

## Novel Lipase Production For Conversion Of Algal Oil To Bio-Fuel

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**Abstract:** By Screening of mesophiles and extremophiles for lypolytic potential Lipases production from the most promising isolates is characterized and Immobilization of selected best lipases on suitable matrices specifically for Conversion of Crude oil to diesel by Enzymatic trans- esterification process, Oil is extracted from cultivated Algae in Photo reactors. The Fuel oil conversion from crude Algal/Bio oil is positively more efficient and Environmental friendly and non conventional , renewable source of Energy

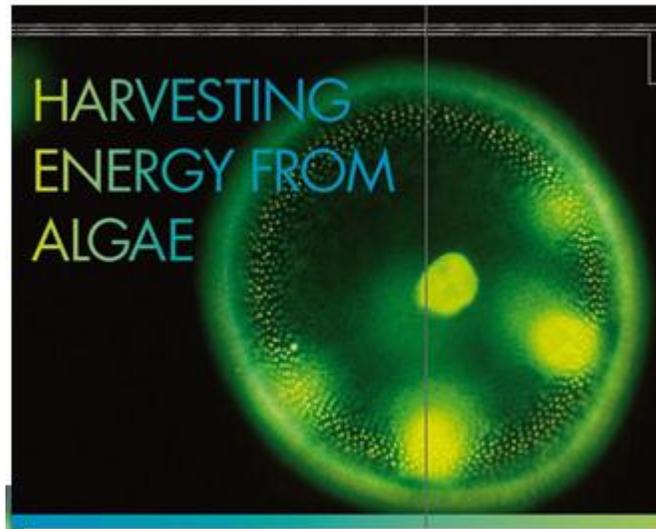
**Keywords:** Photo Reactor, Fermentation, Lipase ,trans-esterification, Algae cultivation, Bio mass,Acrimeto bacter/- *Fusarium oxysporum*f. sp. NCIMB-13260, *Schizochyridium* & *Thrausyritum* sps.

### 1. Introduction

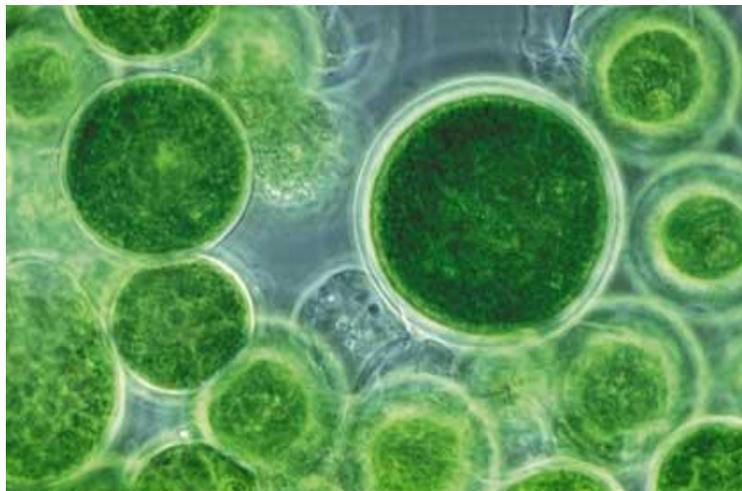
Welcome to 2015 Int'l Conference on Biological and Environmental Science ( BIOES-2015). The Conference is a primary international forum for scientists and technicians working on topics relating to Natural Science so Taking to this esteemed opportunity I present here most vital R& D applied project to execute widely can be ventured by aspiring Government and private Entrepreneurs alike Globally as its loud and clear that We need now alternative Energy source and Fuel to substitute conventional earth Fuel like Petrol, Diesel etc. Densely accumulating Lethal particulates in air and consumed rapidly. Bioremediation is only answer but a methodology and perfect process is required to manufacture efficient Fuel in large scale. So its not a just research academic Paper to publish and put archives. Its a Project by Bio tech Industry in House R&D.

#### Steps of the Research

- Develop an Efficient Technology to manufacture Green crude oil from Algae
- Trans esterification of Green Crude to Bio diesel by Enzymatic resolution
- Unique Lipase Enzyme to be produced by Microbial Fermentation
- Substitute to conventional earth fuel by Bio High yielding production of crude green Oil by Fermentation process of selective Algae and characterization of oils
- Characterization of Oil produced
- Screen mesophiles and extremophiles for lypolytic potential
- Lipases from the most promising isolates will be characterized
- Immobilization of selected best lipases on suitable matrices
- Conversion of Crude oil to diesel by Enzymatic trans esterification process
- Algae can also be used to clean up waste water.
- Blue green algae are vitamin supplements. This business sector has been cultivating algae for many years now in huge quantities. So this technology is not exactly new. But cultivation is usually done in open field ,Ponds which is most un controlled and unpredictable. Cultivation is experimented in controlled Pilot scale Photo Reactors so much high yielding and clean cultivation is done.

