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DETERGENCY STUDIES OF PROTEASE F1

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DETERGENCY STUDIES OF PROTEASE F1

By

GUNASEKARAN A/L THEKKAMALAI

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DEDICATION

This work is dedicated to my beloved father who passed away peacefully during the period of this study. Words just fail to express my heartfelt feelings of appreciation, gratitude and indebtedness for his excellent role in my life. If not for him, I will not be the person I am today.

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LIST OF ABBREVIATIONS

AES	Alcohol ether sulphates
AI	Active ingredient
AOS	Alpha olefin sulfonate
APMSF	Amidinophenylmethane sulfonyl fluoride
CAS no.	Chemical Abstract Service number
CMC	Carboxymethyl cellulose
CMS	Carboxymethyl starch
E.C. no.	Enzyme Classification number
EDTA	Ethylene diamine tetraacetic acid
FAS	Fatty alcohol sulfate
FWA	Fluorescent whitening agents
LAS	Linear alkylbenzene sulfonate
NTA	Nitrilotriacetic acid
PMSF	Phenylmethanesulphonyl fluoride
PORIM	Palm Oil Research Institute of Malaysia
RT	Room temperature
STP (STPP)	Sodium triphosphate (Sodium tripolyphosphate)
THI	Total hardness indicator
Tris	Tris(hydroxymethyl)aminomethane
SDS-PAGE	Sodium dodecyl sulphate - polyacrylamide gel electrophoresis
%SR	Percentage soil removal

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DETERGENCY STUDIES OF PROTEASE F1

By

GUNASEKARAN A/L THEKKAMALAI

February, 1996

Chairman : Professor Abu Bakar Salleh, Ph.D.

Faculty : Science and Environmental Studies

Protease F1, a thermostable alkaline protease extracted from *Bacillus stearothermophilus* strain F1 was investigated for its soil removing properties. In this study the general purity of crude Protease F1 was first determined. Protease F1 was found to be free of lipase, cellulase and α-amylase activities.

The washing performance of crude Protease F1 was measured in terms of percentage soil removal and compared to a commercial enzyme, Savinase. The effects of various factors such as types of soil, washing temperature, enzyme activity, water hardness, pH, types of surfactants and builders on the washing performance of Protease F1 were measured.

The washing performance was determined by stirring a type of soiled cloths in 1 liter solution of enzyme (with a fixed amount of activity) for 10 minutes, followed by 2x3 minutes rinsing. The difference in the whiteness of the soiled cloth before and after washing indicate the degree of soil removal (or detergency). Among the various

types of soiled cloth studied, the one soiled with oil, pigment and milk (AS12) gave a better contrast when washed by the enzymes than the cloths soiled by blood or the egg.

Protease F1 washed better than Savinase at all the temperatures studied. The difference in performance was found to be greatest at 70°C. Washing efficacy was found to increase with increase in concentration of the enzymes until a certain level beyond which a drop in the percentage soil removal was observed. The enzymes performed better at lower water hardnesses. Generally Protease F1 was more sensitive to water hardness than Savinase. At room temperature (RT) and 50°C, Savinase performed better at all pH's. However, at 70°C and pH 7 and 8, Protease F1 performed better.

When formulated with surfactants or builders, Protease F1 was found to enhance the performance of surfactants while Savinase, the builders. The best washing performance was when enzymes, surfactant and builders were formulated together and the washings carried out at RT and 50°C.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi syarat untuk mendapatkan Ijazah Master Sains.

KAJIAN PENCUCIAN OLEH PROTEASE F1

Oleh

GUNASEKARAN A/L THEKKAMALAI

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Kajian mengenai ciri-ciri keupayaan mencuci oleh Protease F1, enzim protease alkali termostabil yang dihasilkan oleh *Bacillus stearothermophilus* telah dilakukan. Dalam kajian ini ketulenan Protease F1 kasar secara am ditentukan dan didapati tiada aktiviti enzim-enzim lipase, sellulase dan α-amilase dalam Protease F1 kasar.

Keupayaan mencuci oleh Protease F1 telah diukur dari segi peratus kotoran yang ditanggalkan dan dibandingkan dengan satu enzim komersil, Savinase. Prestasi mencuci oleh beberapa faktor seperti jenis kotoran, suhu, aktiviti enzim, keliatan air, pH dan jenis surfaktan dan 'builder', telah diukur.

