DIURON STATUS IN PORT KLANG AND SCREENING OF INDIGENOUS BACTERIA FOR POTENTIAL DIURON BIODEGRADATION AGENT

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By

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Faculty : Science

Being as one of the main shipping ports in Malaysia, Port Klang is strategically located at the gateway of Malacca Straits and receiving water influx from two major rivers (Langat River and Klang River) and a few small tributaries. Port Klang is the busiest trading ports in Malaysia in terms of sea vessels harboured, container handled and total cargo tonnage handled. With intense shipping activities and water influx from agriculture areas, the coastal water of Port Klang is predicted to receive various organic pollutants (including Diuron) leached out from the coating paint of the moving vessels and also sources from agriculture runoff, industries, and the residential areas nearby. Diuron is one of the alternative biocides used to replace organotin compound in the formulation of antifouling paints, while also actively use as herbicide at cultivated and non-cultivated areas. Considering the physical and chemical properties of Diuron, it is lethal to targeted and non-targeted marine organisms. Thus, present study aimed [1] to assess the current concentration of Diuron from surface sediments, pore water and surface seawater samples, [2] to isolate, characterize and identify potential Diuron-degrader bacteria from sediment samples, and [3] to observe the growth performance of selected isolates exposed to the Diuron-treated media. For the assessment of Diuron, three types of samples were collected from Station 1 to Station 6 that are represent port and non-port area of Port Klang area. Bacteria strains were isolated from the collected sediment samples based on standard isolation protocols. Meanwhile, growth performance of selected isolates were observed for 7 days at 24 hours interval times. Results showed that present Diuron concentration in Port Klang was higher compared to previous Malaysian coastal water studies in both sediment (19.27 µg/kg) and surface seawater (0.53 µg/L) samples and new data recorded of Diuron was traced in pore water samples (12.91 µg/L). These contaminant level are expecting to be increase in next coming years due to growing of vessels and vigorous activities from nearby of Port Klang area. For isolation part, from all 21 isolates screened out, bacteria labelled SZZ 10 and SZZ 19 showed the capability to survive in the maximum permissible limit concentration, 430 ng/L and happened to be an excellent strains which survived up to 1000 µg/L of Diuron. These bacteria were then genetically identified as *Comamonas jiangduensis* (SZZ 10) and *Bacillus aerius* (SZZ 19) and deposited in GenBank with accession numbers KU942479, KU942480, respectively. In growth performance observation, *Bacillus aerius* (SZZ 19) showed the potential as biodegrader of Diuron due to its high resistance and adaptation mechanism toward toxicity of Diuron. In conclusion, Diuron was found to be pollute in the area of Port Klang with increment in concentration due to the various activities surround the Port Klang area. For a simple yet low cost bioremediation approach, an isolation of bacterial species from contaminated area was succeed in reveal the potential of *Bacillus aerius* as a future Diuron-degrader bacteria after several screening procedure was done. It is suggested that future works should include biodegradation of Diuron by strictly controls the crucial parameters in enhancing the bioremediation process in the effort of decontamination of Diuron from the environment soon.

