BIOREGENERATION OF GRANULAR ACTIVATED CARBON LOADED WITH PHENOL AND *O*-CRESOL BY IMMOBILIZED BIOMASS: QUANTIFICATION AND KINETIC STUDIES

by

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LIST OF ABBREVIATIONS

AC	Activated carbon
AIB	Abiotic immobilized bead
BET	Brunauer-Emmett-Teller isotherm model
BJH	Barret-Joyner-Halenda pore size distribution
COD	Chemical oxygen demand
DB	Dried biomass
EDX	Energy Dispersive X-ray
FTIR	Fourier Transform Infrared Spectroscopy
GAC	Granular activated carbon
IB	Immobilized biomass
IPB	Immobilized PAC-biomass
MLSS	Mixed liquor suspended solid
MLVSS	Mixed liquor volatile suspended solid
PAC	Powdered activated carbon
PRH	Pyrolysed rice husk
PVA	Polyvinyl alcohol
SB	Suspended biomass
SBR	Sequencing batch reactor
SEM	Scanning Electron Microscope
UV-Vis	Ultraviolet-visible
XOCs	Xenobiotic organic compounds

LIST OF SYMBOLS

C_t	Residual substrate concentration in the bulk solution at time t (mg/L)
C_e	Equilibrium substrate concentration (mg/L)
C_o	Initial residual substrate concentration (mg/L)
k	Rate constant of biodegradation (1/h)
k_a	Rate constant of adsorption for biotic system (L/mg h)
k_d	Rate constant of desorption for biotic system (1/h)
k_{ir}	Rate constant of irreversible adsorption (1/h)
k'_a	Rate constant of adsorption for abiotic system (L/mg h)
k'_d	Rate constant of desorption for abiotic system (1/h)
K_L	Langmuir constant related to adsorption energy (L/mg) of the binding sites
m	Adsorbent dosage (g/L)
η	The effectiveness factor
R_L	The favorability of adsorption
ϕ	The Thiele modulus
v	Biodegradation rates obtained with immobilized biomass (mg/L h)
v_s	Biodegradation rates obtained with suspended biomass (mg/L h)
Q_t	The amount of substrate adsorbed at time t (mg/g)
Q_α	The experimentally determined maximum amount of adsorbed substrate irreversibly adsorbed on the adsorbent (mg/g)
Q_e	The amount of substrate adsorbed at equilibrium (mg/g)
Q_r	The amount of substrate adsorbed at the reversible sites (mg/g)
Q_o	Initial loading adsorption capacity of the adsorbent (mg/g)
Q_{max}	The limiting adsorption capacity of adsorbent for the substrate (mg/g)

BIOREGENARASI BAGI BUTIRAN KARBON TERAKTIF YANG TERJERAP DENGAN FENOL DAN *O*-KRESOL OLEH BIOJISIM TERPEGUN: KAJIAN KUANTIFIKASI DAN KINETIK

ABSTRAK

Objektif kajian ini adalah untuk (i) membangun satu pendekatan baru bagi bioregenerasi butiran karbon teraktif (GAC) yang terjerap dengan fenol dan *o*-kresol dengan menggunakan biojisim terampai dan terpegun serta serbuk karbon teraktif (PAC)-biojisim terpegun, (ii) menentukan dan membandingkan kecekapan bioregenerasi GAC yang terjerap dengan fenol dan *o*-kresol dengan menggunakan biojisim terampai dan terpegun serta PAC-biojisim terpegun dan (iii) membangun model kinetik untuk menghuraikan bioregenerasi GAC yang terjerap dengan fenol dan *o*-kresol dengan menggunakan biojisim terpegun.

Pengoptimuman keadaan biojisim terpegun telah didapati berdasarkan kajian-kajian seperti saiz manik, larutan pH awal, kepekatan substrak awal dan kepekatan biojisim awal atas kadar biodegradasi bagi fenol dan *o*-kresol, masing-masing, dengan menggunakan biojisim terampai dan terpegun serta PAC-biojisim terpegun. Berdasarkan keputusan yang didapati, biojisim terpegun dalam 4-mm manik dengan kepekatan biojisim 2.0 g/100 mL gel telah dipilih sebagai keadaan optimum untuk pengunnaan dalam proses bioregenerasi.

Mikrograf SEM menunjukkan kehadiran kolonialisasi biojisim atas permukaan GAC bagi GAC terbioregenerasi dengan menggunakan biojism terampai tetapi tiada biojisim pada GAC terbioregenerasi apabila biojisim terpegun digunakan. Keputusan menandakan bahawa teknik pemegunan biojisim telah meringankan masalah pemisahan antara GAC terbioregenerasi dengan biojism serta meminimumkan masalah bio-tempil. Kecekapan bioregenerasi dikuantifikasikan dengan menggunakan teknik pengukuran terus jumlah substrat terjerap pada GAC. Bagi pendekatan penjerapan dan biodegradasi secara berturutan, keputusan menunjukkan bahawa kecekapan bioregenerasi GAC yang terjerap dengan substrat fenolik tertentu adalah sama pada asasnya sama ada biojisim terampai atau terpegun yang digunakan. Bagi pendekatan penjerapan dan biodegradasi secara serentak, kecekapan bioregenerasi GAC yang terjerap dengan *o*-kresol adalah lebih rendah berbanding dengan GAC yang terjerap dengan fenol. Ini menunjukkan bahawa pecahan penjerapan *o*-kresol yang takterbalik adalah lebih tinggi disebabkan oleh pempolimeran teroksida. Kesan bermanfaat dengan menggunakan PAC-biojisim terpegun adalah penambahan kadar penyingkiran sebatian fenolik melalui penjerapan serta pemendekan tempoh masa bioregenerasi.

