ABSTRACT

Contamination of the environment due to heavy metals and organic waste is a serious problem nowadays. These types of contaminants are toxic and can be dangerous to human health. Heavy metal contaminants include copper, lead and hexavalent chromium while phenol, benzene and toluene are examples of organic pollutants. A treatment of these contaminated waste chemicals must be carried out before they can be released into the environment. Conventional techniques for the treatment of these contaminants are not cost - effective as they are chemical and energy - intensive. Thus, the emerging biological methods for treating these wastes i.e. bioremediation, is more favorable. In this study, a locally isolated bacterium, *Acinetobacter haemolyticus* was chosen for the removal of Cr (VI) and phenol using batch and column systems. Cr (VI) and phenol removal studies were carried out separately and simultaneously using batch and column systems. When treated separately, about 90% and 95% of Cr (VI) could be removed using the batch and column system, respectively. However, for phenol, 30 – 65% and 50 – 80% of the organic compound could be removed using the batch and column system, respectively. In the simultaneous treatment process, the mixed waste effluent of Cr (VI) and phenol was also treated using the same system. About 70% and 85% of Cr (VI) could be removed in the batch and column system respectively while for phenol, about 30 – 40% was removed using the batch system and 50 – 60% for the column system. The batch system also required a longer retention time compared to the column system. This study has demonstrated the ability of *Acinetobacter haemolitycus* to remove Cr (VI) and phenol both from separate and mixed effluent, thus offering a promising alternative method for the detoxification of both pollutants prior to releasing the effluent into the environment.

ABSTRAK

Pencemaran yang disebabkan oleh sisa – sisa logam berat dan organik adalah satu masalah serius ketika ini. Bahan – bahan pencemar seperti ini adalah berbahaya dan membimbangkan berikutan kesan toksiknya ke atas kesihatan manusia. Bahan pencemar logam berat termasuk kuprum, plumbum dan kromium heksavalen manakala fenol, benzena dan toluena pula merupakan contoh sisa – sisa organik. Rawatan untuk sisa air yang mengandungi bahan – bahan pencemar mesti dijalankan sebelum ianya dilepaskan ke alam sekitar. Teknik konvensional yang digunakan untuk rawatan bahan – bahan pencemar ini adalah tidak kos efektif disebabkan penggunaan bahan kimia dan tenaga yang intensif. Oleh itu, kemunculan teknik biologikal di dalam merawat sisa – sisa ini seperti bioremediasi adalah lebih diutamakan. Dalam kajian ini, bakteria pencilan tempatan, *Acinetobacter haemolyticus* telah dipilih untuk menyingkirkan Cr (VI) dan fenol menggunakan sistem kelompok dan kolum. Kajian penyingkiran Cr (VI) dan fenol dijalankan secara berasingan dan serentak menggunakan sistem yang sama. Untuk Cr (VI), 90% dan 95% Cr (VI) telah disingkirkan menggunakan kedua – dua sistem tersebut. Dalam kajian penyingkiran fenol, 30 – 65% dan 50 – 80% fenol telah disingkirkan menggunakan sistem yang sama. Dalam proses rawatan secara serentak, sisa campuran Cr (VI) dan fenol juga dirawat menggunakan sistem ini. Dengan rawatan secara serentak, 70% dan 85% Cr (VI) boleh disingkirkan melalui kedua – dua sistem manakala untuk fenol, 30 – 40% telah disingkirkan menggunakan sistem kelompok dan 50 – 60% pula untuk sistem kolum. Sistem kelompok memerlukan masa remediasi yang lebih lama jika dibandingkan dengan sistem kolum. Dari kajian ini, *Acinetobacter haemolyticus* menunjukkan kemampuan untuk mengasingkan Cr (VI) dan fenol secara berasingan atau serentak, lalu memberikan kaedah alternatif untuk penyahtoksikan kedua – dua pencemar ini.

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CHAPTER 1

INTRODUCTION

1.1 Water Pollution

Water pollution can be described as a range of adverse effects on lakes, rivers, oceans and also ground water which is caused by human activities. Many factors can contribute to water pollution. Industrial waste is one of the sources of water pollution when the waste water is released into the environment. Industrial discharge contains a variety of pollutants such as heavy metals, organic waste, oil and solids.

1.1.1 Heavy Metal Pollution

Exposure to heavy metals is of concern as it can cause deleterious effects to human. Heavy metals are transition elements with incompletely filled *d* orbital with a density above 5 g/cm^3, which provides heavy metals cation the ability to form complex compounds. At higher concentration, heavy metal ions can form unspecific complex compounds in cell, which will lead to the toxic effects (Nies, 1999).

Heavy metals and their compounds can be adsorbed through the air passage and also alimentary canal with food and drinking water. These heavy metals are generated by certain industries such as textile, electronic and fertilizer industries which needed to be treated before being released into the environment. Unlike organic contaminants which are easily degradable, heavy metal contaminants are

stable and will still remain in the industrial effluent until it has been treated. These heavy metals have an etiological effect on hypertension, cancer and lung diseases. Examples of the heavy metals that are dangerous to humans are Hg, Co, Cd, Cr and others (Thacker *et. al*., 2006a).

1.1.2 Organic Waste Contamination

Contamination caused by organic material is frequently observed in the water system. Organic contamination can originate from many sources such as household (detergent, washing liquid), cosmetic industry, petroleum industry, agriculture, mining industry and many more (Zawala *et. al*., 2007).

Some organic compounds are dangerous and can be harmful to organisms because of the toxicity effect. Also, a few of the organic chemicals are mutagenic and carcinogenic such as residues of polycyclic aromatic hydrocarbon (PAH) (Chen *et. al*., 2005).

