Objectives of the Project:

The main objective of the proposed investigation is to produce biolubricant components from vegetable oils.

- In this study, biolubricant components shall be produced via enzymatic hydrolysis of oils followed by chemical/enzymatic esterification.
- To choose proper catalysts (enzyme/chemical) for carrying out the desired conversions.
- To study the effects of different physico-chemical parameters like system pH, temperature, catalyst concentration, nature of feedstock etc., to establish optimum conditions of the process parameters for both the steps. The objective function in terms of non-interactive independent variables will be optimized using classical optimization techniques.
- To carry out kinetic investigation by identifying the nature of the reactions and follow the reaction rate. To identify the nature of the esterification reaction.
- To make a comparison between the chemical and enzymatic esterification routes.
  To compare the properties of the biolubricant produced with that of the conventional ones.

WHETHER OBJECTIVES WERE ACHIEVED

The project objectives have been well-achieved as is enumerated below:

➢ In this study, biolubricant components (i.e. the base oil) has been produced via enzymatic hydrolysis of oils followed by chemical as well as enzymatic esterification.

➢ Both the catalysts Amberlyst 15H (chemical) as well as Novozyme (enzymatic) were identified as potential candidates for carrying out the desired esterification reactions.

➢ The effects of different physico-chemical parameters like system pH, temperature, catalyst concentration, etc., were investigated to establish optimum conditions of the process parameters for both the steps of hydrolysis and esterification. The objective function in terms of non-interactive independent variables were optimized using classical optimization techniques.

➢ Detailed kinetic investigation of the esterification reaction via the chemical route as well as the enzymatic route have been carried out and the nature of the reactions have been identified and the reaction expressions and the kinetic parameters have been evaluated.

➢ A detailed comparison between the chemical and enzymatic esterification routes have been done.

➢ The properties of the biolubricant thus produced have been investigated and its probable area of application has been identified.

ACHIEVEMENTS FROM THE PROJECT

Effective biolubricant base oil components as an alternative to conventional fossil-based lubricant base oils from a cheap and renewable feedstock (Waste cooking oil) were produced using a two-step process. The advantages of the novel two-step process developed in the current study (to produce biolubricant components) over the reported single-step alkali/acid or enzymatic transesterifications are: feedstock flexibility i.e., acceptance of feedstock with any percentages of FFA and water. The work was further developed with the optimizations and kinetic modeling of the reactions involved. Fatty acid long-chain esters were produced as novel biolubricant components, which were characterized, tested and given a suggested classification under lubricants.

The results obtained from the investigation undertaken were published in **five (5)** International Peer-Reviewed Journals and in **three (3)** Conferences given in details in PUBLICATIONS OUT OF THE PROJECT.

One Research Scholar Ms. AVISHA CHOWDHURY had been appointed as the project fellow of this project and she has been awarded her Ph.D degree from the University of Calcutta based on this project work. Two M.Tech students have carried out their Research Project based on this work.

SUMMARY OF THE FINDINGS

This project has presented a structured research on

1. The development of biolubricant components from a renewable bio-material (Vegetable Oil, VO).

2. Waste cooking oil (WCO) is inexpensive and finds limited usage other than environmental disposal. Thus, raw-material used in the present research being a waste biomass (waste cooking oil), scores higher in sustainability quotient than virgin VOs. Additionally, the use of WCO will not curtail the share from the food supply resources.

3. The adopted technology comprises of a two-step process of enzymatic hydrolysis of WCO followed by chemical/ enzymatic esterification of FFA with a higher alcohol (octanol). The processes are substantially feasible and productive.

4. The estrification reactions have been ratified with supportive kinetic models which are likely to aid in the large-scale productions of biolubricant components.

5. The product octyl esters have been characterized and tested at length to be confirmed of its apposite usage.

Countries like India who depend at large on offshore oil recovery and fall short in economic prosperity need to act fast in finding an alternative. India being an agricultural land with a rich diversity of plant species has high potential to produce indigenous VOs. Keeping in mind such prospective, the current work has been undertaken and accomplished.

CONTRIBUTION TO THE SOCIETY

The current study has explored the potential of WCO to produce biolubricant components through a two-step process. The work was directed in pursuance of sustainable development ensuring minimization and reutilization of waste (waste cooking oil). Everyday a large quantity of WCO is generated by several restaurants, food stalls and snack industries which finds no immediate utilization. They are often being dumped in the drainage/ grounds/ water bodies or sometimes sold to soap manufacturers at nominal rates. Dumping of oil to native ecosystems like open grounds or aquatic systems disrupts and even alters the natural ecological balance for a certain time. On the other hand, the consumption of WCO is detrimental since it may result in abnormal changes of (i) body weight (ii) serum enzyme like serum glutamate pyruvate transaminase (SGPT), serum glutamic oxaloacetic-transaminase (SGOT), alkaline phosphatase (ALP) and (iii) histopathological characteristics. Another advantage of using WCO over virgin VO, specifically over edible VO is that it can overdo with the debate of food versus fuel in terms of arable land allocation. Thus a value added product has been generated from an otherwise waste or detrimental raw material.

PUBLICATIONS OUT OF THE PROJECT

Journal

1. Biolubricant synthesis from waste cooking oil via enzymatic hydrolysis followed by chemical esterification, **Journal of Chemical Technology and Biotechnology** 88(2012)139-144.

2. Synthesis of biolubricant components from waste cooking oil using a biocatalytic route, **Journal of Environmental Progress and Sustainable Energy** 33(2014)933-940.

3. Optimization of the product parameters of octyl ester biolubricant using Taguchi's design method and physico-chemical characterization of the product **Industrial Crops and Products** 52 (2014) 783-789.

4. A Kinetic Study on the Novozyme 435-Catalyzed Esterification of Free Fatty Acids with Octanol to Produce Octyl Esters, **Biotechnology Progress** 31(2015)1494-1499.

5. Esterification of free fatty acids derived from waste cooking oil with octanol over Amberlyst 15H: Process optimization and kinetic modeling, **Chemical Engineering & Technology** 39, 4(2016)730-740.

Conference Papers

1. Oral presentation on " Biocatalytic synthesis of biolubricants from waste cooking oil" at International Congress of Environmental Research, from 22nd to 24th Nov. 2012.

2. Oral presentation on "Biolubricants-A Step towards Sustainable Development" IIChE, Kolkata- 700032, on 2012.

3. Poster presentation on "Production of Biolubricants from Waste Cooking Oil: Optimization of the process parameters" 100th Indian Science Congress, 2013.

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002.

Final Report of the work done on the Major Research Project.

1. **Project report No.** : 3rd/Final

2. **UGC Reference No.** : **F. No. 40-9/2011 (SR)**, dated June 29, 2011

3. **Period of report** : From **01.07.2011 to 30.12.2014**

4. **Title of research project** : **Production of fatty acid derivatives as components of biolubricants**

5. **(a) Name of the Principal** : Dr. DEBARATI MITRA

   Investigator

   (b) Department : Chemical Technology

   (c) University/College where: University of Calcutta

   work has progressed   92, A.P.C. Road, Kolkata – 700009, India

6. **Effective date of starting** : 1st July, 2011

   of the project

7. **Grant approved and expenditure incurred during the period of the report:**

   a. **Total amount approved**   Rs.13,32,632 /-

   b. **Total expenditure**   Rs.12,36,997 /-

   c. **Report of the work done** : **(Please attach a separate sheet)**

i. **Brief objective of the project:**   Annexure – I

ii. **Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication):**

Annexure – II

iii. **Has the progress been according to original plan of work and towards achieving the objectives, if not state reasons.**

The work has progressed as per proposed original plan of work to achieve objectives.

iu. **Please indicate the difficulties, if any, experienced in implementing the project.**

The delay in release of the second installment hampered the project work and the fellow received her arear fellowship after about a year.

The main two difficulties are
- a) Administrative delay
- b) Non-availability of the funds

U. **If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet.**

The project work was completed on December 2014.

Ui. **If the project has been completed, please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to University Grants Commission.**

Annexure – III

Uii. **Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any**

Debarati Mitra

Principal Investigator

(Signature with seal)

Dr. (Mrs.) Debarati Mitra
Assistant Professor
Department of Chemical Technology
University of Calcutta
92, A.P.C. Road, Kolkata-700 009

Registrar

(Signature with seal)

REGISTRAR
Calcutta University

01.09.17

01 SEP 2017

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. Title of the Project	:	**Production of fatty acid derivatives as components of biolubricants**
2. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR	:	**Dr. DEBARATI MITRA** Flat-A1, 13/4 Deshbandhu Road, Jadavpur, Kolkata-700032, West Bengal.
3. NAME AND ADDRESS OF THE INSTITUTION	:	**Department of Chemical Technology, University of Calcutta,** 92 A P C Road, Kolkata-700009.
4. UGC APPROVAL LETTER NO. AND DATE	:	**F. No. 40-9/2011 (SR),** dated June 29, 2011
5. DATE OF IMPLEMENTATION	:	**01.07.2011**
6. TENURE OF THE PROJECT	:	Three and half years
7. TOTAL GRANT ALLOCATED	:	**Rs.13,32,632/-**

8. TOTAL GRANT RECEIVED	:	(i) Grant Received	:	Rs. 12,36,949/-
		(ii) Interest Received	:	Rs. 40,601/-
		Total	:	Rs. 12,77,550/-
9. FINAL EXPENDITURE	:	(i) Grant Part	:	Rs. 12,06,776/-
		(ii) Interest Part	:	Rs. 30,221/-
		Total	:	Rs. 12,36,997/-

10. TITLE OF THE PROJECT	:	**Production of fatty acid derivatives as components of biolubricants**
11. OBJECTIVES OF THE PROJECT	:	Annexure - I
12. WHETHER OBJECTIVES WERE ACHIEVED	:	Annexure -II
13. ACHIEVEMENTS FROM THE PROJECT	:	Annexure - III
14. SUMMARY OF THE FINDINGS	:	Annexure - IV (IN 500 WORDS)
15. CONTRIBUTION TO THE SOCIETY	:	Annexure – V
16. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT	:	**The Project fellow Ms. Avisha Chowdhury has been awarded Ph.D. degree from the University of Calcutta based on the work of the said Project. Provisional Certificate of Ph.D degree is attached.**
17. NO. OF PUBLICATIONS OUT OF THE PROJECT	:	Annexure - VI (ATTACHED)

Debarati Mitra

(PRINCIPAL INVESTIGATOR)

Dr. (Mrs.) Debarati Mitra
Assistant Professor
Department of Chemical Technology
University of Calcutta
92, A.P.C. Road, Kolkata-700 009

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REGISTRAR
Calcutta University
01.09.17

01 SEP 2017

SCHOOL OF COMMUNITY SCIENCE AND TECHNOLOGY
INDIAN INSTITUTE OF ENGINEERING SCIENCE AND TECHNOLOGY (IIEST),
SHIBPUR
(Formerly BENGAL ENGINEERING AND SCIENCE UNIVERSITY, SHIBPUR)
P.O.Botanic Garden, Howrah- 711 103, West Bengal, India
Phone No- 033-26684561/62, E-mail: socsat1@gmail.com

EVALUATION OF FINAL REPORT OF UGC MAJOR RESEARCH PROJECT

The UGC Major Research Project titled '**Production of fatty acid derivatives as components of biolubricants**' under Dr. DEBARATI MITRA has been carried out successfully. The adopted technology comprises of a two-step process of enzymatic hydrolysis of waste cooking oil (WCO) followed by chemical/ enzymatic esterification of free fatty acid (FFA) with a higher alcohol (octanol) for producing biolubricant components. The processes are substantially feasible and productive and are quite relevant to modern Indian industry. The esterification reactions have been ratified with supportive kinetic models which are likely to aid in the large-scale productions of biolubricant components. The product octyl esters have been characterized and tested at length and a suitable area of application has been duly identified. WCO is inexpensive and finds limited usage other than environmental disposal. Thus, raw-material used in the present research being a waste biomass (waste cooking oil), scores higher in sustainability quotient than virgin vegetable oil. Additionally, the use of WCO will not curtail the share from the food supply resources. The overall project work has been carried out successfully and offers a promising technique of producing biolubricant components from waste material.