Model kinetik bagi bioregenerasi GAC yang terjerap dengan fenol dan *o*-kresol telah dibangunkan bagi kedua-dua pendekatan. Model kinetik dipadankan dengan baik ($R^2 > 0.8$) kepada data eksperimen mengenai perjalanan masa bagi jumlah substrat terjerap pada GAC dan kepekatan baki substrak dalam larutan pukal. Didapati bahawa penyahjerapan adalah langkah penentu-kadar bagi bioregenerasi GAC hanya pada dos GAC yang tinggi (2.0 – 3.0 g/L) dan kepekatan substrat awal yang rendah (150 mg/L fenol dan 200 mg/L *o*-kresol) bagi kedua-dua pendekatan penjerapan dan biodegradasi secara berturutan dan serentak.

BIOREGENERATION OF GRANULAR ACTIVATED CARBON LOADED WITH PHENOL AND *O*-CRESOL BY IMMOBILIZED BIOMASS: QUANTIFICATION AND KINETIC STUDIES

ABSTRACT

The objectives of this study were to (i) develop a novel approach to bioregenerate GAC loaded with phenol and *o*-cresol, respectively, using immobilized biomass as well as immobilized PAC-biomass, (ii) quantify and compare the bioregeneration efficiencies of GAC loaded with phenol and *o*-cresol, respectively using suspended and immobilized biomasses as well as immobilized PAC-biomass, and (iii) develop kinetic models to describe the bioregeneration process of GAC loaded with phenol and *o*-cresol, respectively, using the immobilized biomass.

The optimization conditions of biomass immobilization technique were obtained based on the studies of bead size, initial pH solution, initial substrate concentration and initial biomass concentration in beads on the biodegradation rates of phenol and *o*-cresol, respectively, using the suspended and immobilized biomasses, as well as immobilized PAC-biomass. Based on the results, immobilized biomass in 4-mm bead with biomass concentration of 2.0 g/100 mL gel was chosen as the optimized condition for application in the bioregeneration process.

The SEM micrographs showed the presence of the biomass on the surface of GAC for bioregenerated GAC using suspended biomass but no biomass was found on the bioregenerated GAC when immobilized biomass was applied. The result indicated that biomass immobilization technique has successfully minimized the biofouling problem and solved the separation problem between the bioregenerated GAC and biomass. Bioregeneration efficiency was quantified using direct measurement of the amount of adsorbed substrate on GAC. Under the sequential adsorption and biodegradation approach, the results showed that the bioregeneration efficiency of GAC loaded with the respective phenolic substrate was basically the same irrespective of whether suspended or immobilized biomass was used. Under the simultaneous adsorption and biodegradation approach, lower bioregeneration efficiency of *o*-cresol-loaded GAC compared to phenol-loaded GAC indicated higher fraction of irreversible adsorption for *o*-cresol due to oxidative polymerization. The beneficial effect of using PAC in immobilized PAC-biomass was the enhancement of the removal rate of the phenolic compounds via adsorption and the shortening of the bioregeneration duration.

Kinetic models were developed to describe the bioregeneration of phenol- and *o*-cresol-loaded GAC for both approaches. The kinetic models gave a relative good fit ($R^2 > 0.8$) to the experimental data. The desorption process was found to be the rate-determining step in bioregeneration only at high GAC dosage (2.0 – 3.0 g/L) and at low initial substrate concentration (150 mg/L phenol and 200 mg/L *o*-cresol), respectively, for both sequential and simultaneous approaches.

CHAPTER 1

INTRODUCTION

1.1 Background

Adsorption onto activated carbons is recognized as an efficient, promising and well-established technology in air pollution control, metal recovery and wastewater treatment. Usually, the activated carbon is incinerated or discarded in a landfill when the adsorption capacity is exhausted. Despite its excellent adsorptive capacity in water purification, the biggest limitation of activated carbon is its relatively high preparation cost. Therefore, regeneration of spent activated carbons is desirable to minimize operational expenditures. There are three commercial disposal methods for the spent activated carbon such as thermal regeneration, landfill deposition and incineration.

For thermal regeneration, the spent adsorbent is heated at temperatures up to 900 °C and steam or inert gas serves as the transport medium in reducing the concentration of the desorbed components (Wen et al., 2011). Yet, thermal regeneration is expensive in terms of energy consumption, time-consuming and suffers from 5% to 15% carbon loss due to oxidation and attrition (Robers et al., 2005). Landfilling deposition is less expensive than incineration and is the preferred option if the spent carbon is not suitable for thermal regeneration. In fact, the traditional disposal method in Malaysia is via open-dumped landfilling practices but it is gradually being limited due to the lack of landfill management aspects (Samsudin and Don, 2013). Incineration is the most expensive of the three options but the one with the least potential liability. Up to the present, Kualiti Alam Sdn. Bhd., one of the waste management companies in Malaysia, is permitted to operate the incinerator in Malaysia. In fact, the first Kualiti Alam Modularised Incinerator plant was set up at the company's waste management centre in Bukit Nenas near Seremban, Negeri Sembilan, Malaysia in 2009. Yet, insufficient proof of safety of incineration technologies was highlighted through the media to the public as the escape of hazardous emissions could potentially lead to another environmental issue which have received nation-wide attentions from many of authorities and ordinary citizens (Abd Kadir et al. 2013).

The offline-bioregeneration of the spent activated carbon is perhaps the most eco-friendly and cost effective option to regenerate the spent activated using microbes. The offline-bioregeneration was also known as sequential adsorption and biodegradation processes. Under the offline-bioregeneration approach, the exhausted adsorbent collected from wastewater treatment plant was re-suspended into biodegradation vessel for bioregeneration and biodegradation processes by microbes. In fact, the involvement of biomass in the offline-bioregeneration provides the potential of complete bio-mineralization, and of eliminating the secondary pollution as occurred in the thermal regeneration and the landfilling deposition as well as the incineration process. As such, it may have a direct bearing on the future directions for the solution of the spent activated carbon using a more environmentally friendly approach. Thus, the use of biomass in regenerating the activated carbon was actively explored in this project.

