UNIVERSITI PUTRA MALAYSIA

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By

MAZURIN BINTI MAHAMOOD

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

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

BIODEGRADATION OF SODIUM DODECYL SULPHATE USING LOCALLY ISOLATED *Pseudomonas aeruginosa* sp. STRAIN D1

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February 2012

Chairman : Professor Mohd Arif Syed, PhD

Faculty : Biotechnology and Biomolecular Sciences

Surfactants are synthetic organic chemicals that are formulated to have cleansing or solubilisation properties. With the development of the industrial economy and increase in population density, surfactants have become one of the most widely disseminated toxic substances to enter the aquatic environment, creating a serious environmental problem. High concentration of SDS in the environment may give negative influences to the folding of a polypeptide chain and changes the surface charge of the molecule in the organisms and thus will disrupt the ecosystem. Their toxicities to organisms have been demonstrated previously by many reserchers (Ying, 2006; Singh *et al*., 2002; Lewis, 1991; Utsunomiya *et al.*, 1997; Mori *et al.*, 2002). Therefore, biodegradation of SDS is important in order to ensure low concentration of SDS in the environment. Local microorganisms were used in this study as it has high ability to adapt with the local environment such as temperature, humidity and so on. The main

objective of this study was to isolate, characterize and finally immobilize a local bacterium with the potential to degrade Sodium Dodecyl Sulphate (SDS), a widely used anionic surfactant. Samples for this study were collected from detergent-contaminated area from several locations in Malaysia including from car wash and laundry's outlets, drains, sludge and soil samples as mentioned in methodology (section 3.2). Screening was carried out by the conventional enrichment culture technique and the bacterium was tentatively identified as *Pseudomonas aeruginosa* sp. strain D1 HM852751 using BiologTM GN plates and partial 16S rRNA phylogeny. The optimal growth conditions in minimal medium and for degradation of SDS by *Pseudomonas aeruginosa* sp. strain D1 HM852751 were at 30°C and at pH 6.5 using phosphate buffer system. Sodium nitrate; at 8 gL^{-1} was found to be the best nitrogen source. The isolated strain exhibited optimum growth at SDS concentration of 1 gL^{-1} but can tolerate up to 14 gL^{-1} SDS, indicating that this isolate was able to survive in a relatively high concentration of SDS. 100% of 1 gL^{-1} SDS was completely degraded after 5 and 2 days of incubation before and after optimization, respectively. Encapsulation or immobilization of microorganisms of interest is a new technique and has proven to be more efficient in biodegradation of pollutants. Hence the *Pseudomonas aeruginosa* sp. strain D1 HM852751 was immobilized using gellan gum to enhance the degradation of SDS by the selected isolate. Optimizations of different immobilization parameters were carried out. The optimum gellan gum concentration for immobilized *Pseudomonas aeruginosa* sp. to degrade SDS ranged from 0.8% to 0.85%. The optimum cell density was between 40 gL^{-1} to 50 $gL^{-1}$and the optimum bead size was 4.5 mm with the

initial cell loading of 250 beads. 1 gL^{-1} of SDS was successfully degraded within 8 hours by immobilized cells compared to 20 hours by the freely suspended cells which was a substantial reduction in the degradation time. The immobilized cells can be used up to 20 cycles with approximately 100% reduction of SDS. These findings indicates *Pseudomonas aeruginosa* sp. strain D1 have high ability to degrade SDS when it was immobilized in gellan gum and has high potential for future research.

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

BIOPENGURAIAN SODIUM DODECYL SULPHATE MENGGUNAKAN *Pseudomonas aeruginosa* sp. STRAIN D1 TEMPATAN.

Oleh

MAZURIN BINTI MAHAMOOD

Februari 2012

Pengerusi : Profesor Mohd Arif Syed, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Surfaktan adalah bahan kimia organik sintetik yang diformulasi untuk mempunyai sifat pelarut. Dengan peningkatan ekonomi perindustrian dan peningkatan populasi, surfaktan menjadi salah satu bahan toksik yang tersebar secara meluas yang masuk ke persekitaran akuatik menyebabkan masalah pencemaran persekitaran yang serius. Kepekatan SDS yang tinggi di persekitaran akan memberi kesan negatif pada ikatan rantai polipeptida dan mengubah permukaan cas pada molekul dalam organisma and seterusnya akan mengganggu ekosistem. Kesan toksik kepada organisma telah dilaporkan sebelum ini oleh ramai penyelidik. (Ying, 2006; Singh *et al*., 2002; Lewis, 1991; Utsunomiya *et al.*, 1997; Mori *et al.*, 2002). Oleh itu, biodegradasi SDS adalah penting untuk memastikan kepekatan SDS adalah rendah di persekitaran. Mikroorganisma tempatan digunakan memandangkan ia mempunyai kebolehan yang tinggi untuk menyesuaikan diri dengan persekitaran tempatan seperti suhu,