Apart from PAH, organochlorine pesticides (OCPs), phtalic acid ester (PAEs), hexachlorocyclohexanes (HCHs) and dichlorodiphenyltrichloroethane (DDTs) are called the persistent organic pollutants (POP) and are always used in the pesticides industry. Other organic pollutants that can be found in the water system are phenol, benzene, tetrahydrofuran and many more (Ma *et. al.*, 2003).

1.1.3 Water Quality

To prevent contamination of the water system, many authorities around the world have introduced laws and regulations. Enforcements such as laws and regulation is necessary to ensure that contamination from waste water or industrial effluent will not occur in the water systems.

In Malaysia, local authorities have a set of by - laws which regulate industries to meet the limits of effluent discharged before discharging the waste water. Table 1.1 shows the parameter limits of effluents for standard A and B (Environmental Quality Act and Regulation Handbook, 1996).

Table 1.1: Parameter Limits of Effluent for Standard A and B.

	Parameter	Unit	Standard	
			A	B
(i)	Temperature	^{o}C	40	40
(ii)	pH value	-	6.0 – 9.0	5.5 – 9.0
(iii)	BOD_5 at $20^{o}C$	mg/L	20	50
(iv)	COD	mg/L	50	100
(v)	Suspended solids	mg/L	50	100
(vi)	Mercury	mg/L	0.005	0.05
(vii)	Cadmium	mg/L	0.01	0.02
(viii)	Chromium, hexavalent	mg/L	0.05	0.05
(ix)	Arsenic	mg/L	0.05	0.10
(x)	Cyanide	mg/L	0.05	0.10
(xi)	Lead	mg/L	0.10	0.5
(xii)	Chromium, trivalent	mg/L	0.20	1.0
(xiii)	Copper	mg/L	0.20	1.0
(xiv)	Manganese	mg/L	0.20	1.0
(xv)	Nickel	mg/L	0.20	1.0
(xvi)	Tin	mg/L	0.20	1.0
(xvii)	Zinc	mg/L	2.00	2.0
(xviii)	Boron	mg/L	1.00	4.0
(xix)	Iron	mg/L	1.00	5.0
(xx)	Phenol	mg/L	0.001	1.0
(xxi)	Free chlorine	mg/L	1.0	2.0
(xxii)	Sulphide	mg/L	0.50	0.5
(xxiii)	Oil and grease	mg/L	Not detectable	10.0

Besides the limits in industrial discharge, the Malaysian government also formulated a water quality standard for marine water according to ASEAN Marine Quality Criteria as shown in Table 1.2 in order to protect aquatic life.

Table 1.2: ASEAN Marine Quality Criteria

Parameter	**Criteria Values**	**Note**
Ammonia (NH_3-N)	70 µg/L	
Cadmium	10 µg/L	
Chromium (VI)	50 µg/L	Criteria value proposed by CPMSII is 48 µg /L. The Meeting recommended to adopt 50 µg /L, following the existing national standards of member countries
Copper	8 µg/L	As the proposed value 2.9 µg/L is too stringent, the Meeting agreed to use round-up value of 7.7 µg /L, the product of the lowest LOEC from a chronic study 77 µg /L for reproduction for *Mysidopsis bahia* and a safety factor of 0.1
Temperature	Increase not more than 2°C above the maximum ambient temperature	
Cyanide	7 µg/L	
Dissolved oxygen	4 mg/L	
Lead	8.5 µg/L	
Mercury	0.16 µg/L	
Nitrate (NO_3-N)	60 µg/L	A single criteria value should be derived for nitrate and nitrite combined in future.
Nitrite (NO_2-N)	55 µg/L	
Oil and grease	0.14 mg/L	Other related parameter, e.g. PAH, should be proposed in the future
Total phenol	0.12 mg/L	
Phosphate	15 µg/L (Coastal 45 µg/L (Estuarine)	
Tributyltin	10 mg/L	
Total suspended solids	Permissible 10% maximum increase over seasonal average concentration	

For Human Health Protection

Parameter	**Criteria Values**	**Note**
Bacteria	100 faecal coliform/100 mL 35 enterococci/100 mL	Coastal water quality for recreational activities

1.2 Chromium

Chromium is one of the metals which are essential for growth of many organisms. Pure chromium is a steel-gray, lustrous and hard crystalline metal which occupies the 24^{th} position in the Periodic Table and belongs to group VIB. Chromium has a melting and boiling point of $1875^{o}C$ and $2680^{o}C$ respectively (U.S EPA, 1984).

Chromium has nine valency states, ranging from -2 to +6. Amongst these valency states, only the trivalent chromium and hexavalent chromium show significant occurance in the environment. Trivalent chromium is the most stable form of the element as it forms kinetically inert complexes with water, ammonia and some other materials. Hexavalent chromium is the most commercially and environmentally important state for chromium and always linked to oxygen, which gives the property of an oxidizing agent. The ground state electron configuration for chromium is $[Ar].3d^5.4s^1$ (Guertin *et al*., 2005).

Trivalent chromium is important as it is required for the metabolism of fat and glucose and also for proper functioning of insulin (Thacker *et. al.*, 2006b). Even though chromium is defined as a micronutrient, at higher concentration it is considered toxic. This metal is considered as a priority pollutant by the US EPA (Thacker *et. al.*, 2006a).

1.2.1 Sources of Chromium

Pure chromium is not naturally found. Chromium occurs primarily in nature as a member of spinel mineral group in the form of chromite ore or chrome iron ore. The ideal chromite ore is the one with the composition of $FeO.Cr_2O_3$ which contains about 46% of chromium (US EPA, 1984).

World's chromite supply comes from South Africa, Philippines, Finland and Russia. Chromite ore deposits are also found in other countries such as United States of America, but the concentration is so low and is not economically feasible for the mining process.

