Synthesis and Optimization Studies of Fructose Palmitate

by

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A thesis submitted
In fulfillment the requirements for the degree of
Master of Science (Bioprocess Engineering)

School of Bioprocess Engineering
UNIVERSITI MALAYSIA PERLIS
2010

AKNOWLEDGEMENTS

Alhamdulillah, all praises to Allah the Al-Mighty for His strengths and blessings towards the completion of this my master's research project.

First and foremost, I am especially grateful to my supervisor, Associate Professor Dr. Mohamed Zulkali Mohamed Daud for his guidance and advice. His constructive comments and suggestions have assisted me in making this project a success. I would like also to express my genuine appreciation to my co-supervisor, Associate Professor Dr. Dachyar Arbain for his incessant support, guidance and constructive comments, during this period of study.

My deepest gratitude to my husband, Mr. Zulhillizan Othman and my son Amier Zuhairi whose love, understanding, prayers, patience and endless support have motivated me to work harder through the whole course of this study. I would also like to express my heartfelt gratitude to my mother Pn. Nurhapifahwati Ahmad and my family in law, for their continuous support and encouragement.

Special thanks to all the respective lecturers, staffs and PLVs, School of Bioprocess Engineering for their cooperation and support. Last but definitely not least, thanks to all my beloved friends, Alina, Najwa, Linda, Mai, Shira, Wani and Azira for their help, kindness, concern and moral support. Thanks for the friendship and memories, hope we all have a very bright future. I would also like to thank MOSTI for providing the Pascasiswazah financial support.

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LIST OF SYMBOLS

Symbol		Unit
α	Alpha (axial distance from center point which makes the design rotatable)	
β_0	regression coefficients for the intercept coefficient	
β_i	regression coefficients for the linear coefficient	
β_{ii}	regression coefficients for the quadratic coefficient	
β_{ij}	regression coefficients for the interaction coefficient	
σ	Standard deviation	
χ_i , χ_j	coded independent variables	
ε	residual associated to the experiments	
3A	3 Ångstrom	
4A	4 Ångstrom	
2^k	Two level full factorial design	
A	immobilized enzyme loading	%(w/w subs.)
AB	interaction between immobilized enzyme loading and substrate molar ratio (sugar to fatty acid)	
AD	interaction between immobilized enzyme loading and reaction time	
B	substrate molar ratio (sugar to fatty acid)	M
BC	interaction between substrate molar ratio (sugar to fatty acid) and reaction temperature	
C	reaction temperature	^oC
D	reaction time	h
F.A	Fatty acid	
k	number of variable	
N	number of measurement	
n	Sample size	
$r_{A,obs}$	initial mass transfer rate of substrate	mg/ml of fatty acid.min
rpm	Rotation per minute	
T	Temperature	K

LIST OF ABBREVIATION

2M2B	2-methyl-2-butanol
ANOVA	Analysis of variance
AlO_4	tetrahedras sodium alumino silicate
CCL	*Candida rugosa* immobilized on acrylic resin
CCD	Central Composite design
CCRD	Central composite rotatable design
DoE	Design of Experiment
DMF	dimethylformamide
DMP	dimethylpyrrolidone
DMSO	dimethylsulfoxide
K_2CO_3	Potassium carbonate
log *P*	partition coefficient of solvent between water and octanol in two-phase system
Lipolase 100L	*Humicola Lanuginosa* immobilized on celite
Lipolase 100T	*Humicola Lanuginosa* immobilized on acrylic resin
MEK	methyl ethyl ketone
MMIM	*Mucor miehei* lipase immobilized on a macroporous anion-exchanger resin of phenolic type
N435	*Candida antarctica* B immobilized on acrylic resin
PCL	*Penicillium chrysogenum* immobilized on celite
PCP	*Pseudomonas cepacia* immobilized on toyonite -200-P
RMIM	*Rhizomucor miehei* immobilized on anion-exchange resin
RSM	Response surface methodology
SP382	*Candida antarctica* B immobilized on acrylic resin
tert-BuOH	2-methyl-2-propanol
TLIM	*Thermomyces Larnuginosa* is silica granulated lipase