Prestasi mencuci telah ditentukan dengan mengacau sejenis kain kotor dalam 1 liter larutan enzim (dengan sejumlah aktiviti tertentu) selama 10 minit, diikuti oleh bilasan 2x3 minit. Perbezaan dalam kebersihan pada kain kotor sebelum dan selepas mencuci menunjukkan tahap penanggalan kotoran(atau pencucian). Di antara jenis

kain kotor yang dikaji, kain yang dicemari minyak, pigmen dan susu (AS12) memberi perbezaan yang lebih jelas bila dibasuh oleh kedua enzim daripada kain yang dikotori darah atau telur. Protease F1 mencuci lebih baik daripada Savinase pada semua suhu yang dikaji. Perbezaan dalam prestasi didapati paling besar pada 70°C. Keupayaan mencuci didapati meningkat dengan meningkatnya kepekatan enzim sehingga ke tahap tertentu. Kepekatan enzim yang lebih dari tahap itu menjejaskan pencucian dan mengurangkan penanggalan kotoran. Enzim-enzim itu mencuci lebih baik pada keliatan air yang rendah. Secara am Protease F1 lebih sensitif daripada Savinase kepada keliatan air. Pada suhu bilik dan 50°C, Savinase mencuci lebih baik pada semua pH. Walaubagaimanapun, pada 70°C dan pH 7 dan 8, Protease F1 mencuci lebih baik.

Apabila diformulasi dengan surfaktan dan 'builder', enzim Protease F1 didapati meningkatkan keupayaan mencuci oleh surfaktan dan Savinase meningkatkan keupayaan mencuci oleh 'builder'. Prestasi pencucian terbaik berlaku apabila enzim-enzim, surfaktan dan 'builder' diformulasi bersama dan pencucian dilakukan pada suhu bilik dan 50°C.

CHAPTER 1

INTRODUCTION

Detergents belong to the group of consumer products which are indispensable for the maintenance of cleanliness, health and hygiene. Their economic importance worldwide is considerable, although consumption varies markedly from country to country.

Use of enzymes in detergents was first described by Otto Röhm. He had found that fabrics could be cleaned more easily and at lower temperatures when penetrated with fat and protein digesting enzymes (Dambmann *et al.*,1971). However such enzyme-containing detergents failed to play a major role in the following decades since the only available proteolytic enzyme preparation, a pancreatic extract obtained from slaughtered animals, was too sensitive to the alkaline and oxidative components of detergents. In 1932, enzymes were utilized in a soap composition and were found to enhance the cleansing action of soap greatly (McCarty,1971).

Preparation of proteolytic enzymes by fermentation using specific strains of bacteria (*Bacillus subtilis*, later on *Bacillus licheniformis*) became possible in the early 60's. These enzymes were highly resistant to alkali (active over a broader pH spectrum, from 7.5 to 10), stable to oxidizing agents of the perborate type and showed adequate stability at temperatures as high as ca.65°C for the time period required by normal wash processes (Jakobi and Löhr, 1987).

Commercial production of detergent enzymes experienced rapid expansion in the years that followed. By 1968, detergent enzymes had already taken root in detergent formulations in Europe while in the United States they just started appearing in their detergent powders after an initial resistance to their incorporation into detergents (Davidsohn and Milwidsky,1986) due to inhalation of enzyme dust which resulted in allergenic reactions in workers.

The practical use of enzymes in detergent formulations was facilitated by two major developments. One was the reduction of builders such as sodium tripolyphosphate in detergents to solve environmental problem. The other was the move towards lower washing temperatures as a result of the growing use of synthetic fibres and to save energy (Starace,1981; Maase and Tilburg,1983; KrÜsmann and Bercovici, 1991). Both these factors contributed significantly towards higher detergent enzyme consumption since the addition of enzymes partially compensated for the loss in detergency suffered as a result of the two developments.

Gradual development over the years has made enzymes an indispensable ingredient in detergents (Dambmann *et al.*,1971). Having been marketed for more than 25 years, they have now become well established as normal ingredients in both powder and liquid detergents all over the world (Christensen *et al.*, 1986). They account for approximately 25% of the total worldwide enzyme production and represent one of the largest and most successful commercial large scale applications of modern biotechnology (Godfrey and Reichelt,1986).

Table 1 provides a brief historical review of the production and application of detergent enzymes.

Table 1

Historical review of the preparation and use of detergent enzymes

Year	Enzyme	Enzyme-containing detergents
1913	Otto Röhm claims the use of tryptic enzymes for detergents	detergents containing pancreatic enzymes
1927		optimized detergents containing pancreatic enzymes
1960	Alcalase	
Post - 1960	Microbial proteases made available on a commercial scale by Novo Industri, Copenhagen	first commercial product containing microbial proteases (presoak and wash pretreatment agent)
	Other producers followed, e.g., Maxatase from Gist en Spiritusfabrieken N.V., Delft; Nagase from Nagase Co.; Monlase 110 from Monsanto; Esperase from Novo	
1968		first heavy duty detergent with microbial proteases (presoak and wash pretreatment agent)
1969		microbial proteases contained in 80% of all detergents in the Federal Republic of Germany
1970		severe setback of addition of microbial proteases due to public criticism (the "allergy debate")
1972	Additional microbial enzymes suggested for use in detergents (amylases, lipases, pectinases, nucleases, oxidoreductases, etc.)	enzymes in detergents declared to be safe by the German Federal Health Agency
1975		market share of enzyme-containing detergents stabilizes in Germany at 80%

Source: Adopted and modified from Berg and Boeck (1976) cited by Jakobi and Löhr (1987).

