ADSORPTION OF TERRAMYCIN ON ACTIVATED CARBON USING RESPONSE SURFACE METHODOLOGY (RSM)

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by

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LIST OF SYMBOL

	Symbol	**Unit**
Y	Response for RSM equation	-
b	Constant for RSM equation	-
x_1	pH	-
x_2	Initial concentration	ppm
x_3	Temperature	°C
C	Concentration	mg/L
V	Volume	L

LIST OF ABBREVIATIONS

WAC	Wood-based activated carbon
HCl	Hydrochloric acid
H_2SO_4	Sulphuric acid
HNO_3	Nitric acid
$Cu(NO_3)_2$	Copper (II) nitrate
KOH	Potassium hydroxide
RSM	Response Surface Methodology
CCD	Central composite design
AC	Activated carbon
BrO_3^-	Bromate
CLAC	Cotton linter fibers activated carbon
GO	Graphene oxide
GO-MPsb	Graphene oxide-magnetic particles
LSAC	Lotus stalks-based activated carbon
Mic-LSAC	Microwaved-LSAC
M	Merck commercial activated cabon
S	Sorbo commercial activated cabon
ANOVA	Analysis of variance
ARG	Antibiotic resistance gene
ARB	Antibiotic-resistant bacteria
WWTP	Wastewater treatment plant
AOP	Advanced Oxidation Process
DOE	Design of Experiment

OFAT	One-factor-at-a-time
PRESS	Prediction Error Sum of Squares
IUPAC	International Union of Pure and Applied Chemistry
CAS	Chemical Abstracts Service
UV	Ultraviolet
CCF	Central composite design face-centered
rpm	Rotation per minute

PENJERAPAN TERRAMYCIN KE ATAS KARBON TERAKTIF MENGGUNAKAN KAEDAH TINDAK BALAS PERMUKAAN (KTBP)

ABSTRAK

Penjerapan terramycin ke atas karbon teraktif berasaskan kayu (KTK) disiasat dalam proses penjerapan kelompok. Keberkesanan penyingkiran terramycin ke atas KTK yang tidak diubahsuai dibandingkan dengan KTK yang diubahsuai. Karbon teraktif diubahsuai dengan menggunakan kaedah pengisitepuan menggunakan lima jenis larutan iaitu HCl, H_2SO_4, HNO_3, $Cu(NO_3)_2$ dan KOH. Kapasiti penjerapan maksimum terramycin ke atas karbon teraktif yang diisitepu dengan peratus berat 1 $Cu(NO_3)_2$ telah memperoleh keberkesanan penyingkiran sebanyak 99.85%. Penjerapan optimum dengan menggunakan Kaedah Tindak Balas Permukaan (KTBP) yang mengambil kira beberapa parameter termasuk nilai pH (3-7), kepekatan awal (5-25 mg/L) dan suhu (30-50 °C). Terdapat tiga set parameter optimum untuk penjerapan terramycin ke atas KTK yang tidak diubahsuai dan diubahsuai berdasarkan Reka Bentuk Komposit Pusat (RBKP) dengan menggunakan RSM (pH 7, 25 ppm, 50 °C; pH 7, 5 ppm, 50 °C; pH 3, 5 ppm, 50 °C) (pH 7, 20 ppm, 36.4 °C; pH 6.5, 20 ppm, 42.3 °C; pH 6, 23 ppm, 39.3 °C). Persamaan-persamaan linear dan polinomial kuadratik telah dibina untuk meramal peratusan keberkesanan penyingkiran terramycin. Nilai keputusan eksperimen menunjukkan nilai yang munasabah dengan nilai yang diramalkan. Berdasarkan nilai statistik ANOVA, nilai kebarangkalian yang rendah (<0.05) menunjukkan penjerapan terramycin ke atas KTK adalah sangat signifikan. Maksimum nilai keberkesanan penjerapan terramycin ke atas KTK yang tidak diubahsuai dan diubahsuai dengan peratus ralat terendah adalah 93.85% dan 99.63% berdasarkan model yang telah disahkan ke atas keadaan optimum.

ADSORPTION OF TERRAMYCIN ON ACTIVATED CARBON USING RESPONSE SURFACE METHODOLOGY (RSM)

ABSTRACT

The adsorption of terramycin onto wood-based activated carbon (WAC) is investigated in a batch adsorption process. Removal efficiency of terramycin onto non-modified WAC is compared with the modified WAC. Modified WAC is prepared by impregnation method using five types of solution which are HCl, H_2SO_4, HNO_3, $Cu(NO_3)_2$, and KOH. The maximum adsorption capacity of terramycin on WAC impregnated with 1 wt % of $Cu(NO_3)_2$ is obtained with 99.85% removal efficiency. The adsorption is optimized by using response surface methodology (RSM) considering various parameters including pH value (3-7), initial concentration (5-25mg/L) and temperature (30-50 ^{o}C). Three sets of optimum conditions are found to be optimum for terramycin adsorption on non-modified and modified WAC based on the Central Composite Design (CCD) using RSM (pH 7, 25 ppm, 50 ^{o}C; pH 7, 5 ppm, 50 ^{o}C; pH 3, 5 ppm, 50 ^{o}C) (pH 7, 20 ppm, 36.4 ^{o}C; pH 6.5, 20 ppm, 42.3 ^{o}C; pH 6, 23 ppm, 39.3 ^{o}C), respectively. One linear and one quadratic polynomial equations are developed to predict the percentage of removal efficiency. The experimental results indicated reasonable agreement with the predicted values. Based on ANOVA statistical value, the adsorption of terramycin onto WAC has been found to be highly significant, with low probability (p) values (<0.05). The maximum experimental terramycin removal efficiencies with the least error percentage of 93.85% and 99.63% for non-modified and modified WAC, respectively are obtained based on the validation model of optimum conditions.