ABSTRAK

SINTESIS DAN KAJIAN PENGOPTIMUMAN FRUKTOS PALMITAT

Fruktos palmitat (gula ester) jika dibandingkan dengan surfaktan tanpa ionik yang lain, adalah antara yang terbaru dalam kelasnya. Kebolehannya sebagai surfaktan kebolehbiodegradasi, ketoksikan yang rendah dan keberkesanannya pada suhu, pH serta kemasinan yang melampau telah meningkatkan kegunaannya dalam pelbagai bidang. Penghasilan gula ester secara skala besar telah didominasi oleh proses kimia konvensional sejak sekian lama. Walau bagaimanapun, proses kimia konvensional ini meninggalkan impak kesan yang buruk kepada manusia dan alam sekitar. Berbanding dengan proses sentesis secara enzim, proses ini menawarkan kaedah lain yang lebih selamat dan mudah. Dalam kajian ini, penghasilan gular ester secara kaedah enzim telah dibangunkan yang mana ia telah mengurangkan kebanyakan kelemahan dari proses kimia konvensional. Gula yang tidak dilindung dan asid lemak yang tidak diaktifkan digunakan secara terus sebagai bahan pemula. Kombinasi antara larutan gula terlampau tepu dibawah keadaan terhidrat dan penambahan molekul penapis sebagai penyerap air secara berskala sewaktu tindakbalas berlaku adalah kaedah paling sesuai untuk meningkatkan kadar tindakbalas dan penukaran asid lemak. Melalui kaedah ini, pengaruh beberapa parameter telah dikaji sebagai asas untuk proses pengoptimuman. Keputusan dari kajian asas ini digunakan untuk pengoptimuman dan analisis esterifikasi fruktos palmitat (gula ester) menggunakan metadologi permukaan sambutan (RSM) berdasarkan reka bentuk komposit berpusat (CCD). Sejumlah 98.58 ± 0.52% penukaran asid lemak yang optimum telah diperolehi dengan menggunakan sebanyak 11.92% (berat/berat bahan pemula) kuantiti enzim tersekatgerak, 0.50 M kepekatan asid palmitik dalam 10 jam masa tindak balas pada suhu 53.67^{o}C. Kebolehgunaan semula enzim tersekatgerak menunjukkan hasil penukaran asid lemak yang bagus, dimana lebih 88% penukaran asid lemak dapat diperolehi selepas 10 kali kitaran tindak balas tanpa perlu melakukan penambahan rawatan terhadap enzim tersekatgerak tersebut.

ABSTRACT

SYNTHESIS AND OPTIMIZATION STUDIES OF FRUCTOSE PALMITATE

Fructose palmitate (sugar ester) is a relatively new class of nonionic surfactants. Their excellent biodegradability and low toxicity surfactant as well as effectiveness at extreme temperature, pH and salinity show their increasing importance in numerous areas of application. For a long time, large scale production of sugar ester was dominated by conventional chemical processing. However, the conventional chemical process leaves out bad impact to the human and environment. Compared to the enzymatic synthesis, this process offers a safer and easier alternative. In the present work, sugar ester production was developed by a novel and effective enzymatic method which can reduce the advantages of conventional chemical process. Direct unprotected sugar and non activated fatty acid were used as a starting material. Combination of supersaturated sugar solution under anhydrous condition and stepwise addition of molecular sieve as water absorbent agent during the reaction were found to be a suitable method in increasing the reaction rate and fatty acid conversion. In this method, influences of several parameters were investigated as a screening to the optimization process. Results from screening were used to optimize and analyze fructose palmitate (sugar ester) esterification using a response surface methodology (RSM) based on central composite design (CCD). The 98.58 ± 0.52% of optimum fatty acid conversion was determined by 11.92% (w/w of substrates) immobilized enzyme loading, 0.50M fatty acid concentration, 10.0h reaction time and 53.67^{o}C of reaction temperature. The reusability of the immobilized enzyme was shown good conversion, were greater than 88% of fatty acid conversion after 10th reaction cycles without additional treatment of the immobilized enzyme.