1.2 Phenolic compounds

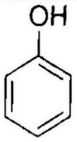
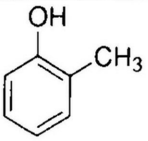
Phenolic derivatives are considered as the XOCs which are present in the wastewater from industries that the involved in the manufacture of a variety of chemicals such as plastics, dyes, cleaning agents and some pharmaceutical products (Vasu, 2008). Some of the phenolic derivatives have been listed as the priority pollutants by US Environmental Protection Agency (USEPA). Proper handling and treatment of phenolic derivatives in wastewater are therefore essential to retard the environmental damage. In this study, phenol and *o*-cresol were chosen due to their widespread industrial applications. Some of the common physical and chemical properties of phenol and *o*-cresol are shown in Table 1.1. Phenol and its methylated phenolic derivatives such as cresol isomer (*o*-, *m*-, and *p*-cresol) are widely involved in the industrial applications such as phenolic resin and coal conversion plants (Ho et al., 2010).

1.3 Removal processes for phenolic compounds

1.3.1 Biodegradation by suspended biomass

Biological remediation technology has the potential to completely mineralize phenol and cresol isomers at relatively low cost. The term biodegradation is defined as complete biomineralization of the phenolic compounds into simple by-

Table 1.1: Basic information concerning phenol and *o*-cresol (Dąbrowski et al., 2005).

Compound	**Phenol**	***o*-Cresol**
Structure		
Molar mass (g/mol)	94.11	108.14
Physical observation	White crystalline solid, strong phenol-like odour	Liquid , strong phenol-like odour
Boiling point (°C)	182	191
pK_a at 25 °C	9.89	10.20
Aqueous solubility at 25 °C (g/L)	93	25

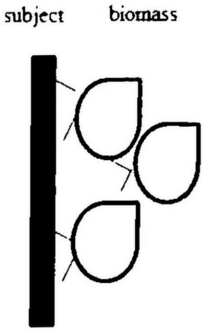
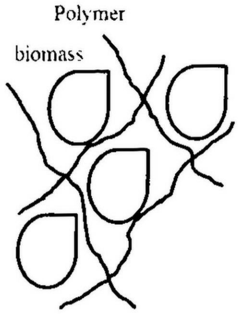
products such as CO_2, H_2O, NO_3 and other inorganic compounds. The presence of the attached functional groups such as methyl group in cresol, severely lowers its biodegradability as compared to phenol. As demonstrated by other reports, suspended microorganisms are significantly inhibited by phenol and cresol at relatively high concentrations (Maeda et al., 2005; Bai et al., 2007; Saravanan et al., 2008; Ho et al., 2010).

1.3.2 Biodegradation by immobilized biomass

Over the years, the involvement of suspended biomass in biodegradation process has been shown to be eco-friendly, practical and economical as complete mineralization of phenolic compounds in the wastewater is feasible. However, at relatively higher concentrations, phenol and cresol are difficult to be treated due to inhibitory effect exerted by them. Despite the fact that adequate level of phenol can be treated successfully by suspended biomass, many operational limitations still remain (Massalha et al., 2010).

In recent years, biomass immobilization technique was developed by which the suspended biomass was immobilized using a porous polymer gel (El-Naas et al., 2009; Massalha et al., 2010; Al-Zuhair and El-Naas, 2011). There are three basic methods for biomass immobilization and the relative merits and demerits are summarized in Table 1.2 Among the three methods, entrapment is the most widely employed techniques for biomass immobilization due to the flexibility and fast approach involving simple mixing of the biomass and polymeric solution. It is usually employed because of several merits, including protection of the bacteria from the direct exposure of high initial phenolic concentrations, ease of separation and potential of reutilization of the biomass (Chen et al., 1998; Song et al., 2005;

Table 1.2: Comparison of the relative merits and demerits among the biomass immobilization techniques (Bickerstaff, 1997).

Immobilization	Image	Description
Adsorption		The forces involved : -mostly weak -electrostatic -van der Waals force -ionic or hydrogen bonding Advantages: -little damage to biomass -simple, and cheap Disadvantages: -Leakage of biomass -Reversible of the bonding -Non-specific binding -Long period of incubation for biomass attachment
Entrapment		The biomass is restricted in lattice polymeric gel by mixing the biomass and the polymeric solution and then polymerized into gel. The porosity of the gel allows transfer of substrate and product. Advantages: -Rigidity and porosity of gel is controllable by its monomer -High loading of cell is allowed -Desired shape and size of gel can be obtained via chemical polymerization Disadvantages: -Mass transfer limitation -Some polymerization methods are toxic to biomass (Chen et al., 1998)
Encapsulation		It is achieved by encapsulating the biomass within various form of semipermeable membrane. Advantages: -Co-immobilization of biomass Disadvantages: -Not suitable for growth type biomass, as rupture of membrane due to fast growth of biomass -Mass transfer limitation -Low density of biomass

El-Naas et al., 2009; Al-Zuhair and El-Naas, 2011).

Mass transfer process between the substrates and the immobilized biomass in the entrapment technique is the crucial issue for the biodegradability efficiency. Although the biomass immobilization allows high loading of biomass concentration per unit bead, permits reusability of immobilized bead and prevents biomass from washout in a continuous process, mass transfer resistance of substrate in immobilized biomass remains a serious problem and is difficult to be solved. Many studies on diffusion modeling on the biodegradation of phenol by entrapped biomass in a porous gel have been reported (Aksu and Bülbül, 1999; Chung et al., 2003; Hsieh et al., 2008; Tepe and Dursun, 2008). Based on their studies, a simplified mathematical model that incorporates simultaneous diffusion and the biodegradation processes with some basic assumptions was developed to determine the effective diffusion coefficient of phenol within the immobilized bead. The basic assumptions are as follows:

1. All the immobilized beads are spherical in shape with same radius at isothermal conditions.
2. There is uniform distribution of biomass within the immobilized bead.
3. Leakage of biomass into the bulk solution is negligible.
4. No partition of the phenol are present between outer and inner of the bead, at which the external mass transfer barrier is negligible due to fast shaking of beads in the nutrient solution.
5. The biodegradation rate is expressed by using the first-order kinetics which is the right assumption at relatively low concentration.

