UNIVERSITI PUTRA MALAYSIA

APPLICATION OF ONE FACTOR AT A TIME (OFAT) AND RESPONSE SURFACE METHODOLOGY (RSM) FOR OPTIMIZING PHENOL-DEGRADING PARAMETER BY ISOLATE 1

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FBSB 2015 76

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Dissertation submitted in partial fulfilment for the requirement for the course of
BCH 4999
Project in the Department of Biochemistry,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia.

June 2015

PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk "Application of One Factor at a Time (OFAT) and Response Surface Methodology (RSM) for Optimising Phenol-degrading Parameters by Isolate 1" telah disiapkan serta dikemukakan kepada Jabatan Biokimia oleh Seha anak Mamat (163910) sebagai syarat kursus BCH 4999 projek.

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ABSTRACT

Global warming has always been a great concern especially in Antarctica due to pollution. Phenol pollution is one of the well-known pollution that highly affects the ecosystem in Antarctic soil and water. Due to it being widely used in various industries, excessive phenol is discharged into rivers and soil which might affect living things. The purpose of this study is to optimise the conditions for phenol-degrading bacteria Isolate 1 to degrade phenol at low temperatures using one factor at a time (OFAT) approach and response surface methodology (RSM). Four major parameters that can affect phenol degradation are pH, nitrogen source, temperature and salinity. From OFAT approach, Isolate 1 was found to have a high capability to degrade phenol with an optimum pH of 7.5 in phosphate buffer, ammonium sulphate as its best nitrogen source with concentration of 0.40 g/L, temperature of 15°C and salinity of 0.10 g/L. Meanwhile, during optimisation using RSM, Isolate 1 shows the highest phenol degradation percentage through interaction between 2 factors which are salinity with optimum sodium chloride (NaCl) concentration of 0.15 g/L and pH of 7.65. The greatest achievement in RSM is that Isolate 1 which is classified as cold-tolerant bacteria can degrade 0.50 g/L phenol up to 98.32% within two days.

ABSTRAK

Pemanasan global sentiasa menjadi kebimbangan terutamanya di Antartika disebabkan oleh pencemaran. Pencemaran fenol adalah salah satu pencemaran yang terkenal yang sangat memberi kesan kepada ekosistem tanah dan air Antartik. Oleh kerana ia digunakan secara meluas dalam pelbagai industri, lebihan fenol akan dilepaskan ke sungai dan tanah yg mungkin memberi kesan kepada hidupan. Tujuan kajian ini adalah untuk mengoptimumkan keadaan untuk bakteria pengurai fenol Isolat 1 untuk mengurai fenol pada suhu rendah menggunakan metodologi pendekatan satu faktor pada satu masa (OFAT) dan tindak balas permukaan (RSM). Empat pemboleh ubah utama yg boleh memberi kesan degradasi fenol adalah pH, sumber nitrogen, suhu dan kemasinan. Dari pendekatan OFAT, Isolat 1 mempunyai keupayaan untuk menguraikan fenol dengan optimum pH 7.5 dalam penimbal fosfat, ammonium sulfat sebagai sumber nitrogen yang terbaik dengan kepekatan 0.40 g/L, suhu 15 darjah celsius dan kemasinan 0.1 g/L. Sementara itu, semasa pengoptimuman menggunakan RSM, Isolat 1 menunjukkan peratusan kemerosotan tertinggi fenol melalui interaksi antara 2 faktor iaitu kemasinan dengan optimum kepekatan natrium klorida (NaCl) 0.15 g/L dan pH 7.65. Pencapaian terbesar dalam RSM ialah Isolate 1 yang mengelaskan bakteria bertoleransi sejuk boleh degradasi 0.50 g/L fenol sehingga 98.32% dalam masa dua hari.

ACKNOWLEDGEMENT

First of all, I would like to express my appreciation towards my supervisor, Dr Siti Aqlima Ahmad, who gave the permission for me to do this project and also for her endless support, comments and guidance through the process of completing my thesis project.

I would also want to thank my teammates especially Tengku Nur Saleha, Syirhan and Syahir for their consistent help in the experiment, opinion and knowledge with my thesis and also for being very supportive during the completion of my thesis project. Next, I would like to also give my thanks to my seniors in lab 204 for their guidance and always prepare for my every need in thesis work.

