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BIODEGRADATION OF ACRYLAMIDE BY A NEWLY ISOLATED Bacillus sp. strain ZK34

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By

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

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BIODEGRADATION OF ACRYLAMIDE BY A NEWLY ISOLATED *Bacillus* sp. strain ZK34

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Chairman : Mohd Arif bin Syed, PhD
Faculty : Biotechnology and Biomolecular Sciences

Acrylamide is a toxic and carcinogenic compound which may cause cancer and genetic mutation, and also irritation to the skin and respiratory tract. The main sources of acrylamide pollution are the release of monomer residues from polyacrylamide which are widely used for water treatment and from the formulation of herbicide glyphosate. To overcome this problem, a potent and efficient acrylamide-degrading bacterium has been isolated from agricultural soil. The isolate was tentatively identified as *Bacillus* sp. strain ZK34 based on 16S rRNA molecular phylogeny and was deposited at the GenBank under the accession number KC433533. *Bacillus* sp. strain ZK34 grew optimally in the range of pH 7.0 and pH 8.0, and at 28°C. The test on effects of carbon sources on the growth of the bacterium was carried out using carbon sources such as glucose, sucrose, fructose, lactose, maltose, mannitol, citric acid, dextrin and glycerol at the initial concentration of 1.0% (w/v) with acrylamide as the sole nitrogen source. The results showed that glucose was the best carbon source for bacterium growth. The effects of different aliphatic amides on the growth of strain ZK34 using 1.0% (w/v) glucose as the carbon source showed that acrylamide, propionamide, methacrylamide, nicotinamide, and acetamide supported growth with increasing assimilative capability from methacrylamide to propionamide while 2-cloroacetamide did not support growth. The optimum concentration of acrylamide for the growth of *Bacillus* sp. strain ZK34 was at 0.5 g/L. *Bacillus* sp. strain ZK34 could degrade 0.5 g/L of acrylamide in three days of incubation with concomitant cell growth. Strain ZK34 was immobilized in gellan gum and the degradation of acrylamide was compared between freely-suspended and immobilized cells. Optimization for immobilization procedures found 0.75% (w/v) of gellan gum, 300 beads/100 mL of BSM and 3 mm of bead size gave optimum degradation of acrylamide. *Bacillus* sp. strain ZK34 which has been immobilized in gellan gum beads showed enhanced degradation of elevated concentrations of acrylamide (3.0 g/L) compared to the free cells (2.0 g/L) and could be reused for at least 8 complete cycles. Kinetics study revealed that immobilized cells suited Yano model which indicated the acrylamide was not toxic to the cells even though the acrylamide concentration was high, while free cells fitted to Luong kinetic model where acrylamide was toxic whether at low or high concentrations. Heavy metals and pesticides showed less inhibition of acrylamide degradation in immobilized cells than the free cells. The outcome of this study will contribute to additional knowledge on a

new source of more efficient microbe in acrylamide degrading process and has high potential to be used in the contaminated sites.