D. K. Mukherjee
18/8/2017

Prof. D. K. Bhattacharyya
School of Community Sc. & Tech.
IIEST, Shibpur

From:
Prof. (Dr) Pinaki Bhattacharya, FICS
Emeritus Professor and Head (Research)

Date: Aug 16, 2017

Evaluation of UGC Major Research Project

The submitted report of the project titled '**Production of fatty acid derivatives as components of biolubricants**' under Dr. DEBARATI MITRA presents a comprehensive work on the production of biolubricant components from fatty acids.Cheap waste cooking oil has been selected as feedstock which results in a new route to utilization of waste materials . Biolubricant synthesis via chemical/biochemical esterification followed by thorough kinetic study of the reactions has been carried out. Response Surface Methodology, a modern statistical tool has been applied for process optimization.

Product isolation and characterization provide information about the properties of the biolub and hence the area of application has been identified. The presentation of the results is clear and concise. The interpretation of the findings is logical and acceptable. The project contributes to the knowledge in the area of green materials and technology.

(Pinaki Bhattacharya)

RESEARCH WORK SUMMARY

The conventional lubricants originating from petroleum base stocks are toxic to the environment, making it increasingly difficult for safe and easy disposal. Moreover, the depleting reserves of mineral oils with an increasing crude oil price have aggravated the thought for alternative lubricant base oil. Vegetable oils (VO) can be identified as an excellent substitute to the mineral oil based lubricants if few of their shortcomings (like low thermal, oxidative and hydrolytic stabilities and poor low temperature characteristics) can be modified. The high price of virgin VOs also limits their large-scale application. This thesis encompasses a novel approach in establishing the use of cheap waste vegetable oil more specifically waste cooking oil (WCO) as a source of biolubricant base oil.

WCO was chosen as the low-cost feedstock for producing potential biolubricant components. The feedstock was characterized prior to use. A two-step cost-effective sustainable technology was developed to formulate an environment-friendly value-added product from waste oil. After initial sets of pilot experiments, a reactor was fabricated to carry out further reactions in a batch mode. In the initial step WCO was hydrolyzed by an enzyme (*Candida rugosa* lipase at a concentration of 1 g L^{-1} for 30 h) to form free fatty acids (FFA). Since hydrolysis of VOs has been extensively studied and reported in literature, this part of the study was limited to a brief investigation. The molar ratio of WCO: water with time was optimized to get a satisfactory conversion of triglycerides to FFA with a minimum enzyme concentration. The subsequent step of the study was the esterification of generated FFA with octanol using both chemical (Amberlyst 15H) and enzyme catalyst (Novozyme 435) to bring about satisfactory conversion of ~98% and ~95% respectively. The effect of various physico-chemical parameters like temperature, catalyst amount, molar ratio of reactants, agitation speed, initial water content and

desiccants on the chemical/ enzymatic esterification were investigated. The process parameters were optimized following two schools of experimentation- 'the classical one-parametric approach' and 'statistical designing approach'. Response surface methodology was employed to optimize the chemical esterification while Taguchi's design method was used for enzymatic esterification. Among the various process parameters, catalyst dosage and temperature was found to have higher effect on both chemical/ enzymatic esterification. According to classical approach of parametric study the optimum process conditions were octanol: FFA molar ratio= 3: 1, temperature= 353 K, Amberlyst 15H= 2 g for chemical esterification and octanol: FFA molar ratio= 3: 1, temperature= 333 K, Novozyme 435= 5 wt% of FFA for enzymatic esterification. On the other hand the optimal conditions based on statistical design analysis were temperature= 354 K, catalyst loading= 1.85 g and molar ratio= 3.2:1 for chemical and; temperature=333 K, enzyme loading=5 wt% of FFA, molar ratio of octanol: FFA=2.5: 1 for enzymatic esterification.

A brief comparative was drawn between the chemical and enzymatic esterification reaction based on the process conditions, reusability of catalyst, catalyst productivity and cost of catalyst. Although both the catalysts were found comparable and effective in bringing about successful esterification with satiable conversions, it is suggested on economic perspective, that the use of chemical catalyst Amberlyst 15H will be more feasible for large-scale production since cost of enzyme catalyst is quite high.

The next phase of the work included the study of kinetics of both chemical and enzymatic esterification reactions. Both the catalysts were physically characterized prior to kinetic modeling. A pseudo-second order (PH) second order kinetic model was developed and validated (R^2=0.98) to describe the kinetic behavior of the chemical esterification. The kinetic parameters viz. rate constants, activation energy, frequency factor were evaluated. Besides Ping-

pong bi-bi kinetics was found to fit (R^2=0.99) with the enzymatic esterification reaction. Along with, the values of various pertinent kinetic parameters viz. K_m, V_{max}, etc. have also been reported.

The final phase of the research was the product isolation, characterization and property testing. Octyl esters were the product of the esterification reaction which are the desired biolubricant components. The product was isolated from the end reaction, purified and subjected to characterization tests using FTIR and NMR analysis. After the ester chemistry of the product was verified by the mentioned analytical methods, it was tested for its physico-chemical and few tribological attributes. It was found that the synthesized biolubricant has ameliorated the inherent short comings of VOs as against the raw material WCO. Based on the properties evaluated, the biolubricant components produced by this two-step process has been suggested to fall under API grade V (engine oils), ISO 3448 VG 46 (industrial oils) and SAE J300- 40 (engine oils). Hence, the objective of the current research was accomplished with the production of biolubricant components from a renewable low-cost bio-material using a sustainable two-step process.

Project Title:

Production of fatty acid derivatives as components of biolubricants

Submitted By

Dr. Debarati Mitra

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1. Introduction

The word 'Lubrication' implies to the technique of reducing friction and wear. In the early ages, our ascendants were hooked to vegetable oils and animal fats as the sources for lubricants but the dawn of technological revolution coupled with petroleum discovery shifted the feedstock priority from vegetable oils and fats to mineral oils. Since every technology upgradation affect people and the environment, commercial petroleum-based lubricants aided with chemical additives used in transportation, agriculture, mining and manufacturing are no exception. About 50% of the consumed lubricants have been reckoned to end up in the environment due to leakages, spills or spent release.[1] Such problems cannot be regulated only by efficient waste processing technologies, hence alternation in lubricant basestock selection is highly recommended. Moreover, depleting mineral oil resources with increasing crude oil prices has highlighted the urgent need to manufacture non-mineral oil based lubricants.

Lately bio-based oils have been recognized as an important part of new strategies, policies, and subsidies, which aid in the reduction of the dependence on non-renewable mineral oil. Vegetable oils (VOs) provide an excellent lubricant basestock which is mainly imputable to their 'environmentally compatible' nature. Conventional mineral oil based lubricants gained primacy over the biolubricants due to their low-cost and efficacy. Although the petro-based lubricants met most of the demands to be an effective lubricant basestock, their main disadvantages lie in being non-renewable and toxic to the environment with poor bio-degradability. VOs are mainly triglycerides. The advantages associated with VOs are:

(i)   high viscosity indices,

(ii)   good anticorrosion properties,

(iii)   high lubricity

(iv)   high flash and fire points,

(v)   low volatility,

(vi)   good additive compatibility,

(vii)   high affinity to metal surfaces and

(viii) low ecotoxicity owing to rapid biodegradability.

All these significant attributes present them as an ideal alternative to be used in lubricant formulations. VOs exhibit few short comings like sensitivity towards hydrolysis, low thermo-oxidative stability and poor low temperature behavior. But these drawbacks can be successfully improvised by chemical modification of the VOs to produce feasible biolubricant components.

According to a recently published report by Global Industry Analysts, Inc., the global market for lubricating oils and greases will reach 10.9 billion gallons by 2017. In India we are expected to consume more than 100 million tons per annum of petroleum products before the dawn of the 21st century and we can visualize consumptions of around 2.0 million tons per annum of lubricants.[2] Latest literature reports on lubricant market show that some manufacturers now market environmentally acceptable bio-lubricants in the United States, Europe, and Asia. US patent numbers 6,278,006 August 21, 2001,[3] 6,420,322 July 16, 2002,[4] 20050150006 July 7, 2005,[5] US20080293602 November 27, 2008,[6] disclose lot of work done on bio-based lubricants. Development of more than 30 viable soybean oil based lubricants, grease and metal working fluid formulations including the high-performance multi-grade hydraulic fluid, brand named BioSOYTM, a patented electrical transformer fluid named BioTRANSTM, chainsaw bar oil called SoyLINKTM, a rail curve lubricant called SoyTrakTM, and Soy TRUCKTM, a semi truck fifth-wheel grease has been reported by Honary, 2010.[7] Several companies have been prompted to get involved in producing VO based lubricants such as Mobil chemicals, Shell oil company, British petroleum, Etc. In India this type of work has been done at Indian Oil Corporation Faridabad, Indian Institute of Petroleum, Dehradun. Indian Institute of Chemical Technology, Hyderabad, Defense Materials and Stores Research & Development Establishment Kanpur, BPCL and HPCL India.[2]

In the current research work waste cooking oil (WCO) has been chosen as the feedstock for the preparation of biolubricant components. WCO can serve as an excellent feedstock reducing the initial investment cost for high priced virgin/ refined vegetable oils. At the same time the work focused on the 'sustainable development' ensuring minimization and reutilization of waste (waste cooking oil). Restaurants and food stalls generate large quantities of WCO which otherwise finds no immediate utilization. They are often being dumped or sometimes sold to soap manufacturers at low rates. Waste cooking oil containing high percentage of water and free fatty acids can be successfully utilized for the proposed biolubricant synthesis.