The effective factor, η in Eq. (1.1) is defined as the ratio of the biodegradation rate using immobilized biomass (v) with diffusion limitation to the biodegradation rate using suspended biomass (v_s).

$$\eta = \frac{v}{v_s} \tag{1.1}$$

The biodegradation rate is determined from the gradient of the time course plot of the residual substrate concentration in the bulk solution at each of the substrate concentrations. The quantity of η represents the extent of diffusion limitation in the immobilized biomass.

The Thiele modulus, ϕ, is related to η as shown in Eq. (1.2) (Hsieh et al., 2008; Tepe and Dursun, 2008).

$$\eta = \frac{3}{\emptyset}[\frac{1}{\tanh \emptyset} - \frac{1}{\emptyset}] \tag{1.2}$$

Hence, for small values of ϕ or η approaches 1, there is little effect of internal diffusion limitation on the rate of the biodegradation of immobilized biomass. On the other hand, the larger the quantity of ϕ or η approaches 0, the lower is the biodegradation rate of immobilized biomass, as internal diffusion is dominant.

1.3.3 Adsorption

In addition to the removal of phenolic compounds by biodegradation, adsorption offers an effective technology in removing phenolic compounds from aqueous solutions. Activated carbon (AC) is considered as one of the most widely used adsorbent due to its high adsorption capacity for phenolic compounds. Commercial activated carbon is available either in the granular (GAC) or powdered form (PAC). The excellent adsorptive capability of AC is usually related to their properties, such as high surface area, pore volume and porosity. In addition, there are at least three important factors affecting the adsorption capacity of AC for phenolic compounds:

- The physical nature of the AC, such as pore structure, specific surface area, functional groups available on AC and the activation method.
- The nature of the phenolic compounds, its pK_a , polarity, molecular weight, size, and the presence of functional groups attached to aromatic ring.
- The solution environment, such as pH, initial phenolic concentration, oxygen level (oxic or anoxic) and ionic strength.

Generally, the porous structure and specific surface area of AC are often subjected to the type of parental carbon material used, the activation process and extent of the activation process. Two activation processes, namely physical steam activation and chemical activation are generally employed to prepare good quality AC. For the steam activation process, the precursor carbon materials first undergoes a carbonization process in an inert environment, followed by partial gasification of the resulting carbon material with steam at high temperature (Rodríguez-Reinoso et al., 1995; Dąbrowski et al., 2005). In the chemical activation process, the carbon material is soaked in chemical dehydrating agents such as phosphoric acid or zinc chloride to prevent formation of tar before undergoing pyrolysis.

Over the years, the application of AC is plagued by relatively high cost associated with its preparation. During the adsorption process, its adsorptive porosity becomes progressively saturated by the pollutants from the aqueous medium. Once it is exhausted, the activated carbon has to be disposed of by incineration or deposited in a landfill. The regeneration of spent activated carbon is of great interest to researchers.

1.3.3.1 Adsorption isotherms

Langmuir and Freundlich isotherms are the most frequently used models to describe the adsorption equilibrium. The basic assumptions of the Langmuir model are as follows:

- The adsorption process is limited to monolayer.
- There is no lateral interaction between the adsorbed substrate.
- Adsorbent is of homogeneous and uniform surface.

The Langmuir isotherm model (Langmuir, 1916) is given in Eq. (1.3)

$$Q_e = \frac{Q_{max} K_L C_e}{1 + K_L C_e} \qquad (1.3)$$

where C_e is the equilibrium residual concentration of substrate (mg/L), Q_e is the substrate adsorbed at equilibrium (mg/g), Q_{max} is the limiting adsorption capacity of adsorbent for the substrate (mg/g) and K_L is the Langmuir constant related to adsorption energy (L/mg) of the binding sites. The Langmuir constant is very useful in determining the favorability of adsorption (R_L) through the equilibrium parameter as indicated by Eq. (1.4).

$$R_L = \frac{1}{1 + K_L C_o} \qquad (1.4)$$

where C_o is the initial concentration of substrate (mg/L). In this context, lower R_L value reflects that adsorption process is more favorable. The R_L value indicates the adsorption behavior to be either unfavorable ($R_L > 1$), linear ($R_L = 1$), favorable ($0 < R_L < 1$) or irreversible ($R_L = 0$).

The Freundlich Isotherm (Freundlich, 1906) is used to describe adsorption to heterogeneous surfaces and is given by:

$$Q_e = K_F C_e^{\frac{1}{n}} \qquad (1.5)$$

where C_e and Q_e have the same meaning as above, K_F is related to the adsorbent capacity $[(mg/g)(L/mg)^{1/n}]$ and n is the affinity of interaction between adsorbate and adsorbent. For $1/n = 1$, the adsorption is linear; For $1/n < 1$, the adsorption is said to be favorable as the incremental change in amount adsorbed decreased with increasing concentration; For $1/n > 1$, the adsorption is unfavorable because the reversible process is true.

1.4 Regeneration of spent adsorbent

The regeneration of spent adsorbent is defined as the renewal of the adsorptive capacity of the adsorbent through certain regeneration processes such as thermal, chemical and biological regeneration. The critical selection of a suitable regeneration process greatly depends on several factors such as types of adsorbate, types of adsorbent, condition and costs of regeneration.