CHAPTER ONE

INTRODUCTION

1.1 Background

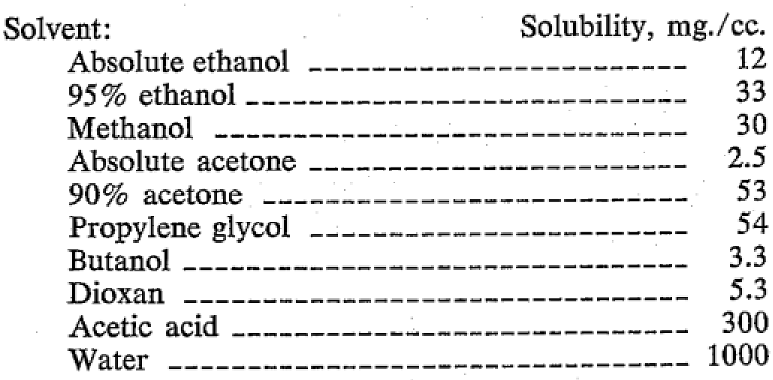
1.1.1 Terramycin

Terramycin is the commercial name of oxytetracycline. This type of tetracycline antibiotic has been discovered in year 1948 as first-generation tetracycline antibiotic with a chemical name 4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (Borghi and Palma, 2014). Figure 1.1 shows different members of tetracycline class. Tetracycline antibiotics inhibit protein synthesis by avoiding the attachment of aminoacyl-tRNA to the ribosomal acceptor (A) site (Chopra and Roberts, 2001). They are broad-spectrum agents which exhibit activity against an extensive range of gram-positive and gram-negative bacteria such as chlamydiae, mycoplasmas, and rickettsiae, and protozoan parasites. Terracyclines are derived from *Streptomyces rimosus* (bacteria) by a fermentation process (Borghi and Palma, 2014).

Generic name	Trade name	Yr of discovery	Status	Therapeutic administration
Chlortetracycline	Aureomycin	1948	Marketed	Oral
Oxytetracycline	Terramycin	1948	Marketed	Oral and parenteral
Tetracycline	Achromycin	1953	Marketed	Oral
Demethylchlortetracycline	Declomycin	1957	Marketed	Oral
Rolitetracycline	Reverin	1958	Marketed	Oral
Limecycline	Tetralysal	1961	Marketed	Oral and parenteral
Clomocycline	Megaclor	1963	Marketed	Oral
Methacycline	Rondomycin	1965	Marketed	Oral
Doxycycline	Vibramycin	1967	Marketed	Oral and parenteral
Minocycline	Minocin	1972	Marketed	Oral and parenteral
Tertiary-butylglycylamidominocycline	Tigilcycline	1993	Phase II clinical trials	

Figure 1.1 Members of tetracycline class (Borghi and Palma, 2014)

Terramycin can be presented as atmospheric base compound, hydrochloride salt or quaternary ammonium salt complex. Hydrochloric salt is the familiar form in parental with terramycin as oxytetracycline hydrochloride. Oxytetracycline hydrochloride is soluble in various solvents as shown in Figure 1.2. The solubility of oxytetracycline hydrochloride in water is the highest among other solvents which is 1000 mg/cm^3 (Neumann et al., 1959). Hence, terramycin hydrochloride has a high concentration in wastewater stream which is originated from the effluent of municipal wastewater treatment plants, as well as the effluent from pharmaceutical manufacturing plants.



Solvent:	Solubility, mg./cc.
Absolute ethanol	12
95% ethanol	33
Methanol	30
Absolute acetone	2.5
90% acetone	53
Propylene glycol	54
Butanol	3.3
Dioxan	5.3
Acetic acid	300
Water	1000

Figure 1.2 Solubility of oxytetracycline hydrochloride in various of solvents (Neumann et al., 1959)

1.1.2 Effect of terramycin in wastewater

Disturbance function of terrestrial ecosystem occurs when terramycin or antibiotic is used extensively. The world is facing global public health challenge due to antibiotic–resistance which infectious organisms such as bacteria become resistant to an antibiotic. This reduces the effectiveness of terramycin in treating the bacterial diseases (WHO, 2014). All of this becomes an indicator that large amounts of terramycin are being released to the environment either from human and animal excretion or waste from a factory producing terramycin.

Since terramycin is widely used in animal intensive farming and aquaculture, it has a high potential for the disturbance in the environment. This is due to its difficulty to be biologically degraded by conventional wastewater treatment plant due to its toxicity and high stability. Moreover, terramycin is classified as recalcitrant bio-accumulative compounds (Huang et al., 2011). Recalcitrant bio-accumulative compounds are highly resistant to bio-degradation and can produce microbial death or other serious problems in wastewater treatment plants (Candal et al., 2012). Hence, terramycin can be regarded as a hazardous chemical which is harmful to the environment when it comes to treatment in wastewater thus results in contamination of aquatic ecosystems.

Not only terramycin itself, but some degradation by-products of this type of antibiotic have higher toxicity than their own compounds (Huang et al., 2011). This shows antibiotics can be seriously dangerous and harmful to the environment. Thus, terramycin needs to be treated either physically or chemically to preserve the environment. There are many techniques to remove antibiotic from wastewater such as biological processes, filtration, coagulation, flocculation, sedimentation, advanced oxidation processes (AOPs), adsorption and membrane processes (Homem and Santos, 2011). These techniques have many advantages and disadvantages. However, adsorption is the most efficient and economical technique to remove antibiotics from the industrial and municipal effluent.

