IMMOBILIZATION OF LIPASE ENZYME FROM *CANDIDA ANTARCTICA* ON SUPERPARAMAGNETIC MAGHEMITE NANOPARTICLES AND ITS BEHAVIOUR IN AQUEOUS AND ORGANIC CATALYSES.

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ABSTRACT

Biocatalysis has emerged as a green technology that is able to replace hazardous and extreme conditions faced in chemical based catalysis. By using magnetized nanomaterials, enhancement on the downstream processing is evident as it eases the immobilized enzyme separation from reaction mixture without having to interfere reaction cavity directly and is able to prepare the enzyme for wide working environment applications. Immobilization of lipase enzyme on superparamagnetic iron oxide nanoparticles is important to maintain the lipase open form as its active sites lie within a conserved catalytic triad which occurs naturally in a closed state. Optimization on synthesis of nanomagnetic materials was conducted using 2.45 GHz microwave. The nanoparticles were synthesized in an aqueous solution of $FeCl_3.6H_2O$ as precursor and NH_3 as nucleating agent. Optimization runs were designed and statistically analyzed using face centered central composite design in Minitab® software. The optimized conditions for microwave assisted synthesis of nanomagnet materials were 100 ^oC reaction temperature, 20 minutes reaction time at 631 W microwave power producing 0.371 g of magnetic nanoparticles. Based on the characteristic studies done on synthesized nanomagnets by using X-Ray diffraction crystallography, field emission scanning electrom microscopy, attenuated total reflectance – fourier transform infrared spectroscopy and vibrating sample magnetometer, the nanoparticles possessed the same structure as standard maghemite with good magnetic properties. Subsequent maghemite complex cross-linking with glutaraldehyde provide suitable environment for the enzyme to be immobilized. Optimization on the conditions for lipase immobilization was carried out using response surface methodology experimental design to obtain the precise optimized condition for the process. Selected process variables involved were incubation time, reaction temperature and glutaraldehyde content and optimized conditions obtained for lipase immobilization were at 5 hour incubation time, 44 ℃ incubation temperature and 11 % (v/v) glutaraldehyde content. The optimized immobilized lipase activity in an aqueous based catalysis was 1.49 ±0.05 U. Developed immobilized lipase complex was then subjected for biodiesel production using local sourced refined cooking palm oil as substrate. The work was performed under microwave irradiation to further speed up catalysis reaction. Effects on microwave treatment towards process efficiency were investigated quantitatively using fractional factorial experimental design. It was found that microwave power input, reaction time, immobilized lipase loading and methanol to feed palm oil ratio, affect the biodiesel yield significantly. The highest biodiesel recovery achieved from microwave assisted immobilized lipase catalysed transesterification of palm oil was 70.2 %. The physical properties of produced biodiesel was evaluated and fulfilled the ASTM general requirement for fuels. Based on the findings, the constructed immobilized lipase from *Candida antarctica* onto maghemite nanoparticles managed to elevate the versatility of immobilized enzymes into wide range of applications by easing the downstream processing with high substrate tolerance and protein stability.

