UNIVERSITI PUTRA MALAYSIA

FORMULATION AND EVALUATION OF AN AUTOMATIC DISHWASHING DETERGENT USING LOCAL ENZYMES

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FBSB 2017 4

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

January 2017

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

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January 2017

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Due to the concern towards the environment, the detergent industry changed their approach to a more environmental friendly, which includes biodegradable chemicals and enzymes. Hence, a locally isolated enzyme, T1 lipase was used to formulate an automatic dishwashing detergent,however the efficiency in the hard water was very low. Consequently, enzymes such as Rand protease and maltogenic amylase were incorporated to formulate a new automatic dishwashing detergent with a better efficiency. All three enzymes have been studied, produced and evaluated for automatic dishwashing detergent (ADD) formulation. Lipase, protease and amylase were isolated from *Geobacillus zalihae* strain T1, *Bacillus subtilis* strain Rand, and *Geobacillus* sp. SK70, respectively and all are thermophilic enzymes. These enzymes were produced in shake flask scale and the enzymatic activities were assayed.

Compatibility tests of these enzymes with different detergent components were carried out. These enzymes were mostly stable in nonionic surfactants, especially those that are made of polyhydric alcohols. All enzymes were also stable in a mixture of sodium carbonate and glycine, at pH 9.25. These enzymes are also stable in sodium citrate.

Each free enzyme was evaluated for its highest performance and the amount of enzymes to reach the significant performance was noted and used in detergent formulation. The dishwashing performance of the formulated ADDs was evaluated in term of percent soil removed using the Leenert‘s Improved Detergency Tester. Formulated detergent consists of alkyl polyglucoside (7%) , sodium bicarbonate (3%), polyacrylate (5%), sodium citrate (3%) containing the lipase, protease, amylase. The pH was adjusted using glycine which is approximately 7%. T1 lipase was then spray-dried whereas Rand protease and maltogenic amylase were freeze dried using additives. The addition of three different enzymes did improve the

dishwashing efficiency in both soft and hard water. Detergent with encapsulated enzymes showed a better washing than free enzymes in both soft water and hard water. In 350ppm $CaCO_3$, the maximum dishwashing performance was achieved at 20 and 30 minutes of washing using free and encapsulated enzymes respectively. The washing efficiency of formulated detergent containing enzymes was proven better than previous detergent formulation in both soft and hard water. In additiom, performance of these formulated detergents is as efficient as commercial detergent, Finish® at 50 °C.

In conclusion, the best working conditions for both formulated detergents are 50 °C and 1.5% detergent concentration. In addition, detergent containing encapsulated enzymes has higher washing efficiency compared to detergent containing free enzymes in most conditions. Futhermore, these detergents should be considered to make it into powder form for a longer storage.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

FORMULASI DAN PENILAIAN DETERGEN UNTUK MESIN PENCUCI PINGGAN MANGKUK AUTOMATIK YANG MENGANDUNGI ENZIM TEMPATAN

Oleh

ASHWINI A/P NAGANTHRAN

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Disebabkan oleh keprihatinan terhadap alam sekitar, industri detergen dikehendaki mengambil halatuju yang lebih mesra alam dalam formulasi detergen, termasuk menggunakan bahan kimia terbiodegradasi dan enzim. Oleh yang demikian, lipase T1 enzim yang diekstrak daripada bakteria yang terdapat di Malaysia untuk membuat formulasi ADD, tetapi effisiensi detergen ini dalam air yang mengandungi garam sangat rendah. Seterusnya, enzim seperti protease Rand dan amylase maltogenik telah digabungkan ke dalam pencuci pinggan mangkuk yang mengandungi lipase T1. Kesemua enzim ini telah dikaji secara mendalam, berjaya dihasilkan dan telah dinilai sebagai enzim-enzim untuk formulasi detergen untuk pencuci pinggan mangkuk automatik (ADD). Kesemua enzim ini adalah enzim yang boleh tahan panas. Lipase, protease dan amylase telah dihasilkan daripada *Geobacillus zalihea strain* T1, *Bacillus subtilis* strain Rand dan *Geobacillus* sp. SK70.