1.1.3 Adsorption

Adsorption is the process through which a substance, originally present in one phase, is removed from that phase by accumulation at the interface between that phase and a separate (solid) phase. Adsorption can occur at either gas–solid interface or liquid-solid interface. Two important terms in adsorption process are adsorbate and adsorbent. The material being adsorbed is called as adsorbate while solid material being used as the adsorbing phase is adsorbent. Adsorption process is the best technique to treat industrial wastewater which contains substances that are difficult to remove via conventionally secondary treatment, toxic or hazardous, volatile and cannot be transferred to the atmosphere, have the potential for creating noxious vapors or odors, or for imparting color to the wastewater and are present is very small concentrations that make their removal via other methods difficult and present in very small concentrations that make their removal via other methods difficult (Armenante, 2000).

Adsorption is the most appropriate treatment to remove terramycin from the wastewater. Simple design and low investment of initial cost and land required makes adsorption process as preferable wastewater treatment than other processes (Rashed, 2013). The efficiency of an adsorption process is highly influenced by the characteristics of adsorbent and composition of the wastewater stream. Activated carbons (ACs), carbon nanotubes (CNTs) especially multiwalled carbon nanotubes (MWCNTs), natural clay materials, for instance, bentonite, ion exchange materials and bacterial celluloses (BCs) are examples of adsorptive materials for antibiotic removal. AC is a popular adsorbent that is used more recently in removing antibiotic from wastewater (Ahmed et al., 2015).

1.1.4 Activated carbon (AC)

The expected global growth for AC in 2016 is projected to increase at the rate of 10.3% per year. 39% of the world demand which is the highest, is dominated by Asia pacific region (Freedonia Group, 2010). AC has been extensively used to remove distinct types of pollutants from contaminated wastewater stream. This is because it has benefits of low cost, high adsorption capacity and easy to dispose of (Carabineiro et al., 2011). AC can be obtained in granular and powdered forms. The granular form has a large internal surface area and small pores which make it easy to regenerate or reuse after saturation while powder form has larger pore diameters and a smaller internal surface area which make it more efficient (Bansal and Goyal, 2005; Calisto et al., 2015).

AC not only has porous and crystalline structures, they also have the chemical structure as well. This chemical structure is strongly influencing the adsorption capacity of AC. Among all heteroatoms presence in an AC, carbon-oxygen surface groups are the most important surface groups that affect the surface characteristics such as wettability, polarity, and acidity, and physic-chemical properties such as catalytic, electrical, and chemical reactivity of AC (Bansal and Goyal, 2005). Oxygen and hydrogen atoms are important components of an active carbon with good adsorptive properties (Kipling, 1956). In the application of treating wastewater, properties of AC make it as a good adsorbent.

1.1.5 Response surface methodology (RSM)

The main objective of Response Surface Methodology (RSM) is to determine the optimum settings of the control variables that result in a maximum or a minimum response over a certain coefficient of determination, R^2 (Khuri and Mukhopadhyay, 2010). RSM develops an empirical model which can predict optimal performance as a function of some quantitative variables that affect performance outcome (Williges, 2006). The statistical experimental design is portrayed in the form of an empirical model in which the response (Y) is predicted as a function of several significant factors (x) that affect the shape of the response surface. The influence of the various x is determined by the order of the empirical model (Williges, 2006).

There are two important models that widely used in classical RSM. The first-order model is presented in Equation 1 below,

$$Y = b_0 + \sum_{i=1}^{n} b_i x_i \tag{1}$$

and the second-order model is shown in Equation 2,

$$Y = b_0 + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n} b_{ii} x_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} x_i x_j \tag{2}$$

Where Y is the response (dependent variable), b_0 is the constant coefficient, b_i is the coefficient for the linear effect, b_{ii} is the coefficient for the quadratic effect, b_{ij} is the coefficient for the interaction effect, x_i and x_j are the independent variables (factors).

The first-order designs are design for fitting first-degree models and the second-order designs are design for fitting second-degree models. The most frequent first-order designs are 2^k factorial (k is the number of control variables), Plackett-Burman and simplex designs while the most common second-order designs are 3^k factorial, Central Composite Design (CCD), and the Box-Behnken designs (Williges, 2006).

The CCD is a better alternative to the full factorial three-level design since it requires a smaller number of experiments while providing comparable results (Benredouane et al., 2016). The typical parameters chosen as the factors affecting removal efficiency in adsorption process are pH, initial concentration, adsorbent dosage, and temperature (Mourabet et al., 2015). The application of RSM in adsorption process has benefits in improving the product yields, reducing the process variability, making the output response close to target requirements and reducing the operating time (Mourabet et al., 2015).

1.1 Problem statement

Antibiotics which can treat and prevent bacterial infections have been fall to resistance due to extensive use of antibiotics from day to day. Increasing prescribe of antibiotics can lead to increase antibiotic content in wastewater as contaminants. Antibiotics are discharged into the aquatic environment either through effluent of municipal wastewater treatment plants or pharmaceutical manufacturing plants (Moussavi et al., 2013; Sun et al., 2012). Since antibiotics have high stability and toxicity to the microorganism in wastewater treatment, antibiotics are difficult to be biologically degraded or eliminated in wastewater treatment plants (Huang et al., 2011).

One of the most familiar groups of antibiotics is tetracycline. Terramycin is the most persistence than other types of tetracycline antibiotics (Huang et al., 2011). Techniques such as adsorption using AC, membrane filtration and advanced oxidation processes (AOPs) can treat and remove terramycin from wastewater. Even though high removal efficiency can be attained when using AOPs or membrane filtration, unfortunately, these methods are restricted due to high cost and harsh conditions. Thus, adsorption presents as an efficient and economical technique because it is unaffected by toxicity as well as inexpensive (Peng et al., 2016).

