

**ADSORPTION-BIODEGRADATION OF
PHENOL BY IMMOBILIZED *Candida tropicalis*
RETL-Cr1 ONTO NATURAL AND MODIFIED
ZEOLITE**

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UNIVERSITI MALAYSIA SABAH

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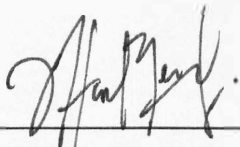
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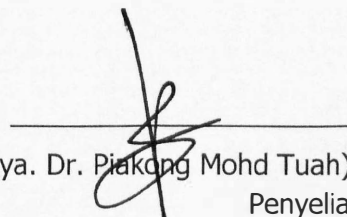
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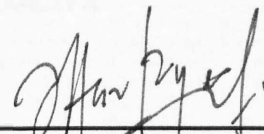
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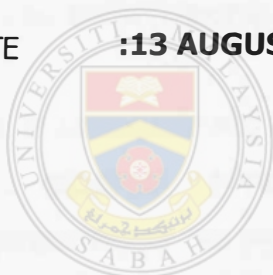
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ABSTRACT

Phenol has been produced since 1860s. The application of phenol and its derivatives is worldwide. However, phenol is highly toxic and able to retain in environment for a long period. Despite being toxic, *C. tropicalis* RETL-Cr1 is capable of consuming phenol as their carbon sources. Remarkable performance in free cells systems makes *C. tropicalis* RETL-Cr1 valuable to the removal of toxic pollutant like phenol. The application of natural and modified zeolite in toxic pollutant treatment is familiar. Therefore, the main goal of this research is to determine the potential of *C. tropicalis* RETL-Cr1 in an immobilized system and the feasibility of natural zeolite (NZ) and surfactant modified zeolite (SMZ) as solid carrier and adsorbent in the adsorption-biodegradation of phenol. NZ is modified with the various concentration of cationic surfactant to form SMZ. The modification is confirmed by the FTIR, XRD, TGA and SEM analysis. Various parameters such adsorbent dose, particle size, initial cell loading and incubation time had been optimized for the immobilization protocol of *C. tropicalis* RETL-Cr1 onto NZ and SMZ. SMZ immobilized significantly higher cells than NZ by 3.8 folds. Cells retention of 3.85×10^{10} CFU/g and 1.09×10^{10} CFU/g were obtained by SMZ and NZ respectively. Various factors influencing the phenol biodegradation by immobilized yeast cells onto NZ and SMZ is optimized and applied to two different modes of continuous phenol biodegradation; simultaneous adsorption-biodegradation and separate adsorption by NZ and SMZ then biodegradation by free cells (FC) in suspension. The removal percentages of phenol from free cells to immobilized cells were improved from 81% to 99% in the 7mM of Phenol. This indicates that the degradation of phenol using immobilized cells is more effective than when utilizing FC. SMZ recorded 99% removal efficiency when phenol concentration is increased to 7mM with the removal rate of 26.35mg/L.hr. While the phenol biodegradation by FC and immobilized cells onto NZ shows 81% and 75% removal. Accumulation of catechol is monitored, with maximum production of 15.75 and 32.33 mg/L when NZ and SMZ are used, respectively. The kinetic model of phenol adsorption-biodegradation by immobilized yeast cells onto NZ and SMZ can be described by pseudo-first (physisorption) and pseudo-second (chemisorption) order respectively. An intraparticle diffusion model proves it is not the only rate controlling phase for the process. When SMZ is applied in continuous system, at the concentration of 16mM, simultaneous and separate system of adsorption-biodegradation took only 34 and 32 hours to complete the phenol removal with the removal rate of 44.29 and 47.00 mg/L.hr respectively with the maximum catechol production of 33.40mg/L for both systems. These results show significant improvement compared to previous report on phenol removal in continuous flow system. *C. tropicalis* RETL-Cr1 perform better when immobilized and applicability of NZ and SMZ as the carrier matrix for phenol removal is confirmed. This study has therefore provided further substantial knowledge regarding versatility of *C. tropicalis* RETL-Cr1 roles in the environment. Finally, it is possible to apply this simple and economic treatment in large scale at wastewater treatment plant containing high concentration of phenol.

