OPTIMIZATION OF AQUEOUS REMAZOL BRILLIANT BLUE R (RBBR) DECOLORIZATION BY *TRAMETES* SP. PELLETS IN FLUIDIZED BED BIOLOGICAL REACTOR (FBBR)

LIZA FERINA

DISSERTATION SUBMITTED IN FULLFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ENGINEERING SCIENCE

DEPARTMENT OF CIVIL ENGINEERING
FACULTY OF ENGINEERING
UNIVERSITY OF MALAYA
KUALA LUMPUR

2013

ABSTRACT

Decolorization of synthetic dye Remazol Brilliant Blue R (RBBR) by *Trametes* sp. pellets in fluidized bed biological reactor (FBBR) was studied. Initially, the decolorization process was performed using shaken flasks which contained 100 ml of RBBR aqueous solution and fungal pellets. The process was followed for 48 hours and the decolorization was analyzed at a visible spectrum of 590 nm.

Response surface methodology (RSM) employing Box Behnken design at three factors i.e. initial concentration of RBBR, mass of pellet and pH was used to optimize decolorization process in shaken flasks. As the result, initial dye concentration was found to be a significant main factor to decolorization process compared to mass of pellets and pH. Meanwhile, maximum decolorization efficiency 67.9 ± 5.4% was achieved when 50 ppm initial concentration of dye, 4 gram of pellet and pH 5.6 were ran.

Kinetic of RBBR decolorization in batch flask with optimum values of variables was also studied. The closest model to explain the kinetic of decolorization was the first order model with kinetic constant (k) = 0.071 h^{-1} and R^2=0.964. Furthermore, the optimized variables in batch flask study to gather with various hydraulic retention time (HRT) and air flow rate were operated in continuous FBBR for 72 hours. From graphical analysis, the highest decolorization was recorded at 24 hr of HRT for all air flow rate tests.

The effects of HRT and air flow rate in continuous reactor were analyzed by second order polynomial model. For the main and interaction factor, both of HRT and air flow rate were found to be significant on RBBR decolorization. The maximum decolorization was obtained at 24 hr of HRT and 0.6 l/min of air flow rate. Kinetic study in FBBR was

studied by comparing plug flow and mixed flow model. Based on kinetic constants from batch study (0.071 hr^{-1}) and both of models, the exit concentration of aqueous RBBR could be predicted. The plug flow model was chosen as the fit model to illustrate the observation process in FBBR, especially for 0.6 l/min of air flow rate.

Keywords: decolorization, *Trametes* sp, response surface methodology, kinetic study, fluidized bed biological reactor.

ABSTRAK

Kaji selidik terhadap proses penyahwarnaan warna 'Remazol Brilliant Blue R (RBBR)' oleh *Trametes* sp. telah dijalankan dengan menggunakan '*fluidized bed biological reactor'* (FBBR). Proses penyahwarnaan RBBR dilakukan dengan menggunakan kelalang goncang yang mengandungi 100 ml larutan RBBR dan pelet kulat. Proses ini dilaksanakan selama 48 jam dan kehilangan warna RBBR dianalisis pada spektrum 590 nm cahaya nampak.

Dengan menggunakan model 'Box Behnken' dalam menjalankan penyelidikan untuk mencari titik optimum penyahwarnaan yang berkesan, tiga faktor yang terlibat secara terus dalam sistem telah dikaji. Faktor-faktor tersebut adalah kepekatan awal RBBR, berat sel kulat dan pH larutan. Daripada hasil kajian, hanya kepekatan awal RBBR sahaja yang memberi kesan penting terhadap sistem penyahwarnaan RBBR yang dijalankan. Keberkesanan penyahwarnaan yang optimum telah dicapai dengan 67.9 ± 5.4% daripada 50 ppm kepekatan awal RBBR, 4 g sel kulat dan pH 5.6.