AC is chosen as the most appropriate adsorbent due to its porosity and very high specific surface area and capacity to remove terramycin from wastewater streams (Moussavi et al., 2013). There is limited study has been made on the adsorption behaviour of terramycin onto AC. Hence, the focus of this paper is to evaluate the adsorption behaviour of terramycin on non-modified AC and modified AC using Response Surface Methodology (RSM).

1.2 Research objectives

The main objectives of this study are:

1. To investigate and compare the adsorption of terramycin on non-modified activated carbon and modified activated carbon by using Response Surface Methodology (RSM)
2. To determine the parametric interaction of terramycin with non-modified activated carbon and modified activated carbon as functions of pH, initial concentration and temperature of terramycin solution
3. To develop model quadratic regressions by optimization study of adsorption of terramycin on non-modified activated carbon and modified activated carbon

1.3 Organization of thesis

This thesis consists of five main chapters and each chapter contributes to the sequence of this study. The following are the contents for each chapter in this study:

Chapter 1 introduces the usage of terramycin antibiotic in industries, the effect of terramycin in wastewater, definition of adsorption, activated carbon and RSM, problem statement, research objectives, organization of thesis and research scope.

Chapter 2 discusses the literature review of this study. An insight into the occurrence of antibiotic in the environment, effect of wastewater treatment processes on activated carbon, discussion on activated carbon and modified activated carbon. Moreover, RSM and central composite design (CCD) are included as well.

Chapter 3 covers the experiment materials and the details of methodology. It discusses on the description of equipment and materials used, batch adsorption experiment, experimental procedure and description of factors affecting the adsorption process.

Chapter 4 clarifies about the result of this research. The performance comparison of the adsorption of terramycin on non-modified and modified activated carbon, its parametric interaction in terms of pH, initial concentration and temperature, and the model developed using RSM will be discussed.

Chapter 5 gives a brief about overall conclusion about our research studies based on result and discussion in Chapter 4 whether the research objectives have been achieved successfully or not. Recommendation and improvement of this study will be conducted prior to the conclusion.

1.4 Research scope

Wood-based activated carbon (WAC) is used as an adsorbent for adsorption of terramycin. The performance of non-modified WAC is compared with the modified WAC. The WAC undergoes modification on its surface by impregnating with several acid and base solution which are nitric acid, sulphuric acid, hydrochloric acid, copper nitrate and potassium hydroxide. Screening experiment is conducted to obtain the best modified WAC that can achieve maximum terramycin removal efficiency. These two distinct WAC will be tested for adsorption of terramycin with different parameters which are pH, initial concentration and temperature of terramycin solution. Adsorption study is optimized using Response Surface Methodology (RSM). This experimental design technique is very helpful to investigate and understand the interactions among parameters that have been optimized by developing model quadratic regression. Optimum conditions of those parameters are determined from the model quadratic regression itself. Then, the output response from the adsorption of terramycin on non-modified and modified WAC is compared based on the model developed and optimum parameters obtained.

CHAPTER TWO

LITERATURE REVIEW

2.1 Occurrence of Antibiotics in the Environment

Antibiotics are health care substances that can treat and prevent bacterial infections either by preventing the growth of bacteria or killing the bacteria to promote human health as well as animal and plants (Nami et al., 2015). However, antibiotic resistance genes (ARGs) carried by antibiotic-resistant bacteria (ARB) has become a growing significant public-health threat to worldwide which occur due to extensive prescribe antibiotics as it reduces the effectiveness of antibiotics (Laxminarayan et al., 2017; WHO, 2014).

Figure 2.1 shows how antibiotics can enter the terrestrial environment through antibiotic application towards animals, humans and aquaculture and through antibiotic production waste (Kumar et al., 2005). Antibiotics not only can be detected in the higher concentration levels in hospital effluents but also in the lower concentration levels in municipal wastewater, surface, sea and groundwater (Homem and Santos, 2011). Wastewater treatment plants (WWTPs) is the main anthropogenic sources of the continuous ARGs release into the environment (Zhang, 2016). This can threaten aquatic and terrestrial ecosystems and increase antibiotic resistance among microbial populations (Moussavi et al., 2013; Sun et al., 2012).

Zhang and Li (2011) reviewed about six classes of antibiotics have been widely detected in sewage, activated sludge, digested sludge, and effluents from different WWTPs worldwide. There are ß-lactams, sulphonamides, quinolones, tetracyclines, macrolides and others. These variety classes of antibiotics presence in WWTPs are due to the different origins of antibiotics have been discharged including domestic, clinical and industrial (Zhang, 2016).