CHAPTER I

INTRODUCTION

1.1. Introduction to sugar ester surfactant

Surfactant or surface active agents are amphiphilic organic compounds. For decades, surfactant such as a general cleaning agent has been used in daily applications. However, progress in the area of surfactant has ultimately widened its possibilities. The product, which proved to be only marginally useful as detergents, showed good emulsifying, wetting solubilizing and foaming characteristics. The characteristics and applications of the surfactant differed by the charged groups in its heads. There were ionic and non-ionic surfactants. Among all surfactants, nonionic surfactants were the fastest in terms of growth with about 45% share of the overall industrial production. This was due to their increased use in the mentioned field (Patel, 2004).

In the past decade, glucoside (sugar based) head groups of nonionic surfactants has been introduced in the market. Sugar esters were one of the glucoside nonionic surfactant, which contained both hydrophobic tail groups (fatty acids) and hydrophilic head groups (sugars). The sugar base of nonionic surfactants structure also mimicked the glycolipids type of biosurfactant (Desai & Banat, 1997). Due to the close structure similarity, these sugar esters has the same characteristics as the biosurfactant, such as its biodegradability, biocompatibility, digestibility and low toxicity, which make them suitable for use in pharmaceuticals, cosmetics and food stuffs production (Deleu & Paquot, 2004; Ferrer et al., 2005; Holmberg, 2001; Karmee, 2008; Kosaric, 1992).

These various applications of the sugar ester surfactants brought about large scale production by most chemical company.

In 1996, over 5 billion pounds of surfactant were produced. In the Asia-Pasific region, the total surfactant consumption grew at an annual rate of 3.9% with a projection of 5.8 million tons in 2010. From the global perspective, the consumption and proportion of surfactants exhibited a different pattern for the North American and Western European region compared with the Asia-Pasific region. However, in the past decades, a new biodegradable surfactant which was known as sugar-based surfactant, has gained significant interests and increased market shares (John, 2001).

1.2. Synthesis of sugar ester

The synthetic sugar ester surfactants was usually produced by a chemical processing method (traditional method) using a base or acid catalysts and performed by toxic solvent such as dimethylformamide (DMF), dimethylsulfoxide (DMSO) or dimethylpyrrolidone (DMP) as the mutual solvent for solubilizing sugar. Potassium carbonate, lithium and sodium soup were also used as the solubilizing agent. This method utilized high temperature and pressure (Adamopoulos, 2006; Yan, 2001). This traditional method often led to major disadvantages such as high energy consumption, degradation of reactants, coloring of products and low selectivity. These disadvantages could cause difficulty in the production separation process. Moreover, certain processes of chemical synthesization of sugar ester were found to be toxic and not readily biodegradable. Thus their application in the cosmetic, food and pharmaceuticals

industry was limited (Chang & Shaw, 2009; Karmee, 2008; Sabeder et al., 2006; Soultani et al., 2001; Tarahomjoo & Alemzadeh, 2003; Yoo et al., 2007).

The use of enzymatic synthesis has been developed as an alternative route to the conventional chemical process. In enzymatic synthesis, a sugar ester was produced using a lipase enzyme as a biocatalyst. The enzymatic synthesis provided work under mild condition, easy recovery, reusability of the catalyst. Moreover, the level of contaminations in both the final products and the environment was also low (Deleu & Paquot, 2004; Desai & Banat, 1997). The enzymatic synthesis gave out regeoselectivity, fewer isomers and side-products (Adamopoulos, 2006; Chua, 2005; Coulon et al., 1999). According to Desai *et al.,* (1997) and Deleu *et al.,* (2004), surfactant obtained through enzymatic synthesis was also known as natural surfactant and it was supported by the Food and Drug Administration (Chua, 2005; Deleu & Paquot, 2004; Desai & Banat, 1997).