Dengan menggunakan titik optimum penyahwarnaan, penyelidikan terhadap kinetik penyahwarnaan RBBR juga telah dijalankan dengan menggunakan reaktor kelompok. Model kinetik yang paling sesuai dalam menjelaskan mekanisma penyahwarnaan RBBR oleh sistem yang diselidik adalah model linear dengan pemalar kinetiknya (k) =0.071/jam dan R^2=0.964. Dengan menggunakan titik optimum pada setiap faktor yang telah diselidik, penyelidikan terhadap *hydraulic retention time* (HRT) dan halaju aliran udara juga telah dijalankan selama 72 jam dengan menggunakan sistem FBBR yang berterusan. Daripada analisis grafik, telah didapati penyahwarnaan RBBR adalah tertinggi pada 24 jam HRT untuk kesemua halaju aliran udara yang dikaji.

Analisis terhadap kesan HRT dan kelajuan udara dalam reaktor berterusan telah dilakukan dengan menggunakan model polinomial tahap kedua. Kedua-dua HRT dan

halaju aliran udara telah memberi kesan penting terhadap penyahwarnaan RBBR. Penyahwarnaan RBBR yang tertinggi telah didapati pada 24 jam HRT dan kelajuan udara 0.6 l/min. Analisis kinetik dalam FBBR telah dilakukan dengan mengaplikasikan model aliran palam dengan model aliran campuran. Daripada pemalar kinetik daripada sistem kelompok (0.071/jam) dan berpandukan kepada kedua- dua model, anggaran kepekatan larutan keluar RBBR dapat dijalankan. Model gerakan aliran palam dalam sistem dipilih sebagai model terbaik untuk menerangkan proses penyahwarnaan dalam FBBR terutama ketika halaju udara 0.6 l/min digunakan.

Kata kunci: penyahwarnaan, *Trametes* sp, '*response surface methodology'*, analisis kinetic, reaktor biologi '*fluidized bed'*.

AKNOWLEDGEMENT

First and foremost, I wish to acknowledge with deepest gratitude to Allah SWT for all the blessings and grace are best owed so that I can complete the research and writing of this thesis well. On this occasion, I would also like to express our appreciation and heartfelt thanks to those who have provided help and support in the implementation and completion of this research.

I would like to express special thanks and appreciation to my thesis research supervisor, Prof. Shaliza binti Ibrahim and Dr Mohamad Suffian bin Mohamad Annuar, for the encouragement and guidance of his outstanding research in the field of Decolorization by white-rot fungi. The entire guidance and support in the form of innovative ideas and intellectual spectacular really stimulate my interest and enthusiasm in exploring this research. Thank you for all your support and sharing many valuable experiences in helping me on write this thesis.

Furthermore, I would like to thank Universiti Malaya, especially all colleagues at the Biotechnology and Environmental Engineering Department for all their help and support facilities are not limited to equipment used in this study.

I am also very grateful to my family, especially for my lovely parents Jafri (deceased) & Darmilis, my husband Mulya Andhika Putra, and my marvelous children Akhdan Thalib Asyrafi (deceased) & Rana Syakira Utami, for all the attention, patience, help and tremendous support to me in completing the writing of this thesis research. Without support of them, I can not imagine this success can be achieved. Last but not least, I would like to thank all those who have helped me directly or indirectly in completing research towards this thesis. Hopefully this research can provide benefits to the development of research in the field of Wastewater Technology.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

COD	Chemical Oxygen Demand
GYMP	Glucose Yeast Malt Peptone
HRT	Hydraulic Retention Time
FBR	Fluidized Bed Reactor
LiP	Lignin Peroxidases
MnP	Mangan Peroxidases
RBBR	Remazol Brilliant Blue R
RSM	Response Surface Methodology
WRF	White Rot Fungi

CHAPTER I

INTRODUCTION

1.1 Background

There are several different classes of dyes that are manufactured worldwide, such as acidic, reactive, basic, disperse, azoic, diazole, anthraquinone-based, and metal complex dyes. It has been estimated that 10,000 dyes and pigments are produced with total market value of more than 7×10^5 tons per year (Doble & Kumar, 2005). More than 80,000 tonnes of reactive dyes are produced and consumed every year (Hessel, Allegre, Maisseu, Charbit, & Moulin, 2007). Approximately 5 to 10% of them are lost during coloration and colored natural water bodies (Doble & Kruthiventy, 2007; Yesilada, Asma, & Cing, 2003). The effluents from the textile industries containing dyes are highly coloured and may significantly affect photosynthesis activity (Kilic, Nielson, Yuce, & Donmez, 2007). These effluents are often carcinogenic, mutagenic, and highly harmful to the environment (Banat, Nigam, Singh, & Marchant, 1996).