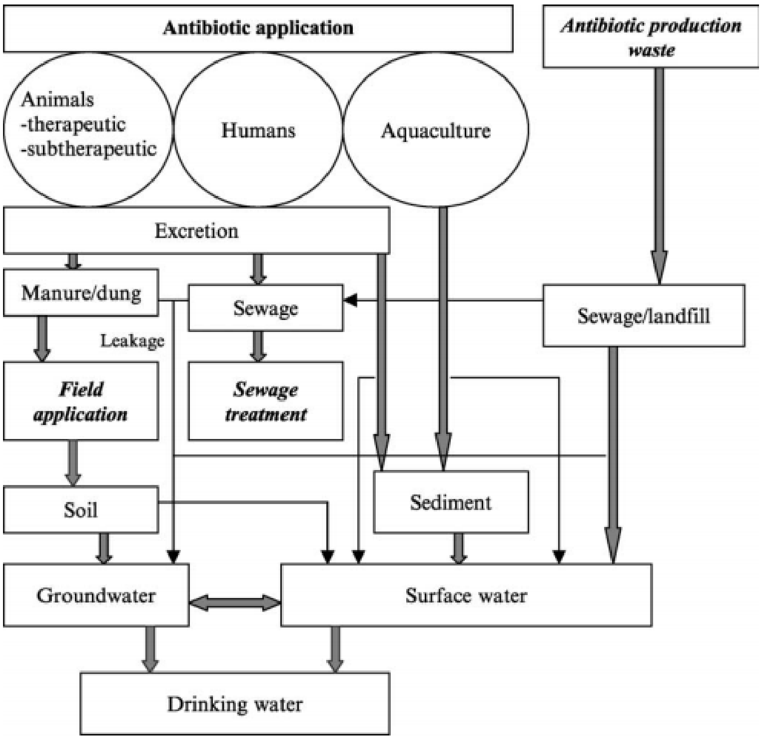


Figure 2.1 Application of antibiotic (Kumar et al., 2005)

Tetracyclines are one of the antibiotic’s groups that have significant concentration in influent and effluent of WWTPs worldwide (Zhang and Li, 2011). Percentage of tetracycline in 2014 is 4.6% of total antibiotics prescribed in primary care clinics in Malaysia (Rahman et al., 2016). Although tetracycline has less amount of consumption compared to other antibiotics, but they can be seriously dangerous for a large number of living organisms. There are five types of tetracycline antibiotics detected in WWTPs as shown in Table 2.1. Yuan et al. (2011) investigated that oxytetracycline is frequently detected in the aquatic environment and WWTPs effluents.

Table 2.1 Members of tetracycline class detected in WWTPs (Chopra and Roberts, 2001; Zhang and Li, 2011)

Chemical Name	Generic Name	Trade Name
Tetracycline	Tetracycline	Achromycin
5-Hydroxytetracycline	Oxytetracycline	Terramycin
7-Chlortetracycline	Chlortetracycline	Aureomycin
6-Demethyl-7-chlortetracycline	Demethylchlortetracycline	Declomycin
6-Deoxy-5-hydroxytetracycline	Doxycycline	Vibramycin

Hydrochloride salt is the most common form in parental of oxytetracycline and it is very soluble in water (1000 mg/cm^3) and organic solvents (Tantiyaswasdikul, 1996). Oxytetracycline its trade name as terramycin is a broad-spectrum antibiotic which frequently used in medicine and agriculture (Jácome-Acatitla et al., 2014). Huang et al. (2011) claimed about 150 tonnes of high concentration wastewater is produced from terramycin production plants and it is hard to treat and eliminate this type of antibiotic by using traditional wastewater procedure. Adsorption is the simplest and efficient technique as compare to others but there is limited research has been conducted on adsorption behaviour of terramycin onto AC.

2.2 Effect of Wastewater Treatment Processes on Antibiotics

The presence of antibiotics in the environment has led to plentiful studies investigating the role of water treatment processes towards treating and eliminating these compounds. However, there is a limited study in removing terramycin from wastewater treatment plant (WWTPs). Peng et al. (2016) reviewed various remediation techniques to remove antibiotics which have been considered as disseminate pollutants such as conventional techniques (biological processes, filtration, coagulation, flocculation and sedimentation), advanced oxidation processes (AOPs), adsorption, membrane processes and combined methods.

Terramycin has severe resistance toward biological degradation process as it is recalcitrant bio-accumulative compounds (Huang et al., 2011). Therefore, terramycin is not suitable to be removed using biological treatment. Physical or chemical treatments are more appropriate methods to treat them. Common examples of chemical treatment on terramycin are chemical oxidation and advanced oxidation process (AOP). However, Zhang et al. (2016) claimed that the removal of low concentration of terramycin is not efficient when using chemical oxidation.

Meanwhile, AOP such as photo-Fenton process producing toxic intermediates. Other than that, Liu et al. (2012) reviewed other AOP like electron pulse radiolysis, photoelectrolysis and redox with nano zero valent iron are high cost and have complicated operation during practical application even though they can treat wastewater containing tetracycline antibiotics efficiently. Frequent methods of physical treatment are membrane filtration and adsorption. Membrane filtration has advantages in requiring minimum addition of aggressive chemicals and produces zero problematic by-products as investigated by Yoon and Lueptow (2005) and has disadvantage on its high cost as reviewed by Peng et al. (2016).

Comparing terramycin removal techniques and strategies like chemical oxidation, AOP, membrane filtration and adsorption, adsorption is the most favourable technology due to its easy operation and practically feasible. Other than that, this process has a benefit towards terramycin due to its high hydrophobicity that has big Van der Waals force with the adsorbent. Furthermore, adsorption technique is more economical because it is inexpensive and the operation cost usually depends on the types of adsorbents being used. There are numerous adsorbents can be used for adsorption process including AC, aluminium oxide, graphene oxide, chitosan and others (Liu et al., 2012).

2.3 Activated carbon (AC)

A variety of adsorbents has been used for the adsorption of oxytetracycline and oxytetracycline hydrochloride as shown in Table 2.2. Comparing all kind of adsorbents, AC of different bases provides the highest adsorption capacity which lead to highest removal efficiency of oxytetracycline from wastewater especially oxytetracycline hydrochloride. ACs are suitable adsorbent to treat pollutant that difficult to be degraded such as terramycin (Huang et al., 2011). They have high adsorptive surface area range between 500 to 1500 m^2g^{-1} with pore volume ranges between 0.7 to 1.8 cm^3g^{-1}. This large surface area is due to its porous surface which able to adsorbs and retains solutes. Hence, large amount of material will be possibly adsorbed on AC (Çeçen and Aktaş, 2011).