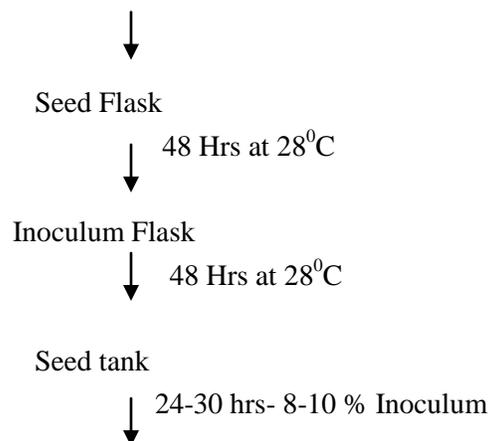


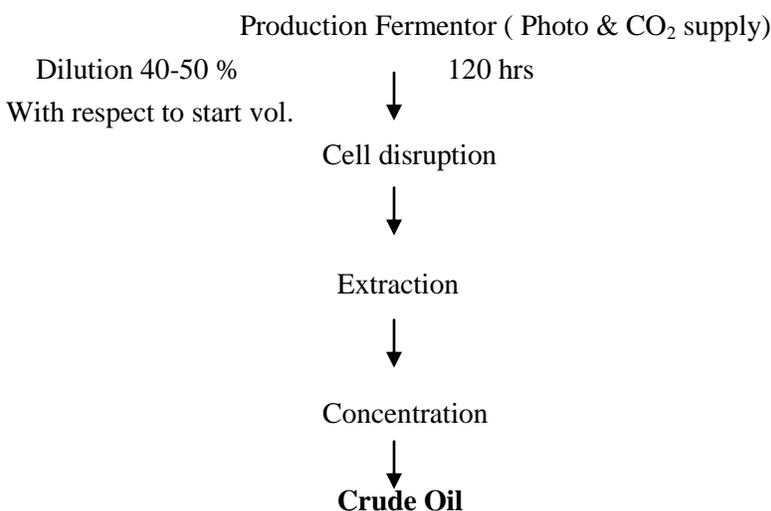
- **Organism identified and developed:** *Schizochyridium* & *Thrausyritum* sps
- **Oil identified:** Omega 3 fatty acid with a 22-carbon chain
- **Molecular Weight determined:** 328 Daltons



Algal growth

Process Flow: Slant





**Specific Lipase Production:** Lipase producing microbe *Acrimeto bacter-barmanni- NCIMB-13260* isolated and screened on tributyrine oil emulsion agar. Lipase activity values will be performed by either titrimetric using oil emulsion or colorimetric using p-Nitrophenol palmitate (pNPP) substrate and the activity will be measured in terms of released free fatty acid or p-Nitrophenol (pNP), respectively. Fermentation medium components and process parameters is optimized by rational or statistical approach. Lipase is characterized using various electrophoresis and chromatographic methods. Produced lipase then immobilized using adsorption or entrapment techniques. oils produced by Algae was characterized using ASTM standard procedures. oils will be trans esterified by free or immobilized enzymes for biodiesel production. Produced biodiesel will be characterized using ASTM standard procedures. The work will be directed towards use of alternative renewable sources for cost effective, eco-friendly and bio-friendly bio-diesel. In this Algal oil, will be readily sources of bio-diesel. Diesel as fuel for this country and this approach could also save huge export currency of this country. Compared with the aforementioned enzymes, lipases obtained in this study through submerged fermentation had higher thermostability and may have applications in industrial processes that require high temperatures. Enzymatic processes that occur at higher temperatures have higher reaction rates. It may be possible to use thermostable lipases in the synthesis of biopolymers, pharmaceuticals, agrochemicals, cosmetics, biodiesel, and aromas. According to Diaz et al even for identical lipases produced by different methods of cultivation (submerged and solid-state), there may be thermostability differences caused by the binding of nonprotein compounds derived from the culture medium through noncovalent bonds to the lipases, changing their physical and chemical properties.

1) Enzyme thermo stability may be affected by production conditions, such as the producer microorganism, the method of cultivation, and the medium used. Thermostability is the result of the protein's amino acid sequence, which provides a more rigid conformation to the enzyme through intramolecular interactions, with the internalization of hydrophobic residues and superficial exposure of hydrophilic residues. Lipase thermostability may also be affected by the presence of compounds such as short-chain alcohols, metals, and ions as Ca<sup>2+</sup> and Mg<sup>2+</sup> which bind to the surface of enzymes whose binding sites are generally formed by negatively charged groups [48]. According to Iyer and Ananthanarayan thermal stabilization of lipases may be caused by the presence of divalent ions, anions, or cations and were present in the lipase production culture medium, which, if not consumed by the fungus for growth and synthesis, remain soluble after the separation of cells, and become part of the lipolytic extract. That may explain the thermostability of the produced enzymes. However, if this enzyme extract containing lipases were purified for further use, causing the removal of these ions of the culture medium, the study of the stability of the purified protein would be needed.