Several technologies are available for decolorization of textile dye effluents, such as adsorption, irradiation, ion exchange, oxidation, coagulation and precipitation, aerobic process, and anaerobic process; but the problem has not been solved because of high cost, low efficiency, sludge handling problems, less microbial resistant to the pollutant etc (Anjaneyulu, Chary, & Raj, 2005). Recently, many studies of biological decolorization utilizing fungal strains have been reported (Deveci, Unyayar, & Mazmanci, 2004). White rot fungi have been shown to degrade a wide variety of recalcitrant organic pollutant (Young & Yu, 2007). Several fungal strains were found to possess the potential to decolorize commercial reactive dyes e.g. *Bjerkandera adusta*, *Trametes versicolor* and *Phanerochaete chrysosporium* (Heinfling, Bergbauer, & Szewzyk, 1997; Swamy & Ramsay, 1999)

In this study, *Trametes* sp pellets were used for decolorizing reactive dye Remazol Brilliant Blue R (RBBR). RBBR is usually used in the production of polymeric dyes. This dye is an anthraquinone derivative which represents a class of toxic and organopollutant materials (Deveci, et al., 2004; Wesenberg, Kyriakides, & Agathos, 2003).

1.2 Objectives of study

This research aims:

1. To investigate the effect of process variables (initial dye concentration, pH, and mass of fungal pellets) on the RBBR dye decolorization using *Trametes* sp and the kinetics of dye decolorization in the batch process;
2. To Investigate the effect of HRT and air flow rates on the RBBR decolorization in *Trametes* sp fungal pellet continuous fluidized bed biological reactor.

1.3 Outline of Thesis

Chapter One introduces the background of the research, followed by the objectives of the study. Chapter Two, the current technologies for dye removal and the literatures of dyes decolorization by white rot fungi are reviewed. The use of response surface methodology (RSM) to design and analyze the experimental data is outlined. This chapter also examines fluidized bed biological reactor (FBBR) process and the kinetic models available.

Chapter Three describes the materials and methodologies used; the statistical design of aqueous batch studies by RSM Box Behnken design method; and the experimental runs in FBBR studies.

The result of all experiments and analysis are reported in Chapter Four. The results were discussed and compared with available literature. Chapter Five concludes the finding of the studies and implications for further studies are discussed.

CHAPTER II

LITERATURE REVIEW

2.1 Classification of Dyes

Dyes are substances for coloring materials which become an integral part of the materials, which cannot be removed by rubbing or washing (Thakur, 2006). There are two major sources of dyes, natural dyes and synthetic dyes. Natural dyes are taken from plant sources, minerals, or animal sources. Synthetic dyes are man-made colorants produced in a laboratory or factory. The first synthetic dye was discovered by William Henry Perkin in 1856 (Aspland, 1997).

Dyes are classified into two categories according to their chemical structure and applications. Based on their application and the methods used to apply them, dyes are classified as shown in Table 2.1.

Table 2.1 Classification of Dyes Based on Its Applications

Type of Dyes	Characteristic	Application
Acid Dyes	Usually sodium salt of sulphuric acid or carboxylic acid	Applied to wool, silk, nylon, etc.
Basic Dyes	Contain salts of amino or subsituted amino groups, which in acid solution forms water-soluble cations.	Used to dye modified nylon and polyesters.
Direct Dyes	Can be directly applied to the fabrics from an aqueous solution, very useful for those fabrics which can form hydrogen bonds	Used to dye cotton, rayon, wool, silk, and nylon.