Table 2.2 Maximum adsorption capacities of various adsorbents for oxytetracycline antibiotics

Adsorbent	Oxyetracycline Antibiotic	Adsorption capacity (mg/g)	Reference
Graphene	Oxytetracycline	336.0	(Zhang et al., 2016)
CLAC	Oxytetracycline hydrochloride	1340.82	(Sun et al., 2012)
GO	Oxytetracycline	212.0	(Gao et al., 2012)
GO-MPsb	Oxytetracycline	45.0	(Lin et al., 2013)
LSAC	Oxytetracycline hydrochloride	564.97	(Huang et al., 2011)
Mic-LSAC	Oxytetracycline hydrochloride	561.8	(Huang et al., 2011)
M	Oxytetracycline	471.1	(Rivera-Utrilla et al., 2013)
S	Oxytetracycline	375.4	(Rivera-Utrilla et al., 2013)

2.3.1 Wood-based activated carbon (WAC)

Huang and Cheng (2008) stated that wood-based activated carbons contained more mesopores than coconut- and coal-based activated carbons. Mesoporous activated carbon resulting in the adsorption of more adsorbents. They proved that more bromate (BrO_3^-) as adsorbents are adsorbed on the mesoporous activated carbon. BrO_3^- is an anion with negative charge meanwhile terramycin is an amphoteric compound which able to react both as a base and as an acid (Douvas et al., 1951). This shows that terramycin has high tendency to be adsorbed on the WAC. Moreover, based on Huang and Cheng (2008) study, WAC has high oxygen content than coconut- and coal-based carbons. The chemical structure of AC has significant influenced to the adsorption performance especially the adsorption capacity. Kipling (1956) and Bansal and Goyal (2005) stated that oxygen atoms are important components of an AC with good adsorptive properties in terms of wettability, polarity, acidity and physic-chemical properties.

2.3.2 Modified activated carbon

One of the unique feature of AC is its surface can be modified to change its adsorption characteristics and to tailor-make carbons for specific application. The modification of AC's surface can be conducted by the formation of different types of surface groups such as carbon-oxygen, carbon-hydrogen, carbon-nitrogen, carbon-sulphur and carbon-halogen. Formation of carbon-oxygen surface groups by oxidation of the carbon surface with oxidising gases or solutions, carbon-hydrogen surface groups by treating with hydrogen gas at high temperatures, carbon-nitrogen surface groups by treating with ammonia, carbon-sulphur surface groups by treating with compound which has sulphur such as carbon disulphide and sulphur dioxide and carbon-halogen surface groups by treating with halogen in gaseous or solution phase (Bansal and Goyal, 2005).

Besides formation of different types of surface groups, modification of the carbon surface can be also carried out by impregnating the surface with metals such as silver, copper, aluminium and iron. These metals have significantly high adsorption capacity. The main reasons impregnation method is used are to optimise the existing properties of AC through enhancement of its in-built catalytic oxidation capability, promote synergism between AC and impregnating agent to increase adsorption capacity and improve the capacity of AC as inert porous carrier (Bhatnagar et al., 2013).

2.4 Design of experiment (DOE)

An experiment is a sequence of tests conducted in a systematic and orderly manner to increase and enhance the understanding of an existing process or to explore a new process. Meanwhile, design of experiment (DOE) is a tool to develop an experimentation strategy in statistical way that maximizes learning using a minimum of resources (ReliaSoft Corporation, 2015). A well-designed experiment is vital because the data collected gives big impact to the results and conclusion drawn from the experiment (Montgomery, 2012). DOE has benefit in maximizing yield and decreasing variability which widely used by engineers and scientists in the improvement of manufacturing processes as it can develop new products and processes in a cost effective and confident manner (ReliaSoft Corporation, 2015).

An experiment often involves several factors and how these factors influence the output response of the system becomes the main objective of the experiment. Common strategy of experimentation used by the experimenter is one-factor-at-a-time

(OFAT). OFAT method begins with selecting a starting point as baseline set of levels for each factor. Then, each factor is varying within its range with the other factors held constant at the baseline level. Although OFAT experiment is easy to understand as compare to DOE, but it fails to consider any possible interaction between all factors (Montgomery, 2012). Considering interaction effects are more important than individual factors effect because there are numerous factors presence together in the application environment of the process instead of stick to occurrence of one factor at different times (ReliaSoft Corporation, 2015).

DOE experiments are frequently conducted in five stages which are planning, screening, optimization, robustness testing and verification. Planning is important to achieve the objective of the experiment before continuing with the process of testing and data collection. Screening step is used to identify the factors that have greatly affect the process of the experiment. Example of screening experiments are general full factorial designs, two level full factorial design, two level fractional factorial design, Placket-Burman design and Taguchi’s orthogonal arrays. Optimization experiment such as response surface methodology (RSM) is conducted to determine the best settings of the factors to achieve an optimum value of the response (ReliaSoft Corporation, 2015).

Robustness testing is significant to make the process of experiment insensitive to noise factors which are factors that affect the process but are beyond the control of the analyst. Verification is the last stage of DOE experiment which conduct a few follow-up experiment runs to validate that the system functions and objectives are follow as desired (ReliaSoft Corporation, 2015). In this study, screening stage is excluded as the experiment does not involve many factors to investigate the effect of interactions. Hence, DOE experiment is mainly using RSM as one of the objectives of this experiment is to develop model quadratic regressions by optimization study of adsorption of terramycin on WAC.

