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PHENOL DEGRADATION AND MOLECULAR ANALYSIS OF PHENOL HYDROXYLASE FROM Acinetobacter sp.

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ABSTRACT

Phenol is the simplest member of a family of compounds in which an -OH group is attached directly to a benzene ring, can be found either naturally or man-made. Phenol are classified as highly hazardous chemical due to their toxic, mutagenic, and carcinogenic characteristic. Bioremediation was chosen to solved the phenol pollution due to environmental friendly, rapid and cost effective. *Acinetobacter* sp. is one of the bacteria that can degrade phenol. The key enzyme for the degradation of phenol in *Acinetobacter* sp. is phenol hydroxylase. The highest degradation rate of phenol by *Acinetobacter* sp. is 3.57 mg/h at concentration 600 mg of phenol. The molecular analyse result confirmed the presence of phenol hydroxylase in *Acinetobacter* sp.

ABSTRAK

Phenol tergolong dalam keluarga paling ringkas yang mana satu kumpulan –OH terikat secara langsung kepada gegelang benzene, ianya boleh dijumpai secara neutral ataupun buatan manusia. Phenol dikelaskan sebagai bahan kimia yang sangat berbahaya disebabkan kerana sifatnya yang toksik, mutagen, dan boleh menyebabkan kanser. Bioremidiasi telah terpilih bagi menyelesaikan masalah pencemaran phenol kerana ianya mesra alam, cepat, dan sangat efektif. *Acinetobacter* sp. merupakan salah satu bakteria yang boleh mendegradasi fenol. Enzim utama bagi fenol degradasi yang ada pada *Acinetobacter* sp. adalah enzim phenol hidroksilase. Kadar degradasi fenol yang paling tinggi oleh *Acinetobacter* sp. adalah 3.75 mg/j iaitu pada kepekatan 600 mg fenol. Hasil daripada analisis molekular membuktikan kehadiran enzim fenol hidroksilase pada *Acinetobacter* sp.

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LIST OF ABBREVIATIONS

%	Percent
µl	microlitre
4-AAP	4-amino antipyrine
bp	Base pair
dH_2O	Distilled water
DNA	Deoxyribonucleic acid
g	gram
g/l	gram per liter
kb	Kilobase
L	liter
mg/L	milligram per liter
ml	mililiter
ml	miligram
MS	Mineral salt
nm	nanometer
O.D	Optical density
^oC	Degree Celsius
PCR	Polymerase Chain Reaction
rpm	Revolution per minute
TAE	Tris-acetate-EDTA

CHAPTER 1

INTRODUCTION

Phenol compounds are widely distributed from a natural as well as a man-made aromatic product. Natural phenolic compounds and their derivatives are present everywhere in the environment all around the world. Phenol is a troublesome contaminant that is harmful to human race, generating a strong interest in strategies to decrease this phenol pollution. In the last 20 years, studies have been performed on the study for bioremediation to degrade the phenol and their derivative such as both aerobic and anaerobic treatment of aromatic pollutants by using pure microorganisms or mixed cultures.

The increasing of environmental pollution leads to progressive deterioration in the quality of human life. These circumstances obligate the world's scientific community to look for effective technique of environmental remediation with the purpose of human being and nature preservation. One of the remediation method that become increasingly popular nowadays is bioremediation.

Bioremediation which can be defined as the cleaning up technique that transforms hazardous chemical to less hazardous chemical by biological system, continues to be the preferred and suitable method for household waste recycling, toxic chemical and pollutant removal. Bioremediation includes three main processes, first is transformation or insignificant alteration of the molecule; second is fragmentation or degradation of the molecule to simpler compounds; and third is the mineralisation or conversion of the complex compound into simpler one.

Phenol can be degraded by various microorganisms such as *Acinetobacter*, *Pseudomonas*, and *Alcaligenes*, which utilises phenol as the sole carbon source for

the growth of the organisms. A number of both aerobic and anaerobic phenol degrading microorganisms have been isolated and characterised. Microorganisms that are capable of aerobic phenol degradation were described as early as 1908. In addition to bacteria, fungi are known for their diversity and remarkable ability to degrade phenolic compounds. Because of their specialities, they are able to grow under environmentally stressed and extreme conditions such as low nutrient availability, low water activity, low temperature and at low pH values. Although both aerobic and anaerobic organism are able to degrade phenol and its derivative, aerobic process may be preferred (Sahinakaya and Dilek, 2002). A typical pathway for metabolizing an aromatic compounds is to dihydroxylate the benzene ring to form a catechol derivative and then to open the ring through ortho or meta oxidation.

1.1 Objectives

1) To evaluate the ability of *Acinetobacter* sp. to degrade phenol
2) To determine the degradation rate of phenol by *Acinetobacter* sp.
3) To analyse the phenol hydroxylase gene from *Acinetobacter* sp.

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