1.2.2 Chromium in the Environment

Chromium is being released into the environment through 2 major routes. The first route is via direct processes that either produce or consume chromium or use chromium compounds to manufacture products. Examples of such processes are like leather tanning, refractory production, chromium plating, steel production and others (US EPA, 1984).

The second route is the indirect emission through processes that do not produce chromium or its compounds but chromium was present as an impurity in the raw or materials used. For example, the chromium released during combustion of fossil fuels because it was present as a constituent of the fuel burned (US EPA, 1984). Other examples of indirect processes are cement production, asbestos mining and sewage sludge incineration.

Nowadays, chromium has been widely used in the production of pigments, fungicides, magnetic tapes, catalysts and in leather tanning. Significant amount of this heavy metal is released into the environment by these industries (Thacker *et. al.*, 2006a).

1.2.3 Hexavalent Chromium and Its Toxicity

Hexavalent chromium is one of the valence states of chromium. Hexavalent chromium is considered a dangerous pollutant, as it is believed to be mutagenic and carcinogenic. Compared to trivalent chromium which is less soluble, hexavalent

chromium is easily dissolved and in one hundred times more toxic than the trivalent state (Liu *et. al.*, 2006). When dissolved in water, hexavalent chromium is present in the forms of divalent oxyanions, CrO^{2-}_4 or dichromate, $Cr_2O^{2-}_7$ depending on the pH of the solution (Shen and Wang, 1994). According to US EPA, hexavalent chromium is one of the 17 chemicals posing the greatest threat to humans (Cheung and Hu, 2007).

Due to leakage, poor storage and improper disposal, hexavalent chromium has become one of the most frequently detected contaminants at waste dumping sites. This has led to increased awareness about the toxicity of this metal and its danger to humans. Hexavalent chromium may cause lung cancer, chromate ulcer, perforation of nasal septum and kidney damage (Thacker *et. al.*, 2006a).

The permissible levels of hexavalent chromium especially in industrial effluents and water systems are set by the authorities to meet the regulated standards. In Malaysia, the level of chromium in industrial discharge is set at 0.20 mg/l (standard A) and 1.0 mg/l (standard B) as shown in Table 1.1 (page 3). In marine water enviroment, the presence of hexavalent chromium is limited to 50 µg/L as shown in Table 1.2 (page 4).

1.3 Phenol

Phenol is an aromatic compound with an OH group attached to the benzene ring structure. It is also known as carbolic acid or hydroxybenzene. Phenol can be found in both solid and liquid form. In crystal form, phenol forms is white or colorless and sometimes pink. It can also form a thick liquid (ATSDR, 2006). Phenol has a characteristic acrid smell and sharp burning taste. In liquid state, phenol is clear, colorless, and has a low viscosity. Its melting point is 43^{o}C while the boiling point is 182^{o}C. Phenol is highly soluble in water and in most organic solvents. The structure of phenol is shown in Fig. 1.1.

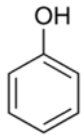

Figure 1.1: Structure of phenol

Phenol is usually used as solvent and starting material in industries such as refineries, pesticides, pharmaceutical, pulp and paper mills and others.

1.3.1 Sources of phenol

There are three sources of phenol in the environment, i.e. natural source, man made source and endogenous source.

In nature, phenol can be produced from the decomposition of organic material such as burnt woods, animal manure and others. Phenol also can occur as a constituent of coal tar in the soil environment (Murray, 1977).

Phenol can be produced from certain activities such as fossil fuel extraction and chemical manufacturing process. Oil refineries, petrochemical plants, coking plants and phenol resin plant are among the industries that produce and release phenol together with other related aromatic compounds into the environment (Haleem *et al*., 2002).

Endogenous source is an important additional source of phenol. Phenol can be formed from several xenobiotics such as benzene under the influence of light. (Hoshino and Akimoto, 1978).

1.3.2 Phenol in the environment

Phenol and its derivatives are among the most common pollutants in rivers, industrial effluents and landfill runoff water.

Phenol can also be released into the atmosphere mainly from the processing facilities. During manufacturing, phenol is released from storage tank vents during transportation. Phenol released into the atmosphere are also linked to wood burning, car exhaust, emission from waste incinerator plant and cigarette smoke (Haleem *et al.*, 2002).

Processing facilities are also major sources of phenol that eventually end up in the water system. Most industries such as resins, plastics, fiber and paper industries release phenol into the water system through waste effluents which are slightly treated or untreated. Many reports conclude that phenol can be detected in certain effluents discharged from industries (Tuah, 2006).

In soil, phenol is believed to have come from manufacturing processes. During production, transportation and loading of phenol, spills can occur which will contaminate the soil environment. Phenol also can be released into the soil when it leaches from hazardous chemical dumping wastes site or from landfill (Xing *et al.*, 1994).

1.3.3 Toxicity of phenol

Phenol and its derivatives when released into the environment through industrial effluents are toxic and persistent. They accumulate in the environment and affect organism living in the surrounding areas. Phenolic compounds are dangerous and toxic upon inhalation, contact or ingestion, even at low concentration (Yang and Lee, 2001).

At concentrations higher than 50 ppb, phenol is toxic to some of the aquatic life. For human, an oral dose of 1 g may be lethal. Exposure and continuous ingestion of phenol can cause mouth sores, diarrhea, excretion of dark urine and impaired vision (Goldfrank, 2002). The chemical also affects the nervous system and organs. In some reports, people living near rivers contaminated with phenol have been shown to suffer from some health problems such as headache, nausea, vomiting, diarrhea and abdominal pain (ATSDR, 1998).

Repeated exposure of phenol on skin may result in onychronosis (yellowing of the skin), skin irration and eruption as well as dermal inflammation and necrosis. Inhalation may lead to gastrointestinal effects such as anorexia, weight loss, excess production of saliva, muscle pain and general weakness (ATSDR, 1998).

