UNIVERSITI PUTRA MALAYSIA

BIODEGRADATION OF SODIUM DODECYL SULPHATE BY A LOCALLY ISOLATED BACTERIUM, *Klebsiella oxytoca* DR.Y14

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By

WAN SURINI BINTI WAN HUSIN

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

June 2006

Dedicated to my father Wan Husin Sapien,, my mother
Khasiah, Isa, sisters and brother

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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Chairman: Professor Mohd Arif Syed, PhD

Faculty : Biotechnology and Biomolecular Sciences

This study was conducted on sodium dodecyl sulphate (SDS) biodegradation. Bacteria capable of utilizing SDS as a sole source of carbon were isolated from water samples exposed with surfactants. Enrichment culture yielded several isolates capable of metabolizing SDS. Of these, Isolate S11 was selected for further studies based on its biodegradative capability as determined using methylene blue active substance (MBAS) assay. The isolate was identified as *Klebsiella* sp. using Biolog^{TM} identification system and was confirmed using 16S rRNA molecular phylogenetic analysis. Isolate S11 exhibited optimum growth at 37 °C in media containing high SDS concentrations (up to 1.0 g/L SDS), and is able to degrade 99% of 1.0 g/L SDS in 3 days. It requires minimal nitrogen source as low as 0.5 mg/L ammonium sulphate for optimum growth consistent with the *Klebsiella* genus ability to fix atmospheric nitrogen. Partially purified alkylsulphatase from S11 showed optimum enzyme activity at 80°C and at pH 8 using Tris-HCl buffer when tested using MBAS assay. The apparent K_m and apparent V_{max} of the SDS-degrading enzyme were determined to be 0.232 mM and 1.391 µmol per minute per mg protein respectively. The enzyme was found to be stable at room temperature for 50 days in Tris-HCl buffer at pH 7.5.

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

PENGURAIAN SODIUM DODECIL SULFAT DARIPADA BAKTERIA TEMPATAN, *Klebsiella oxytoca* DR.Y14

Oleh

WAN SURINI BINTI WAN HUSIN

June 2006

Pengerusi: Profesor Mohd Arif Syed, PhD

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Kajian mengenai bio-penguraian natrium dodesil sulfat (SDS) telah dijalankan. Bakteria yang berupaya menggunakan SDS sebagai sumber tunggal karbon telah dipencilkan daripada sampel air yang terdedah dengan surfaktan. Beberapa isolat pengurai SDS telah diperolehi daripada teknik kultur pengkayaan. Isolat S11 telah dipilih untuk kajian seterusnya berdasarkan kebolehannya mengurai SDS setelah diuji melalui asai MBAS (Methylene Blue Active Substance). Isolat ini telah dikenalpasti sebagai *Klebsiella* sp. menggunakan sistem pengenalpastian Biolog^{TM} dan telah dipastikan dengan lebih lanjut lagi menggunakan analisis filogenetik molekul 16S rRNA. Isolat S11 telah mempamerkan pertumbuhan yang optimum pada suhu 37 ºC dalam media yang mengandungi kepekatan SDS yang tinggi sehingga 1.0 g/L dan berupaya mengurai 99% daripada amaun ini dalam masa 3 hari. Untuk pertumbuhan optima, ia memerlukan sumber nitrogen yang amat minimal serendah 0.5 mg/L ammonium sulfat sebagai sumber nitrogen terbaik seiring dengan kebolehan genus *Klebsiella* untuk mengikat nitrogen daripada udara. Penulenan separa alkilsulfatase daripada S11 menunjukkan aktiviti optima pada 80 ºC

menggunakan penimbal Tris-HCl pada pH 8.0 apabila diuji menggunakan asai MBAS. Nilai K_m dan V_{max} yang diperolehi daripada enzim pengurai SDS ini ialah masing-masing 0.232 mM dan 1.391 µmol per minit per mg protein. Enzim ini didapati stabil pada suhu bilik selama lebih 50 hari di dalam penimbal Tris-HCl pada pH 7.5.

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I certify that an Examination Committee has met on 21st June 2006 to conduct the final examination of Wan Surini binti Wan Husin on her Master of Science thesis entitled "Biodegradation of Sodium Dodecyl Sulphate by a locally isolated bacterium, *Klebsiella oxytoca* Dr.Y14" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotation and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

WAN SURINI BINTI WAN HUSIN

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LIST OF ABBREVIATIONS

°C	degree Celsius
%	percent
bp	base pair
CFU	colony forming unit
dH_2O	distilled water
DNA	deoxyribonucleic acid
EDTA	ethylene diamine tetraacetic acid
GPS	global positioning system
HPLC	High Performance Liquid Chromatography
IUPAC	International Union of Pure and Applied Chemistry
kb	kilobase
kDa	kilodalton
K_m	Michaelis-Menten Constant
$K_{m(\mathrm{app})}$	Apparent Michaelis-Menten Constant
µl	microlitre
µM	micromolar
M	molar
mA	milliampere
mAu	milli absorbance unit
MBAS	methylene blue active substance
mg	milligram
mM	millimolar
nm	Nanometer

OD	optical density
PBS	phosphate-buffered saline
pH	-log concentration of H^+ ion (*Puissance hydrogene)*
PCR	polymerase chain reaction
PMSF	phenylmethylsulfonylfluoride
RNA	ribonucleic acid
rpm	revolutions per minute
SDS	sodium dodecyl sulphate
TCA cycle	Tricarboxylic acid cycle
PAGE	polyacrylamide gel electrophoresis
UV	ultraviolet
w/v	weight/ volume
v/v	volume/ volume
V_{max}	Maximum velocity
$V_{max(\mathrm{app})}$	Apparent Maximum velocity
WWTP	wastewater treatment plant

CHAPTER 1

INTRODUCTION

Study on surfactant biodegradation has assumed importance as a consequence of the chemical revolution which occurred in the detergent industry during the decade centering on 1950 (Swisher, 1987). Historically, potential surfactant contamination of the environment followed the shift from the use of soap-based detergent to synthetic surfactants (Scott and Jones, 2000).