ABSTRAK

Biomangkin telah muncul sebagai teknologi hijau yang boleh menggantikan keadaan lampau dan berbahaya yang dialami dalam kimia yang berasaskan mangkin. Dengan menggunakan nanobahan bermagnet, peningkatan pemprosesan hiliran sebagai bukti kerana ia memudahkan pemisahan enzim yang tidak boleh gerak daripada campuran tindakbalas tanpa mempunyai gangguan rongga tindakbalas secara terus dan mampu menyediakan enzim untuk penggunaan yang lebih luas. Proses sekat gerak enzim *lipase* menggunakan partikel *superparamagnet* nano adalah penting untuk memastikan enzim berada dalam keadaan terbuka disebabkan tapak aktif enzim secara naturalnya berada dalam keadaan tertutup. Pengoptimunan sintesis bahan nanomagnet telah dijalankan dengan menggunakan ketuhar gelombang mikro 2.45 GHz. Nanopartikel tersebut telah disintesiskan melalui tindakbalas menggunakan larutan akues $FeCl_3.6H_2O$ sebagai prapenanda dan NH_3 sebagai agen pengnukleusan. Kajian pengoptimunan direkabentuk menggunakan program statistik rekabentuk komposit berpusat dalam perisian Minitab®. Keadaan optimum tindak balas menggunakan ketuhar gelombang mikro dalam penghasilan nanomagnet adalah 100 ^oC, 20 min tempoh tindak balas, 631 W kuasa ketuhar gelombang mikro serta dapat menghasilkan sebanyak 0.371 g nanomagnet. Berdasarkan keputusan kajian pencirian nanomagnet menggunakan kristalografi pembelauan x-ray, mikroskopi elektron pancaran medan, spektroskopi pantulan cahaya inframerah jelmaan Fourier dan magnetometer getaran sampel, nanopartikel mempunyai struktur yang sama seperti *maghemite* piawai dengan mempunyai sifat magnet yang baik. Tidak balas silang *maghemite* bersama larutan glutaraldehida menyediakan sekitaran yang sesuai untuk enzim tidak boleh gerak. Pengoptimunan terhadap keadaan yang diperlukan untuk tidak boleh gerak enzim *lipase* secara statistik telah dijalankan menggunakan kaedah sambutan permukaan untuk mencari keadaan optimum proses. Tiga parameter proses tidak boleh gerak enzim *lipase* telah dipilih iaitu tempoh inkubasi, suhu inkubasi dan kandungan glutaraldehida dan didapati keadaan optimum untuk tidak boleh gerak enzim *lipase* adalah pada tempoh inkubasi 5 jam, suhu inkubasi 44 °C dan kepekatan glutaraldehida 1.1 %. Aktiviti optimum enzim dalam akues bermangkin adalah 1.49 ± 0.05 U. Enzim *lipase* tidak boleh gerak yang dihasilkan kemudian menjadi mangkin dalam penghasilan biodiesel menggunakan minyak kelapa sawit bertapis sebagai substrat. Ketuhar gelombang mikro telah digunakan untuk mempercepatkan tindak balas mangkin. Kesan rawatan gelombang mikro terhadap kecekapan proses telah dikaji secara kuantitatif menggunakan reka bentuk eksperimen pecahan faktoran. Didapati kuasa masukan gelombang mikro, tempoh tindakbalas, kuantiti enzim tidak boleh gerak yang digunakan serta pecahan mol metanol kepada minyak sawit yang digunakan mempengaruhi secara ketara kebolehupayaan penghasilan biodiesel. Perolehan biodiesel tertinggi dicapai daripada gelombang mikro berbantukan transesterifikasi bermangkin *lipase* tidak boleh gerak minyak sawit adalah 70.2%. Ciri – ciri fizikal biodiesel yang terhasil telah dinilai dan didapati memenuhi spesifikasi ASTM untuk bahan bakar. Berdasarkan penyelidikan yang telah dijalankan, pembentukan enzim *lipase* tidak boleh gerak daripada *Candida antarctica* yang dirangkap kepada nanopartikel *maghemite* meningkatkan keupayaan enzim tidak boleh gerak dalam penggunaan yang luas dengan memudahkan proses hiliran yang mempunyai protin yang lebih stabil serta toleransi tinggi kepada pelbagai substrat.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

MWA	-	Microwave Assisted
Cs	-	Chitosan
Glu	-	Glutaraldehdye
ASTM	-	American Society for Testing and Materials
FFD	-	Full Factorial Design
RSM	-	Response Surface Methodology
ANOVA	-	Analysis of Variance
SAED	-	Selected Area (Electron) Diffraction
FAME	-	Fatty Acid Methyl Esters
SPION	-	Superparamagnetic Iron Oxide Nanoparticles
XRD	-	X – RAY Diffraction