As phenol is considered to be toxic to many living things and receives attention from various authorities, the discharge limit for phenol must be set. For Malaysia, the limit for phenol in industrial discharge is 0.001 mg/l (standard A) and 1.0 mg/l (standard B) as shown in Table 1.1 (page 3), while based on Table 1.2 (page 4), the limit of phenol level in marine water is 0.12 mg/l (Environmental Quality Act and Regulation Handbook, 1996).

1.4 Treatment of heavy metal contaminants

1.4.1 Conventional method

Heavy metal pollution is a critical environmental issue. The treatment of potential pollutants is carried in several ways such as precipitation, ion exchange and adsorption on alum, kaolinite and ash. Conventional physical and chemical methods for treating heavy metals are not cost effective and are not recommended for full scale treatment. Treating metal-contaminated industrial effluents using physico-chemical methods are relatively chemical and energy intensive. The maximum achievable chromium removal is not sufficient to meet the desired level of treated effluent quality standards for the disposal by the industries. Treatment of industrial

effluent using conventional method will also generate chemical sludge as a by - product, which also can cause contamination (Zhu *et al.*, 2008).

1.4.2 Bioremediation

The use of biological methods to treat heavy metal-contaminated waste is an emerging area of environmental conservation. Biological treatments for example bioremediation and biosorption could reduce the cost for chemicals and energy used compared to conventional methods.

Bioremediation is a process which uses living microorganisms to eliminate or reduce environmental hazard caused by toxic chemicals or waste. The basis of bioremediation is the utilization of naturally occurring or genetically engineered bacteria to transform organic and inorganic compounds to less harmful by - products.

For example, in the remediation of hexavalent chromium in contaminated environments, the reduction of chromium from hexavalent state to the trivalent state is important. Bacterial reduction of hexavalent chromium offers a potential cost effective remediation strategy (Rege *et. al*., 1997). Several bacterial species have been shown to have the chromate reducing ability, which can lead to an economical and environmentally treatment process.

1.4.2.1 Bacteria used in bioremediation.

Extensive research has been conducted using several types of bacteria to treat industrial waste especially waste containing heavy metals. Among the bacteria used are species from the genera *Arthrobacter*, *Bacillus*, *Enterobacter* and *Escherichia* (Zhu *et. al*., 2008).

1.4.2.2 Bacteria used in remediation of hexavalent chromium.

Many workers have reported the ability of bacteria to reduce hexavalent chromium to trivalent chromium (Table 1.3).

Table 1.3: Example of hexavalent chromium reducing bacteria.

Bacteria	Reference
A. haemolyticus	Zakaria *et al*., 2006
Achromobacter sp. Strain Ch 1	Zhu *et al*., 2008
Bacillus sp.	Liu *et al*., 2006
Providencia sp.	Thacker *et al*., 2006b

Providencia sp., a gram negative bacteria, can grow and reduce Cr (VI) at concentrations ranging from 100 – 400 mg/L. The other reported strains are *Bacillus* sp. which can reduce 0.33 mM CrO_4^{2-} within 22 hours, *Achromobacter* sp. strain Ch 1 with a maximum reduction capacity of 54.2 mM and *A. haemolyticus,* which can tolerate and reduce Cr (VI) up to 100 mg/L.

1.4.3 Microbial reduction of hexavalent chromium

The other dominant form of chromium which is less toxic and soluble is Cr (III). The insolubility of Cr (III) can facilitate its precipitation and removal, thus the biotransformation of Cr (VI) to Cr (III) has been considered as an alternative way for the treatment of Cr (VI) – containing waste water (Cheung and Hu, 2007).

After the discovery of microbes with the ability to reduce Cr^{6+} in the 1970s, studies on Cr^{6+} reduction has been intensified. These studies include the search for Cr^{6+} reducing bacteria in both aerobic and anaerobic conditions.

1.4.3.1 Aerobic Cr (VI) reduction

Many reports have been published on the aerobic reduction of Cr (VI) by bacteria such as *Arthrobacter* sp., *Bacillus* sp. (Megharaj *et al*., 2002), *Streptomyces* sp. (Laxman and More, 2002) and *Brucella* sp. (Thacker *et al*., 2006a).

The mechanism of aerobic reduction is usually associated with the soluble fraction of the cell which utilizes NADH as the electron donor (Thacker *et al*., 2006b). In aerobic condition, Cr^{6+} reduction occurs as two or three step processes. It starts with Cr^{6+} reduced to short lived intermediates Cr^{5+} and/or Cr^{4+} before further reduction to a stable end product, Cr^{3+}. But it is unclear whether the reduction of Cr^{5+} to Cr^{4+} and Cr^{4+} to Cr^{3+} were spontaneous or enzyme - mediated (Czako-Ver *et al*., 1999).

From the report by Cheung and Hu (2007), Cr (VI) reductases ChrR (identified from *Ps. Putida* MK 1) reduces Cr^{6+} with one electron shuttle to generate Cr^{5+}, followed by two electron transfer to form Cr^{3+}. Reducing enzyme, YieF (identified from *E. coli* chromosome), a unique enzyme which can catalyze the reduction of Cr^{6+} to Cr^{3+} through four electron transfer, where three electrons were consumed in reducing Cr^{6+} and one electron transferred to oxygen.

1.4.3.1 Anaerobic Cr (VI) reduction

A number of bacteria were found to have the ability to reduce Cr (VI) in the absence of oxygen. *E. cloacae*, a reducing facultative anaerobe, is an example where in the presence of oxygen, the Cr (VI) reduction is inhibited. However, the reduction process is resumed when the oxygen content is removed (Laxman and More, 2002). Other reported strains for the anaerobic reduction are *Shewanella putrefaciens* MR -1 (Myers *et. al*., 2000), *Desulfovibrio desulfuricans* (Tucker *et al*., 1998) and *Shewanella oneidensis* (Daulton *et al*., 2006).