2. Literature Review

Current researchers are motivated enough to work in biomass utilization to achieve sustainable development. Vegetable oil-based lubricants and derivatives possess excellent lubricity and biodegradability, for which they are being investigated as a base stock for lubricants and functional fluids.[8] Investigations toward biolubricant preparation have been conducted by using a number of vegetable oils as feedstock, such as sunflower,[9] soyabean,[10] castor,[11] rapeseed,[12] palm,[13] jatropha,[14] etc. WCO has been identified as a cheap and effective feedstock for production of biodiesel by many workers [15-17]. Talukdar *et al* (2010) converted waste cooking oil to biodiesel via enzymatic hydrolysis of WCO to generate FFA followed by chemical esterification of FFA with lower alcohols to produce esters.[18] Kulkarni *et al* (2005) has also showed hydrolysis of castor oil with lipase enzyme from *Aspergillus oryzae*.[19]

The fatty acids produced from VOs can be converted to their corresponding esters with higher alcohols (C8 to C14) in presence of suitable catalyst, for use as lubricants.[20] Chemical modifications such as epoxidation, estolides formation, and transesterification of plant oils with polyols have been shown to achieve optimal characteristics for extreme applications as lubricants.[21] Synthesis of oleochemical diester as biolubricant components by 3 step process including epoxidation, ring opening (using p-toluenesulfonic acid as catalyst) and esterification (using 10 mol% H_2SO_4 as catalyst) has been carried out by investigators.[23-25] Biolubricant has also been prepared in an integrated system by an esterification reaction of fusel oil and oleic acid using immobilized Novozym 435 lipase enzyme.[26] Ecofriendly lubricant formulations with improved kinematic viscosity and viscosity thermal susceptibility was developed by Quinchia *et al* (2009).[9] They blended a high oleic sunflower oil with polymeric additives at different concentrations thereby improving the low viscosity values of the oil.

Both chemical as well as enzymatic catalysts have been found to be effective for desired biolubricant production. Workers have proven successful utilization of sulphuric acid as catalyst for esterification of free fatty acids with alcohols.[27,28] Studies with alkaline catalyst like 15% potassium hydroxide/ aluminium oxide to produce biolubricant has also been reported.[29] A novel application of Fe–Zn double-metal cyanide (DMC) complexes as solid catalysts in the preparation of fatty acid alkyl esters (biodiesel/ biolubricants) from vegetable oils was reported by Sreeprasanth *et al* (2006).[30] Carmo *et al* (2009) has worked with mesoporous aluminosilicate Al-MCM-41 as catalyst for esterification of palmitic acid.[31]

Transesterification of edible and nonedible vegetable oils with alcohols (to produce biodiesel/ biolubricants) using heteropolyacids supported on clay (K-10) as catalyst is being reported by Bokade and Yadav (2007).[32] Various transesterification and esterification reactions with Amberlyst 15 H has also been reported in the literature.[27,33-35]

Lipase enzymes have been used widely for undergoing esterification reactions. n-Octyl oleate has been synthesized by Laudani *et al* (2006) using immobilised lipase from *Rhizomucor miehei* as biocatalyst.[36] Commercial lipase from *Candida antarctica* (Novozym 435), immobilized on a macroporous anionic resin has been used by Koszorz *et al* to carry out enzymatic esterification of oleic-acid and i-amyl alcohol to produce i-amyl-oleate (bio-lubricant).[37] Several other workers like Lerin *et al*,[38] Liu *et al*,[39] Duan *et al*,[40] Åkerman *et al* [27,41] has undertaken esterification reactions using Novozyme 435.

Dörmő *et al*, 2004, have suggested from the tribological properties of their produced product (from fusel oil and oleic acid) that the biolubricant can be used at high speed and low load regime of the tribological circumstances. It can be applied in mechanical industry as a cooling lubricant compound for metalworking processes and in mist lubrication, chain lubrication, launch engine lubrication, where lubricant loss may occur.[26] The jatropha biolubricant synthesized by Ghazi *et al*, 2010, has been classified under the ISO VG46 that serves for most light gears, automotives and industrial gear applications.[42] TMP-caprylic acid esters are used for biolubricant application requiring high viscosity and high stability, and can also be used as dielectric coolants and as rail/wheel lubricants[43,44] while TMP oleate is the most widely used biolubricant product for hydraulic fluids.[27]

Esterification kinetics involving various substrates has been investigated quite often. Many reported literature are available on the kinetic study of esterification of different fatty acids with primary alcohols like methanol, ethanol, propanol, etc.[45-47] In most of the cases the esterification kinetics, which is actually heterogeneous has been explained by the pseudo-homogeneous (P-H) model for the sake of mathematical simplicity. Lipase enzyme catalyzed esterification reactions of acid and alcohols has been widely explained by Ping-Pong Bi-Bi mechanism by several workers.[48-50]

Coming to conducting experiments, researchers have always supported the classical method of parametric approach to identify the effects of process factors. This method consist of selecting a starting point, or baseline set of levels, for each factor and then successively varying each factor over its range with the other factors held constant at the baseline level. Later a series of graphs are constructed showing how the response variable is affected by varying each factor with all other factors held constant. But very recently there has been a

surge in adopting a new way of experimentation using statistical tools. The disadvantages of the one-factor-at-a-time strategy are that it fails to consider any possible interaction between the factors and it requires many observations (data) leading to more expense in terms of time and money. On the other hand statistical design of experiments refers to the process of planning the experiment so that appropriate data will be collected and analyzed by statistical methods, resulting in valid and objective conclusions requiring minimum experiments.[51] Thus the current scientists are more devoted in designing there experiments using various models of statistical designs viz. Response surface methodology, Taguchi's design, factorial designs etc.

3.   Objectives

The research work has been initiated with the following objectives:

1. Development of a suitable and sustainable technology of producing biolubricant components from a cost-effective feedstock using appropriate catalyst.
2. Reactor fabrication to carry out the esterification experiments in a batch mode.
3. Investigation on the effects of different physico-chemical parameters on biolubricant production.
4. Optimization of process parameters to get maximum biolubricant yield using classical method of parametric approach as well as statistical tools like Response surface methodology (RSM) and Taguchi's analysis.
5. Batch mode kinetic study of the reaction processes.
6. Isolation and characterization of the product ester (biolubricant components)
7. Property testing to evaluate the efficiency of the product as an effective biolubricant baseoil.

4. Work pursued

The following work has been performed to achieve the objectives:

1. **Adopted technology for biolubricant production**: A two-step process of enzymatic hydrolysis of WCO to produce FFA followed by chemical/enzymatic esterification of FFA with higher alcohol (octanol) to produce biolubricant components (octyl esters) has been undertaken

2. **Reactor fabrication**: A batch-reactor has been modeled to undergo the esterification reactions.

3. **Physico-chemical parametric study**:

   a. The effect of various physico-chemical process factors like temperature, catalyst amount, molar ratio of reactants, agitation speed, initial water content and desiccants on the chemical/ enzymatic esterification process has been studied based on the classical approach of parametric study (one parameter at a time).

   b. Parametric study and process optimization of the chemical/ enzymatic esterification has also been done using statistical techniques like RSM and Taguchi's analysis.

4. **Kinetic study**: The kinetics of chemical esterification has been studied and established by a model.

5. **Product analysis and characterization**: The product (biolubricant components) has been isolated and characterized.

6. **Biolubricant testings**: The property testing of the product has been conducted to establish its utility as an effective biolubricant.

5. Experimental

5.1.   *Materials*

5.1.1. Feedstock procurement: WCO was supplied by local food stall.

5.1.2. Chemicals obtained:

Catalysts: *Candida rugosa* lipase was supplied by Sigma Aldrich, USA.

Novozyme 435 (lipase from *Candida antartic*a immobilized on macroporous anionic resin) was obtained from Novozymes South Asia, Bangalore, India.

Amberlyst 15H was supplied by HiMedia Laboratories Ltd., Mumbai, India

<u>Other analytical chemicals</u>: 1-Octanol (99% pure), ethanol, chloroform, deuterochloroform, n-hexane, acetone, ethanol, anhydrous sodium sulphate, potassium hydroxide, oxalic acid and all other analytical chemicals were purchased form Merck Specialties Ltd.

5.1.3. Instruments used to accomplish the work:

- Digital balance (CP 225 D, Sartorius),
- Gas chromatograph (Agilent 6890, version N.05.05) equipped with FID detector.
- Fourier Transform Infrared (FTIR) Spectrophotometer fitted with a DLATGSs detector (JASCO FTIR-670 Plus),
- Bruker AVANCE 600 MHz spectrometer with TCI cryoprobe,
- Mastersizer 2000, Malvern Instruments Ltd.,
- Quantachrome make NOVA 2000
- PoreMaster-60 (Quantachrome)
- Laminar flow chamber (1103 Klenzaids, India)
- Hot-plate associated magnetic stirrer (MT-400, Minato Corporation)
- Shaker incubator (LJE-Orbitek),
- Rotary vacuum evaporator (EYELA, Tokyo Rikakikai Co. Ltd. Japan),
- Centrifuge (R-24, REMI), and
- A self designed batch reactor (locally manufactured by Marconi Instruments Pvt. Ltd.) equipped with the following features: a jacketed reaction vessel containing thermic fluid that served as the heating medium; an electrically driven stirrer with a digital display of rpm; a thermostat to maintain the desired temperature (digital display), allowing temperature variation within ± 0.5 °C; a condenser to condense back the water vapor generated during the reaction; a sample inlet duct and a sampling point equipped with a valve.

5.2.   Methods
5.2.1. Feedstock characterization

WCO was filtered each time it was supplied by the local restaurant and its acid value (AV) was determined following a previously established method.[18] The saponification value (SV) and water content of WCO was determined following ASTM D5558 and ASTM D95[52] respectively. The fatty acid composition of the WCO was determined using a gas chromatograph (Agilent 6890, version N.05.05) equipped with FID detector. Various

physico-chemical property testings of the feedstock (WCO) has been done and reported in Table 5.

5.2.2. Catalyst characterization

Amberlyst 15H, a commercial ion-exchange resin catalyst was employed to facilitate the chemical esterification reaction between FFA and octanol. Various characterization tests were conducted to estimate the physical properties of the Amberlyst 15H viz. density, surface area, pore size, pore volume, porosity and tortuosity.

5.2.3. Hydrolysis of WCO

WCO was taken in glass stoppered bottles and the reaction was initiated by adding a specified amount of *C. rugosa* lipase solution at 303 K and 250 rpm in a shaker incubator. The bottles were incubated for 30 h to attain equilibrium. *C. rugosa* lipase was dissolved in deionized water to prepare the lipase solution. The enzyme concentration was kept constant at 1.0 g L^{-1} (1 g of lipase powder dissolved in 1 L of water). The water to WCO ratio (v/v) was varied in the range 0.5: 1 to 5: 1.

5.2.4. Separation of FFA

An excess of n-hexane was added to the reaction mixture after 30 h of reaction. The mixture was centrifuged at 4000 rpm and 303 K for 15 min. After an initial centrifugation of 10 min the upper layer of free fatty acids in hexane was separated and then again centrifuged for another 5 min for complete separation. The solvent phase (n-hexane) containing the FFA was separated from the glycerol-water layer at the bottom. Anhydrous sodium sulphate was added to the fatty acid–solvent phase to remove any trace of water. The solvent layer containing fatty acids was separated and hexane was removed by distillation. The initial acid value for the generated FFA was determined before starting esterification.

