
    d. Alkali / Acid (Sodium hydroxide and Potassium hydroxide / Sulphuric acid)
4. To characterize the biodiesel thus obtained and studying its components by GC-MS and testing its Performance characteristics of engine and emission.
5. Cost effectiveness of lipase catalyzed biodiesel production.
6. RNA sequence analysis of high lipase producing microbe for authentication.