Table 2.1 (continued)

Type of Dyes	Characteristic	Application
Azole Dyes	Insoluble azo dyes which are produced by a chemical reaction on the fabric itself. The reaction involve the coupling of fabrics with diazonium salt.	Used for cotton, silk, polyesters, and nylon.
Disperse Dyes	Dyes in which the minute particle are dispersed or spread in suitable reagent before applying to the fabric.	Used for nylon, polyesters, and polyacrylonitrile.
Fiber Reactive Dyes	Attach to the fibre by irreversible chemical reaction, then dyeing process takes little time and the colour is retained for a long time.	Applied in cotton, wool, and silk.
Vat Dyes	These are insoluble coloured compounds that reduced to colourless soluble form (leuco) and oxidised to an insoluble coloured dye by exposure to air or an oxidising agent.	Can be applied to most fabrics.
Mordants Dyes	Require additional substances for fixing, generally a metal ion, and then forms a link with metal ion which binds to the fabric	Used mainly for wool
Solvent Dyes	Solubility in organic solvent. The molecules are typically non polar and insoluble in water	Used for wood staining, producing coloured lacquers, solvent ink, colouring oils, waxes, and fats.

(Thakur, 2006)

The classification of dyes are published in Color index (C.I.) by the Society of Dyers and Colourists (United Kingdom) in cooperation with American Association of Textile Chemists and Colorists (AATC), which indicates their application class, the hue and a number that reflects the chronological order in which the colorants were introduced commercially (Bank, Environment, & Organization, 1999; Christie, 2001). The classification of dyes according to their chemical structures is shown in Figure 2.1.

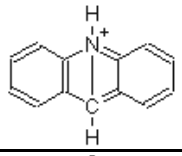
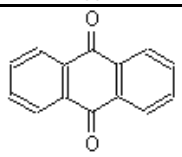
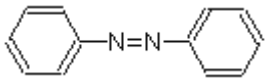
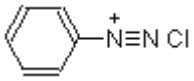
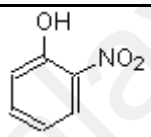
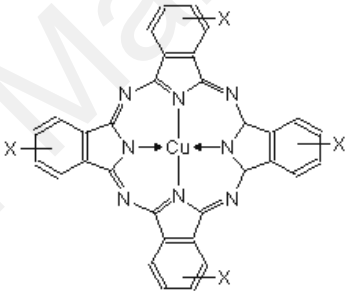
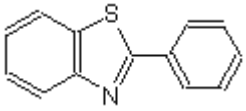
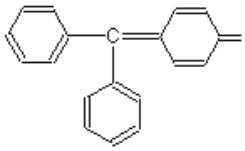
Class	General Formula
Acridine	
Anthraquinone	
Azo	
Diazonium	
Nitro	
Phthalocyanine	
Thiazole	
Triarylmethane	

Figure 2.1 Chemical Structure of Dyes

2.2 Environmental Impact of Dye

Many industries in the whole world have used synthetic dye in their production process, because it is easier, cheaper, and more stabile to light, detergent, and microorganism. On the other hand, synthetic dyes affect the environment as a consequence of inefficiency in dying process, poor handling of effluents, and insufficient treatment of industrial dyestuff wastes (Bhatt et al., 2000). One of industries which used synthetic dyes at prodigious amount is textile industry and its processes.

Textile industries also contributed a large amount of pollutant substances. Its pollutant load generally contains salts, detergents, organic acids, and dyestuffs. Although dyestuffs are not a significant load, they are the main pollutant for the effluent colouration, which may decrease light transmission to aquatic life (Hessel, et al., 2007). Besides that, some of the dyes and their degradation products have proven to be toxic, mutagenic, and carcinogenic in nature. Based on any research, these dyes cannot be removed by aerobic microbial degradation in wastewater treatment plant because of some factors like resistance to chemical, and light induced oxidative fading, high water solubility, high molecular weight, and complex aromatic ring structures which inhibit permeation through organism cell membranes. Thus, development of technology to removes dyes from effluent has been important (Keharia & Madamwar, 2004).