Besides, I also want to thanks my academic supervisor, Prof. Mohd Arif Syed and other supervisor, Dr Adeela binti Yasid for their comments and guidance given in my thesis progress presentation.

Lastly, I would like to thanks my family, especially my parents, Mamat anak Angkah and my mother, Maliah anak Jemat for their moral and financial support throughout this project. Not to forget all those who contributed to my thesis directly or indirectly, I want to give my thanks for your support and advices. I am very grateful for everyone who concern about my thesis project.

Seha anak Mamat, 2015

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LIST OF ABBREVIATIONS

%	Percent
$(NH_4)_2SO_4$	Ammonium sulphate
µg	Microgram
µl	Microlitre
4-AAP	4-amino antipyrene
cm^3	Centimetre cubes
et al.,	And friends
$FeSO_4.H_2O$	Ferrous sulphate monohydrate
G	Gram
H_2O	Water
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
hrs	Hours
$K_2Fe(CN)_6$	Potassium ferric cyanide
K_2HPO_4	Di-potassium hydrogen phosphate
KH_2PO_4	Potassium dihydrogen phosphate
L	Litre
mg	Miligram
$MgSO_4$	Magnesium sulphate
min	Minutes
ml	Millilitre
mM	Milimolar
$MnSO_4.H_2O$	Manganese sulphate
mol	Moles
MSM	Minimal salt medium
NaCl	Sodium chloride
$NaMoO_4.2H_2O$	Sodium molybdate dehydrate
NaOH	Sodium hydroxide
NH_4Cl	Ammonium chloride
nm	Nanometer
O_2	Oxygen
ºC	Degree of celcius
rpm	Revolution per minute
β	Beta

CHAPTER 1

INTRODUCTION

Antarctic island is one of the last pristine regions of planet (Vodopivez *et al*., 2015) which facing pollutions issues. In cold climate such as Antarctica, location where there are human habitats or any traces of human activity are mostly increases in chances of soil and water contamination (Litova *et al*., 2014). Biodegradation and bioremediation by using bacteria are most focusing study in Antarctica during early hydrocarbon pollution (Polmear *et al*., 2015) due to it safety and harmless rather than chemical clean-up procedure (Luz *et al*., 2006).

Phenol is an organic compound that is being widely used in agricultural chemicals, pesticides, petrochemicals, pharmaceuticals, textiles and steel industries (Basha *et al*., 2010; Mahiudddin *et al*., 2012). The presence of phenol wastes from sewage and industrial discharge have become a great concern because of their toxicity and persistent in environment (Bui *et al*., 2012). Industrialisation country especially Malaysia is having problems with the unsafe levels of phenol as it can affects the communities' health (Ahmad *et al*., 2011). Even at low concentration, continuous exposure towards phenol can lead to serious damage on our urinary system especially kidneys and central nervous system with only via inhalation, direct contact or ingestion (Suhaila *et al*., 2013).

To overcome this situation, a numbers of studies have demonstrated the method of treating phenol wastewater biologically without causing harmful effects (Chen *et al*., 2002). Several measurements and factors are crucial in the biodegradation of phenol includes temperature, pH, incubation periods, carbon and nitrogen sources (Suhaila *et al*., 2013). Mesophilic bacteria is usually used for phenol biodegradation in tropical country such as Malaysia in which its temperature range is between 20-37 ºC for optimum degrading activity (Ahmad *et al*., 2011). Thermophilic bacteria usually have the optimum temperature to degrade phenol between 60-65 ºC (Margesin *et al*., 2005). In cold climates, it is important for cold-adapted microorganisms have ability to degrade organic

contaminants under cold condition (Margesin *et al*., 2005). According to Bergauer *et al*., (2005), psychrophiles or cold-tolerant phenol degraders are able to degrade phenol at low temperature due to their adaptation to cold environment.

At high concentration of phenol wastewater, bacteria will reach optimal degradation capability by using response surface methodology (RSM). RSM is a statistical and mathematical technique that is used to achieve the optimal response for phenol-degrading bacteria to degrade phenol (Huang *et al*., 2013). Central composite design is one of the examples of experimental design that used in RSM (Suhaila *et al*., 2013).

Objectives of this research:

1. To identify phenol-degrading bacteria, Isolate 1.
2. To characterise the potential of phenol-degrading bacteria using different optimisation methods: one factor at a time approach (OFAT) and response surface methodology (RSM).

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