Anaerobic Cr (VI) reduction, was initially considered as a fortuitous process since it provides no energy for the microbial growth. However, a SRB isolate was discovered later, which can utilize the energy generated from the anaerobic reduction for its growth. In the absence of oxygen, Cr (VI) can act as the terminal electron acceptor in the respiratory chain for a large array of electron donors such as protein, carbohydrate and hydrogen (Cheung and Hu, 2007).

1.5 Organic waste treatment

1.5.1 Conventional method

Like heavy metals, organic wastes are also physic – chemically treated using filtration, flotation, ion exchange and adsorption. However, these techniques are costly and energy intensive, beside used large amount of chemical and create secondary pollution.

1.5.2 Biodegradation

Biodegradation can be described as a natural way of recycling waste. It involves the breaking down of organic matter into nutrients that can be used by other organisms. "Degradation" means decay while "bio" prefix means that the decay process is carried out by assortment of bacteria, fungi, algae, insect and other organisms which consume certain materials and will be recycled into another form.

Biodegradation processes vary greatly in extent, but in the end, the final product of degradation is almost the same, which is carbon dioxide or methane. Organic material can be degraded in both aerobic and anaerobic conditions.

The term biodegradation is often related to certain aspects such as ecology, waste management and environmental remediation. Biodegradation is the aspect of waste management and can be defined as the breaking down of organic contaminants into smaller compounds which can also reduce toxicity and leads to a solution in solving pollution problems. Biodegradation is a key process in the natural attenuation of contaminants in areas contaminated with hazardous chemicals.

1.5.2.1 Microorganisms involved in biodegradation

Biodegradation process can be carried out by many types of microorganisms including bacteria, fungi, algae and some organisms such as worms. In natural environment, the rates of degradation processes depend on certain factors; physical, chemical and biological factors which vary among the different ecosystems (van Agteren et al., 1998).

1.5.2.2 Microorganisms in degradation of phenol

Phenol degrading microorganisms have been reported as early as 1908 (Evans, 1947). Phenol can be degraded by many species belonging to various genera. Many reports have been published regarding the degradation of phenol by microorganisms including bacteria, fungi, algae and yeast (Table 1.4).

Table 1.4: Examples of phenol degrading microorganisms

Microorganism	Reference
A. Bacteria	
A. calcoaceticus	Nakamura and Sawada, 2000
Brevibacillus spp. Strain P-6	Yang and Lee, 2001.
Pseudomonads spp.	De Lipthay *et al*., 1999
Rodococcus spp	Margesin *et al*., 2004
B. Fungi	
Coprinus spp.	Guiraud *et al.*, 1999
Geotrichum candidum	Garcia *et al.*, 2000
C. Yeast	
Candida tropicalis	Bastos *et al*., 2000
D. Algae	
Ochromonas danica	Semple and Cain, 1995

1.5.3 Phenol Biodegradation

Phenol can be degraded by bacteria under two conditions, aerobic or anaerobic. In aerobic condition, phenol is degraded into carbon dioxide while under anaerobic condition is degraded into carbon dioxide or methane (Tay and Show, 2006). During the degradation process, many intermediates are produced in the pathway before the final product, carbon dioxide or methane is produced. Intermediates in the phenol degradation process are benzoate, catechol, cis, cis – muconate, β – ketodipate, succinate, acetate (Knoll and Winter, 1987).

Phenol can be degraded in its free form or after adsorption onto soil or sediment, which may result in lower rate of degradation process (Knoll and Winter, 1987). According to a report by Howard (1989), the highest rate for aerobic phenol degradation is in sewage (>90% in 8 hours of retention time), followed by fresh water (biodegradation less than 1 day), soil (complete degradation in 2 – 5 days) and sea water (50% degradation in 9 days). In anaerobic degradation, the rate is lower than in aerobic condition (Baker and Mayfield, 1980).

1.5.3.1 Aerobic degradation

In aerobic condition, oxygen will be used as the electron acceptor in electron transfer process. During the transfer of electron between the electron donor and acceptor, a substrate is essential to create and maintain biomass. In this case, phenol is the main substrate and must be available in order to create active biomass in the degradation process. From the study by Ritmann and Sàez (1993), once the active biomass is present, any biotransformation activity can take place with the microorgasnism which possess enzyme that can catalyze the reaction. These enzymes usually define the range of substrates that can be transformed through certain metabolic pathways (Pieper and Reineke, 2000).

In aerobic metabolism, the first step is the hydroxylation of phenol into catechol by phenol hydroxylase, an NADPH dependent flavoprotein. During the hydroxylation, the aromatic ring will be incorporated with one oxygen atom to form catechol. The second step is the catalyzation process by catechol 1,2 – dioxygenase or catechol 2,3 – dioxygenase. After a series of subsequent steps, the product will be incorporated into the Kreb cycle or tricarboxylic acid cycle (TCA). It has been established that the degradation process of phenolic compound is metabolized by various strain in either the *meta* or *ortho* cleavage pathways (Shingler, 1996). The aerobic degradation of phenol is shown in Figure 1.2.