2.3 Technologies for Dye Effluent Treatment

There are many technologies known to decolorize all types of dyes. Dyes could be decolorized by physical, chemical, and biological method. Physically, dyes can be removed without occurs degradation of molecular. Chemically, decolorization occurs by adding chemical compound, such as fenton agents and ozonation. This chemical reaction will modify the chromophore which is part of molecule causing color. Biological decolorization involved biological mechanism like the biosorption, degradation or accumulation.

2.3.1 Physical Method

2.3.1.1 Adsorption

Adsorption process is known as one of the most effective methods in water and wastewater treatment. This process can removes pollutans from aqueous or gaseous phase onto solid phase (Venkat, Khrisna, & Karthikeyan, 2000). In decolorization process, collaboration of adsorption and ion exchange is occured (Slokar & Marechal, 1998), and it is influenced by

many factors, i.e dye/adsorbent interaction, contact time, pH, surface area of sorbent, temperature, and size of particle (M. Kumar, Sridhar, Bhavani, & Dutta, 1998).

The most commonly method of dye removal is activated carbon. Activated carbons using sawdust-based and coal-based material have been proved to effectively removing reactive dye (Vijayaraghavan, Won, & Yun, 2008). However, the activated carbon is considered too expensive. The alternative low cost technologies of adsorption are using natural waste adsorbent e.g. bark, rice husk, waste ash, and coffee grounds (McKay, 1983; Nakamura, Tokimoto, Tamura, Kawasaki, & Tanada, 2003; Smelcerovic, Dordevic, Novakovik, & Mizdrakovic, 2010).

2.3.1.2 Electrocoagulation

Another method used widely in water and wastewater treatment is electro coagulation that is categorized as an easy and efficient method. The electrocoagulation uses two electrode material, i.e aluminium or iron. These electrode materials create direct electric current to adsorb pollutant in decolorization process (Merzouk et al., 2009).

The important key of electrocoagulation is flocculants forming process. These flocculants were generated by electrical current from the anode (Dubrow, Boardman, & Michelsen, 1996; Robinson, McMullan, Marchant, & Nigam, 2001). Flocculants would adsorb pigment aggregates and/or dissolved dye in textile effluent (Essadki et al., 2008; Zidane et al., 2008). Finally, these flocs could be removed from wastewater by sedimentation or flotation. Unfortunately, these technologies produce a large volume of sludge and require sufficient space and capacity for disposal (Dubrow, et al., 1996; Robinson, et al., 2001).

2.3.2 Chemical Methods

2.3.2.1 Fenton reagent

Fenton reagent is one of the most effective methods of organic pollutants oxidation. The Fenton reagent has been found effective in treating various industrial wastewater components including aromatic amines, and a wide variety of dyes (Barbusinski, 2005). Fenton reagent is a combination of hydrogen peroxide (H_2O_2) and ferrous iron (Fe^{2+}) in solution (Alshamsi, Albadwawi, Alnuaimi, Rauf, & Ashraf, 2007; Ay, Catalkaya, & Kargi, 2009; Sun et al., 2009). The reaction mechanism is as follow.

1. $Fe^{2+} + H_2O_2 \longrightarrow OH^o + OH^- + Fe^{3+}$
2. $Fe^{3+} + H_2O_2 \longrightarrow OOH^o + H^+ + Fe^{2+}$

Further, dyes are oxidized by hydroxyl (OH^o) and peroxyl (OOH^o) radicals generated from those reactions. Fenton's reagent is acceptable for toxic wastewaters which inhibit growth of the microbial biomass in the sludge. But, this technology also produces a large volume of suspended solids due to flocculation and requires space and capacity (Robinson, et al., 2001; Slokar & Marechal, 1998; Vandevivere, Bianchi, & Verstraete, 1998).