Keywords: Port Klang, Diuron, Biocides, Bacteria, Resistant, Biodegrader

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

STATUS DIURON DI PELABUHAN KLANG DAN SARINGAN BAKTERIA ASAL SEBAGAI POTENSI EJEN BIODEGRADASI DIURON

Oleh

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Sebagai salah sebuah pelabuhan terpenting di Malaysia, Pelabuhan Klang terletak pada kedudukan yang strategik di pintu masuk Selat Melaka dan menerima kemasukkan air daripada dua buah sungai utama (Sungai Langat dan Sungai Klang) dan dari beberapa buah anak sungai. Pelabuhan Klang adalah sebuah pelabuhan perdagangan yang tersibuk di Malaysia dari segi pelabuhan kapal laut, pelabuhan kontena dan jumlah tan kargo yang dikendalikannya. Dengan kepadatan aktiviti perkapalan dan kemasukkan air dari kawasan pertanian, kawasan perairan di Pelabuhan Klang diramalkan menerima pelbagai pencemar organik (termasuk Diuron) yang terbebas keluar daripada cat sadur pada kapal yang bergerak dan juga sumbernya daripada sisa pertanian, industri dan kawasan perumahan yang terdekat. Diuron merupakan salah satu alternatif biosida yang digunakan untuk menggantikan penggunaan sebatian organotimah di dalam cat kapal. Melihatkan kepada ciri-ciri fizikal dan kimia Diuron, ianya boleh membunuh organisma sasaran dan bukan sasaran. Oleh itu, kajian ni dilakukan bertujuan [1] untuk menilai kepekatan Diuron semasa daripada sampel permukaan sedimen, air liang dan sampel permukaan air laut, [2] untuk memencilkan, mencirikan dan mengenal pasti bakteria yang berpotensi sebagai bakteria mendegradasi Diuron daripada sampel sediment, dan [3] untuk memerhatikan prestasi pertumbuhan isolat yang dipilih diuji ke atas media yang ditambah dengan Diuron. Bagi penilaian Diuron, tiga jenis sampel telah di ambil dari Stesen 1 hingga Stesen 6 yang mewakili kawasan pelabuhan dan bukan pelabuhan di kawasan Pelabuhan Klang. Tambahan pula, bakteria telah di pencilkan dari sampel sedimen yang telah diambil. Sementara itu, prestasi pertumbuhan bagi bakteria yang terpilih adalah dalam pemerhatian selama 7 hari dalam selangan masa 24 jam. Keputusan menunjukkan bahawa kepekatan Diuron di Pelabuhan Klang tinggi berbanding dengan kajian yang telah dijalankan di kawasan perairan Malaysia yang lepas di dalam kedua-dua sampel sedimen (19.27 µg/kg) dan sampel permukaan air laut (0.53 µg/L) dan data pertama telah direkodkan bagi penemuaan Diuron di dalam sampel air liang (12.91 µg/L). Tahap pencemar ini dijangka akan meningkat bagi tahun berikutnya berikutan aktiviti perkapalan yang pesat di Pelabuhan Klang. Bagi bahagian pemencilan, dari 21 isolat yang telah dipilih, bakteria dengan label SZZ 10 dan SZZ 19 telah menunjukkan keupayaan untuk bertahan hidup dalam kepekatan Diuron maksima yang dibenarkan,

430 ng/L dan menjadi strain yang terbaik yang mampu bertahan pada kepekatan Diuron 1000 µg/L. Bakteria ini kemudiannya telah dikenal pasti secara genetik, dikenali sebagai *Comamonas jiangduensis* (SZZ 10) dan *Bacillus aerius* (SZZ 19) serta didepositkan di GenBank dengan nombor penyertaan KU942479, KU942480 masing-masing. Untuk itu, *Bacillus aerius* telah menunjukkan potensinya sebagai pembiodegradasi Diuron atas daya tahan yang tinggi dan mekanisma adaptasi terhadap ketoksikan Diuron. Kesimpulannya, Diuron telah ditemui mencemarkan kawasan Pelabuhan Klang dengan peningkatan kepekatan akibat dari pelbagai aktiviti di kawasan sekitar Pelabuhan Klang. Pendekatan yang mudah lagi menjimatkan bagi bioremediasi, pemencilan bakteria dari kawasan yang tercemar telah berjaya mendedahkan potensi *Bacillus aerius* sebagai bakteria biodegradasi Diuron di masa hadapan selepas beberapa saringan dilakukan. Adalah dicadangkan supaya kerja-kerja berkaitan dengan biodegradasi Diuron dijalankan di masa hadapan iaitu dengan mengawal ketat parameter yang penting untuk meningkatkan proses bioremediasi dalam usaha dekontaminasi Diuron dari persekitaran kelak.

Kata Kunci: Pelabuhan Klang, Diuron, Biosida, Bakteria, Bahan pencemar, Pembiodegradasi

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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 the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS / SYMBOLS

IMO	International Maritime Organisation
DT_{50}	Degradation half time
PSI	Photosystem I
PSII	Photosystem II
ATPs	Adenosine triphosphate (s)
NADPHs	Nicotinamide adenine dinucleotide phosphate (s)
Cu_2O	Cuprous oxide
CuSCN	Cuprous thiocyanate
LOD	Lower of Detection
3,4-DCA	3,4-dichloroaniline
DCPMU	1-(3,4 dichlorophenyl)-3-methylurea
DCPU	1-(3,4-dichlorophenyl)urea
LLE	Liquid-to-liquid extraction
LC	Liquid chromatography
UPLC	Ultra performance liquid chromatography
MS	Mass spectrometry
MRM	Multiple Reaction Monitoring
TEUs	twenty-foot equivalent units
DCM	Dichloromethane
$NaSO_4$	sodium sulphate anhydrous
USDA	United States Department of Agriculture
ANOVA	Analysis of Variances
PBS	Phosphate buffer saline
NaCl	sodium chloride
$NaNO_3$	Sodium nitrate
KCl	Potassium chloride
$MgSO_4.7H_2O$	Magnesium sulfate heptahydrate
$FeSO_4$	Ferum sulphate
$ZnSO_4$	Zinc sulphate
$NaHPO_4$	disodium hydrogen phosphate
KH_2PO_4	monopotassium phosphate
NA	Nutrient agar
NB	Nutrient broth
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium dye
MSM	Mineral salt media
N_2	Nitrogen gas
S.D	Standard deviation
CO_2	Carbon dioxide
Q_B	Quinone B protein
mPa	Megapascal
%	Percentage
Kg ac/ha	Kilogram per acre/hectare
w/w	Weight per weight
ng/L	Nanogram per litre
µg/kg	Microgram per kilogram
ppb	Part per billion
ppt	Part per trillion
ng/g	Nanogram per gram