Thermal regeneration is considered as the most widely employed regeneration method which involves pyrolysis and oxidation process at high temperature (Nahm et al., 2012). Other methods involve the application of electrochemical, wet air oxidation and ultrasonic regeneration (Weng and Hsu, 2008). For chemical regeneration, extraction agent is used to desorb the adsorbate from the spent adsorbent, thus restoring the adsorptive capacity of adsorbent. Despite the high regeneration efficiencies for these methods, there are several disadvantages, such as high energy consumption, production of secondary waste from by-product and loss of adsorbent due to outgassing at very high temperature. Owing to relatively high carbon footprint and associated cost, biological regeneration of adsorbent has been actively explored in this project.

1.4.1 Irreversible adsorption

Over the last few decades, conflicting observations have been made by researchers concerning the low regeneration yield of phenolic-loaded adsorbent due to irreversible adsorption. The term irreversibility of adsorption is used to describe a phenomenon in which the adsorbed substrate cannot desorb from adsorbent into the bulk solution, even by heating at high temperature. The fate of irreversible adsorption on AC has been reported by many researchers (Grant and King, 1990; Vidic et al., 1993; Cooney and Xi, 1994; Osei-Twum et al., 1996). Grant and King (1990) perhaps is the first report to clarify the nature and mechanism of the irreversible adsorption of phenolic compounds. Several operational conditions such as (i) contact time, (ii) pH, (iii) temperature, (iv) dissolved oxygen (DO) and (v) nature of phenolic adsorbate, had been investigated in the study. The study showed that the polymerization of phenolic compounds on AC's surface originated from the oxidative coupling reaction. Cooney and Xi (1994) showed that the presence of the functional group attached to aromatic ring influenced the oxidative coupling reaction in the order $OCH_3 > CH_3 > Cl > H > COOH > CHO > NO_2$. In fact, identification of dimer and trimer by oxidative coupling reaction had been conducted by several researchers (Abuzaid and Nakhla, 1994; Zeid et al., 1995). Hence, the selection criteria for adsorption process should be chosen and designed properly in order to obtain higher percentages of desorption for the purpose of regeneration and to mitigate the collateral consequences from the oxidative coupling reaction.

1.5 Bioregeneration of spent adsorbent

As physical and chemical regeneration methods are associated with high cost and large carbon footprint, many investigations in extending the lifetime of the adsorbents have been attempted by researchers over the years (de Jonge et al., 1996a; Lee and Lim, 2005; Aktaş and Çeçen, 2007a; Ng et al., 2009; Oh et al., 2011; Al-Amrani et al., 2012). Bioregeneration of spent adsorbent is defined as a process to renew the exhausted adsorbent through microbial action (de Jonge et al., 1996a; Ng et al., 2009). Bioregeneration is recognized as an eco-friendly and cost effective way of regenerating the adsorbent as compared to chemical and physical regeneration methods. The involvement of biomass in bioregeneration provides the potential of complete bio-mineralization, and of eliminating the chances of producing secondary pollution as in chemical or thermal regeneration, in which additional cost is required to treat the pollution. However, the biodegradation ability of biomass is subjected to environmental changes as suspended biomass is severely inhibited under extreme conditions. Bioregeneration of adsorbent can be achieved under either simultaneous or sequential adsorption and biodegradation approaches.

1.5.1 Simultaneous adsorption and biodegradation

In simultaneous adsorption and biodegradation, both adsorption and biodegradation processes are allowed to occur at the same time, by practically mixing biomass together with the adsorbent and substrate. Concentration of substrate in the bulk solution is reduced through adsorption onto the adsorbent and biodegradation by the biomass. The synergistic incorporation of bioregeneration is found in the biological activated carbon (BAC) and powdered activated carbon treatment (PACT) continuous systems. The synergistic combination of adsorption and biodegradation processes in enhancing the treatment of various types of wastewater has been reported in many studies (Ivancev-Tumbas et al., 1998; Ha et al., 2000; Lim and Er, 2000; Klimenko et al., 2003; Lee and Lim, 2003; Loo et al., 2010; Lee and Lim, 2005; Muhamad et al., 2013).

The bioregeneration of granular activated carbon (GAC) loaded with phenol and 2,4-dichlorophenol was evaluated in the BAC supplemented sequencing batch reactor (SBR) (Ha et al., 2000). Based on the finding, desorption of substrate was biologically induced due to concentration gradient between the bulk solution and GAC or biomass, respectively. In another study by Lee and Lim (2003) with similar adsorbent supplemented into SBR, the synergistic effect to treat phenol and alkyl phenols was observed in the comparative study. In another quantitative study conducted by Lee and Lim (2005), the bioregeneration of PAC in the treatment of alkyl-substituted phenolic compounds for simultaneous adsorption and biodegradation processes was investigated. Loo et al. (2010) used SBR supplemented with PAC and pyrolysed rice husk (PRH), to treat *p*-nitrophenol (PNP) as a sole organic carbon source. The result showed that improvement in the performance of the SBR was observed with the addition of PAC.

Oh et al. (2011) studied the bioregeneration of chlorophenol-loaded GAC in the simultaneous adsorption and biodegradation processes involving 4-chlorophenol (4-CP) and 2,4-dichlorophenol (2,4-DCP), respectively, by direct measurement of the amount of chlorophenol adsorbed onto GAC. Based on the finding, the highest bioregeneration efficiency was achieved provided that the initial substrate concentration was lower than the acclimated concentration. When the initial substrate concentration was higher than the acclimated concentration, the highest bioregeneration efficiency was achieved if excess adsorbent was used.