An enzymatic synthesis of sugar esters was a reaction between hydroxyl groups of sugar and carboxylic groups of fatty acid. Both sugar and fatty acid was joined by the ester bond to form sugar fatty acid esters or sugar esters. The enzymatic synthesis was carried out by lipase enzyme under nonaqueous conditions. In nonaqueous conditions, lipases were able to catalyze the reverse reaction to form ester bond and this process was known as esterification. There was a considerable number of studies on the lipase-catalyzed production of sugar fatty acid esters in organic solvent (Adachi & Kobayashi, 2005; Kobayashi & Adachi, 2004), ionic liquids (Ganske & Bornscheuer, 2005b; Lee et al., 2008), supercritical CO_2 system (Habulin et al., 2008; Tai & Brunner, 2009) and in solvent free system (Xu et al., 2003) under nonaqueous condition.

1.3. Problem statement

The rapid advances in the enzymatic synthesis of sugar esters has led to a considerable interest being generated in the development of this method for the manufacturing of surfactants and other value-added compounds on an industrial scale (Bommarius & Riebel, 2004; Chang & Shaw, 2009; Karmee, 2008; Zhang, 1999). However, a limited source of enzyme and the low rate of production has made enzymatic synthesis quite unfavorable amongst large industry.

The problem posed by the enzyme catalyzed process is the high cost of the lipase used as the catalyst due to its limited availability. However, the high operational stability of an immobilized enzyme made recycling possible in a batch and continuous system. These has been reported by many researchers (Halim, 2008; Kim et al., 2004; Mat et al., 2005).

Another problem in enzymatic approach for sugar ester production was low production caused by the negative effects of low sugar dissolution in the reaction medium and the present of water as a by-product. The water could lead to ester hydrolysis that is the reverse reaction of esterification. Therefore, the water should be removed while the reaction was in progress in order to increase the yield (Hari & Divakar et al., 2001; Sekeroglu et al., 2004; Yu et al., 2008). Several methods of water removal during reaction has been reported namely the vacuum pressure (Dang, 2004; Zhang, 1999), azeotropic distillation (Yan et al., 1999; Yan, Bornscheuer, & Schmid, 2001) and membrane pervaporation (Bufi-Bak et al., 2002; Sakaki et al., 2006; Yan,

Bornscheuer, Stadler et al., 2001). However, the possibility of solvent loss, clogging and mass transfer restriction accuring during the evaporation process was high. This may increase the production cost in a large scale production set up. Many researchers used desiccants or drying agent such as molecular sieve, silica gel and anhydrous salt to remove the water content (Cauglia & Canepa, 2008; Chaiyaso et al., 2006; Sabeder et al., 2006; Yoo et al., 2007; X. Yu et al., 2004). The molecular sieve was commonly used as an absorbent but it has limited water absorbing capacity (Chua, 2005; Yan, 2001). Thus, a stepwise addition of molecular sieve was proposed.

Most esterification of sugar ester was carried out in intermediate-polarity organic solvents. These solvent mediums played an important role in dissolving the two different substrates (sugars and fatty acids) so that esterification can occur. High yield was achieved in a solvent system compared to a solvent-free condition. Nevertheless, the production was still low. In this case, a low solubility of the sugar in the organic solvent system contributed to the low level of sugar ester conversion (Adachi & Kobayashi, 2005; Kobayashi & Adachi, 2004). Several methods have been developed in order to solve this problem. They included the use of a highly polar organic solvents (DMSO, DMF or Pyridine) and a mixture of highly polar solvent with an intermediate-polarity solvent (Chang & Shaw, 2009; Karmee, 2008; Kennedy et al., 2006).

Unfortunately the method that has been proposed to increase solubility of the sugar has also created another problem with regards to the final products and the enzyme activity. A mixture of toxic solvent should be removed so that it will not contaminate the product. To solve the negative effect of dangerous solvent, moderate polar organic solvent such as tertiary alcohol has been proposed. Many researchers has

shown that the use of tertiary alcohol which is a non-toxic solvent, has shown a high solubility rate of sugar as well as good stability of lipase enzyme (Cauglia & Canepa, 2008; Kim et al., 2004; Yoo et al., 2007).