kelembapan dan lain-lain. Objektif utama kajian ini adalah untuk memencilkan dan mencirikan bakteria tempatan dan seterusnya menyekat-gerak bakteria yang mempunyai potensi untuk menguraikan Sodium Dodecyl Sulphate (SDS), iaitu satu surfaktan anionik yang digunakan secara meluas. Sampel untuk kajian ini diambil daripada kawasan yang tercemar dengan bahan pencuci dari beberapa lokasi di Malaysia termasuk dari tempat membasuh kereta dan kedai dobi, longkang, lumpur dan sampel tanah seperti yang dinyatakan di dalam metodologi (seksyen 3.2). Pemilihan dijalankan menggunakan teknik pengayaan kultur konvensional dan bakteria tersebut dikenali sebagai *Pseudomonas aeruginosa* sp. strain D1 HM852751 menggunakan plate BiologTM GN dan filogeni separa 16S rRNA. Keadaan optimum bagi pertumbuhan dan degradasi SDS dalam media minimal oleh *Pseudomonas aeruginosa* sp. strain D1 HM852751 adalah pada 30°C dan pH 6.5 menggunakan sistem penimbal fosfat. Natrium Nitrat pada 8 gL^{-1} merupakan sumber nitrogen yang terbaik. Bakteria ini menunjukkan pertumbuhan optimum pada kepekatan 1 gL^{-1} SDS tetapi masih boleh bertahan sehingga 14 gL^{-1} SDS, menunjukkan bakteria ini berkemampuan untuk hidup dalam kepekatan SDS yang tinggi. Degradasi 1 gL^{-1} SDS sebanyak 100% berlaku selepas inkubasi selepas 5 dan 2 hari pada sebelum dan selepas proses pengoptimuman. Kaedah sekat-gerak mikroorganisma terpilih merupakan teknik yang baru dalam membiodegradasi bahan tercemar dan terbukti lebih efisien dalam biodegradasi bahan-bahan tercemar. Oleh itu, *Pseudomonas aeruginosa* sp. strain D1 HM852751 disekat-gerak menggunakan gellan gum untuk meningkatkan degradasi SDS oleh isolat yang telah dipilih. Pengoptimuman parameter untuk

prosedur sekat-gerak dilakukan. Kepekatan gellan gum optimum untuk *Pseudomonas aeruginosa* sp. yang disekat-gerak untuk degradasi SDS adalah di antara 0.8% to 0.85%. Ketumpatan sel optimum adalah di antara 40g L^{-1} to 50g L^{-1} dan saiz butir manik optimum adalah 4.5mm dengan permulaan bilangan sel pada 250 butir manik. 1 gL^{-1} SDS telah berjaya diuraikan dalam masa 8 jam oleh sel yang disekat-gerak berbanding 20 jam oleh sel bebas. Sel yang disekat-gerak ini juga boleh digunakan sehingga 20 kitaran dengan penurunan hampir 100% SDS. Penemuan ini menunjukkan *Pseudomonas aeruginosa* sp. Strain D1 mempunyai keupayaan yang tinggi dalam degradasi SDS apabila ia disekat gerak menggunakan gellan gum dan ia mempunyai potensi yang tinggi untuk penyelidikan di masa hadapan.

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APPROVAL SHEET 1

I certify that a Thesis Examination Committee has met on **13 February 2012** to conduct the final examination of **Mazurin Binti Mahamood** on her Master of Science thesis entitled **Biodegradation of SDS By Locally Isolated *Pseudomonas aeruginosa* sp. strain D1** in accordance with the Universities and Universiti College 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.

Members of the Examination Committee were as follows:

Prof. Madya Dr. Foo Hooi Ling, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
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Y. Bhg. Prof Dr. Mohd Ali Hassan, PhD
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_____

Prof. Dr. Zulkarnain Zainal Prof. Dr. Seow Heng Fong
Professor and Deputy Dean
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Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of Supervisory Committee were as follows:

Mohd Arif Bin Syed, PhD
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BUJANG BIN KIM HUAT, PhD
Professor and Dean
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Date:

DECLARATION

I declare that the thesis is my original works except for quotations and citations which have been duly acknowledge. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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MAZURIN BINTI MAHAMOOD

Date: 13 February 2012

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