µg/g	Microgram per gram
µg/L	Microgram oer litre
µL	microlitre
mg	Milligram
ml	Milliliter
mm	Millimeter
rpm	Revolution per minute
ºC	Degree Celsius
mg/L	Milligram per litre
g	Gram
µm	micrometre
mM	millimolar
µg	Microgram

CHAPTER 1

INTRODUCTION

1.1 Background study

In the recent decade, scientists have highlighting the presence of organic pollutants in bodies of water that could cause deleterious effects to aquatic organisms, especially to non-targeted and commercially important organisms. One of the organic pollutants that is well-known to demonstrate this effect is the Organotin (OT). OT was used in the formulation of antifouling paints and applied on sea vessels. Due to its persistency and deleterious effects, OT has been globally banned since 2008. Thus, alternative biocides have been used to replace OT for similar functions. One of the commonly used alternative biocides is Diuron.

Diuron can be found resulting from agricultural activities while also acting as biocides in the coating paint of sea vessels. Diuron, chemically named as 3-(3,4-dichlorophenyl)-1,1-dimethylurea is a urea herbicide group actively applied in the agricultural sector. This broad-spectrum herbicide plays a role in killing unnecessary weeds by interrupting the photosynthesis process (Laisk & Oja, 2013). In Malaysia, many studies related to Diuron have been conducted and focused on its application in the agricultural sector. The fate of Diuron residues in plant compartments has widely been investigated especially in oil palm plantations (Muhamad *et al*., 2013; Badrul Hisyam, 2012).

Besides, Diuron is one of the 18 active ingredient biocides in the copper-based formulated paint (Arai *et al*., 2009). It is designated to replace the organotin-based paints and has been proposed to be globally used. Playing a vital role as an antifoulant in the formulation of paint, Diuron has high possibility to harm aquatic organisms as it leaches out from the ship hulls during ship movements (Mohamat-Yusuff *et al.*, 2011). As the point of sources of Diuron, this coating paint can be found occurring at significantly high concentration in the area of the sea ports. The newest records on Diuron were found to be conducted at Peninsular Malaysia in the area of the aquatic ecosystem (Ali *et al*., 2014). It was revealed that Malaysia's coastal water has been contaminated with the pollutant herbicide, Diuron. Increase in shipping activities encourages the tons of vessels to the water bodies and also the modernized development of shipping port in Malaysia. The fact of Diuron sinking into the water column and ending up in the sediment has been doubted for many years. Like many other organic pollutants, this hydrophobic biocide is persistent in the environment especially in the sediment depending on the particle size and characteristics of the medium and having half-life from one month up to one year (Thomas & Langford, 2009).

Due to its persistence, the compound transformation significantly contributes to the availability of Diuron in the environment. However, the disappearance of this herbicide or cleavage of compound (degradation) is meant to be by either abiotic or biotic process (Giacomazzi & Cochet, 2004). Abiotic process naturally occur by photolysis and hydrolysis (Hussain *et al*., 2015) while, biotic degradation is through the action of an organism's enhancements. Instead of using the abiotic approach with high cost and time consuming, microbial degradation is the easiest, convenient and to date way in reducing the availability of any organic pollutant from the environment. Bacteria or fungus degrader are excellent decomposers and have been proven from many studies (Ellegaard-Jensen *et al*., 2013). Isolated from a great variety of soil and sediment type from a different country, bacteria has revealed their capabilities in the degradation of Diuron. This is proven from the great response of resistance and degradation of bacteria species towards phenylurea herbicide family (Ngigi *et al*., 2011). With great works from the microbial entity, it could help in cleaning up contaminants from the chemical environmental hazards.

1.2 Objectives of the study

Thus, this present study was conducted:

[1] To assess the current concentration of Diuron from surface sediments, pore water and surface seawater samples.
[2] To isolate, characterize and identify the potential bacteria-degrader towards pollutant, Diuron.
[3] To observe the growth performance of selected isolates exposed to the Diuron-treated media.

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