1.5.2 Sequential adsorption and biodegradation

Under the sequential adsorption and biodegradation approach, the substrate is initially adsorbed onto the adsorbent's surface, followed by the desorption of the adsorbed substrate into the nutrient medium due to the inverse concentration gradient build-up between the surface of adsorbent and in the bulk liquid, thus renewing the exhausted active sites. The desorbed substrate is then degraded by microbial action, resulting in further desorption. This type of bioregeneration approach is also known as offline bioregeneration (Aktaş and Çeçen, 2007b).

Vinitnantharat et al. (2001) evaluated the effects of different bioregeneration duration and biomass concentration on bioregeneration of lignite-based AC loaded with phenol and 2,4-DCP, respectively, using direct measurements of substrate loading through reloading of the bioregenerated GAC. The results showed that increase of initial biomass concentration from 100 to 300 mg/L could only shorten the bioregeneration period but has less effect on the bioregeneration efficiency.

The feasibility of bioregeneration of AC after adsorption of surfactants from aqueous solutions was studied (Klimenko et al., 2003). The bioregeneration of spent AC by surfactant-degrading bacteria was evaluated under static and dynamic conditions, in which higher bioregeneration efficiency of AC was noticed for dynamic systems in a short period of adsorption process. In another quantitative study conducted by Ng et al. (2009), the bioregeneration efficiencies of PAC and PRH loaded with phenol and 4-nitrophenol were determined at different adsorbent dosages using respirometric techniques. Based on the limiting adsorption capacity as estimated from Langmuir isotherm model, the condition of adsorbent at different adsorbent dosages, was classified either excess or non-excess adsorbent in the system. The use of excess PAC in a simultaneous adsorption and biodegradation approach was found to resemble the sequential approach with comparable extent of bioregeneration. The impacts of adsorbent size (granular or powder form) and carbon activation (thermal or chemical activation) on the bioregeneration of adsorbents loaded with phenolic compounds were extensively studied (Aktaş and Çeçen, 2006a, 2006b, 2007a, 2009, 2010). From their results, both powdered and granular forms, chemical activation of the adsorbent could yield higher bioregeneration efficiency due to lower degree of irreversible adsorption (hysteresis). For the treatment of 2-chlorophenol, Aktaş and Çeçen (2007a) recommended adsorption alone without applying a biological process due to difficulty in biodegrading 2-chlorophenol. Aktaş and Çeçen (2010) investigated the extent of cometabolic bioregeneration of AC loaded with 2-nitrophenol in the presence of phenol in lab-scale batch activated sludge reactors. From their results, the AC loaded with 2-nitrophenol could only be partially bioregenerated cometabolically in the presence of phenol as the growth substrate. The cometabolic bioregeneration efficiency of chemically treated AC was higher in accordance with higher desorbability as compared to thermally treated AC indicating the desorption process was the rate-determining step in bi-solute bioregeneration of phenol and 2-nitrophenol.

Quantitative investigation of the role of biomass in the bioregeneration of mono-amine modified silica (MAMS) and GAC loaded with Acid Orange 7 (AO7) dye had been reported (Al-Amrani et al., 2012). The results showed that considerably higher bioregeneration efficiency of AO7-loaded MAMS as compared to AO7-loaded GAC was attained due to greater desorption of MAMS for AO7. The progressive loss of lifetime of MAMS for AO7 with multiple subsequent cycles of use suggested that possible chemical and microbial fouling of the adsorption sites. Bioregeneration of spent resin was evaluated in multiple cycles by several researchers in recent years (Venkatesan et al., 2010; Sharbatmaleki and Batista, 2012). The bioregenerated resin could then be reused up to at least three cycles, rather than being incinerated.

1.5.3 Mechanisms of bioregeneration

There are two proposed mechanisms to describe the bioregeneration of adsorbent.

1.5.3.1 Exoenzymatic reaction

According to the proposed mechanism, the degradation of adsorbed substrate by exoenzymatic reaction occurs on the surface of adsorbent. The hydrolytic byproducts from the reaction desorb into the bulk solution for biodegradation process. The mechanism emphasizes that the biomass is able to produce the exoenzyme which can enter into the pores of the adsorbent. However, exoenzyme reactions occurring on adsorbent remains inconclusive. Some researchers (Zhang et al., 1991) disagreed with this mechanism and argued that the size of the enzyme is larger compared to the size of micropores, thus limiting the accessibility of the enzyme to react with the adsorbed substrate in micropores. They estimated that the pore diameter (∅) must be at least three times greater than the enzyme size for a proper enzyme catalytic reaction. Considering a monomeric enzyme (molecular weight between 13,000 and 35,000) with a diameter of around 31 – 44 Å, they concluded that the pore diameter must be greater than at least 10 nm which exclude the micropores (∅ < 2 nm) and some of the mesopores (2 < ∅ < 50 nm) of adsorbent. Yet, some researchers (Sirotkin et al., 2001, Klimenko et al., 2003; Aktaş and Çeçen, 2006a) suggested that the space constraint was not a critical issue for the occurrence of exoenzymatic reaction as the exoenzyme activity depends on the type of carbon activation and porosity of the AC used.

1.5.3.2 Concentration gradient

This mechanism describes the release of the substrate from the adsorbent due to the concentration gradient between adsorbed substrate and in the bulk liquid (de Jonge et al., 1996a; Lee and Lim, 2005; Ng et al., 2009; Aktaş and Çeçen, 2010; Oh et al., 2012; Al-Amrani et al., 2013; Ren et al., 2013). The build-up of the concentration gradient occurs whenever the concentration of the substrate in the bulk solution becomes lower due to microbial biodegradation. The bioregeneration of adsorbent due to occurs when the adsorbed substrate is further biodegraded in the bulk solution. Thus, maintaining a high concentration gradient during the bioregeneration process is the key factor for this mechanism. With a high concentration gradient, further desorption can be induced resulting in a higher bioregeneration efficiency.

1.5.4 Factors affecting bioregeneration

Bioregeneration of adsorbent depends on the characteristics of the substrate and adsorbent as well as on the operational factors. Changes in any of the factors may affect the extent and rate of the bioregeneration.