2.4.1 Response surface methodology (RSM)

Response surface methodology is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes (Benredouane et al., 2016). Montgomery (2012) stated that response surface is usually represent graphically in three-dimensional shape as in Figure 2.2, where expected yield (ŋ) is plotted against temperature (x_1) and pressure (x_2). Figure 2.3 shows a contour plot of the response surface which help to visualize the shape of response surface. Each contour which is the curves or lines represents a specific value of expected yield defined for combinations of the levels of the factors (Khuri and Cornell, 1996)

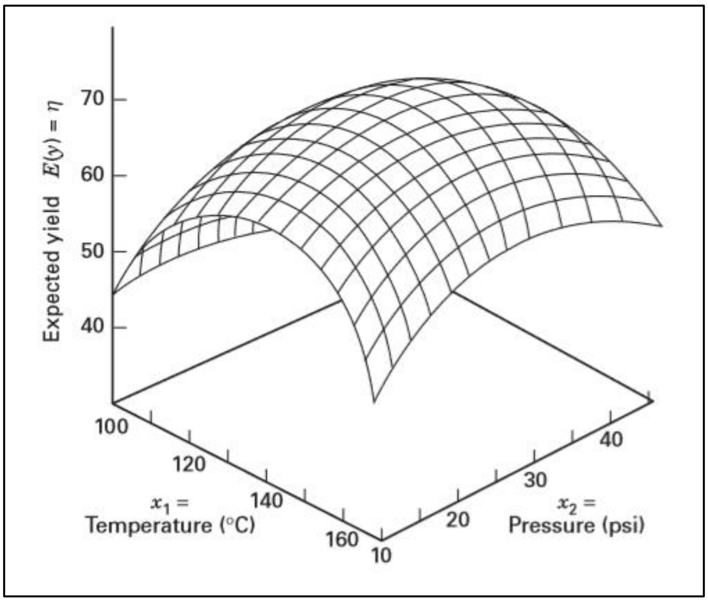


Figure 2.2 A three-dimensional response surface showing the expected yield (ŋ) as a function of temperature (x_1) and pressure (x_2) (Montgomery, 2012)

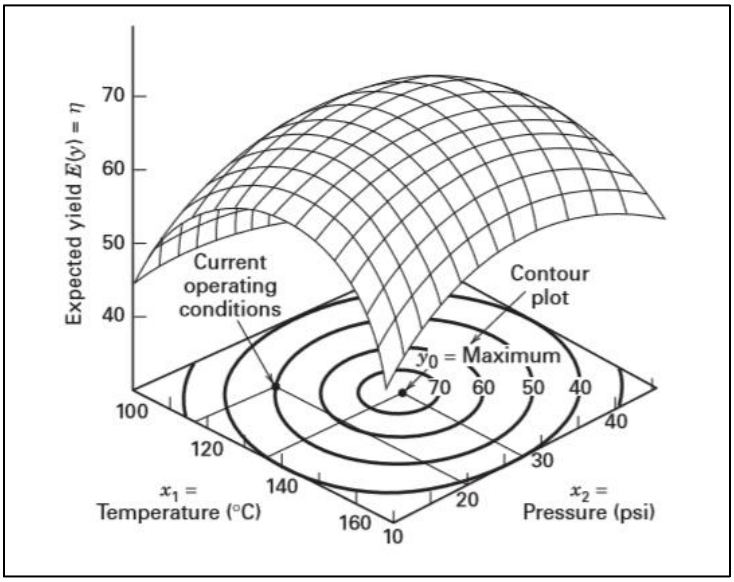


Figure 2.3 A contour plot of a response surface (Montgomery, 2012)

Khuri and Cornell (1996) has defined that RSM as a set of methods which includes several important things before the optimal settings of the experimental factors that produce optimum value of output response can be obtained. RSM will design a set of experiments that will yield adequate and reliable response. Then, the data collected from the set of experiments will be used to develop a mathematical model that best fits those data. RSM has several design experiments including central composite design (CCD), Box-Behnken design, and 3-Level Factorial. However, among these designs, CCD is the most favourable design in most application including adsorption process. This is because CCD requires a significantly smaller number of experiments than full three level factorial design and CCD provide good and reliable results than Box-Behnken design as shown in Table 2.3.

Table 2.3 Advantages and disadvantages of RSM designs (Rakić et al., 2014)

Type of RSM design	Advantages	Disadvantages
Full three level factorial design	• Enable good and reliable results	• Require of large number experimental runs which will produce unwanted, high order interactions
Central composite design (CCD)	• Enable good and reliable results	• Time consuming with large number of factors
Box-Behnken design	• Avoids extreme conditions of experiments	• Has insufficient reliable results

2.4.2 Central composite design (CCD)

Central composite design (CCD) is the most popular class of designs used for fitting second-order model (Montgomery, 2012). This is because it gives a better alternative to the full factorial three-level design as it demands a smaller number of experiments and providing comparable results (Benredouane et al., 2016). CCD consists of three portions which are a complete 2^k factorial design whose factors levels are coded as $-1, 1$, this is called the factorial portion, 2^k axial or star runs, and n_0 center runs (Montgomery, 2012). Hence, the total number of design points in a CCD is $n = 2^k + 2^k + n_0$. The values of distance α of the axial runs from the design center and n_0, the number of center-point replications are important parameters that must be specified in the design so that the CCD can obtain certain desirable properties such as orthogonality and rotatability (Montgomery, 2012; Benredouane et al., 2016).