## 2. pH Stability

The pH stability of enzymatic extracts obtained through submerged and solid-state fermentation was determined treating these extracts with different buffers for 24 h and after the enzymatic activity was determined using the optimized pH and temperature for the enzymes of each fermentation process .

Reresidual lipolytic activity as a function of pH for the enzymes produced through solid-state and submerged fermentation. Lipases produced through submerged fermentation by *Aspergillus flavus* were stable at pH ranging from 3.5 to 6.5 for 24 h, with residual activities greater than 80%. At pH 7 to 10 there was a reduction in the stability of enzymes with residual activity of around 50%.

Grown on Nutrient Agar and Broth and then cultivated as follows; Physico-chemical parameters for lipase production by using microorganisms Growth Activity Growth medium .Optimization of physio-chemical parameters for the growth of microbial cells and lipase production has been achieved for different microorganisms by several researchers. The optimum values of pH and temperature for growth and lipase production have been reported and the effect of other parameters, like carbon and nitrogen source, mineral salts and surfactants have been studied. Concluded to most efficient Microbe, so all experiments arried out by this only to avoid time consuming traditional exercises. *Fusarium oxysporum. sp.*

We standardized all parameters by altering it in multiple shake Flask run concluded to leaving some space for further improvement:

Temp 30<sup>0</sup> C ,pH - 7.0

Carbon Source: Dextrose

Nitrogen source : Yeast Extract and Corn steep liquor

Optimal Environ mental Parameters maintained:

- Aerations: 0.5 vvm
- DO2 – 40%
- Ph -7.0
- Cycle: 55 hrs
- Temperature: 30 DC

Fermented Broth is then clarified by centrifugation or Filtration followed by Ultra filtration( RO) of 20 Kdal Molecular cut off pore size to concentrate the Enzyme to high activity the Liquid concentrate may be further concentrated by Spray drying making powder for more stability.

We have tried to immobilize also to make most efficient device for trans esterification of Oil.

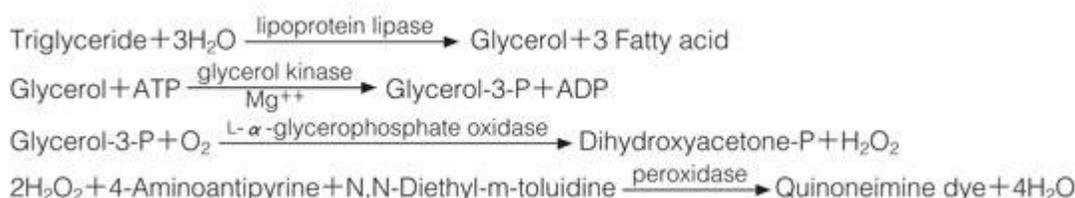
So till now in this paper context, We had isolated with reference potent strains and propagated as follows:

1	Liso Bacter	MTCC-9497	Glucose, S.B.meal, Olive oil	37 0c	7.0	44 hrs	255 u/ml
2	Acrimetobacter	NCIM-11764	Soyaoil/Triacyl glycerol	37 0 c	6.5	50	1455 u/ml
3	Deauneria sp.	NCIM-1216	Palm oil	35	7.0	35	182 u/ml
3	Aspergillus flavus	isolate	S.B.meal, Dextrose,Soya oil	32	5.5	72	299 u/ml

All the strains run on shaker flask with different substrate, temp, Ph and RPM, so above table only the best found result of each is mention. So We selected Acrimeto bacter the best for our application. So standardized process in Lab 5 liter Fermentor. Which given all data to scale up.

Maximum Fermented Broth Activity achieved :1455 lu/ml

## Assay Procedure for Lipase Principle



The appearance of quinoneimine dye is measured at 545nm by spectrophotometry.

## Unit definition

One unit causes the formation of one micromole of glycerol (half a micromole of quinoneimine dye) per minute under the set conditions.