Enzim-enzim ini dihasilkan secara besar-besaran pada skala kelalang makmal dan aktiviti enzim untuk setiap enzim telah diukur. Enzim-enzim kemudiannya diperiksa keserasiannya dengan komponen-komponen detergen dengan melakukan ujian kestabilan. Mengikut pemerhatiaan, ketiga-tiga enzim ini sangat stabil di dalam surfaktan yang tidak bercas, terutamanya alkohol polihidrik. Enzim-enzim itu juga stabil dalam campuran natrium carbonat dan glicin, yang memberi pH 9.25. Tambahan pula, enzim-enzim ini agak stabil di dalam citrat.

Setiap enzim telah dinilai untuk prestasi pencucian yang tertinggi dan kuantiti enzim yang digunakan untuk mencecah prestasi itu telah direkod dan digunakan di dalam formulasi detergen. Prestasi pencucian pinggan mangkuk oleh detergen yang diformulasikan dinilai dengan peratus berat kotoran yang berjaya ditanggalkan menggunakan Penguji Keberkesanan Pencucian Rekaan Leenert yang telah

dimajukan. Detergen yang diformulasi mengandungi glucopon (7%) , sodium bikarbonat (3%), polyacrylate (5%), natrium sitrat yang mengandungi enzim lipase, protease, amylase. pH telah diubah menggunakan glycine yang kira-kira 7%. Seterusnya, lipase T1 mentah disembur-kering manakala Protease Rand dan amylase maltogenik telah dikering-sejukbekukan dengan bahan tambahan. Kombinasi ketiga-tiga enzim menunjukkan peningkatan prestasi pencucian di dalam air yang mengandungi tidak mengandungi dan mengandungi garam. Prestasi cucian yang dicapai oleh enzim serbuk-kering adalah lebih tinggi daripada enzim mentah.. Di dalam 350ppm $CaCO_3$, prestasi cucian maksima telah dicapai pada masa minit ke 20 dan 30 menggunakan enzim mentah dan serbuk-kering. Effisiensi pencucian menggunakan formulasi detergen yang mengandungi enzim terbukti lebih baik dalam di dalam air yang tidak mengandungi dan mengandungi garam daripada detergen yang diformulasi sebelumnya. Tambahan pula, effisiensi detergen dirumuskan juga didapati secekap detergen komersial, Finish® di suhu air 50 °C.

Kesimpulannya, keadaan kerja yang terbaik untuk kedua-dua formulasi ialah 50 °C dan kelikatan detergen 1.5%. Detergen yang mengandungi enzim serbuk-kering mempunyai kecekapan basuh lebih tinggi berbanding dengan deterjen yang mengandungi enzim mentah dalam kebanyakan keadaan. Tambahan pula, detergen ini perlu dipertimbangkan untuk membuat ia ke dalam bentuk serbuk untuk penyimpanan yang lebih lama.

ACKNOWLEDGEMENTS

I wish to express my since appreciation to the chairman of my supervisory committee, Prof Dr Raja Noor Zaliha Raja Abdul Rahman for giving me the opportunity to work on this very interesting project, and her valuable guidance and constant motivations. My true appreciation is also extended to my co-supervisor, Associate Prof. Dr Mohd Shukuri Mohamad Ali for his ideas and suggestions in this study. I would like to extend my gratitude to Prof Abu Bakar Salleh for his advice and criticism which improved me alot.

I would like to thank my family for their prayers and support in finishing my studies. I would like also to thank my colleagues, Siti Farhanie, Nurshakila Musa, Noor Aishah Wahab and many more for a constant friendly support throughout the research done. They also never failed to share their knowledge with me. And also, rest of my colleagues in Enzyme Technology group (EMTech) for their contributions towards the progress of this study.

Last but not least, I would like to thank my family, for their prayers, unconditional love and support throughout this study.