5.2.5. Esterification of FFA by chemical catalysis

Initially pilot esterification reactions were carried out in a 500 ml beaker fitted over a hot-plate associated magnetic stirring device (MT-400, Minato Corporation). Later a batch reactor was fabricated to perform further reactions. FFA and octanol were esterified in the reactor vessel using Amberlyst 15H catalyst. Experiments were conducted changing one process parameter and keeping others constant. The parameters evaluated were molar ratio (FFA: octanol =1: 1 to 4: 1), catalyst amount (1 to 15 wt% of FFA i.e., 0.2 to 3g), reaction

temperature (333K to 383 K) and stirrer speed (450 to 650 rpm). Desiccants like magnesium sulphate, sodium sulphate and silica gel powder were added to the reaction mixture at 50% of the weight of FFA in order to investigate their effects on the reaction rate.

The chemical esterification reaction was also conducted following statistical design of experiments to see the interactions among the process variables and to find the statistically significant optimum condition to yield maximum biolubricant. RSM based analysis was carried out using the Design Expert (version 7.0) package. Temperature (K), catalyst loading (g) and molar ratio of octanol: FFA (M) was chosen as three independent process parameters. A five level, three factor orthogonal quadratic design (OQD with $\alpha=1.525$) scheme was employed requiring 20 experiments.[51] The optimum process condition was determined by fitting the most suitable regression model in the three dimensional parametric space followed by Analysis of Variance (ANOVA).

The data generated from the chemical esterification reactions was also used to model the reaction kinetics.

5.2.6. Esterification of FFA by enzymatic catalysis

Esterification of FFA with octanol was also conducted using Novozyme 435 in the batch reactor with a varying octanol to FFA molar ratio (1:1 to 4:1), catalyst amount (1.5 to 10 wt % of FFA) and reaction temperature (313K to 343K). In all sets of experiments the agitation speed was kept constant at 250 rpm (determined from preliminary pilot experiments) Additionally, the effect of initial water content on the conversion efficiency of Novozyme 435 was investigated at a range of 0.25 -1 wt % of FFA.

The Taguchi's L_9 orthogonal array[53] was employed to examine the four factors (temperature, enzyme amount, molar ratio and agitation speed) at three levels (all experiments were performed in triplicate) in order to maximize % conversion of octyl ester. The L and the subscript 9 represent the Latin square and the number of experimental runs, respectively. The software MINITAB-16 (Minitab Inc. USA for Windows7) was employed to undertake the Taguchi design method. The S/N ratio values corresponding to the conversions were calculated, using the 'larger-the-better' characteristics, since the aim of the work was to maximize the response (FFA conversion).

5.2.7. Isolation of product; Analysis & Product characterization

The esters produced from the reaction with FFA and octanol was the desired biolubricant (octyl esters). The product was withdrawn from the sampling point of the reactor from time to time and tested for its AV. The excess/ unreacted octanol was recovered by vacuum distillation and the product was isolated for characterization. Fourier transform infrared spectroscopy (FT-IR) and nuclear magnetic resonance (NMR) analyses were conducted to confirm that the product formed is ester. FT-IR scans were carried out in a Jasco-670 Plus FT-IR fitted with a DLATGS detector. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in the Bruker AVANCE 600 MHz spectrometer with TCI cryoprobe using $CDCl_3$ solvent.

The physico-chemical properties of the product were determined following standard methods to ensure its usability as a lubricant. The kinematic viscosity and viscosity index were measured using ASTM methods D 445 and D 2270 respectively.[52] The pour point was determined following ASTM D 97 [52] standard method. The flash point and oxidation stability of the product were obtained following ASTM D 93 [52] and IP 48 [54] respectively. The relative biodegradability of the product ester (biolubricant) was determined according to the most commonly used standard test CEC-L-33-A-93.[55] All the above mentioned tests were run in triplicate and the average values were reported.

6.   Results and Discussions

6.1.   Feedstock characterization

AV of WCO was found in the range of 2-14 mg KOH g^{-1} of WCO. The SV was found to be in the range of 227–229 mg of KOH g−1. The water content was found to be 0.35-0.6 wt%. Oleic acid, palmitic acid, linoleic acid, stearic acid and traces of myristic and erucic acids were found as the constituent fatty acids of the WCO. The average molecular weight of the total FFA from WCO was 271.66 g $mole^{-1}$.

6.2.   Amberlyst 15H characterization

The Typical properties of Amberlyst 15H dry ion-exchange resins have been reported in Table 1.

Table 1. Physico-chemical attributes of Amberlyst 15H

Physical form	Spherical opaque beads
Ionic form	H^+
Average particle size (m)	0.51×10^{-3}
Surface area (m^2 kg^{-1})	45×10^3
Porosity (%)	0.29
Average pore size (Å)	250
Density (Kg m^{-3})	1.09×10^3
Pore volume (m^3 kg^{-1})	0.25×10^{-3}
Tortuosity (dimensionless)	3.45
Maximum operating temperature[*] (K)	398

[*]Provided by the catalyst manufacturing company

6.3.   Effect of water on hydrolysis

The effect of different water to WCO ratio on the extent of hydrolysis was investigated. *Candida rugosa* lipase has been reported as one of the most efficient lipase enzymes for hydrolysis of oils and fats.[18,56,57] This enzyme was used for hydrolyzing WCO to FFA. Lipase enzyme has a characteristic interfacial activation.[19] Figure 1 shows that with increasing water to oil ratio from 0.5: 1 to 5: 1 the percentage yield of FFA

Figure 1. Hydrolysis of WCO with 1g L^{-1} *C.rugosa* lipase solution at different water: WCO ratio.

significantly increased, due to increase in water to oil interfacial area, reaching a maximum of 92% at water to oil ratio of 4: 1 with 1 g L ^{1} enzyme concentration in 30 h. This reaction is reversible. A large amount of water is therefore essential to shift the equilibrium towards hydrolysis.[58,59] Further increase in the water to oil ratio from 4: 1 to 5: 1 resulted in reduction of FFA yield. This can be attributed to the fact that higher level of water has a negative effect

on lipase activity. Shimada *et al.* reported the dilution effect of lipase enzyme at higher water level which reduces the efficiency of the enzyme to hydrolyze.[59] The main objective of the work being the investigation of esterification reaction, the hydrolysis step was not studied in detail and only a single concentration of enzyme was taken into account that gave sufficient yield of FFA.

6.4.   Esterification of FFA by chemical catalysis

6.4.1. Classical approach of parametric study

6.4.1.1.   Effect of molar ratio of substrates

The reaction temperature was set at 353K, agitation speed was maintained at 550 rpm and 2 g catalyst was employed to execute the experiments at different molar ratio of octanol: FFA. The conversion increased with increase in the octanol: FFA molar ratio (Fig. 2). Similar results have been reported by others.[26,60] Higher amounts of alcohol shifts the esterification equilibrium to the right thereby increasing the ester yield.[18] Moreover the rate of ester synthesis also increased with time at higher molar ratios. The maximum conversion of 98%

Figure 2. Esterification reaction with varying molar ratio of octanol: FFA, temp. 353 K catalyst 2g, 550rpm. (n= 3, mean ± S.D)

was obtained at an octanol: FFA ratio of 3: 1 in 3 h. Increasing the molar ratio to 4: 1 did not show any significant increase in reaction rate to attain equilibrium. Hence, 3: 1 was chosen as the optimum molar ratio.

6.4.1.2.   Effect of catalyst amount

The effect of varying amounts of Amberlyst 15H has been presented in Fig.3. The rate of conversion significantly increased with increase in catalyst amount. 0.2 g catalyst gave a maximum conversion of 83.3% after 8 h of esterification, while 2 g of catalyst brought 98% conversion in just 3 h. Therefore it is evident that esterification reaction is very much

dependent on the amount of catalyst loading. More catalyst reveals more active sites which participate in the reaction and catalyze the production of lubricant.[18,61] With further increase in Amberlyst 15H to 3 g the rate of fatty acid conversion remained the same, i.e. the reaction rate reached a maximum for Amberlyst 15H loading of 2 g.
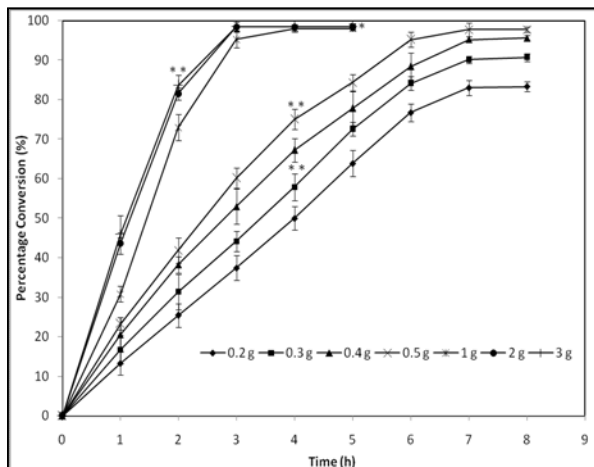

Figure 3. Esterification reaction with varying amount of catalyst, octanol: FFA 3:1, temp. 353 K, 550 rpm (n= 3, mean ± S.D).

6.4.1.3. *Effect of temperature*

The ester conversion at different temperatures has been reported in Fig.4. The esterification reaction is endothermic hence increase in temperature resulted in an increase in conversion of FFA to esters.[28,62] At higher temperatures the rate of reaction was also found to increase giving 98% conversion in minimum time. Maximum conversion was obtained at 383 K in 1.5 h whereas it took 3h to achieve almost the same conversion at 353 K. The color of the final lubricant product mixture was found to be affected by the temperature as well. The color

Figure 4. Esterification reaction with varying temperature, octanol: FFA 3:1, catalyst 2g, 550 rpm (n= 3, mean ± S.D).

gradually darkened from light yellow to dark brown from 353 to 383 K. Similar findings have been reported by Özgülsün *et al.*[60] Since, the product darkened at higher temperatures, 353 K was chosen as the optimal experimental temperature.
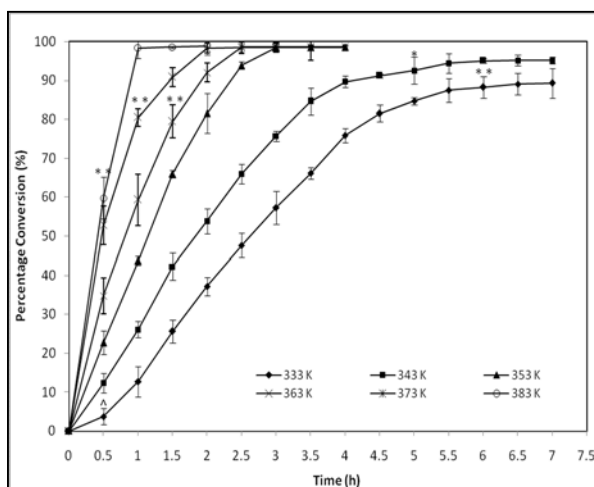
6.4.1.4. *Effect of stirrer speed*

The influence of external resistance to mass transfer was assessed by varying the stirrer speed from 450 to 650 rpm keeping other reaction conditions the same (353 K, 2 g

catalyst, alcohol : FFA ratio 3 : 1). The increase in agitation speed did not significantly affect either the final conversion percentage of the reaction or the reaction rate. 550 rpm was selected as the optimum stirring speed.