ABSTRAK

PENJERAPAN-BIODEGRADASI FENOL OLEH *C. tropicalis* RETL-Cr1 TERSEKAT GERAK DALAM ZEOLIT SEMULAJADI DAN ZEOLIT DIUBAHSUAI

Fenol telah dihasilkan sejak tahun 1860-an. Penggunaan fenol dan terbitannya adalah di seluruh dunia. Walau bagaimanapun, fenol adalah sangat toksik dan boleh kekal dalam persekitaran untuk jangka masa yang lama. Walaupun bersifat toksik, *C. tropicalis* RETL-Cr1 mampu menggunakan fenol sebagai sumber karbon mereka. Prestasi luar biasa dalam sistem sel bebas menjadikan *C. tropicalis* RETL-Cr1 istimewa dalam penyingkiran bahan cemar toksik seperti fenol. Penggunaan zeolit semulajadi dan diubah suai dalam rawatan bahan cemar toksik adalah biasa. Oleh itu, matlamat utama penyelidikan ini adalah untuk menentukan potensi *C. tropicalis* RETL-Cr1 dalam sistem pegun dan ketersauran zeolit semulajadi (NZ) dan zeolit diubahsuai surfaktan (SMZ) sebagai pembawa pepejal dan penjerap dalam penjerapan-biodegradasi fenol. NZ diubahsuai dengan pelbagai kepekatan surfaktan kationik untuk membentuk SMZ. Pengubahsuaian ini disahkan oleh analisis FTIR, XRD, TGA dan SEM. Pelbagai parameter seperti dos penjerap, saiz zarah, muatan sel awal dan masa inkubasi telah dioptimumkan untuk protokol pemegunan *C. tropicalis* ke dalam NZ dan SMZ. SMZ mememegunkan bilangan sel-sel yang jauh lebih tinggi daripada NZ sebanyak 3.8 kali ganda. Pemegunan sel 3.85×10^{10} CFU/g dan 1.09×10^{10} CFU/g masing-masing didapati oleh SMZ dan NZ. Pelbagai faktor yang mempengaruhi biodegradasi fenol oleh sel-sel yis pegun dalam NZ dan SMZ dioptimumkan dan digunakan dalam dua mod biodegradasi fenol berterusan; penjerapan-biodegradasi serentak dan penjerapan berasingan oleh NZ dan SMZ kemudian biodegradasi oleh sel bebas (FC). Peratusan penyingkiran fenol dari sel-sel bebas ke sel-sel pegun bertambah baik dari 81% hingga 99% pada 7mM fenol. Ini menunjukkan bahawa degradasi fenol menggunakan sel-sel pegun adalah lebih berkesan daripada menggunakan FC. SMZ mencatatkan 99% kecekapan penyingkiran apabila kepekatan fenol meningkat kepada 7mM dengan kadar penyingkiran 26.35mg/L.hr. Biodegradasi fenol oleh FC dan sel-sel pegun dalam NZ menunjukkan 81% dan 75% penyingkiran. Penghasilan maksimum katekol sebanyak 15.75 dan 32.33 mg/L apabila NZ dan SMZ digunakan. Model kinetik penjerapan-biodegradasi fenol oleh sel-sel yis pegun ke dalam NZ dan SMZ boleh dijelaskan oleh tertib pseudo-pertama (fisierapan) dan tertib pseudo-pertama (pengkimierapan) masing-masing. Model resapan intrazarah membuktikan ia bukan satu-satunya tahap pengawal kadar untuk proses itu. Apabila SMZ diterapkan dalam sistem yang berterusan, pada kepekatan 16mM, sistem serentak dan berasingan penjerapan-biodegradasi mengambil hanya 34 dan 32 jam untuk menyelesaikan penyingkiran fenol dengan kadar penyingkiran 44.29 dan 47.00 mg/L.hr masing-masing dengan maksimum pengeluaran katekol 33.40mg/L untuk kedua-dua sistem. Keputusan ini menunjukkan peningkatan yang ketara berbanding dengan laporan sebelumnya mengenai penyingkiran fenol dalam sistem aliran berterusan. *C. tropicalis* RETL-Cr1 berfungsi dengan lebih baik apabila dipegunkan dan kebolegunaan NZ dan SMZ sebagai matriks pembawa untuk penyingkiran fenol disahkan. Oleh itu, kajian ini memberi pengetahuan lanjut mengenai keserbagunaan peranan *C. tropicalis* RETL-Cr1 dalam alam sekitar. Akhir sekali, rawatan sederhana dan ekonomi ini boleh diterapkan secara besar-besaran di loji rawatan air sisa yang mengandungi kepekatan fenol yang tinggi.