Protease F1, a thermostable enzyme produced by *Bacillus stearothermophilus* has high temperature stability and is active at alkaline pH's (Abdul Rahman, 1993). It

was found to have soil removing properties comparable to the established detergent enzyme Savinase (Cheah, 1994). The objective of this study is to confirm this finding and to determine if Protease F1 has suitable properties to be used as a detergent enzyme.

CHAPTER 2

LITERATURE REVIEW

Detergents and their Ingredients

A detergent is strictly anything that cleans, including soap and even water, but the word is normally used only for synthetic detergents which are referred to as surfactant or syndet in the USA whilst in Europe the corresponding term is tenside (for tension active materials).

A detergent is defined as a formulation comprising essential constituents (builders, boosters, fillers and auxiliaries), which is specially devised to promote the development of detergency (Davidsohn and Milwidsky, 1986). The term detergency refers to the theory and practice of dirt removal from solid surfaces by surface chemical means (Shaw, 1985; Adamson, 1990).

The two categories of detergents on the market in various parts of the world are household detergents (for domestic laundries) and institutional detergents (for industrial and commercial use). Among the household detergents are heavy-duty or all-purpose detergents, specialty detergents, laundry aids and aftertreatment aids.

Generally, detergents for household and institutional use are very complex formulations containing several different types of substances which can be categorized into the following major groups: surfactants, builders, bleaching agents and auxiliary

agents. The different components have specific functions in the washing process, although to some extent they have synergistic effects. Apart from these substances, certain additives are made necessary by the production process, whereas other materials are introduced to improve product appearance.

Surfactants

Surfactants constitute the most important group of detergent ingredients and are present in all types of detergents. Generally these are water-soluble surface active agents consisting of a hydrophobic portion (usually a long alkyl chain) attached to hydrophilic or solubility-enhancing functional groups. They can be classified as anionic, cationic, nonionic or amphoteric, depending on the charge present in the molecule after dissociation in aqueous solution. Table 2 provides an overview of the various classes of surfactants.

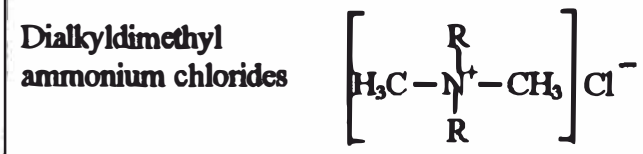
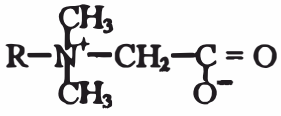
Anionic surfactants are the most common agents in detergents designed for laundry, dishwashing, and general cleansing while nonionic surfactants being efficient wetting agents and effective emulsifiers are also finding broad use at home and in industry (McKenzie,1978). Cationic surfactant use is largely restricted to aftertreatment aids because of the fundamental incompatibility of these materials with anionic surfactants. Amphoteric surfactants, known for their extreme skin kindness, have found an increasing market in the manufacture of toiletries and cosmetics (Palicka, 1991).

When added to water the surfactant molecules align themselves along the interphase (air-water boundary) such that the hydrophilic ends face the water and the hydrophobic ends away from the water. Such an adsorption of surfactants along the

interphase helps reduce the surface tension and improves its wetting properties (Woollatt, 1985). A solution which has sufficiently strong wetting properties in relation to the substrate can penetrate under the soil and gradually ease it away as a droplet.

Table 2

Surfactants of various ionic nature

Surfactant	Formula	Ionic nature
Alkylsulfonates	$R-SO_3^- Na^+$	Anionic
Dialkyldimethyl ammonium chlorides		Cationic
Alkyl poly(ethylene glycol) ethers	$RO-(CH_2-CH_2-O)_nH$	Nonionic
Betaines		Amphoteric

Source: Adapted from Jakobi and Löhr (1987).

Surfactant properties are influenced by the structure of the hydrophobic residue. Adsorption and wash effectiveness generally increase with increasing chain length. For example ionic surfactants bearing n-alkyl groups show a linear relationship between the number of carbon atoms in the surfactant molecules and the logarithm of the amount of surfactant adsorbed on activated carbon or kaolin (Jakobi and Löhr, 1987).

Surfactants with little branching in their alkyl chains generally show good wash effectiveness but relatively poor wetting characteristics, whereas more highly branched surfactants are good wetting agents but have unsatisfactory detergency. For