Many sugars solubility rate was note to be very poor in the tertiary alcohol. Therefore, sugar dissolution rate may limit the ester synthesis. Several researchers used various methods to increase the sugar dissolution rate such as the feed batch addition of solid sugar during esterification (Dang et al., 2005), sugar derivatives (protected sugar) or alkyl glygosides (Ganske & Bornscheuer, 2005b) and amorphous sugar (Dang, 2004). These methods not only require additional steps but can also affect the sugar structure. Thus, the uses of these methods were not a suitable approach for the production of sugar ester on a large scale because it incurred an increase in production costs. Thus, a supersaturated sugar solution has been proposed by several researchers (Cauglia & Canepa, 2008; Flores & Halling, 2002; Flores et al., 2002). In the supersaturated sugar solution, more solid sugar was dissolved compared to a cool solution.

In the present study, the esterification of fructose palmitate using Novozyme 435 as the catalyst in a *tert*-BuOH system was investigated. In this study, an experiment were supersaturated sugar solution under an anhydrous condition was performed. In this system, one step esterification was conducted in a batch process to get the optimum condition for the production of nonionic fructose palmitate surfactant. High yields and product could be obtained, since the problem of low sugar dissolution and water by-product can be solved using the supersaturated sugar solution under an anhydrous condition technique and stepwise addition of molecular sieve during the reaction. More

sugar was dissolved by supersaturated sugar solution under the anhydrous environment. The anhydrous environment was triggered by molecular sieves. Meanwhile, the water by-product was adsorbed by the feed batch addition of molecular sieves during the esterification process. Then, a statistical analysis software which was known as Design of Experiment (DoE) has been applied to the factor affecting the fatty acid conversion in supersaturated sugar solution under anhydrous condition technique, for the optimization and analysis of the parameters.

1.4. Research Objectives

The purpose of this research was to develop a novel and alternative method for sugar ester surfactant production using an enzymatic approach, which was considered safe to be used in food, pharmaceutical and cosmetic industries. In this method, fructose and palmitic acid would be used as reactants to produce a fructose palmitate as sugar ester in enzymatic esterification. The development of the enzymatic synthesis involved the improvement of reaction rate, fatty acid conversion and immobilized enzyme stability via batch system. The present research has the following objective:

General objective:

To study the effect of supersaturated sugar solution under anhydrous condition and feed batch addition of molecular sieve to the esterification of fructose palmitate (sugar ester) in an immobilized enzyme batch flask system.

Specific objectives:

1) To study the effects of process parameters on esterification of fructose palmitate (sugar ester) in an immobilized enzyme batch flask system based on fatty acid conversion and initial reaction rate.

2) To identify the optimum condition for sugar ester surfactant synthesis in a batch flask system using Design of Experiment (DoE).

3) To study the effect of immobilized enzyme recycling to the fructose palmitate (sugar ester) surfactant enzymatic synthesis.

CHAPTER II

LITERATURE REVIEW

2.1. Non ionic sugar ester surfactant

Nonionic surfactants are found today in a large variety of domestic and industrial products. They can be found in powdered forms or liquid formulations. However, the market is dominated by ethoxylates, alkyl benzene sulfonates, alcohol ether sulphates, and alcohol sulphates surfactant products. These traditional surfactants exhibit a low rate of biodegradation and a high potential of aquatic toxicity (Deleu & Paquot, 2004). For these reasons, new green surfactants are promising even if their performances could be slightly inferior or their price more expensive. Among these surfactants, sugar based surfactant such as alkylpolyglycosides (APG) are the most successful at this time (Table 2.1). In the past decade, sugar ester which was one of the alkylpolyglycosides group of surfactants has been introduced in the market due to their low toxicity, which make them more suitable for pharmaceuticals, cosmetics and food products application (Holmberg, 2001; Maag, 1984).