LIST OF SYMBOLS

$FeCl_3.6H_2O$	-	Iron Chloride Hexahydrate
E_a	-	Activation Energy
NH_3	-	Ammonia
Hz	-	Hertz
W	-	Watt
tan δ	-	Loss Tangent
γ- Fe_2O_3	-	Maghemite
Cs	-	Chitosan
Glu	-	Glutaraldehyde
Fe	-	Ferum
His	-	Histidine
ε’	-	Microwave Energy Real Permittivity
ε”	-	Microwave Energy Loss Factor
N	-	Normality
M_s	-	Saturation Magnetization
H_c	-	Coercivity
M_r	-	Remanent Magnetization
Ø	-	Cetane Number
Ƞ	-	Kinematic Viscosity
P	-	Density
δ	-	Higher Heating Value
Oe	-	Oerstad
emu	-	Magnetization per Unit
Å	-	Angstrom
$Jg^{-1}°C^{-1}$	-	Joule per gram per Celcius

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Research Background

Enzymes are the most environmental friendly catalysts that can be utilized in various chemical reactions either *in vivo* or *in vitro*. The urge to shift towards green and sustainable chemistry is increasingly demanding due to presence of many heavy elements and transitional metals in catalysts which in turn caused handling and disposal issues (Brahmachari, 2017). Generally, these enzymes are natural proteins that sourced out from plants down to microorganisms. These biocatalysts are able to work under atmospheric conditions with high specificity and not substantially consumed post reactions. Such characteristics promotes these bio-based catalysts as important alternatives to chemical catalysts (Dwevedi & Kayastha, 2011). Ability for enzymes to speed up reactions is just like chemical catalysts where they lowered the activation energy (E_a) of any chemical reaction hence are able to reach the substrate-enzyme transient stage (Krishtalik, 1985). This transient stage is the most important part in determining the success of any catalysed reactions. In such stage, interaction between enzyme-substrate occurred through various mechanisms depending on the biological and chemical natures of both enzymes and substrates. Pathway for enzymatic catalysis is shown in Figure 1.1.

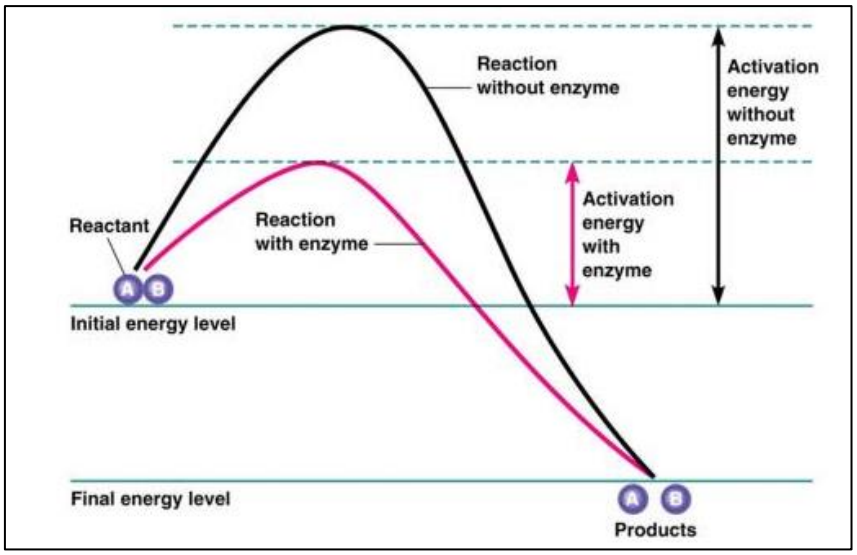

Figure 1.1 Enzymes as biocatalysts and ability to reduce activation energy of reaction (Bretz & Linenberger, 2012) .