I certify that a Thesis Examination Committee has met on 24 January 2017 to conduct the final examination of Ashwini a/p Naganthran on her thesis entitled "Formulation and Evaluation of an Automatic Dishwashing Detergent using Local Enzymes" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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TABLE OF CONTENTS

		Page
ABSTRACT		i
ABSTRAK		iii
AKNOWLEDGEMENTS		v
APPROVAL		vi
DECLARATION		viii
LIST OF TABLES		xii
LIST OF FIGURES		xiii
LIST OF ABBREVIATIONS		xiv
CHAPTER		
1	**INTRODUCTION**	1
2	**LITERATURE REVIEW**	3
	2.1 Detergent	3
	2.1.1 Enzymes in detergents	3
	2.2 Dishwashing product	3
	2.2.1 Hand dishwashing detergent	4
	2.2.2 Automatic dishwashing detergent	4
	2.2.2.1 Finish® automatic dishwashing detergent	5
	2.3 Detergent Components	5
	2.3.1 Surfactants	5
	2.3.2 Water Softeners	6
	2.3.3 Bleaching Agents	7
	2.3.4 Dispersing Agents	8
	2.3.5 Auxiliary Agents	8
	2.4 Detergent Enzymes	9
	2.5 Formulation of an Enzyme Detergent	9
	2.6 Detergent Performance	11
	2.7 Water hardness effect	12
	2.8 Enzyme Encapsulation	12
	2.8.1 Spray drying	13
	2.8.2 Freeze drying	14
	2.9 Research Background	14
3	**MATERIALS AND METHODS**	16
	3.1 Experimental Approach	16
	3.2 Enzyme Production	17
	3.2.1 T1 lipase	17
	3.2.2 Rand protease	17
	3.2.3 Maltogenic amylase	17
	3.3 Enzyme Analysis	18
	3.3.1 T1 lipase assay	18
	3.3.2 Rand protease assay	18
	3.3.3 Maltogenic amylase assay	18
	3.4 Protein assay	19

	3.5	Enzyme Encapsulation	19
		3.5.1 T1 lipase	19
		3.5.2 Rand protease	19
		3.5.3 Maltogenic amylase	20
	3.6	Enzyme compatibility test	20
	3.7	Detergent formulation	20
	3.8	Hard water preparation	21
	3.9	Dishwashing Tests	21
		3.9.1 Efficiency of formulated detergent	21
		3.9.2 Amount of enymes	22
		3.9.3 Comparison of free and encapsulated enzymes detergent	22
		3.9.4 Effect of detergent concentration on removal of soil	22
		3.9.5 Effect of washing time on removal of soil	22
		3.9.6 Comparison of formulated detergent and commercial	22
	3.10	Statistical Analysis	23
4	**RESULTS AND DISCUSSION**		24
	4.1	Compatibility of Detergent Enzyme	24
		4.1.1 Surfactants	24
		4.1.2 Bleaches, dispersing agent and water softeners	25
		4.1.3 Alkalinity agents	26
	4.2	Enzyme residual activities	28
		4.2.1 T1 lipase	28
		4.2.2 Rand protease and amylase	28
	4.3	Performance of Detergent Formulation	30
		4.3.1 Efficiency of formulated detergent	30
		4.3.2 Amount of enzymes added into detergent	32
		4.3.3 Comparison of detergent with free and encapsulated enzymes	34
		4.3.4 Effect of washing time on soil removal with commercial	36
		4.3.5 Detergent concentration	38
		4.3.6 Comparison of formulated detergent containing enzymes with commercial	38
		4.3.7 Summary of Detergent Formulations	40
5	**CONCLUSION AND RECONMENDATIONS FOR FUTURE RESEARCH**		42
REFERENCES			43
APPENDICES			51
BIODATA OF STUDENT			83
PUBLICATION			84

LIST OF TABLES

Table		Page
1	Typical Formulation of phosphate containing ADD	10
2	Phosphate free ADD	10
3	Detergent formulation (U1)	11
4	Stability of enzymes in various detergent components	26
5	Stability of enzymes in various alkalinity agents	27
6	Enzymatic activity of spray dried T1 lipase	28
7	Enzymatic activity of freeze dried Rand protease	29
8	Enzymatic activity of spray dried maltogenic amylase	29
9	Effect of detergent concentration on soil removal	38
10	Detergent formulation (A and B)	40