6.4.1.5.   Effect of desiccants

Water produced from the esterification reaction could facilitate the backward reaction. Various desiccants (50% of the weight of the FFA) were tried to remove this water. Esterification reaction with this amount of desiccants exhibited high level of conversion. Silica gel powder was found to give the best conversion followed by magnesium sulphate and sodium sulphate. However, the reaction mixture was found to be turbid with the use of desiccants and it was rather difficult to isolate the ester.

6.4.1.6.   Optimization of process factors

The present experimental design was done following the classical approach of parametric study (one parameter at a time). The optimum physico-chemical conditions to achieve
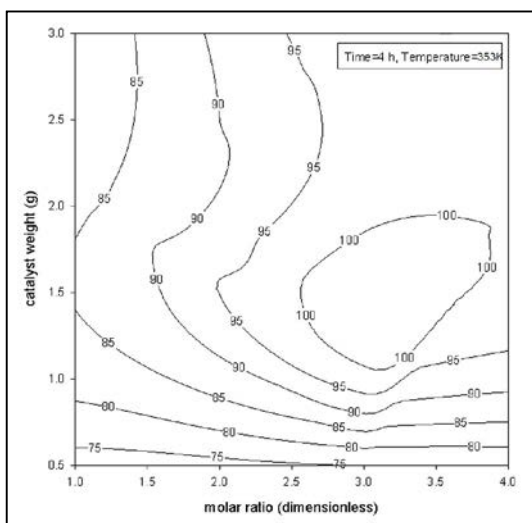

Figure 5a. Contour plot showing the effect on % conversion with the variation of molar ratio and catalyst weight.
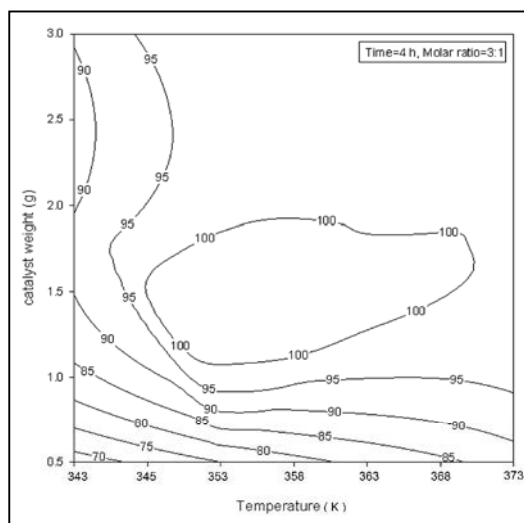
Figure 5b. Contour plot showing the effect on % conversion with the variation of temperature and catalyst amount.

maximum conversion for such esterification reaction were found to be octanol: FFA molar ratio = 3: 1, temperature = 353 K, catalyst = 2 g. Figures 5a and 5b show contour plots generated by Sigma Plot (with two variables at a time) which support the optimum conditions of the present work.

6.4.2. *Application of response surface methodology to optimize the chemical esterification.*

Different standard models, which include (i) linear (ii) quadratic (iii) 2FI, and (iv) cubic were tested to sort out the best regression model to represent the response (% conversion) as a function of independent/ regressor variables. The models were compared in terms of (i) standard deviation (ii) R^2 and adjusted R^2 (iii) PRESS, and (iv) p-value for lack of fit. The results clearly pointed out that the present system can be best described by quadratic model. Though the cubic model slightly improved the regression accuracy, still it was avoided because of its inherent mathematical complications. After selecting the quadratic model, detail ANOVA was carried out to evaluate the effect of each parameter and their respective interactions. The results are summarized in Table 2 which confirms effective applicability of the model to describe the studied reaction. The final model equation generated in terms of actual factors is as follows

$$\% \, Conversion = -237.64241 + 4.62975T + 58.32023W + 35.12978M - 0.10429TW$$
$$- 0.075875TM - 0.63696WM - 0.021564T^2 - 8.99235W^2 - 4.66794M^2$$

$$(1)$$

Table 2. Analysis of Variance (ANOVA) for Orthogonal Quadratic Model (OQD)

Source	Sum of squares	Degree of freedom (df)	Mean square	F-value	p-value prob $>F$
Model	11242.78	9	1249.198	360.7246	< 0.0001
T (temperature)	3213.274	1	3213.274	927.8809	< 0.0001
W(catalyst amount)	3820.651	1	3820.651	1103.27	< 0.0001
M (molar ratio)	587.055	1	587.055	169.5209	< 0.0001
TW	46.03201	1	46.03201	13.29243	0.0045
TM	41.45051	1	41.45051	11.96945	0.0061
WM	9.658013	1	9.658013	2.788895	0.1259
T^2	804.6105	1	804.6105	232.3433	< 0.0001
W^2	1528.437	1	1528.437	441.3591	< 0.0001
M^2	1192.136	1	1192.136	344.2471	< 0.0001
Residual	34.63024	10	3.463024		
Lack of Fit	28.67571	5	5.735142	4.815778	0.0548
Pure Error	5.954533	5	1.190907		
Cor Total	11277.41	19			

Table 2 shows large F-values associated with the $W(=1103.09)$, $T(=927.88)$ and $W^2(=447.36)$. This represents higher significance of the same compared to the other terms.[63] Additionally, low F-values of mixed quadratic terms reflects comparatively smaller interactive influence of the process parameters relative to their individual influence on the response i.e., % conversion.

Three different contour plots along with the corresponding response surfaces according to the quadratic regression model are shown in Fig. 6(a-f). However, a complete characterization about the true nature of the response cannot be done only with the contour/ response surface plots. Therefore a more formal canonical analysis was done to reveal the true nature. The model equation (Eq. (1)) may be represented in a more compact form[51]

$$\% \, Conversion = \beta_0 + Xb + XBX^T \tag{2}$$

where β_0= -237.64241, b= [4.62975, 58.32023, 35.12978]^T,

$$B = \begin{vmatrix} -0.021564 & 0.052145 & 0.0379375 \\ & -8.99235 & 0.31848 \\ Sym. & & -4.66794 \end{vmatrix}, \text{ and } X = [T, W, M]^T.$$ the stationary point of the

response (X_S) was located as

$$X_s = -\frac{1}{2} B^{-1}b = [369.16 \quad 2.58 \quad 2.80] \tag{3}$$

The result indicates that the stationary point is well inside the region of exploration. Though the contour/ response surface plots indicates that the stationary point is a maximum, it may be confirmed by checking the signs of Eigen values of the matrix B.[51] Eigen values of the matrix B were found to be $\lambda = [-0.9016 \quad -4.6949 \quad -0.0210]^T$. As all the Eigen values, $\{\lambda_i\}_{i=1,3}$ are negative, X_S is confirmed to be a point of maximum. However, the predicted response (% conversion) at the stationary point $(= \beta_0 + \frac{1}{2}X_S^T b)$ was found to be 110.27, which is beyond the threshold of maximum % conversion ($\sim$ 100). Naturally, the response surface was truncated at 100 and multiple solutions were obtained. Out of 39 feasible solutions, the working optima was selected based on the simultaneous, near-central location of all the independent parameters. Quantitatively this was done by sorting out the specific location, which yielded minimum product of mean deviations from the mean for all the three parameters. The nearest whole number values (to import feasibility in terms of experimental conditions) of the optimized process parameters were found to be temperature 354K, catalyst loading 1.85 g and molar ratio 3: 1(octanol: FFA) achieving 98.52% ester conversion.
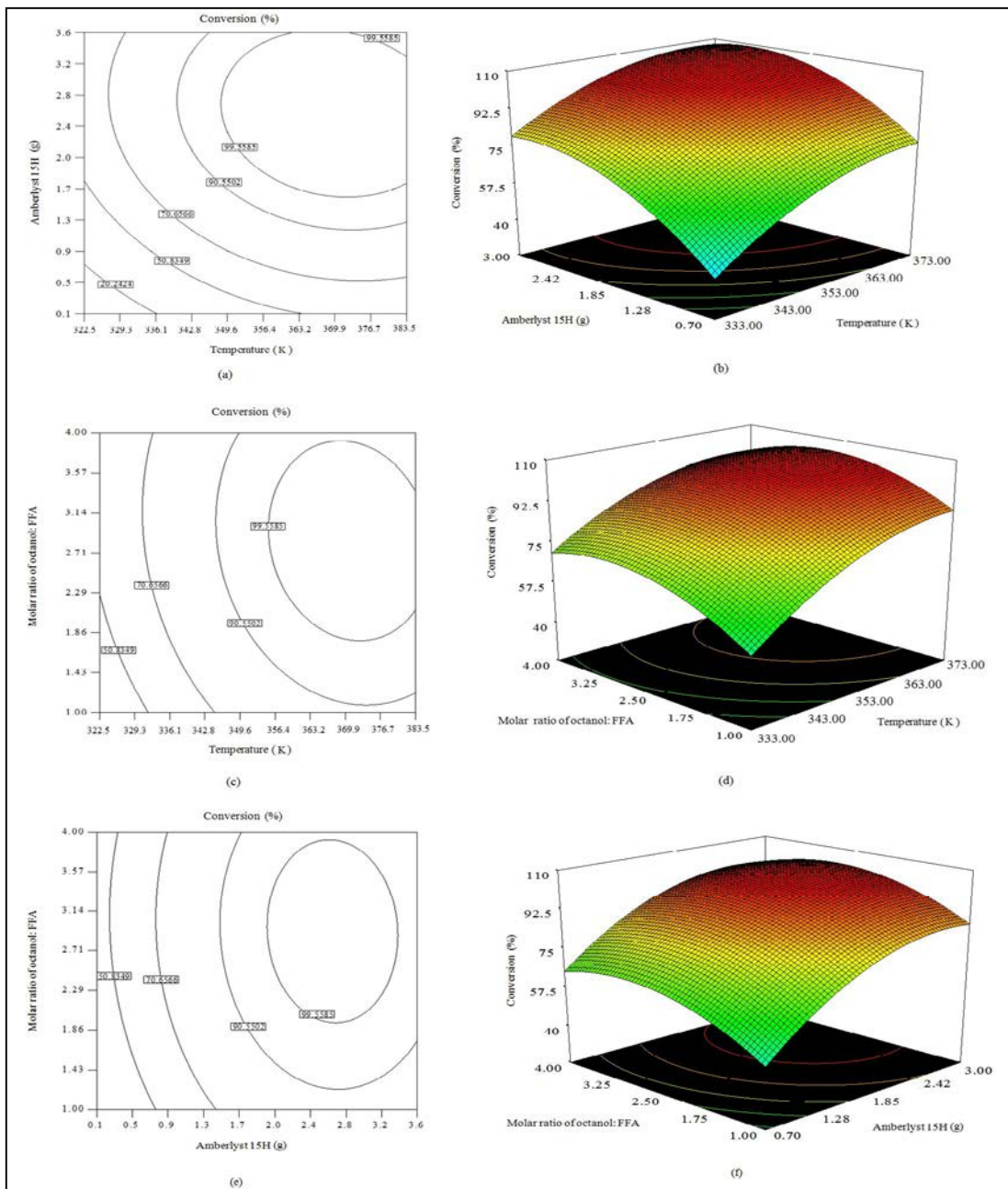
Figure 6. (a) The 2D contour plot for the effect of temperature and Amberlyst15H amount on ester conversion (%) **(b)** The 3D response surface plot for the effect of temperature and Amberlyst15H amount on ester conversion (%) **(c)** The 2D contour plot for the effect of temperature and molar ratio of octanol: FFA on the ester conversion (%) **(d)** The 3D response surface plot for the effect of temperature and molar ratio of octanol: FFA on the ester conversion (%) **(e)** The 2D contour plot for the effect of Amberlyst15H amount and molar ratio of octanol: FFA on the ester conversion (%) **(f)** The 3D response surface plot for the effect of Amberlyst15H amount and molar ratio of octanol: FFA on the ester conversion (%).