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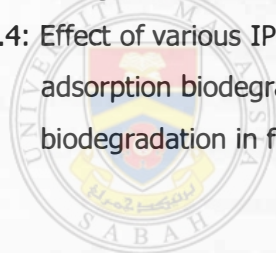
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LIST OF ABBREVIATIONS

ARE	-	Average relative error
CEC	-	Cation exchange capacity
C1,2D	-	Catechol 1,2-dioxygenase
ccMA	-	Cis,cis-muconic acid
CFU	-	Colony forming unit
CMC	-	Critical micelle concentration
ECEC	-	External cation exchange capacity
FTIR	-	Fourier transform infrared
HDTMA- Br	-	Hexadecyltrimethylammonium bromide
2-HMSA	-	2-hydroxymuconic semialdehyde
IPC	-	Initial phenol concentration
HPLC	-	High-performance liquid chromatography
PH	-	Phenol hydroxylase
RM	-	Ramsay medium
rpm	-	Rotation per minute
SMZ	-	Surfactant modified zeolite
sp.	-	Species
SEM	-	Scanning electron microscopy
pH	-	Hydrogen ion concentration
ppm	-	Parts per million
RETL-Cr1	-	Ramsey effluent of Treatment Lagoon-Cream 1
XRD	-	X-ray diffraction
UV	-	Ultra violet

LIST OF SYMBOLS

°C	-	Degree Celsius
C₀	-	Initial Concentration
CFU	-	Colony Forming Unit
cm	-	Centi meter
d	-	Day
g	-	Gram
hr	-	Hour
kv	-	Kilo volt
L	-	Liter
m	-	Meter
M	-	Molar
mA	-	Mili ampere
mA_u	-	Mili Absorbance Unit
meq	-	Mili equivalent
mg	-	Mili gram
min	-	Minute
mL	-	Mili Liter
mm	-	Mili meter
mmol	-	Milimol
mM	-	Mili mol
OD	-	Optical Density
ppm	-	Part per million
rpm	-	Rotation per minute
s	-	Seconds
v	-	Volume
µm	-	Micro meter
θ	-	Angular

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Environmental pollution is a worldwide problem that is faced by developing and developed countries over the year. With urbanization and extensive industrialization, the pollution of the environment with man-made (synthetic) organic compounds has become a major problem (Mohanty, 2012). Environmental pollution is now considered as a side effect of modern industrial society. The United State Environmental Protection Agency (EPA) had published the current list of 126 Priority Pollutants on (EPA, 2013). It was a set of chemical pollutants that were being regulated and have several developed analytical test methods. Among the commonly found wastes are arsenic, benzene, chloroform, cadmium, chromium, lead, phenol, PCB's, trichloroethylene and toluene.

Organic pollutants comprise a potential group of chemicals which can be dreadfully hazardous to human health. As they persist in the environment, they are capable of long range transportation, bioaccumulation in human and animal tissue and bio-magnifications in food chain. Phenolic compounds are hazardous pollutants that are toxic at relatively low concentration (Nair *et al.*, 2008). Phenol or phenolic compounds are widely distributed in the environment partly as a result of natural processes and more importantly, due to human and industrial activities. Phenols, being persistent compounds and due to their toxic, mutagenic and carcinogenic characteristics, are classified as highly hazardous chemicals (Crawford *et al.*, 2008).

Phenol has been classified as an important contaminant, thus most country has created laws regulating the phenol level in drinking water and effluents discharged from factories as pollution prevention action in order to monitor, control and regulate it.

For instance, U.S. Environmental Protection Agency (US EPA) and World Health Organization (WHO) have set a guideline of the maximum permissible level for phenol in environment is 0.1 mg/L (Hsieh *et al.*, 2008). European Council Directive has set up a phenol limit of 0.5µg/L to control the phenol concentration in drinking water (Tziotzios *et al.*, 2005), while Japan Ordinance No 15 law (JEGS, 2012) permitted the phenol level of 5 mg/L in water source. United Arab Emirate also limits the concentration of phenols in industrial effluent to the environment to 0.1 mg/L (Al Zarooni and Elshorbagy, 2006). At Argentina, law 24051 of hazardous residues was established and the level of phenol in drinking water is limited to 2µg/L (Coniglio *et al.*, 2008).

In Malaysia, Department of Environment (DOE) has restricted the limit of phenol as 0.001 mg/L and 1.0mg/L for Standard A and Standard B respectively. This is the guidelines under the Environmental Quality (Sewage and Industrial Effluents) Regulation, 1979 (IWK, 2012). Despite being very harmful to environment and living organisms, phenol is widely used in many industries. These compounds originate mainly from industrial processes such as resin manufacturing, oil refineries, petrochemicals, pharmaceuticals, dyes, textiles and plastic industries (Ahmad *et al.*, 2012).