LIST OF FIGURES

Figure		Page
1	A schematic overview of the experimental stages	16
2	Stability of all three enzymes in surfactant	25
3	Efficiency of detergent formulated	31
4	Detergent performances profiles using three enzymes	33
5	Detergent dishwashing performances with free and encapsulated enzymes	35
6	Effect of washing time on soil removal	37
7	Comparison of formulated detergent with commercial ADD	39

LIST OF ABBREVIATIONS

ABS	Absorbance
ADD	Automatic dishwashing detergent
APG	Alkyl polyglucoside
DE	Dextrose equivalent
DI water	Deionized water
DNSA	Dinitrosalicylic acid
G600	Glucopon ®600 UP CS
GA	Gum Arabic
Hrs	Hours
MD	Maltodextrin
ml	Mililiter
PEG	Polyethylene gycol
s	seconds
SB	Sodium bicarbonate
SC	Sodium carbonate
STPP	Sodium tripolyphosphate
T80	Tween 80
w/w	weight over weight
w/v	weight over volume
v/v	volume over volume

CHAPTER 1

INTRODUCTION

Detergent is a substance that contains any chemical that can remove dirts. Soap was the first man-made detergent and it was made using ashes and fats (Levey M, 1958). However, soaps are not efficient enough in water since water contains cations such as Ca^{2+} and Mg^{2+} (Osorio *et al*., 2005). Interactions between soaps and these metallic substances would create calcium precipitation. However, nowadays detergents consist of highly developed surfactants and water softeners. These detergents are way better than soaps because they can perform better washing than normal soap in the presence of metallic ions. Surfactant which is popularly used, sodium tripolyphosphate, because of its high chelating property. Chlorines, on the other hand, improve washing using active oxygen species in redox reactions. But unfortunately, increasing use of these two chemicals caused ecological issues (McCoy, 2011).

Due to these ecological issues, modern detergents have moved the trend from petrochemical-based to oleochemical-based to be more environmental-friendly. Consequently, enzymes were introduced as additives in detergent formulations. For example is cellulase, which can enhance fabric appearance as well as softening, soil removal and color brightening (Hasan *et al*., 2010). Proteases aid the removal of proteinaceous food stains whereas amylase removes starch based stains (Hmidet *et al*., 2009). Enzymes are better than conventional chemicals, as they can get rid of stubborn stains and degrade before entering the waterways. In this way, enzymes help to alleviate water pollution problems. Thus, there is a need of an environmentally-friendly automatic dishwashing detergent with locally isolated enzymes.

Previously an automatic dishwashing detergent (ADD) formulation was formulated by Rahman *et al*.(2012) using only T1 lipase and it only showed 40% of soil was removed in hard water. In order to improve this formulation, another two different enzymes were added and some modifications on the formulation were done. Thus, previously isolated T1 lipase, Rand protease and maltogenic amylase were used to formulate a detergent formulation. All these three enzymes are thermostable enzymes (Leow *et al*., 2007; Abusham *et al*., 2009, Sulong *et al*., 2015). T1 lipase was proven as a good additive in detergent formulation (Rahman *et al*., 2012). Therefore, combinations of these enzymes will improve efficacy of washing addressing dirt from the 3 substrate categories. The amylase hydrolyzes starchy food, the protease hydrolyzes protein containing stains and the lipase hydrolyzes fatty stains. Free enzymes and encapsulated enzymes are used and compared. Encapsulation of enzymes is to prevent exposure towards chemicals, such as bleaches and proteases and reduce enzyme denaturation during storage.

The detergent formulation containing the all three enzymes and compatible detergent components was evaluated for dishwashing performance. Then, the formulated detergent was evaluated for dishwashing performance based on selected conditions.

The efficiency of formulated detergent was then compared with the commercial detergent.

The objectives of this research were:

1. To evaluate the detergent components that are compatible with T1 lipase, Rand protease and maltogenic amylase based on stability tests
2. To encapsulate the T1 lipase, Rand protease and maltogenic amylase enzymes
3. To evaluate dishwashing efficiency of the formulated detergent based on parameters of soft water, hard water, washing time, detergent concentration and comparison with commercial detergent

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