6.4.3. Kinetic modeling

The Amberlyst 15H catalyzed esterification reaction of free fatty acids and octanol in absence of solvent can be written as follows:

$$RCO_2H(A) + ROH(B) \leftrightarrow RCO_2R(C) + H_2O(D)$$

The reaction may be classified as elememtary, second order, liquid phase reversible reaction. The Reynolds Number (Re) for the batch system was calculated to be ~10,000 for the present system, which ensured complete turbulent flow and complete dispersion of the catalyst in the reaction medium.

The kinetic model for chemical esterification was proposed based on the following assumptions:

(i) Constant volume reaction since it was a liquid-phase reaction system.

(ii) Negligible external mass transfer resistance due to the high Re maintained in the system (~ 10,000).

(iii) Homogeneous reaction system. The justifications are as follows:

    a. The role of internal mass transfer resistance for the present reaction was evaluated by estimating the Weisz–Prater parameter which was estimated to be 4.79×10^{-3} for the present reaction system. It is reported that for any second order, solid catalyzed reaction, internal mass transfer resistance may be neglected if the Weisz-Prater parameter is below the threshold value of 0.3.[64,65]

    b. Next, the average pore size of the catalyst used was 250Å (Table 1). Therefore it is evident to have Knudsen diffusion as the dominant internal transport mechanism. Therefore the effective diffusivity (D_e), to be used in eq. (4) is determined as.[66]

    c. Vigorous agitation, as reflected by high Re ensured no settling and complete dispersion of the catalyst beads in the bulk liquid.

    d. The bulk volume of the used catalyst under different parametric conditions was negligible relative to the total volume of the reaction system.

Thus, the aforementioned assumptions categorized the present reaction as a pseudo-homogeneous (P-H), second order type. The rate equation for such reaction can be given as

$$-r_A = k_1\, C_A C_B - k_2\, C_C C_D$$

or

$$-\frac{dC_A}{dt} = k_1\, C_A C_B - k_2\, C_C C_D \tag{4}$$

where, $-r_A$ is the rate of reaction per unit volume of the reacting phase (L mole^{-1} sec^{-1}), k_1 and k_2 are the forward and backward rate constants (L mole^{-1} sec^{-1}), t is the time (sec) and C_A, C_B, C_C, C_D are the concentration of FFA, concentration of octanol, concentration of ester and concentration of water (mole L^{-1}), respectively.

In terms of fractional conversion of A (X_A), the concentrations of different reactants and products may be expressed as,

$$C_A = C_{A0}(1 - X_A)$$

$$C_B = C_{A0}(M - X_A)$$

$$C_C = C_{A0}\, X_A$$

$$C_D = C_{A0}\, X_A \tag{5}$$

where, C_{A0} is the initial concentration of FFA in mole L^{-1}.

Combining Eq. (4) and Eq. (5), the following rate equation is obtained

$$\frac{dx_A}{dt} = k_1 C_{A0}\{(1 - X_A)(M - X_A) - k_2 C_{A0} X_A^2 \tag{6}$$

where, M is the molar ratio of octanol: FFA.

Now, the equilibrium constant K is

$$K \equiv \frac{k_1}{k_2} - \frac{X_{A_e}^2}{(1 - X_{A_e})(M - X_{A_e})} \tag{7}$$

where, X_{A_e} is the equilibrium fractional conversion of FFA to ester

Replacing k_2 from eq. (7), eq.(6) becomes

$$\frac{dx_A}{dt} = \frac{k_1 C_{A0}}{X_{A_e}^2}(X_A - X_{A_e})\{MX_A X_{A_e} + X_A X_{A_e} - M(X_A + X_{A_e})\} \tag{8}$$

Integrating eq. (8) the final expression is obtained as,

$$\frac{k_1 C_{A_0} t}{X_{A_\varepsilon}{}^2} = \frac{1}{MX_{A_\varepsilon} + \{M - X_{A_\varepsilon}(1+M)\}X_{A_\varepsilon}} \ln\left|\frac{X_{A_\varepsilon}}{X_{A_\varepsilon} - X_A}\right|$$
$$+ \frac{1}{2MX_{A_\varepsilon} - X_{A_\varepsilon}{}^2 - MX_{A_\varepsilon}{}^2} \ln\left|\frac{[MX_{A_\varepsilon} + \{M - X_{A_\varepsilon}(1+M)\}X_A]}{MX_{A_\varepsilon}}\right| = f(X_A)$$

<div align="right">(let) (9)</div>

Eq. (9) represents the working equation for the well known integral method of analysis. According to Eq.9, $f(X_A)$ vs. t plot must be linear with negligible intercept. Fig. 7 represents the corresponding fit. High R^2 value (=0.98) clearly indicates the validity of the proposed P-H model and confirms that the present esterification process is entirely surface reaction controlled. k_2 was determined from Eq. (7). The values of k_1 and k_2 were found to be 0.5 and 0.03 (L mole^{-1} sec^{-1}) respectively, which
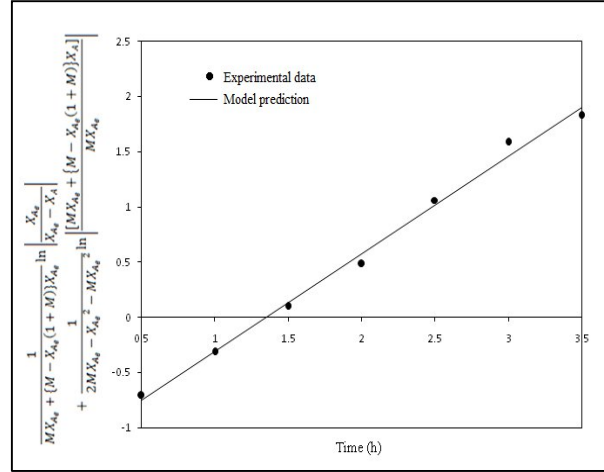

Figure 7. Determination of the kinetic constants by using Eq. (9) under the optimum conditions of octanol: FFA molar ratio=3.2: 1, Amberlyst 15H amount= 1.85 g and reaction temperature=354K.

indicated that the rate of backward reaction is practically negligible compared to forward reaction.

Varying reaction temperature with otherwise unchanged process conditions (catalyst loading and molar ratio), the frequency factor as well as the activation energy for both the forward and the backward reaction were evaluated according to the Arrhenius' model,

$$\ln k = -\frac{E_a}{RT} + \ln A$$

<div align="right">(10)</div>

Where, k is the rate constant (L mole^{-1} sec^{-1}), E_a is the activation energy (kJ mole^{-1}), A is the frequency factor (L mole^{-1} sec^{-1}), R is the universal gas constant (kJ mole^{-1}K^{-1}) and T is the temperature in Kelvin (K). According to Eq. (10), E_a and A for both the reactions were determined by linear regression. The results are presented in Table 3.

Table 3. Activation energy and pre-exponential factor for the esterification of FFA with octanol by Amberlyst 15H

Rate constants	E_a (kJ mole^{-1})	A (L mole^{-1} sec^{-1})	R^2
k_1 (forward reaction)	24.74	2.4×10^3	0.995
k_2 (backward reaction)	15.23	5.06	0.978

On the other hand, the standard heat of reaction (ΔH^0) was estimated from the plot of ln$K$ vs. 1/T by linear regression analysis because

$$\frac{d\ lnK}{d(1/T)} = \frac{-\Delta H^0}{R}$$

(11)

High R^2 value (=0.98) represented that ΔH^0 is nearly insensitive to temperature change over the temperature range of the present study. The value of $\Delta H^0 = 9.47$ kJ mole^{-1} signified low endothermicity of the esterification reaction.

6.5.   Esterification of FFA by enzymatic catalysis

6.5.1. *Classical approach*
6.5.1.1.   *Effect of Initial Water Content*

Initial water content has a profound impact on the enzyme activity. Initial water content up to 0.5 wt % of FFA increased the conversion considerably, whereas a further increase of water content negatively affected the conversion (Fig. 8). Too low (0.1 wt %) and too high (1 wt %) water content retards the reaction rate. Therefore the optimal water content (0.5 wt % in this study) is essential for the immobilized enzyme to hydrate and work proficiently.[67]

The decrease in the conversion rate with increasing water content may be attributed to the fact that at high water content the enzyme particles tend to agglomerate leading to diffusional limitation or it may favor the backward hydrolysis reaction resulting in reduced ester conversion rate.[27]
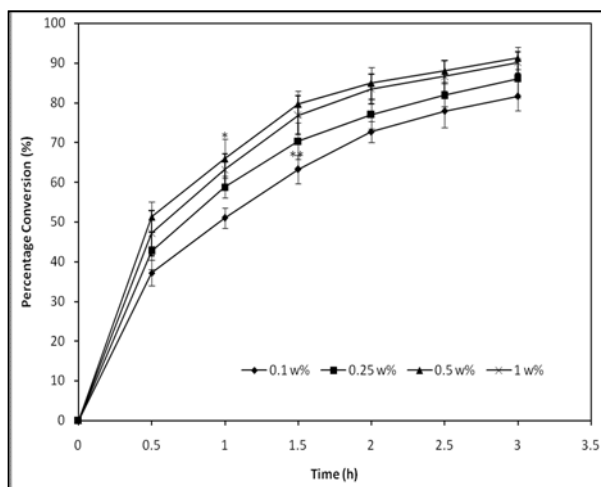
Figure 8. Esterification reaction with varying amount of initial water percentage, temperature 333 K, octanol: FFA 3:1, catalyst 2.5 w% of FFA, 250 rpm (n= 3, mean ± S.D).