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

BIODEGRADASI AKRILAMIDA OLEH BAKTERIA *Bacillus* sp. strain ZK34

By

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Akrilamida adalah sebatian toksik dan karsinogenik yang boleh menyebabkan kanser dan mutasi genetik, iritasi pada kulit dan juga saluran pernafasan. Sumber utama pencemaran akrilamida adalah pembebasan sisa monomer daripada poliakrilamida, yang digunakan secara meluas untuk rawatan air dan daripada formulasi racun ǿglyphosateô. Bagi mengatasi masalah ini, bakteria penguraian akrilamida yang kuat dan cekap telah diasingkan daripada tanah pertanian. Isolat ini telah dikenalpasti sebagai *Bacillus* sp. strain ZK34 berdasarlan filogeni molekul 16s rRNA dan telah didaftar di dalam GenBank di bawah nombor pengaksesan KC433533. *Bacillus* sp. strain ZK34 tumbuh secara optimum dalam lingkungan pH antara pH 7.0 hingga pH 8.0 pada suhu 28°C. Kesan sumber karbon kepada pertumbuhan bakteria telah dijalankan dengan menggunakan sumber-sumber karbon seperti glukosa, sukrosa, fruktosa, laktosa, maltosa, mannitol, asid sitrik, dekstrin dan gliserol pada kepekatan 1.0% (w/v) dengan akrilamida sebagai sumber nitrogen. Hasil kajian menunjukkan bahawa glukosa merupakan sumber karbon terbaik untuk pertumbuhan. Kesan alifatik amida kepada pertumbuhan strain ZK34 dengan menggunakan 1.0% (w/v) glukosa sebagai sumber karbon menunjukkan bahawa akrilamida, propionamida, methakrilamida, nikotinamida dan asetamida membantu pertumbuhan dengan peningkatan keupayaan asimilasi dari methakrilamida kepada propionamida manakala 2-kloroasetamida tidak membantu pertumbuhan. Kepekatan optima akrilamida untuk pertumbuhan *Bacillus* sp. strain ZK34 adalah pada 0.5 g/L akrilamida. *Bacillus* sp. strain ZK34 boleh menguraikan 0.5 g/L akrilamida dalam masa tiga hari pengeraman seiring dengan pertumbuhan sel. *Bacillus* sp. strain ZK34 telah disekat-gerak di dalam gel gellan dan penguraian akrilamida telah dibandingkan di antara sel bebas dan sel tersekat-gerak. Pengoptimuman bagi prosedur sekat-gerak mendapati 0.75% (w/v) gel gellan, 300 butir dalam 100 mL media dan 3 mm bagi diameter gel memberikan penguraian akrilamida yang optima. *Bacillus* sp. strain ZK34 yang tersekat di dalam butir gam gellan menunjukkan penguraian yang lebih baik pada kepekatan akrilamida yang tinggi (3.0 g/L) berbanding sel bebas (2.0 g/L) dan boleh digunakan semula sekurang-kurangnya 8 kitaran lengkap. Kajian kinetik mendedahkan bahawa sel tersekat-gerak sesuai dengan model Yano yang menunjukkan akrilamida tidak toksik kepada sel walaupun kepekatan akrilamida adalah tinggi, manakal sel bebas pula sesuai dengan model Luong dimana akrilamida adalah toksik kepada sel sama ada pada kepekatan tinggi ataupun rendah. Logam berat dan racun serangga memberikan kesan rendah pada penguraian akrilamida oleh sel tersekat-gerak berbanding sel bebas.

Keputusan kajian ini mampu meningkatkan pengetahuan mengenai sumber baru mikrob yang lebih cekap dalam proses penguraian akrilamida dan mempunyai potensi yang tinggi untuk digunakan di tapak pencemaran.

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I certify that a Thesis Examination Committee has met on 25 March 2015 to conduct the final examination of Norzila binti Kusnin on her thesis entitled "Biodegradation of Acrylamide by a Newly Isolated *Bacillus* sp. strain ZK34" 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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Declaration by graduate student

I hereby confirm that:

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celcius
ϐ	Beta
ϡ	Lambda
µg	Microgram
µg/L	Microgram per liter
µL	Microliter
Abs	Absorbance
BSM	Basal Salt Medium
DNA	Deoxyribonucleic acid
DOE	Department of Environment
EDTA	Ethylene diamine tetraacetic acid
EPA	Environmental Protection Agency
et al.	And all
g	Relative centrifugal force
g	Gram
g/cm^3	Gram per centimeter cube
g/L	Gram per liter
g/L hr^{-1}	Gram per liter per hour
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
ICL	Initial cell loading
kDa	KiloDalton
L	Liter
M	Molar
mAu	Mili absorbance unit
mg	Milligram
mg/ L	Milligram per liter
min	Minutes
mL	Milliliter
mM	Milimolar
MW	Molecular weight
n.a	Not available
OD	Optical density
OFAT	One Factor at a Time
ppm	Parts per million
SEM	Standard error of the mean
UV	Ultraviolet
v/v	Volume per volume
w/v	Weight per volume
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Ever since Malaysia embarked on rapid industrialization country, several problems regarding the environment and human health have been raised in many different ways. A number of serious environmental problems have grown and affected natural ecosystems. The most common toxic industrial wastes involved in water, ground water and soil contamination are petroleum, oil, hydrocarbon, heavy metal sludge, textiles, adhesives, pesticides and chemicals (DOE, 2009; Pauzi et al., 2001; Ripin et al., 2014; Mohamed et al., 2014). One of the compounds which is ranked among the most toxic and relatively recalcitrant to biological biodegradation processes is acrylamide (Shah, 2014; Syed et al., 2012; Ahmad et al., 2014).