Based on their favorable physicochemical properties, synthetic surfactants are extensively used in many fields of technology and research for example in pharmacy, cosmetics, textile industry, agriculture and biotechnology (Bizukojc and Bizukojc, 2005). Worldwide production of surfactant increased from 3500 tons in 1950 to approximately 4.3 million tons in 1990 (Jerabkova *et al*., 1999).

Due to extensive application, an appreciable amount of anionic surfactant is released in aquatic and terrestrial environment causing serious water pollution (Cserhati *et al*., 2002). Even though surfactants are essentially nontoxic to humans, there is general agreement that their presence in drinking water is undesirable, (Swisher, 1987; Jerabkova *et al*., 1999). Surfactants caused foaming in aerated bioreactor and decreased the settling ability of the sludge. Besides that, surfactant was reported to be toxic to microorganisms (Jorge and Moreira, 2005).

Taking into account the potential environmental impact of surfactant, many studies concerning biodegradability and toxicity of surfactants have been performed (Petterson *et al*., 2000; Bizukojc *et al*., 2005). Due to current laws on the banning of importation of microbes as well as the highly cautious use of genetically-modified organism (GMO) to be used for the bioremediation of xenobiotics (Walter *et al*., 2003) in Malaysia, it is important to isolate local bacteria for bioremediation of anionic surfactants in Malaysia. Research into bioremediation or the use of microbes or their enzymes to biodegrade the contaminated environment to their original state are currently still in the early ages (Thassitou and Arvanitoyannis, 2001). To date, no such publications exist for isolation of sodium dodecyl sulphate (SDS)-degrading microbes from Malaysia and this work is thus of high importance for fundamental knowledge as well as application. As SDS is the most common surfactant found in detergent, shampoos and cleaning formulations, its biodegradation is vital to be studied compared to other anionic surfactants.

In this study, four major objectives will be accomplished. The objectives of this study are:

1. to isolate and screen local SDS-degrading bacteria.
2. to determine the optimum growth characteristics of the isolated SDS-degrading bacterium.
3. to identify the SDS-degrading bacterium to species level.
4. to partially purify and characterize the SDS-degrading enzyme.

CHAPTER 2

LITERATURE REVIEW

2.1 Surfactants

Anionic surfactants, as major components of synthetic detergents used for both domestic and industrial applications, contribute significantly to the pollution profile of sewage and wastewaters. In 1995, world production of 9.3 million tons anionic surfactants reflects the high demand for this type of surfactant (Douib *et al*., 2003). Anionic surfactant is the major surfactant used as it represents 59% of surfactants used worldwide (Dhouib *et al*., 2003). In terms of environmental issues, the focus of concern has largely been on the effects of the surfactant in detergent formulation. The most widely used surfactants in detergent formulations are those containing anionic group such as alkyl sulphate (Jerabkova *et al*., 1999).

Surfactant is a large group of structurally-diverse molecules and possesses surface-active properties. Surfactant molecules are amphiphiles, contain both strongly hydrophobic and hydrophilic group (Cserhati *et al*., 2002). Thus, they tend to concentrate at the surfaces and interfaces of the aqueous systems including air, oily material and particles (White and Russell, 1994).

Detergent is distinct from surfactant which refers to a commercial formulation or product that is designed with particular cleansing properties (White and Russell, 1994). These formulations contain one-third surfactant, larger amounts of a “builder” which acts as chelating agents and smaller amounts of perfumes, colouring agents, whiteners, enzymes and other components (White and Russell, 1994).

2.2 Classification of Surfactants

Surfactants are classified broadly based on the chemical nature of the polar group as being anionic, non-ionic, cationic or zwitterionic (Figure 1) (White and Russell, 1994). The structural characteristics and surface activity of surfactants are based on simple principles. The molecule of a surfactant is formally constructed by bonding one or more lipophilic groups to one more hydrophilic group (Hummel, 2000). The surface activity of a surfactant is determined by the nature of and relationship between the lipophilic and hydrophilic groups and by their spatial arrangement. The hydrophilic groups can consist of electrically charged (ionic) and also uncharged polar structures (Hummel, 2000).

Anionic surfactants produce negatively charged ions in aqueous solution, originated from sulphate or sulphonate groups. Cationic surfactants produce positively charged ions in solution, frequently quaternary ammonium ions, which constitute less than 10% of the ionics and are used for fabric softening, disinfection and other specialized applications. Zwiterionic surfactants contain both cationic and anionic moieties in the same molecule while non-ionic surfactants contain hydrophobic and hydrophilic that is organic and do not ionize (White and Russell, 1994).

Bioavailability of surfactants changes under anaerobic conditions and may affect the outcome of toxicity and inhibition studies, and to some extent biodegradation or removal rates. The specific chemical structure of some surfactants contributes to a rapid precipitation with water hardness ions (Ca, Mg) into insoluble forms, as well as adsorption to the surrounding solid matrix. This highlights the need to use the real environmental form of a surfactant in inhibition and biodegradation tests, in order to obtain a realistic test result (George and White, 1999).