6.5.1.2. *Effect of temperature*

The effect of temperature on the production of biolubricant was observed at a varying range of temperatures from 308 to 343K (Fig. 9). Similar to the chemical esterification reaction the enzymatic esterification also showed endothermicity i.e., increasing temperature resulted in an increase in the conversion of FFA to esters upto 333 K. A further increase in the reaction temperature did not show any significant increase in conversion. This may be

Figure 9. Esterification reaction with varying temperature, octanol: FFA 3:1, initial water content 0.5 w% of FFA, catalyst 2.5 w% of FFA, 250 rpm (n= 3, mean ± S.D).

attributed to the fact that high temperatures are liable to cause thermal denaturation of the biocatalyst. It is evident from literature reported that the optimal working condition of Novozyme 435 is between 313 and 338K.[68,69] Maximum ester (~95% conversion) was obtained at 333 K in 4.5 h. Thus, 333 K was chosen as the optimal reaction temperature.

6.5.1.3.   *Effect of molar ratio of substrates*

The reaction temperature was set at 333 K, agitation speed was maintained at 250 rpm and 2.5 wt % of Novozyme 435 was employed. The conversion increased significantly with the increase in the octanol: FFA molar ratio (Fig. 10). Similar results have been found in chemical esterification. Further increase in the alcohol: FFA molar ratio after 3: 1 did not show any significant increase either in the ester conversion percentage or in the rate of conversion. Thus, considering the limited use of alcohol, 3:1 was chosen as the optimum molar ratio.
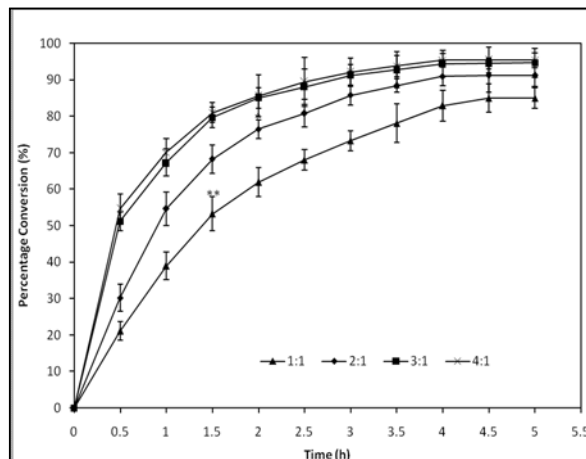
Figure 10. Esterification reaction with varying molar ratio of octanol: FFA, temp. 333 K, initial water content 0.5 w% of FFA, catalyst 2.5 w% of FFA, 250 rpm. (n= 3, mean ± S.D)

6.5.1.4.   *Effect of enzyme amount*

Figure 11 shows the esterification profile with varying amounts of Novozyme 435. The rate of conversion significantly increased with the increase in catalyst amount. 1.25 wt % catalyst gave a maximum conversion of 83.93% after 5.5 h of esterification, while 5 wt % of catalyst brought 95% conversion in just 2.5 h time. As mentioned for chemical catalysis more catalyst reveals more active sites which participate in the reaction and catalyses the production of lubricant. A further increase in
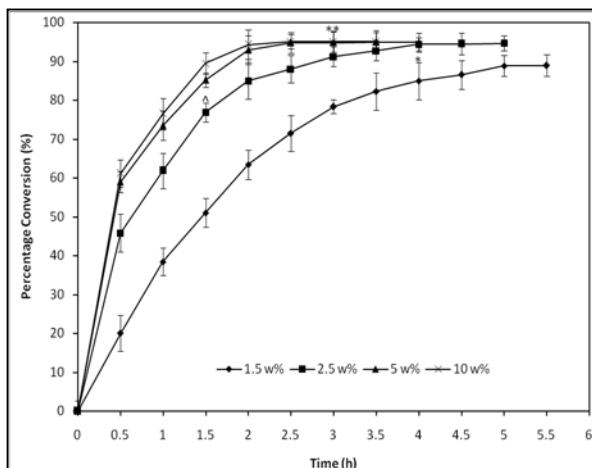
Figure 11. Esterification reaction with varying amount of Novozyme 435, initial water content 0.5 w% of FFA, molar ratio of octanol: FFA 3:1, temp. 333 K 250 rpm. (n= 3, mean ± S.D)

Novozyme 435 amount to 10 wt % although slightly increased the rate of conversion of FFA to ester, but the final conversion percentage remained unchanged. Excess of enzyme present in the reaction medium does not further boost the conversion due to diffusional limitation.[28,70] The final conversion was not found to increase beyond 95% due to the accumulation of water as a by-product of the reaction. The accrued water is liable to shift

the reaction equilibrium towards hydrolysis, ceasing further conversion of FFA to octyl esters.

6.5.1.5.   *Optimization of process parameters*

The optimum values of the reaction parameters to achieve maximum conversion (~ 95%) in minimum time were found to be octanol: FFA molar ratio= 3:1, catalyst amount=5 wt % of FFA and temperature=333 K. Figures 12 a, b and c are the contour plots (with two variables at a time) which indicates the optimum conditions of the enzymatic esterification.
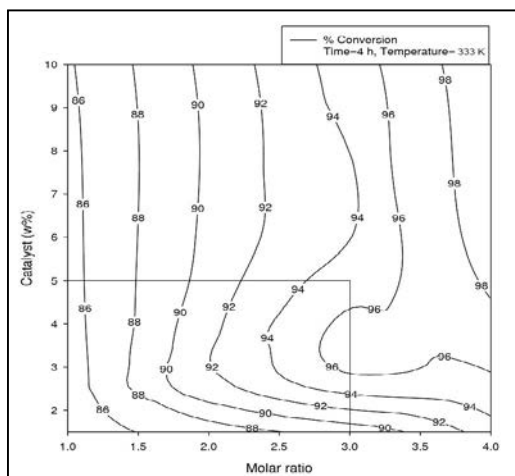

Figure 12a. Contour plot showing the effect on % conversion with the variation of catalyst weight and molar ratio (n= 3, mean ± S.D)
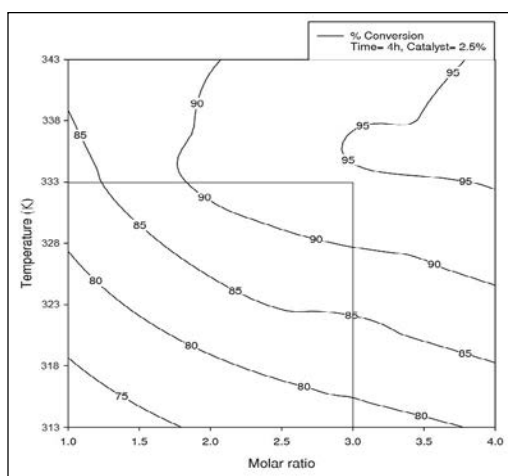
Figure 12b. Contour plot showing the effect on % conversion with the variation of temperature and molar ratio. (n= 3, mean ± S.D)
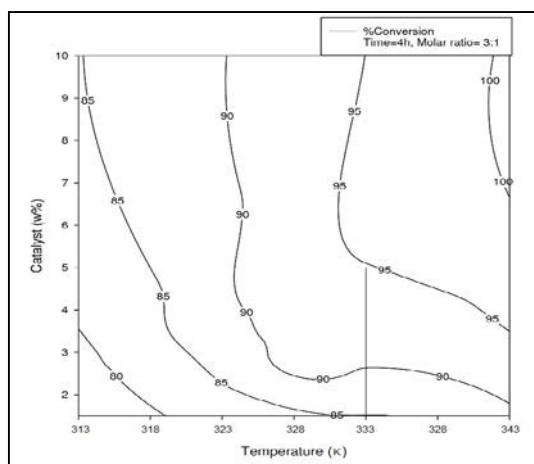

Figure 12c. Contour plot showing the effect on % conversion with the variation of catalyst weight and temperature (n=3, mean ± S.D)

6.5.2. *Application of Taguchi's design method to optimize the enzymatic esterification*

The ANOVA results for the L_9 orthogonal array are shown in Table 4. The results indicated that out of the four process-control factors studied, temperature and catalyst amount have significant effect on the esterification reaction with their P values less than 0.05. The highest F value (Table 4) for the temperature indicates its highest influence on the reaction followed by catalyst amount, time and temperature. Based on the values of S/N ratio (Fig. 10), temperature (L3) and enzyme amount (L3) were identified as the significant parameters.

Table 4 ANOVA results for parameters affecting ester conversion

Factor	DF	Sum of squares	Mean squares	F- value	P-value
Temperature	1	321.348	321.348	54.36	0.002
Catalyst amount	1	45.938	45.938	7.77	0.044
Molar ratio	1	1.279	1.279	0.22	0.666
Time	1	0.086	0.086	0.01	0.910
Error	4	23.648	23.648		
Total	8	392.299			

Although molar ratio and reaction time have insignificant effect on the response according to ANOVA analysis, molar ratio at L2 and time at L2 have been optimized based on the Fig. 13. The predicted optimal S/N ratio for the optimized conditions was computed[71] and was found to be 39.7 db (corresponding predicted conversion = 96.18%). In order to validate the optimal conditions confirmatory runs (triplicate) were conducted that gave 95.19% conversion with 39.35 db. Esterification of FFA with alcohol being an endothermic reaction, temperature has an imperative effect in raising the reaction kinetics and thereby bringing substantial conversion. Temperature and catalyst amount have been found to affect the esterification reaction most significantly. This evidently indicates that the reaction under study is kinetically controlled.
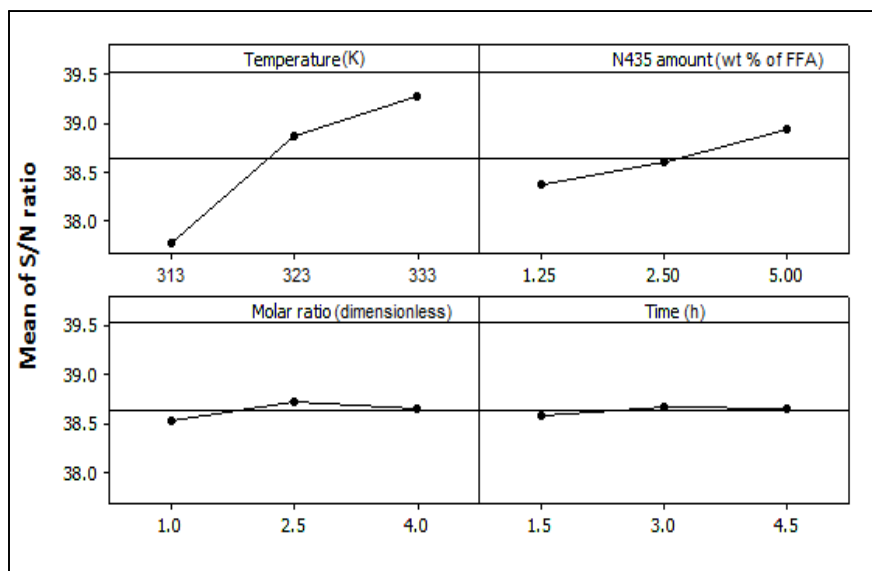
Figure 13. S/N ratio plots for four process control factors studied