Acrylamide (C_3H_5NO) is a white odorless crystalline solid which is soluble in water, ethanol, ether and chloroform. It is an unsaturated amide compound, thus a reactive electrophilic compound. In vivo, it is metabolized to glycidamide through epoxidation process on the double bond (Olesen et al., 2008). It is also known to be carcinogenic, mutagenic and teratogenic as shown in the mammalian studies either in vitro or in vivo (Dearfield et al., 1995; Williams et al., 2014). Acrylamide can cause cancer as it is able to bind to deoxyribonucleic acid (DNA) and form DNA adducts. It is also classified as "probably carcinogenic" toxic compound (Group 2A) to human by International Agency for Research on Cancer (IARC, 1986; Virk et al., 2014; Hogervorst et al., 2014).

Most of the industrial effluents with high acrylamide levels come from the construction of the reservoirs and wells of drinking water, grouting activity, dyes and plastics industries (Igisu et al., 1975; Mona et al., 2001; Imai et al., 2014). These effluents can be treated by physicochemical methods such as adsorption, cavitation, solvent extraction, and chemical oxidation. However, they often suffer from serious drawbacks including high cost and the formation of hazardous by-products (Guezennec et al., 2014; Shah, 2014; Syed et al., 2012; Ahmad et al., 2014)

As an alternative, bioremediation has become a popular approach for the removal of environmental contaminants as it offers complete mineralization and rare possibility of secondary pollution (Shukor et al., 2009b; McLoughlin, 1994; Shah, 2014; Ahmad et al., 2014). There are a few studies concerning acrylamide degradation using microorganisms that were isolated from acrylamide-contaminated soil. For example, *Bacillus sphaericus*, *Nocardia rhodocrous*, and *Pseudomonas putrefaeciens* (USEPA, 1985) have been isolated from contaminated soils and used to remove acrylamide from the environment. Degradation of acrylamide depends on the amidase enzyme which catalyzes acrylamide into ammonium and acrylate/acrylic acid (Syed et al., 2012). This enhances the feasibility of using bioremediation to treat acrylamide compounds. Thus, to treat this matter seriously, many scientists have worked on bioremediation, a method

which exploits microorganism to degrade pollutants (Lakshmikandan et al., 2014; Shah, 2014).

Whole cell immobilization has been shown to have remarkable advantages over free cells, such as increase in metabolic activity and metabolite production (Gadkari, 1990; Saber and Crawford, 1985; Saez et al., 2014), protection from toxic substances (Keweloh et al., 1990; Cassidy et al., 1996; Kuzmenko et al., 2014) and increase in plasmid stability (Nasri et al., 1987). These advantages make immobilization an alternative technology for environmental applications such as wastewater treatment and remediation of toxic chemicals (Surkatti et al., 2014). The most commonly used immobilization techniques is entrapment of cells within polymetric matrices. Amongst the earliest works on acrylamide degradation by microbes involves the use of immobilized *Pseudomonas* for acrylamide degradation using calcium alginate (Nawaz et al., 1993). Maximum attention has been given previously to alginate gels (Ramakrishna and Prakasham, 1999). However, more researchers are currently turning their attention to gellan gum as it gives more advantages in terms of stability and price compared to the studies that used alginate and k-carrageenan gel (Moslemy et al., 2003; Chakraborty et al., 2014; Shi et al., 2014). To accomplish this, there is a need for a potent acrylamide-degrading bacterium with better characteristics compared to previous strain for immobilization purposes. Hence one of major themes is to immobilize a new locally isolated acrylamide-degrading bacterium using gellan gum and to study its efficacy and stability compared to free cells.

To fulfill the above theme, the following objectives are carried out;

1. To screen, isolate and identify a local acrylamide-degrading bacterium.
2. To characterize the growth and acrylamide degradation of the acrylamide-degrading bacterium.
3. To investigate the stability and tolerance to heavy metals and pesticides by freely suspended and immobilized cells during acrylamide degradation.

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