Based on Figure 1.1, in order for enzyme to catalyse a reaction, reactants are required to enter the enzyme's active site for substrate docking or correct confirmation. The weak transient bond that formed provides sites for product formations and this is where pronounced difference in the activation energy (E_a) of non-catalysed and catalysed reactions are seen (Bretz & Linenberger, 2012). The E_a for catalysed reaction is substantially lower than reaction without enzyme thus requires less energy input and shorter reaction time to push the reaction forward. Upon reaction completion, enzymes will release the desired products and return to its native shape to be ready for the next run.

Enzyme lipase of carboxylesterase is one of the biocatalysts vastly utilized in industries due to their versatility. The enzymes are able to work in different polarity reaction medium and are abundantly available in nature. There are no limitation in accepting acyl donors and acceptors with different chain lengths and branching and they have high tolerance towards elevated working temperature (Ghaly, Ghaly, Dave, Brooks, & Budge, 2010). Although exploitation of bio-catalysts in process industries is increasingly demanding, high operational cost seems to be one of major drawbacks especially when free enzymes are used. Direct exposure of free enzymes to the reaction environment may result in biocatalyst inhibition and these results in process instability as well as lacking in selectivity. Process separation during product recovery is also laborious due single phase product formation (Dwevedi & Kayastha,

2011). These free enzymes are known for only one time usage. In order to overcome the challenge, utilization of immobilized lipase as biocatalyst in biodiesel production is seen as one of promising solutions as the enzyme can be used repetitively (Zhong et al., 2020).

Immobilization of enzymes are able to elevate the robustness of biocatalysts in reactions as they can withstand a wide range of reaction conditions due to increase in enzyme stability (D. M. Liu, Chen, & Shi, 2018; Nishida et al., 2018). The process is able to improve product separation and reduce purification time and cost as immobilized enzymes can be separated directly from reaction medium either by filtration, centrifugation, or even just simple decantation. By immobilizing enzymes onto targeted matrices, biocatalysts which are robust can be produced for enhanced applications in various sectors. There are many ways to achieve successful lipase immobilization for different purposes. Immobilization procedures can be performed either physically or chemically. Physical adsorption and enzyme entrapment within insoluble carrier matrices are examples of physical enzyme immobilization whilst covalent attachment and crosslinking are categorised as chemical immobilization (D. M. Liu et al., 2018; Quilles Junior et al., 2016).

Since enzyme immobilization involves two different entities in its construction, apart from the method applied, it is important to choose the correct carrier for successful enzymatic catalysis. Organic, inorganic hybrid or composite are types of materials that have been applied as enzyme immobilizing carriers. Despite reaction targeting enzyme complex, several other carrier characteristics are important to ensure the complex stability and flexibility hence successful catalysis. A small size of carrier is able to create large surface area for enzyme attachment thus will increase the probability of successful substrate - enzyme interaction. Apart from that, the presence of specific chemical moieties within carriers is able to attract enzymes more efficiently hence increase the chances for successful enzyme immobilization. Since bio-catalysed reaction is favoured mostly due to its behaviour, it is also important to ensure the carriers are also those of environmental friendly and non-toxic to the reactions performed (Barbosa et al., 2013; D. M. Liu et al., 2018).

It is of high interest in constructing magnetic nanoparticles carriers from inorganic materials to be utilized in enzyme immobilization. The main interest is because of their ability to induce non- disruptive contactless enzyme separation. Immobilized enzyme on magnetic nanoparticles can be easily separated from reaction medium by applying external magnet on the outer side of reaction vessel. In addition to that, the nano – sized magnetic particles create super large surface areas with high pore volume to ease successfulness in enzyme immobilization (Wenlei & Ning, 2009). This distinct characteristic of magnetic nanoparticles enhances substrate-enzyme successful collisions by reducing substrate diffusional limitation to the enzyme site (Jia, Zhu, & Wang, 2003).