6.6.　Product isolation and characterization

The product was characterized by spectroscopic analysis (FT-IR and NMR). Prior to the analysis the final reaction product was subjected to vacuum distillation in order to remove the excess water and octanol. Fig. 14 illustrates the distinctive changes that were found in the absorption peak of the product (ester) formed compared to the corresponding FFA absorption data. The characteristic peak at 1709 cm^{-1} for C=O stretching of

Figure 14. FT-IR spectra for FFA and product ester

carboxylic acid (FFA) shifted to 1738 cm^{-1} for the ester. Another characteristic CO bond peak for the ester was seen at 1056 cm^{-1} wavelength.[72]
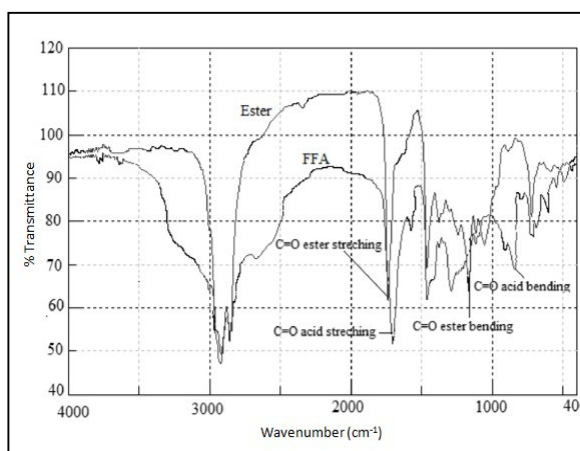
The structure of the synthesized product was further verified with ^{1}H-NMR and ^{13}C-NMR spectroscopy and is presented in Fig. 15 and 16 respectively.^{1}H-NMR spectra shows the significant proton signals at 0.89–0.90 ppm due to the terminal methylenegroups (–CH3) and at 1.19–2.78 ppm due to aliphatic –CH$_2$ groups. The characteristic signals for protons

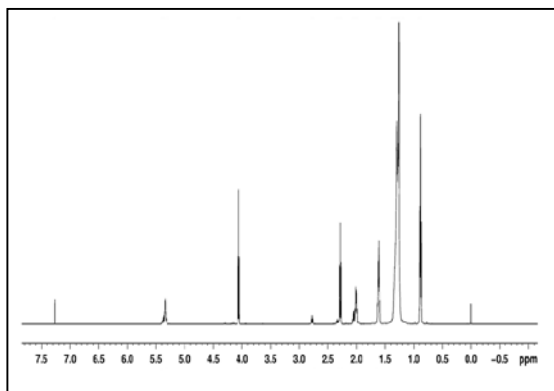**Figure 15.** ^{1}H-NMR spectra for product ester

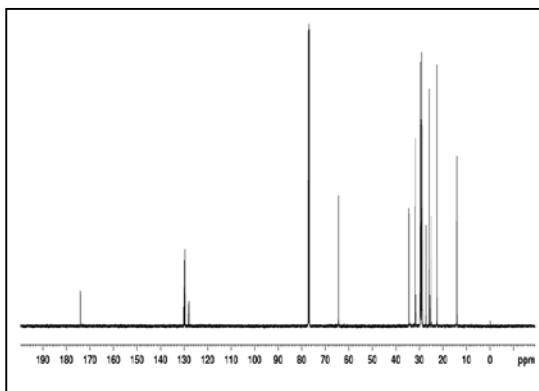**Figure 16.** ^{13}C-NMR spectra for product ester

attached to ester group are prominent at 2.0–2.78 ppm and 4.04–4.07 ppm. Furthermore, the signal at 5.31–5.39 ppm signifies the protons attached to olefinic carbons (–CH=CH–). The ^{1}H spectra show a singlet at 7.29 ppm which is due to the –COOH group (a minimum amount of fatty acid was left in the final product). Fig. 13 (^{13}C-NMR) indicates similar findings showing significant band at 173.95–173.98 which exhibit characteristic signal attributed to ester groups.[73-75] The bands at127.8–130.16 and 22.55–29.75 of ^{13}C spectra refers to the olefinic carbons and aliphatic carbons respectively. A distinctive signal at 64.37 signifies existence of methine carbons.[76]

6.7.   Property testing of product

Physico-chemical properties of the synthesized product (biolubricant components) are summarized in Table 5. The kinematic viscosity of the octyl ester is higher than the WCO. This is possibly because viscosity index (VI) increases with the increasing linearity of the molecule.[77] The product has presented a sufficiently high VI as desirable. The pour point of the product was observed to be much less (+274 K) compared to the raw material WCO (+283 K). The pour point did not show excellent improvement since the saturated fraction (Palmitic acid fraction) was not modified in esterification reaction. The flashpoint of the product is enough and hence guarantees safe operation at high temperatures. The iodine value of a lubricant reveals its degree of unsaturation. The unsaturation of the oil (WCO) was kept unaltered and thus iodine values remained similar for both WCO and for the developed

biolubricant. The oxidation stability of the developed octyl ester was found much higher in comparison to the raw material used. This result is also in agreement with other studies; oxidation stability increases with increasing chain length of the esterified FFA.[78,79] The overall biodegradability of the product was determined which proved that the product esters are biodegradable.

Table 5. Various physico-chemical properties of the product biolubricant (octyl ester)

Physico-chemical properties	WCO	Product ester
Viscosity (mm^2/ sec, 40°C)	46.13	32.35
Viscosity Index	203.14	218.47
Pour Point (°C)	+13	+1
Flash Point (°C)	308.5	324.4
Iodine value (mg I$_2$/ g oil)	36	35
Oxidation Stability (viscosity$_{40°C}$ ratio)	2.93	1.18
Biodegradability (%)	> 95	>90

7.   Conclusions

The research work established the following revelations:

1. The inexpensive WCO can serve as an excellent raw material to generate the FFA.

2. The developed two-step process of enzymatic hydrolysis of WCO (to FFA) followed by chemical/ enzymatic esterification of FFA with octanol to produce octyl ester (biolubricant components) is a cost-effective sustainable technology to formulate an environment-friendly value-added product from waste oil.

3. WCO can be successfully hydrolyzed with *Candida rugosa* lipase to get FFA at an enzyme concentration of 1 g L^{-1} for 30 h.

4. Both Amberlyst 15H and Novozyme 435 catalyzes the esterification of FFA and octanol resulting in satisfactory conversion (~98 %).

5. Various physico-chemical parameters like temperature, catalyst amount, molar ratio of reactants, agitation speed, initial water content and desiccants have effects on the chemical/ enzymatic esterification process.

6. According to classical approach of parametric study the optimum process conditions are octanol: FFA molar ratio = 3: 1, temperature = 353 K, Amberlyst 15H= 2 g for chemical esterification and octanol: FFA molar ratio = 3: 1, temperature = 333 K, Novozyme 435= 5 wt% of FFA for enzymatic esterification.

7. Both response surface methodology and Taguchi's design method has successfully optimized the chemical and enzymatic esterification process respectively. Among various process parameters, catalyst dosage and temperature have higher effect on both chemical/ enzymatic esterification than others. The optimal conditions for chemical and enzymatic esterifications are temperature= 354 K, catalyst loading= 1.85 g and molar ratio= 3.2:1; temperature=333 K, enzyme loading=5 wt% of FFA, molar ratio of octanol: FFA=2.5: 1, respectively.

8. A proposed P-H second order kinetic model can well enough describe the kinetic behavior of the chemical esterification. The kinetic parameters viz. rate constants, activation energy, frequency factor were evaluated. The small heat of reaction (9.47 kJ mole^{-1}) indicates low endothermicity of the chemical esterification reaction.

9. A comparative analysis of the physico-chemical properties of the raw material (WCO) and the biolubricant revealed that the developed ester has ameliorated its properties compared to that of WCO. The produced ester can thus be used as an effective biolubricant baseoil.

8.   Contribution of research to the society: Future scopes

'Sustainable development' with 'wise use of resources' is the call of the time. This could be achieved with the consolidated approach of research and technology as pursued in the current research work. Further work might be undertaken to produce biolubricant keeping the feedstock same but changing the alcohol type (like trimethylolpropane, neopentyl glycol etc.) and catalyst. Catalyst preparation from renewable bio-based material may be emphasized to make the production technology holistically greener and more sustainable. Moreover, the kinetic data generated might be utilized for the design of large-scale industrial flow reactors for the production of octyl esters.

The produced biolubricant components can only be marketed as a finished product when compiled with appropriate additives. Research is needed in formulating environment-friendly additives so that the finished product can meet the biolubricant specifications. The area of applicability of the produced biolubricant is to be identified which will aid in further modification of the product (if needed).

The positive qualities of the bio-based lubricants over non-renewable ones accompanied with the accelerating demand for the mineral oil alternative will definitely promote the use of biolubricants in the recent future. Several European countries have already stepped ahead in accepting biolubricants over petro-based lubricants. The rest of world is also expected to show the prudent urge before long. Industries and stakeholders are yet to realize the long-term benefits of the biolubricants rather than concentrating on the short-term visions, which tend to deter them from affirming biolubricants. The driving forces which could add up an extra momentum to the switch (from non-renewable to renewable lubricants) would be: public awareness about environmental particulars, enactment of strategic policies, rules and regulations by governments, globalization of the biolubricant market and encouragement as well as enforcement of economic incentives and subsidies.

Acknowledgement

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List of publications in international peer-reviewed journals

Journal

1. Biolubricant synthesis from waste cooking oil via enzymatic hydrolysis followed by chemical esterification, **Journal of Chemical Technology and Biotechnology** 88(2012)139-144.

2. Synthesis of biolubricant components from waste cooking oil using a biocatalytic route, **Journal of Environmental Progress and Sustainable Energy** 33(2014)933-940.

3. Optimization of the product parameters of octyl ester biolubricant using Taguchi's design method and physico-chemical characterization of the product **Industrial Crops and Products** 52 (2014) 783-789.

4. A Kinetic Study on the Novozyme 435-Catalyzed Esterification of Free Fatty Acids with Octanol to Produce Octyl Esters, **Biotechnology Progress** 31(2015)1494-1499.

5. Esterification of free fatty acids derived from waste cooking oil with octanol over Amberlyst 15H: Process optimization and kinetic modeling, **Chemical Engineering & Technology** (accepted in press)

Conference Papers

1. Oral presentation on " Biocatalytic synthesis of biolubricants from waste cooking oil" at International Congress of Environmental Research, from 22nd to 24th Nov. 2012.
2. Oral presentation on "Biolubricants-A Step towards Sustainable Development" IIChE, Kolkata- 700032, on 2012.
3. Poster presentation on "Production of Biolubricants from Waste Cooking Oil: Optimization of the process parameters" 100th Indian Science Congress, 2013.