Ferreira et al. (2007) reviewed that the center-point replications at the center point have two main objectives which are providing a measure of pure error and stabilizing the variance of the predicted response. Many replicates are better to obtain a more accurate estimate of error. The important parameters that has mentioned before are based on the number of factors, n in the design, where α is equal to $\sqrt{n}$. The choice of α depends on a great extent on the region of operability and region of interest. The value of the distance α usually varies from 1.0 up to $\alpha = \sqrt{n}$. If the distance from the center of the design space to a factorial point (2^n) is ± 1unit, the distance from the center of the design space to a star point is ± α with $[\alpha] > 1$ (Myers et al., 2009)

Two main varieties of CCD available in most statistical software program by changing α are rotatable and face-centered. Table 2.4 summarizes the properties of the two varieties of CCD and Figure 2.4 illustrates the differences among these varieties. From the illustration, CCC explores the largest process space while CCI explores the smallest process space. CCC is rotatable design but CCF is not rotatable design. In this study, CCF design is used as it only requires 3 level of each factors.

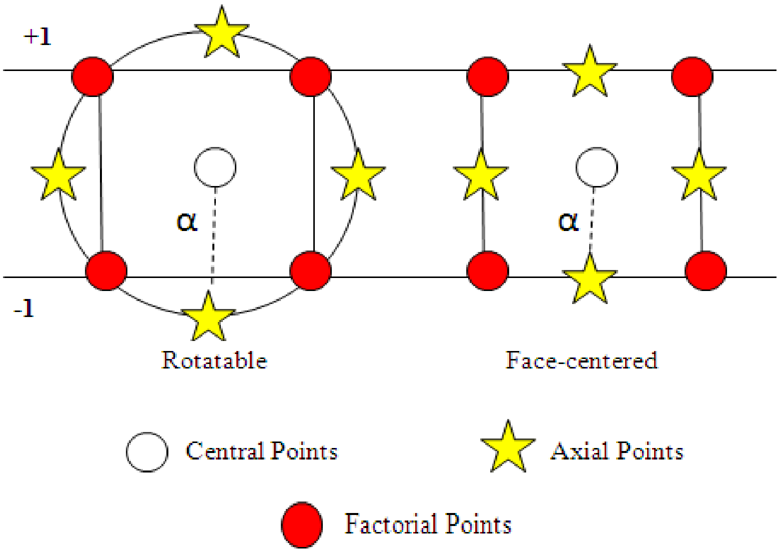


Figure 2.4 Types of central composite design (Croarkin and Tobias, 2014)

Table 2.4 Comparison Types of central composite designs (Croarkin and Tobias, 2014)

CCD Type	Terminology	Descriptions
Rotatable	CCC	• The original form of the central composite design. • The axial points are at some distance α from the center based on the properties desired for the design and the number of factors in the design. • The axial points establish new extremes for the low and high settings for all factors. • The designs have circular, spherical or hyperspherical symmetry. • Require 5 levels for each factor. • Augmenting an existing fractional factorial design with axial points can produces this design.
Face-centered	CCF	• The axial points are at the center of each face of the factorial space, so that $\alpha = \pm 1$. • Requires 3 levels for each factor. • Augmenting an existing factorial design with appropriate axial points can also produce this design.

2.4.3 Analysis of data

CCD is the most popular class of designs used for fitting a quadratic which is second-order model. A complete second-order model is as Equation 3 (Montgomery, 2012):

$$Y = b_0 + \sum_{i=1}^{3} b_i x_i + \sum_{i=1}^{3} b_{ii} x_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} b_{ij} x_i x_j + \varepsilon \qquad (3)$$

Where Y is the response (dependent variable), b_0 is the constant coefficient, b_i is the coefficient for the linear effect, b_{ii} is the coefficient for the quadratic effect, b_{ij} is the coefficient for the interaction effect, x_i and x_j are the independent variables (factors).

Based on analysis provided inside DOE software, there are several things need to be followed to validate a model. Firstly, data is analysed by fitting a response surface model. The suggested model is given whether it is linear, 2FI, quadratic or cubic. However, cubic model is aliased and it does not significantly improve the fit model.

This is because the central composite matrix provides too few unique designs points to determine all the terms in the cubic model (Stat-Ease Inc., 2016).

The quadratic model is usually the best model to fit in as it exhibits low standard deviation, high R-squared values and low PRESS (Stat-Ease Inc., 2016). Prediction Error Sum of Squares (PRESS) is a statistical test that measures the adequacy of the model to predict the response (Granato et al., 2010). Then, the suggested model is used for the stepwise automatic model regression. This procedure allowed the selection of probabilities (*p*-values) for adding, removing or exchanging the model terms. Other types of automatic model regression which are backward and forward only allowed the model terms to be removed and added respectively (Stat-Ease Inc., 2016).

Secondly, analysis of variance (ANOVA) will analyse the chosen model previously. The ANOVA statistics consists of value of R-squared, adjusted R-squared, predicted R-squared, lack of fit test, standard deviation, and others. These statistic keys are important to confirm the adequacy of the chosen regression model. R-squared statistics are very good if it is near to 1. *p*-values which is called as “Prob>F” in the output must be less than 0.05 which indicates that model terms are significant. In addition, the individual terms with probability values greater than 0.10 can be removed from the model. Besides that, if the lack of fit value is significant, the model is not fit (Stat-Ease Inc., 2016).

Thirdly, analysis of data is proceeded with the diagnostic of the statistical properties of the regression model. There are various diagnostic plots shown in the DOE to statistically validate the model. Normal probability plot of the standardized residuals which known as normal plot of residuals is the most important diagnostic. The data points should be approximately linear. A non-linear plot like S-shaped curve