1.5.4.1 Biodegradability of substrate

It is recognized that desorption and biodegradation of substrate are major steps for the bioregeneration of adsorbent. Nonetheless, bioregeneration can also occur for some phenolic compounds which have low biodegradability if the process allows for sufficient contact time with acclimated biomass (Aktaş and Çeçen, 2010; Oh et al., 2011). For AC loaded with a strong persistent compound, such as 2-chlorophenol, it was found that bioregeneration did not proceed at all (Aktaş and Çeçen, 2007a).

1.5.4.2 Desorbility of substrate

According to de Jonge et al. (1996a), PAC loaded with non-phenolic compounds, such as 3-chlorobenzoic acid, was more easily bioregenerated as compared to *o*-cresol-loaded PAC. In addition, the nature of the substituted functional groups on the aromatic ring of the phenolic compounds affects the extent bioregeneration process. Lee and Lim (2005) reported that phenol-loaded carbon was bioregenerated more efficiently compared to carbon loaded with *p*-cresol, *p*-ethylphenol and *p*-isopropylphenol. They contended that the decreasing bioregenerability of the AC loaded with the alkyl-substituted phenols was associated with the lengthening of the hydrophobic alkyl chain. As discussed in Section 1.4.1, the low desorbility for certain type of the phenolic compounds was probably due to the irreversible adsorption of the phenolic compounds caused by the oxidative coupling reaction. Grant and King (1990) found that the high fraction of irreversibly adsorbed *o*-cresol increased with increasing contact time due to the occurrence of oxidative polymerization. In contrast, the desorption of non-phenolic compound was not affected by the contact time.

1.5.4.3 Adsorbent activation

The activation process for the adsorbent affects the extent of bioregeneration. Many studies showed a higher efficiency of bioregeneration for chemically activated-wood-based PAC as compared to thermally activated-peat-based PAC, both of which loaded with *o*-cresol and 3-chlorobenzoic acid, respectively (de Jonge et al., 1996a, 1996b). Bioregeneration of chemically activated carbon of high efficiency over thermally activated carbons was also demonstrated in other studies (Aktaş and Çeçen, 2006a, 2006b, 2007a, 2010).

1.5.4.4 Adsorbent morphology

Reversible adsorption occurs due to van der Waals attraction and weak charge-transfer complexes at adsorptive sites. Irreversible adsorption can be explained by the strong adsorptive energy interaction between the adsorbent and the substrate. The porous structure of AC that consists of micropores, mesopores and macropores is another key factor limiting the degree of bioregeneration. It was known that phenol was preferably adsorbed onto the micropores as compared to mesopores and macropores (Ng et al., 2010). Besides, the diffusion rate of substrate is slower for the micropores as compared to mesopores and macropores (Benedek and Peel, 1983).

1.5.4.5 Initial biomass concentration

Initial biomass concentration affects the extent and the rate of bioregeneration. Vinitnantharat et al. (2001) showed that increasing the initial mixed liquor volatile suspended solids (MLVSS) concentration in bioregeneration process shortened the reaction time that was needed to reach equilibrium yet had little impact on the magnitude of bioregeneration. In contrast, Oh et al. (2013) found no observed effect of varying initial biomass concentrations on the rate and efficiency of bioregeneration of 4-CP-loaded GAC if there was no inhibitory effect due to the substrate in the latter case. However, microbial fouling is a collateral consequence upon increase of initial biomass concentration during the bioregeneration process. Due to relatively long reaction time in bioregeneration, there is a strong possibility of biomass attachment on the surface of adsorbent (Gibert et al., 2013; Muhamad et al., 2013) which could hamper the bioregeneration efficiency especially during the subsequent cycle of use (Venkatesan et al., 2010; Al-Amrani et al., 2012). Al-Amrani et al. (2012) reported that significant amounts of COD concentration was detected in the fouling removal reagent after use, suggesting that the COD concentration might be contributed by the presence of the biomass from the surface of adsorbents.

1.6 Quantification of bioregeneration

Many methods in determining the extent of bioregeneration quantitatively were reported in the literature. These include direct determination of the adsorbed substrates on adsorbent (Klimenko et al., 2003; Aktaş and Çeçen, 2006a; Oh et al., 2011; Al-Amrani et al., 2012), indirect measurement of the production of carbon dioxide (de Jonge et al., 1996a) and the measurement of oxygen uptake by respirometry (Orshansky and Narkis, 1997; Ivancev-Tumbas et al., 1998; Lee and Lim, 2005; Ng et al., 2009). A summary of the results of quantification of bioregeneration are shown in Table 1.3. Most of the studies on the quantification of bioregeneration were based on sequential adsorption and biodegradation approach due to the difficulty in determining precisely the extent of bioregeneration during the simultaneous adsorption and biodegradation approach. According to Lee and Lim (2005) and Ng et al. (2009), the use of indirect oxygen uptake measurement to measure the extent of bioregeneration in simultaneous adsorption and biodegradation requires that the adsorption equilibrium be established prior to biodegradation in

Table 1.3: Quantification of bioregeneration efficiency reported from the literature.