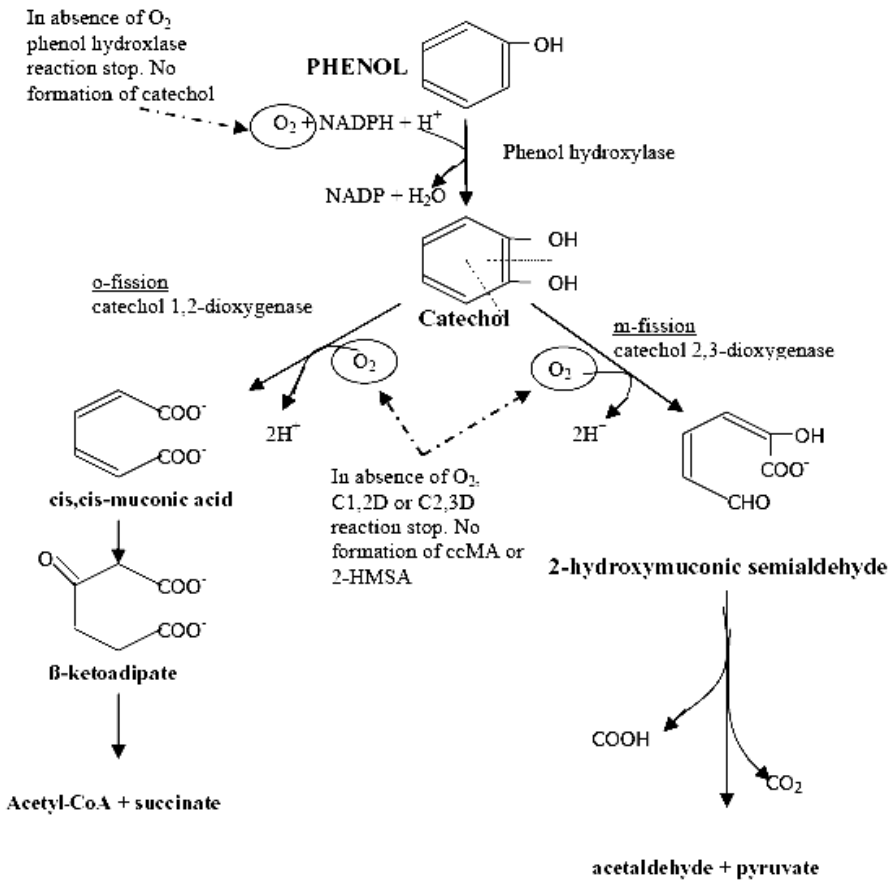

Figure 1.2: The main pathway of phenol degradation under aerobic condition (*ortho* and *meta* fission of the benzene ring) (Krug and Ziegler, 1985). (Note: C1, 2D – catechol 1, 2 – dioxygenase and C2, 3D – catechol 2, 3 – dioxygenase).

Bacteria normally degrade phenol via the *meta* pathway; however some bacteria can degrade phenol via both the *meta* and *ortho* pathway. An example of this is *Pseudomonas* sp. (De Lipthay *et al*., 1999).

1.5.3.2 Anaerobic degradation

Phenolic compounds are one of the most common pollutants in landfill. Landfill is believed to be a habitat for anaerobic microbial species which can degrade many compounds such as toluene and phenol. In landfill area, anaerobic condition

occurs during decomposition of the waste materials and carbon dioxide will be the electron acceptor (Barlaz, 1996).

Various bacteria have been reported to degrade phenol anaerobically (van Schie and Young, 1998; Shinoda *et al*., 2000). Phenol is carboxylated to 4 – hydroxybenzoate and growth of the bacteria on phenol was dependent on the presence of carbon dioxide (Tschech and Fuchs, 1987). Benzoate is a key intermediate for the degradation process for aromatic compounds such as phenol.

The carboxylation process of phenol is a 2 step process involving the formation of phenylphosphate as the first intermediate. Phenylphosphate is postulated as the substrate for second enzyme (E2 – phenylphosphate carboxylase) which requires Mn^{2+} and catalyze the carboxylation of phenylphosphate to 4 – hydroxybenzoate (Lack and Fuch, 1992). The overall anaerobic degradation process of aromatic compound by denitrifying bacteria is shown in Figure 1.3.