Maghemite, magnetite and hematite are three types of superparamagnetic iron oxide nanoparticles (SPIONs) that are widely known for their vast applications. Among those three, magghemite and magnetite are more favoured as they can be synthesized at lower temperature. The superparamagnetic properties of SPIONs further enhance their applications in biological based industries. Their super small nano-sized molecules resulted in the nanoparticle magnetic hysteresis free loop (absence of remnant magnetism in absence of external magnetic field) and sensitive magnetophoretic response (Ha et al., 2018; Sodipo & Aziz, 2016). This allows SPIONs to be applied as enzyme carrier due to fast separation post reaction as well as generation of clean reaction mixture complex.

In order to enhance enzyme immobilization on SPIONs, their outer surface needs modification or grafting with suitable coating agent in order to attract enzyme protein (-NH moiety) efficiently (Magro et al., 2020). Such modification will increase the stability of nanoparticles thus prevent them from aggregation and structure expansion (Sharafi, Bakhshi, Javidi, & Adrangi, 2018). There have been many organic and inorganic polymers applied as grafting agent which in turn determine the surface and physicochemical properties of SPIONs following functionalization post modification (Z. Zhu et al., 2014).

One of prominent application of catalysts can be seen through biodiesel production. The biodiesel production can be achieved through two separate methods which are non-catalysed and chemical or enzymatic catalysed transesterification where two substrates of different nature are efficiently mixed in two media of different polarity. Triacylglyceride as acyl donor is dominantly present in hydrophobic solution and alcohol as acyl acceptor dissolve in denser hydrophilic solution within the same reaction tube. By applying optimum mixing rate and catalysts in the tube, an transesterification occurred as method for biodiesel production. Such process is important for reducing the viscosity of triacylglycerol (which normally uses vegetable oils as sources) towards conventional diesel thus enhancing the engine life by converting triglyceride to three mono-alkyl esters (Math & Chandrashekhara, 2016; Thangarasu & Anand, 2019).

Catalysis in biodiesel production can be further separated into three different ways which are homogenous catalysis, heterogeneous catalysis and enzymatic catalysis. Homogenous catalysis involves both bases and acids catalysed transesterification. Alkaline catalysed esterification is applied when low free fatty acid level is used and acidic catalysed esterification is used at higher level of free fatty acid (Math & Chandrashekhara, 2016). Homogenous catalysed transesterification shows greater efficiency than heterogenous catalysis as it has lower substrate diffusional limitation into the catalyst pore in order to achieve efficient forward transesterification reaction (S. H. Ali, Tarakmah, Merchant, & Al-Sahhaf, 2007; Cipolatti et al., 2014; Jyoti, Keshav, & Anandkumar, 2016).

Despite of its advantageous, homogenous transesterification resulted in some side reactions as well as increase difficulty in downstream product extraction due to catalyst-substrate-product homogeneity (Jyoti, Keshav, Anandkumar, & Bhoi, 2018). The drawbacks of homogenous catalysed transesterification reaction on the other hand became the strength for heterogenous catalysis. The nature for heterogenous catalysts ease product recovery hence increases the biodiesel yield and purity.

Enzymatic catalysed transesterification is currently the most favoured route in biodiesel production due to its high selectivity, ability to produce biodiesel in high purity hence ease the downstream processing as well as its friendliness towards environment and handling (Kaur, Yellapu, & Tyagi, 2019). Furthermore, the structure of the enzymes that used in transesterification is not affected by the reactions hence they can be used repetitively. The method is the most promising in generating complete green technology for betterment. However, introduction of biocatalysis in synthesis industries resulted in reaction sensitiveness in order to maintain the 'living' nature of enzymes f such, the enzymatic catalysed reactions are highly sensitive to their working environment and need proper protection to performance. This can be achieved through enzyme immobilization on suitable matrices as mentioned previously.