Crawford *et al.*, (2008) conclude that phenol is released to the air and water as a result of its manufacture, its use in phenolic resins, and organic synthesis. Phenol is found in petroleum products such as coal tar, and creosote and can be released by combustion of wood and auto exhaust. Phenol may be formed in the environment caused by the natural degradation of benzene, since phenol is a major metabolite of benzene (Crawford *et al.*, 2008) and is found extensively in the environment (ASTDR, 2008).The main source of phenol pollution in water is industrial effluent discharge.

Phenol is generally biodegrade rapidly in soil. However, biodegradation of phenol in water or soil may be hindered or precluded by the presence of high, toxic concentrations of phenol or other chemicals, or by other factors such as a lack of nutrients or microorganisms capable of degrading phenol. If biodegradation is sufficiently slow, phenol in sunlit water will undergo photo-oxidation with photo-

chemically produced peroxy radicals, and phenol in soil will leach to groundwater. Phenol may remain in air, water, and soil for much longer periods if it is continually or consistently released to these media from point sources.

Thus, with the massive urbanization, it is difficult to have free-phenol environment. Phenol presences in environment are toxic in nature and cause various health hazards. Exposure to phenol by any routes; inhalation, oral and dermal can produce various health problems to human and animals. Long-term exposure to phenol at work has been associated with cardiovascular disease. Ingestion of liquid products containing concentrated phenol can cause serious gastrointestinal damage and even death. Application of concentrated phenol to the skin can cause severe skin damage. Investigations on phenol toxicology to the animals have been done in the laboratory. The effect may vary depending on the duration of exposure. Short-term exposure to high levels of phenol has caused irritation of the respiratory tract and muscle twitching. Longer-term exposure to high levels of phenol caused damage to the heart, kidneys, liver, and lungs (ATSDR, 2008). Drinking water with extremely high concentrations of phenol has caused muscle tremors, trouble walking, and death in animals. Short-term application of phenol to the skin has produced blisters and burns (ATSDR, 2008).

Hence, the treatment of wastewater containing phenol is a necessity. Generally there are 3 main methods of phenol treatment; chemical, physical and biological. Phenol removal by chemical treatment arise secondary pollution due to the excessive use of chemical usage. High cost in purchasing chemical reagents and the high electrical energy demand makes chemical treatment for phenol not favorable. Phenol removal by physical method is demanding a good adsorbent. The expensive cost, limited lifetime, hard pretreatment and regeneration process of adsorbent makes the physical treatment less preferable. Biological method is generally preferred due to lower costs and possibility of complete mineralization as mentioned methods have serious drawbacks such as high cost and formation of hazardous by-products (Basha *et al.*, 2010) . Many microorganisms (bacteria, fungi and algae) are capable of using aromatic compounds as the sole source of carbon and energy which includes both aerobic and anaerobic microorganisms. Pure and mixed cultures of the *Pseudomonas* genus are the most commonly utilized biomass

(bacteria) for the biodegradation of phenols (Stoilova *et al.*, 2007) and they are believed to have good potential for different biotechnological applications.

Fungi share a significant part in the recycling of aromatic compounds in the biosphere and several studies have shown that diverse fungi are capable of phenols mineralization. They are capable of consuming a wide variety of carbon sources by enzymatic mechanisms, thus providing possibilities for metabolizing phenols and other aromatic derivatives (Stoilova *et al.*, 2007). The most abundant fungi in polluted environments are yeasts. Some yeasts such as *Candida tropicalis*, *Fusarium flocciferum*, and *Trichosporon cutaneum* are capable of utilizing phenol as the major carbon and energy source (Agarry and Aremu, 2012; Al-Khalid and El-Naas, 2012). This makes them an interesting subject for studies aimed at the development of technologies for purification of contaminated soils and waters.

However, microbial growth will restrain the concentration of phenol at high concentrations (Pradeep *et al.*, 2015). Several strategies have been proposed to overcome substrate inhibition. These include cell acclimatization to higher concentrations of phenol, the use of genetically engineered microorganisms and cell immobilization (Benerjee and Goshal, 2011). An immobilized cell is one of the approaches for incorporating bacterial biomass into an engineering process. The advantages of the process based on immobilized biomass include enhancing microbial cell stability, allowing continuous process operation and avoiding the biomass-liquid separation requirement (Annadurai *et al.*, 2007).

In order to extend the scope of biodegradation, considerable amounts of research have been carried out on the biodegradation of phenol by immobilization technique for its high removal efficiency and low cost. Combined with immobilization technique, the biodegradation process was updated by some advantages including enhancing microbial cell stability, allowing continuous process operation and avoiding the biomass-liquid separation requirement. In addition, this effective technique was also employed to protect the microbe from high phenol concentrations.