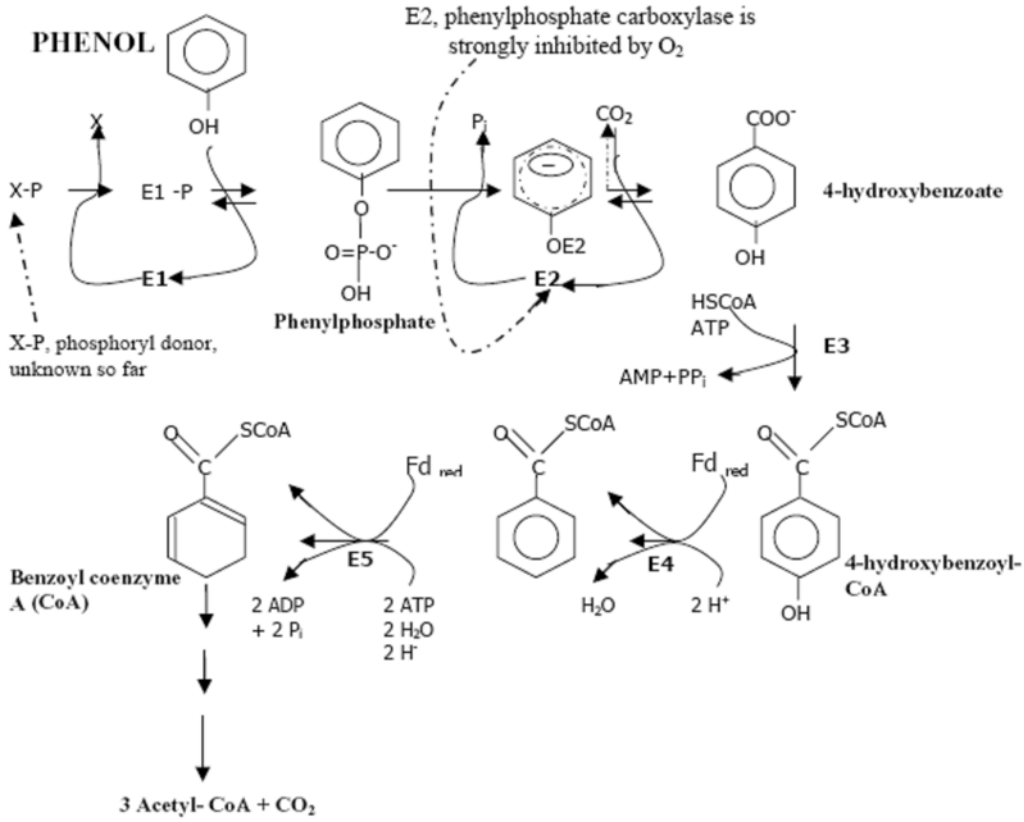

Figure 1.3: Anaerobic phenol degradation process of aromatic compound by the denitrifying bacteria *T. aromatic*. E1 – phenylphosphate synthesis; E2 – phenylphosphate carboxylase; E3 – 4 – hydroxybenzoate CoA ligase; E4 – 4 – hydroxybenzoyl CoA reductase; E5 – benzoyl CoA reductase; Fd – ferredoxin; X – P – phophoryl donor (Breinig *et al.*, 2000).

1.6 Bioaccumulation

1.6.1 Overview of the bioaccumulation process

Bioaccumulation can be defined as a process by which organisms accumulate chemicals both directly from the abiotic environment such as soil, water and soil or from dietary sources (Hodgson, 2004). It is commonly known that the organisms

living in the environment have the ability to adsorb chemicals from their surrounding via their gills, outer protective layer or direct ingestion of contaminated food or water. Bioaccumulation occurs either because the chemicals are taken up faster than it can be used, or due to the inability to break down or metabolize the chemicals. Usually, the level of such chemical contaminants in the environment is quite low. These adsorbed chemicals may be metabolized, excreted or stored within the fatty acid tissue layer of the organism (Robinson and Thorn, 2001).

In bioaccumulation, several steps are involved including direct partitioning between air and water with the living organisms, or more complex transfer processes where the compound is taken up with food and transport internally to other parts of the organism (Schwarzenbach *et al*., 2002). There are several steps involved in the uptake of chemicals by the microorganisms. The process involves the passage of compound through biological membrane, mediated by a carrier or as a single solute. The major uptake process for many organic compounds and metals is the passive diffusion, where the driving force involves the fugacity difference between water and organism (van Leeuween and Vermeire, 2007). Fugacity can be described as the tendency of a substance to prefer one phase (liquid, solid or gas) over the other phase.

Therefore, bioaccumulation is best described through the concept of fugacity. Organisms have the tendency or higher capacity to store xenobiotic compound per unit volume than water. For example, metals which can bind to protein (metallothionein), can be stored in relatively high concentration within the organism. Usually, organic compounds are stored in lipids whereas organometals, can be stored in either lipids or protein. Thus the bioaccumulation may reach high concentrations in the organism (van Leeuween and Vermeire, 2007).

1.6.2 Bioaccumulation of toxic compounds

In the environment, there are various sources of toxic pollutants, which living organisms can accumulate directly or indirectly. One example of chemical

contaminant that can be accumulated by organisms is pesticide. Besides that, many organic compounds originating from certain industries such as automobile, agriculture and pulp and paper industries can also be released into the environment. These contaminants can spread into the soil or small creeks, and eventually find their way into water bodies such as rivers, estuaries and the ocean. Once the toxic pollutants are in the soil or water, they can easily enter the food chain. There are several examples of toxic compounds that can accumulate in living organisms e.g. mercury, silver as well as insecticide such as DDT and pentachlorophenol (Connell, 1990).

1.6.3 Bioaccumulation of phenolic compounds

The presence of organic pollutants in the environment which originated from various sources could affect living organisms. Some of these contaminants have the potentials to be accumulated by aquatic organisms, either directly from the water or via the dietary route of aquatic food chains (Newsted, 2004). Examples of the pollutants which can be accumulated by organisms include phenol and its related compounds. Previous studies reported the bioaccumulation of phenolic compounds, such as the accumulation of phenol by green algae, *Selenastrum capricornutum* (Newsted, 2004) or 2, 4 – dichlorophenol by fresh fingernail clam, *Sphaerium corneum* (Guerrero *et al*., 2007)

1.7 Biosorption

1.7.1 Overview of biosorption

Biosorption can be defined as the passive uptake of toxicants by dead or inactive biological materials or materials derived from biological sources (Vijayaraghavan and Yun, 2008). It can also be described as a property of certain types of inactive, dead microbial biomass which can bind and concentrate metals

from aqueous solution. The special surface properties of the biomass enable them to adsorb different kinds of contaminants from the solution (Aksu, 2005).

Biosorption process holds some advantages over the conventional methods such as low cost operation, high efficiency and selectivity, minimization of chemical or biological sludge, possible metal recovery besides the reuseability of the biosorbent used (Kratochvil and Volesky, 1998). The use of dead cells in the biosorption process itself has their own advantages as they do not require continuous supply of nutrients, not affected by the toxic waste, and their reusable nature. Dead cells can also be stored or used for extended periods at room temperature, besides its abilities to accumulate pollutants to the same or greater extent in comparison to resting or growing cells (Aksu, 2005).

1.7.2 Biosorbent

Studies on the binding capacity by several types of biomass has been conducted since 1985, where some of the biomass types were found to be very effective in accumulating heavy metals and organic substances (Vieira and Volesky, 2000). In general, there are many types of biosorbents that can be used for the removal of heavy metals or organic materials. They can be divided into several categories including bacteria, fungi, algae, industrial waste, agriculture waste and polysaccharide materials.

Several factors should be considered in selecting the biosorbent for clean up purposes such as the availability and the origin, cost, selectivity and reusability besides the effectiveness of the biomass. The examples of the biosorbents that have been used in previous research are listed in Table 1.5.

Table 1.5: Examples of biosorbents used in the biosorption process.