Due to the needs of enzyme catalysed reactions as alternative routes for chemical based catalyses, this research is focused on generating immobilized lipase originated from *C.antarctica* on SPIONs through chemical immobilization. By utilizing chitosan poly [b-(1,4)-linked-2-amino-deoxy-*D*-glucose] polymer to modify the surfaces of SPIONs, a high valued nanoparticle with environmental friendly characteristics such as good biocompatibility, bioactivity, the non-toxicity can be constructed (Xie & Huang, 2018). Above all, the most important characteristic of chitosan that can be exploited in the enzymatic immobilization method is its ability to protects the SPIONs from erosions due to particles agglomeration and provide important functional groups for glutaraldehyde attachment to assist lipase covalent binding (Zhao, Qi, Yuan, Du, & Liu, 2015). The pre-activated SPIONs with glutaraldehyde are then applied as carriers for enzyme lipase. This pre-activation is essential to create simple and highly efficient immobilized lipase complex as it enhanced the enzyme stability by inducing strong multipoint covalent bonding between them (R. M. Barros et al., 2003).

With such aim in mind, this research is focused in targeting the development of immobilized biocatalyst with high stability, enhanced working environment adaptability as well as environmental friendly catalyses. It is important to have continuity to the clean technology during exploitation of biocatalyst in the world of synthesis in order to create a thorough green working path. In order to achieve that, microwave assisted technique (MWA) became the synthesis method of interest for both SPIONs and lipase biocatalysed transesterification of refined palm oil to produce biodiesel due to its rapid reactions and ease of handling.

1.2 Problem Statement

Enzyme lipases (triacylglycerol acylhydrolases, *EC 3.1.1.3*) ability to catalyse both hydrolysis and synthesis of esters with high chemo-, regio- and/or enantioselectivity are highly influenced by their working hydration environment. Such robust properties of enzymes made them the most favoured enzymes in industries (Verma, Azmi, & Kanwar, 2008; Yan, Bornscheuer, & Schmid, 1999). To cater the needs, immobilization of lipase enzymes on nanoparticles has become one of many methods to improve stability of robust (Zhong et al., 2020). Stability of immobilized lipase enzyme on SPIONs is crucial in determining the successfulness on the bio-catalysed reactions.

Chemical structure of solid carriers applied in immobilization will determine type of interactions between lipase and solid support (Miletić, Nastasović 2012). Besides possessing these properties, the ability to separate the biocatalyst from reaction media and enzyme reusability are also another factor to be considered. Thus, attachment of lipases on SPIONs groups provides opportunities for the said advantages. Activation of active sites for specific purposes on lipase is highly dependent on the properties of immobilized enzyme complex construction. Therefore, for enzyme to work successfully in bio-synthesis industry, it is crucial to develop immobilized lipase complex with high enzyme activity and durability.

One of biggest challenges in synthesizing SPIONs is the controlled nanomagnet size distribution as it is the important limiting factor in physicochemical properties determination (Majidi, Sehrig, Farkhani, Goloujeh, & Akbarzadeh, 2016). Chemical co-precipitation method in producing nanoparticles provides several advantages as it can produce homogenous products at high purity, it does not requires extreme heat treatment and to be one of the cheapest method for SPIONs synthesis (Nazari, Ghasemi, Maddah, & Motlagh, 2014). This method however demands long synthesis period (5-6 hours working time). Hence, the need in method improvisation is essential for the process.

Biodiesels production through alkaline transesterification process requires the usage of concentrated alkali, high reaction temperatures and the use of either homogeneous or heterogenous chemical catalysts. Due to unwanted side effects and wastes generated from conventional chemical catalysis (V. Gude, Patil, Martinez-Guerra, Deng, & Nirmalakhandan, 2013)., the drive to use enzymatic catalysed esterification for biodiesel production has become an attractive alternative. MWA lipase biocatalysed biodiesel production allows sufficient excitation energy to overcome energy barrier so reactions proceed faster than conventional heating (Yadav, Hude, & Talpade, 2015). Increase in solvent polarity enhances the microwave energy absorption hence forcing the reaction forward. However, this is unfavourable to lipase biocatalysed biodiesel production as it leads to activation of lipase active sites that is responsible for hydrolysis. The change of solvents to low polarity which favours biocatalysis does not favour microwave irradiation resulting in energy transparency. Tailoring the working reaction solvents is one of major limiting factors in determining successfulness in microwave assisted biocatalyses. Hence, this has become of interest of this study to investigate.