Compound	**Method**	**Adsorbent**	**Carbon features**	**Bioregeneration Efficiency (%)**	**Reference**
o-cresol	By-product	PAC Norit	Thermal	15	de Jonge et al., 1996a
chlorobenzoic acid		PAC Norit	Chemical	85	
phenol	Direct method	PAC Norit	Thermal	58.1	Aktaş and Çeçen, 2006a
		PAC Norit	Chemical	93.6	
		GAC Norit	Thermal	66.6	
		GAC Norit	Chemical	84.8	
Surfactant	Direct method	GAC AG3		12 – 35	Klimenko et al., 2003
		GAC ACPR		11 – 29	
		GAC SKN		8 – 23	
phenol	Respirometic	PAC		77 ± 4	Lee and Lim, 2005
p-cresol				69 ± 4	
p-ethylphenol				68 ± 4	
p-isopropylphenol				58 ± 6	
phenol	Direct method	GAC Sigma		39 – 48	Ha et al., 2000
2,4-dichlorophenol				38 – 43	
phenol	Direct method	GAC Sigma		74.5 – 76.3	Ha and Vinitnantharat, 2000
2,4-dichlorophenol				56.8 – 60.2	
phenol	Direct method	GAC Sigma		67.3 – 83.8	Ha et al., 2001
2,4-dichlorophenol				51.7 – 64.3	
phenol	Direct method	GAC Sigma		31.4	Vinitnantharat et al., 2001
2,4-dichlorophenol				14.3	
phenol	Direct method	PAC Norit	Thermal	23.7 – 27.0	Aktaş and Çeçen, 2009
		PAC Norit	Chemical	55.4 – 65.2	
		GAC Norit	Thermal	50.6	
		GAC Norit	Chemical	81.5	
2-chlorophenol		PAC Norit	Thermal	28.9 – 39.4	
		PAC Norit	Chemical	66.3 – 66.6	
		GAC Norit	Thermal	38.6	
		GAC Norit	Chemical	52.1	
phenol	Direct method	PAC Norit	Thermal	19.5	Aktaş and Çeçen, 2010
		PAC Norit	Chemical	90.0	
		GAC Norit	Thermal	32.6	
		GAC Norit	Chemical	82.3	
2-nitrophenol		PAC Norit	Thermal	64.9	
		PAC Norit	Chemical	64.7	
		GAC Norit	Thermal	84.9	
		GAC Norit	Chemical	89.9	
phenol	Respirometic	PAC Malbon	Thermal	6 – 67	Ng et al., 2009
		PRH	Thermal	52 – 78	
4-nitrophenol		PAC Malbon	Thermal	46 – 61	
		PRH	Thermal	65 – 83	
4-chlorophenol	Direct method	GAC Norit	Thermal	42 – 69	Oh et al., 2011
2,4-dichlorophenol				46 – 68	
AO7	Direct method	GAC Norit	Thermal	0 – 15	Al-Amrani et al., 2012
		MAMS		74 – 97	

order to determine the initial substrate loading accurately. However, for many other systems such as non-PAC adsorbent, the above-mentioned condition is generally not satisfied because biodegradation could have proceeded before adsorption equilibrium is established. For that, the respirometry technique appears to be a poor method as it is difficult to determine the initial substrate loading of the adsorbent.

In other studies, Vinitnantharat et al. (2001) and Aktaş and Çeçen (2009) employed direct determination of the amount of adsorbed substrate on adsorbent after the completion of bioregeneration by reloading the bioregenerated adsorbent. By using direct method, the extent of bioregeneration can be quantified in situations that limit the use of the respirometric method. This has been reported by Oh et al. (2011) and Al-Amrani et al. (2012) in tracking the time courses of amount of adsorbed substrate on adsorbent in bioregeneration process.

1.7 Kinetic models of bioregeneration

Kinetic model provides information on the rate of bioregeneration process which allows the optimization of the process. In earlier studies (Ivancev-Tumbas et al., 1998; Aktaş and Çeçen, 2007a) efforts had been made in the kinetic modeling of bioregeneration. According to those studies, the rate of bioregeneration was assumed to follow the first-order kinetics and was expressed as:

$$-\frac{dQ_t}{dt} = kQ_t \qquad (1.6)$$

Integrating Eq. (1.6),

$$Q_t = Q_o e^{-kt} \qquad (1.7)$$

where Q_t (mg/g) is the amount of adsorbed substrate per unit weight of adsorbent remained on adsorbent at time, t, Q_o (mg/g) is the amount of adsorbed substrate per unit weight of adsorbent initially, and k (1/h) is the first-order rate constant. Based on their approach, in order to determine the bioregeneration rate constant in Eq. (1.7), Q_t was estimated by using Freundlich isotherm as shown in Eq. (1.8):

$$Q_t = K_F C_{2e}^{\frac{1}{n}} - \frac{(C_2 - C_{2e})v}{w} \qquad (1.8)$$

where C_2 (mg/L) is the concentration of substrate after reloading the adsorbent with same condition of substrate from the initial loading, C_{2e} (mg/L) is the equilibrium concentration of the substrate after reloading equilibrium is attained, K_F [(mg/g)(L/mg)$^{1/n}$] and n are the constants of the Freundlich isotherm, v (L) is the volume of sample and w (g) is the weight of adsorbent. Eq. (1.8) was used to determine the concentration of adsorbed substrate at the equilibrium condition. Yet, owing to the difference of the concentration gradient across the dynamic bioregeneration process, the validity of Eq (1.8) to evaluate Q_t is doubtful.

In a recent study, Ng et al. (2010) proposed a two-step first-order kinetic equation, consisting of induced desorption and biodegradation processes, to describe the sequential type of bioregeneration process of PAC and PRH loaded with phenol and 4-nitrophenol, respectively. The proposed equation is given in Eq (1.9):

$$C = C_i e^{-kt} + \frac{m k_d Q_o}{k - k_d}(e^{-k_d t} - e^{-kt}) \qquad (1.9)$$

where C (mg/L) and C_i (mg/L) are the phenolic compound concentrations in the bulk solution at time t and at initial condition, respectively, k_d (1/h) and k (1/h) are the rate constants for biological-induced desorption and biodegradation, respectively, m (g/L) is the dosage of adsorbent used and Q_o (mg/g) is the initial amount of adsorbed substrate on adsorbent. The model fitted well to their findings with $R^2 > 0.9$. Comparing the values of the rate constants, the biological-induced desorption step was found to be the rate determining step.