Our project is based on concluded table given below:

Task 1: Cultivation/Fermentation and design Photo Bioreactor
Task 2: Screening, isolation & identification of lipase producing microbes
Task 3: Lipase production & characterization
Task 4: Enzyme immobilization
Task 5: Free and immobilized enzyme based trans/esterification for biodiesel potential
Task 6: Biodiesel characterization/Large scale production

## 3. Trans Esterification of Omega-3 Fatty acid

Fatty acid (Oil) + Methanol ----- > Bio-diesel + Glycerol  
Comparison of methanol with methyl acetate (MA) as acyl acceptor for Lipase -catalyzed transesterification of palm oil to biodiesel. ► Methanol is more reactive than MA but deactivates Enzyme ► The presence of MA minimizes methanol deactivation of Enzyme ► Combined use of methanol and MA enhances the reaction 3-fold and reduces the enzyme load .Diesel consists of Mono Alkyl Esters

## 4. Acknowledgement

United Alacrity Pte inc Singapore, Rossari Biotech Ltd India, Sequence Biotech Ltd India supporting this research Project.

Al ready many counties are continuously doing extensive Research to find a foolproof process to make Bio fuel its reported time to time.

**Thailand:** For Thailand, biodiesel can be produced from several raw materials such as crude palm oil, palm kernel oil, palm stearin and coconut oil. Oil palm is the highest vegetable oil production in Thailand followed by coconut oil and soybean oil (Pleanjai et al., 2004; Attanatho et al., 2004). In 2003, Thailand consumes about 17,550 million litres of total petroleum based fuel, which was mostly used in transportation and agricultural sectors (www.eppo.go.th). Large-scale commercial production of biodiesel has not yet begun in Thailand. The biodiesel plants use used frying oil and crude palm oil as feedstock. The Government has formulated a strategy to ensure that by 2012 all the diesel sold in Thailand will be 10% biodiesel.

United States: produced Biodiesel is made from soybean oil, which will remain the predominant feedstock, although an increasing share of Biodiesel production will come from other oils, including yellow grease, rapeseed oil, soybean oil, sunflower oil, palm oil, canola oil, cottonseed oil, animal fats and recycled frying oil (Lloyd et al., 1996; Abigor et al., 2000; Moser, 2008).

Brazil: In May 2002 the PROBIODIESEL (Programa Brasileiro de Desenvolvimento Tecnológico de Biodiesel) programme was announced, which will set up the regulatory framework for Biodiesel development and production. Soybean, palm oil and caster oil are important feedstock for biodiesel production in Brazil (Alamu et al., 2007, 2008).

**Canada:** Canada is known for its large rapeseed production (“canola”), but sunflower seed is also grown there. Today Canada is the world’s 4th largest oilseed exporter. Currently vegetable oils, but also recycled frying oils and animal fats are used as feedstock sources (Reaney et al., 2006; Chhetri et al., 2008).

” Lipase Activity Assay

Lipase activity was assayed using p-nitrophenly palmitate (p-NPP) as a substrate. The 30 mg of p-NPP was added into 10ml of 2-propanol and mixed with 90 ml of 5 mM phosphate buffer (pH 8) containing 207 mg of sodium deoxycholate (NaDOC) and 100 mg of gum arabic. The 100 µl of crude enzyme were added in 2ml of \

the reaction mixture and then incubated at 55°C for 15 min. The 2.9 ml of 2 M sodium carbonate (211.8 mg of sodium carbonate in distilled water 1 L) were added to stop the reaction after incubation. The lipase reaction was measured absorbance by spectrophotometer wavelength of 410 nm. One Unit of lipase activity is defined as an enzyme releasing 1 μmol of free p- nitrophenol per minute

## 5. Conclusion

We explored a novel and most desired process to crude generate oil and conversion to efficient and economical Fuel ( diesel) by absolutely Biological process so most environment friendly. Other Institutions and concerned industries are invited to join hands and associate the venture to prevent conventional fuel hazards by emitting toxic gases. Find process to substitute of earth fuel, which is consumed rapidly.

It is triple benefit process by producing oil by algal growth, conversion to Bio-Diesel by bio catalyst ( Lipase) and then Biomass generated as nutrient fodder.

## 5. References

- [1] G. Kouker and K. Jaeger, Specific and sensitive plate assay for bacteria Applied and Environmental Microbiology vol.53, pp. 211-213 9 3)Enzymatic production of biodiesel from canola oil using immobilized lipase,Biomass and Bioenergy vol. 3 pp. 1274-1278,2008
- [3] T Hoshino T. Sasaki, Y. Watanabe, T. Nagasawa, and T. Yamane, Purification and some characteristics of extracellular lipase from fusarium oxysporum f. sp. Lini,Bioscience Biotechnology and Biochemistry vol. 56pp. 660-664, 1992.
- [4] L. Goujard P. Villeneuve, B. Barea, J. Lecomte, M. Pina, and S. Claude, A spectrophotometric transesterification-based assay for lipases in organic solvent Analytical Biochemistry vol. 385, pp. 161–167.2008.
- [5] K. Ban, M. Kaieda, T. Matsumoto, A. Kondo, and H. Fukuda,  
“Whole cell biocatalyst for biodiesel fuel production utilizing rhizopus oryzae cells immobilized within biomass support particles.