1.3 Research Objectives

The objective of this study is to construct immobilized lipase on SPIONs (maghemite) and develop efficient microwave intensification on lipase biocatalysed biodiesel production. In order to achieve the main objective, following objectives need to be fulfilled:

a) To develop an efficient system for SPIONs synthesis under microwave irradiation.

b) To construct SPIONs surface coating complex for efficient lipase enzyme attachment and evaluation on immobilized enzyme properties and activities.

c) To optimize conditions for lipase immobilization on surface modified SPIONs.

d) To evaluate performance of immobilized lipase under microwave assisted catalysed transesterification of refined palm oil as model organic based biocatalysis.

1.4 SCOPES OF THE STUDY

The scope of this study is divided into:

a) SPIONs are synthesized using modified co-precipitation method under microwave irradiation to enhance process efficiency. The process was performed and analysed statistically.

b) Properties of SPIONs synthesized such as size distribution and magnetic properties were determined. The SPIONs thermal profiling was performed.

c) Lipase immobilization on modified SPIONs was conducted and influence of reaction parameters (reaction temperature, contact time and glutaraldehyde content) on process efficiency were investigated statistically.

d) Immobilized lipase catalysed synthesis of biodiesel was performed under microwave irradiation and effect of process parameters (reaction time, microwave intensity, reaction temperature, solvent ratio and enzyme loading) were investigated statistically.

e) Physical properties of produced biodiesel were investigated and evaluated for standard ASTM fuel fulfilment.

1.3 Significance of the Study

The main significance of this work can be seen through development of a thorough green technology in synthesizing high valued products. The work herein intends to develop robust and highly stabilized immobilized lipase that is able to work in both aqueous and organic catalysis using SPIONs which further enhances the reaction downstream processing. The immobilized lipase / SPIONs complex can be easily separated and reuse to increase the system sustainability. This work also improvises the method for biodiesel production to rapid, fast and green technology for immobilized lipase bio-catalysed synthesis. This work combines latest emerging technology with sustainable resources to ensure continuous supply for biofuels.

SPIONs synthesized under microwave irradiation are able to reduce the production period hence increase the production efficiency. Utilization of SPIONs synthesized under microwave assisted (MWA) as enzyme carrier eases the downstream processing with contact-less enzyme removal post reaction.

The immobilization of lipase enzyme from *C.antarctica* through chemical techniques with utilization of common linkers is able to produce running biological catalyst with high stability and efficiency. The study utilized chitosan polymers to surface modified SPIONs nanoparticles in order to prevent agglomeration and glutaraldehyde as linker to the enzyme moiety. The use of immobilized lipase as green catalyst, SPIONs synthesis and biodiesel production through MWA provide another frontier in the world of enzymatic biocatalysis, as it is not just allowing rapid reaction in a clean environment with advantage to both reaction system and lipase enzyme folding stability.

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LIST OF PUBLICATIONS

a) Journal with Impact Factor

1. **Ariffin, Maryam F. K**., Idris, Ani, & Ngadiman, Nor H. A.. (2019). “Optimization Of Lipase Immobilization On Maghemite And Its Physico-Chemical Properties”. Brazilian Journal of Chemical Engineering, 36(1), 171-179. Epub July 15, 2019. https://doi.org/10.1590/0104-6632.20190361s20180168

2. **M. F. Kamel Ariffin, A**. Idris and N. H. A. Ngadiman, "Optimization of One-Pot Microwave-Assisted Ferrofluid Nanoparticles Synthesis Using Response Surface Methodology," in *IEEE Transactions on Magnetics*, vol. 54, no. 6, pp. 1-6, June 2018, Art no. 3200606, doi: 10.1109/TMAG.2018.2816589

b) Book Chapter

1. **Ariffin, Maryam** & Idris, Ani. (2019). Application of Lipase in Non-Conventional Media Based Catalysis.