Name	References
A) Bacteria	
Bacillus sp	Tunali *et al*., 2006
Pseudomonas sp	Ziagova *et al*., 2007
B) Fungal	
Aspergillus niger	Rao and Viraraghavan, 2002
Penicilium	Tan and Cheng, 2003
C) Algae	
Red seaweed	Davis *et al*., 2003
Blue seaweed	Davis *et al*., 2003

1.7.3 Mechanism in the biosorption process

The mechanism of biosorption is a bit complex, as the process may be one or a combination of complexation, ion exchange, coordination, adsorption, electrostatic interaction and microprecipitation. The complex cellular structure of the microorganism provides many ways for the contaminant to be taken up by the microbial cell. The biosorption mechanisms are various and depend on the biomass used, and they are still not fully understood (Ahalya et al, 2003).

In biosorption, as dead biomass was used, a non – metabolic process is involved. The pollutant uptake is mediated by the physicochemical interaction between the metal/organic materials with the functional groups present on the microbial cell surface. This process is based on the physical adsorption, chemical sorption and ion exchange, which are not dependent on cell metabolism. As the uptake process by the inactive cell is extracellular, the chemical functional groups of the cell wall play important roles in the biosorption process. Due to the nature of the cellular components, several functional groups are present on the microbial cell wall,

including the carbonyl, sulphate, carboxyl, amine, phosphate and hydroxyl groups (van der Wal *et al*., 1997).

1.7.4 Biosorption isotherm

The adsorption capacity of a biosorbent can be calculated using an equation. Equation 1.1 is the equation for the measurement of uptake by the biosorbent, where the unit is expressed as milligram of solute sorbed per gram of the biosorbent material (mg/g) (Vijayaraghavan and Yun, 2008).

$$Q = (V_oC_o - V_fC_f)/ M \qquad \text{Equation 1.1}$$

Where, Q - solute uptake (mg/g)

C_o - initial solute concentration in solution (mg/L)

C_f - equilibrium solute concentration in solution (mg/L)

V_o - initial solution volumes (L)

V_f - final solution volumes (L)

M - mass of the biosorbent (g)

1.7.5 Equilibrium modeling of biosorption

Adsorption isotherms are the basic requirement for the designation of biosorption system for the removal of the targeted compound. These empirical models are simple mathematical relationships, characterized by a set of limited number of adjustable parameters. These models give a good description of the experimental behavior over a large range of operating conditions. Some models which are frequently employed and established, usually involves two, three or more parameters to model the isotherm data. Langmuir, Freundlich, Langmuir – Freundlich, Redlich Peterson and Brunauer – Emmet – Teller (BET) and Radke – Prausnitz are amongst the models that are usually used in the biosorption study

which describe the non linear equilibrium between the sorbed materials on the cell (q_{eq}) and the materials in the solution (C_{eq}) at a constant temperature.

1.7.6 Biosorption of organic contaminants

Organic contaminants found in waste water can be degraded or detoxified using several techniques including physical, chemical and biological treatment before being released into the environment. Even though the stated methods can degrade some of the organic pollutants, the byproducts formed during the degradation process could be harmful and dangerous. Some compounds are nondegradable and require alternative removal technique. From current studies, these types of contaminants can be removed from the wastewater using the biosorption process (Aksu, 2005).

Biosorption has emerged as an alternative or supportive technique as a removal process in dealing with organic contaminants in waste water. The examples of organic pollutants which cause great concern are dyes, pesticides and phenolic compounds due to their extreme toxicity and persistency in the environment. Many types of biomass, including live and dead microorganisms have been investigated and used in the biosorption study (Ahalya *et al*, 2003).

1.7.6.1 Phenol biosorption

Phenol is one of the organic contaminants which can be removed using either the conventional or biological methods. Biological methods which can be applied for the removal of phenol from the waste water are biodegradation, bioaccumulation and biosorption.

Many microorganisms have been found to adsorb phenol and phenolic compound including algae, fungi and bacteria. The examples of microorganisms

used for the biosorption of phenol and its related compound are *Aspergillus niger* (Rao and Viraraghavan, 2002), *Rhizopus arrhizus* and *Phanerochaete chrysosporium* (Juan and Yu, 2005). Besides the single type microorganism, the use of a bacterial consortium in biosorption has also been investigated as reported by several researchers (Aksu *et al*., 1999; Antizar – Ladislao and Galil, 2004), where the consortium bacteria was used for the biosorption of phenol with nickel or chlorophenol, respectively.

1.8 *Acinetobacter haemolyticus*

Acinetobacter is a Gram negative bacteria belonging to the phylum Proteobacteria. *Acinetobacter* species are oxidase – negative and occur in pairs when seen under the microscope.

Acinetobacter haemolyticus is strictly aerobic. This species occurs as rods in the early stage of growth and shows coccobacillary morphology in the later stage of their growth. *A. haemolyticus* grows well at a temperature range of 20 – 37^{o}C with the optimum at 33 – 35^{o}C. *A. haemolyticus* are widely distributed in nature and can be found in soil, sewage and water environment (Zakaria, 2006).

1.9 Objectives of the study

The aim of this research is to investigate chromium (VI) bioreduction and phenol biodegradation using the batch and the column systems.

1.10 Scope of the study

The research will focus on five main aspects:

1. Determination of the presence of both contaminants; Cr (VI) and phenol in selected industrial waste

2. Assessing the adaptability of bacteria in simulated waste solution; i) Cr (VI) waste, ii) phenol waste and iii) Cr (VI) and phenol mixed waste.

3. Evaluation of the suitability of a growth medium for the cultivation of bacteria i.e. liquid pineapple waste.

4. Determining the ability of bacteria in treating both contaminants separately and simultaneously using the batch and column system.

5. Investigation on other mechanism for phenol removal from the waste solution by the bacteria i.e. the bioaccumulation or biosorption process.