2.3.2.2 Ozonation

Application of ozone has been found successful in decolorizing of dye solution by several studies (Khadhraoui, Trabelsi, Ksibi, Bouguerra, & Elleuch, 2009; Peralta-Zamora et al., 1999). Ozone can degrade a wide variety of dyes because it is a strong oxidizing agent compared to chlorine and hydrogen peroxide. But, it is usually used in the final step of the treatment process. The high strength of raw textile waste water affects low efficiency of ozonation treatment (Lu, Yang, Chen, & Sun, 2009). So that, ozonation needs additional treatment to obtain an acceptable level of decolorization.

Ozonide radical has the half-life ranges from seconds to hours. It is depending on water quality and is commonly reduced by organic compounds existing in the wastewater (Von, 2007). The pH condition must be controlled because decomposition of ozone is catalyzed by hydroxide anions (Hoign ́e, 1998). This technology is recommended only to effluents with high dye concentrations because the higher capital costs to setup an ozonation facility than other technologies (Robinson, et al., 2001; Vandevivere, et al., 1998).

2.3.3 Biological Methods

Biological decolorization methods are easy and low in operation cost. These methods include:

1. Use of microbial cultures either single or mixed bacterial cultures under aerobic, anaerobic, or mixed condition.
2. Adsorption by living or dead microbial biomass;
3. Decolorization by fungus.

Padmavaty *et al*., (2003) observed mixed bacterial used to decolorize many type of reactive azo dyes aerobically. Azo dyes are mostly used in textile industry. Potential microorganism was identified as *Pseudomonas sps, Bacillus sps, Halomonas sps, Orthobacter sps, Micrococci sps* were mixed to form consortia. COD removal obtained in their research was 75.15-95.9% and decolorization percentage was 37.5-95.6%. Examination for the aerobic mixed culture's potential for decolorization of Remazol Black B dye in batch reactor was also performed by Kumar *et al*., (2009). The decolorization was successful where 98% was achieved at 25 ppm initial concentration of dye after 18 hours incubation period and 75% at 300 ppm after 48 hr incubation period. Color together with COD removal was the advantage of aerobic process. But, generally the azo dyes were resistant to aerobic microbial degradation (Anjaneyulu, et al., 2005).

Decolorization of three reactive azo dyes by mixed cultures isolated from textile effluents under anaerobic condition was observed by Cetin and Donmez (2006). Percentage decolorization was up to 80% with exposure time between 24-48 hr. Similarly observation was achieved by Karatas et al., (2009). They were using mixed microbial culture to decolorize three reactive dyes found in textile industrial wastewater. The dyes consist of diazo, azo and anthraquinone dye with differential initial concentrations after 24-72 hr incubation. High decolorization efficiencies were obtained up to 90% for azo and diazo dyes after 24 hr incubation. The anthraquinone dye was decolorized lower than 21% after 72 hr. That indicated that decomposition of anthraquinone was difficult compare with azo group.

Generally, anaerobic decolorization by microbe was successful to be applied. But, the initial step in bacterial azo dye metabolism under anaerobic condition involved the reductive cleavage of azo linkage, which results in dye decolorization and the formation of hazardous colorless aromatic amines. Under anaerobic, these amines were not degraded and accumulated (McMullan et al., 2001). It was suggested to combine aerobic and anaerobic for dye decolorization.

Combination of anaerobic and aerobic method was examined by Franciscon *et al*., (2009). They were successful to decolorize four azo dyes in sequential microaerophilic-aerobic treatment by facultative *Klebsiella sp*. Anaerobic and aerobic treatment not only removing of dye but also degraded aromatic amines. Decolorization rates achieved by this treatment were up to 92% with decolorization time ranged between 72 hours till 168 hours or about 3-7 days. The presence of aromatic amines was detected after microaerophilic stage, but the significant reduction of them was observed after aerobic stage. Anaerobic and aerobic method was also performed by Supaka *et al*., (2004); Sandhya et al., (2005)

2.4 Decolorization of Dye by White Rot Fungi

White rot fungi are a heterogeneous group of organisms which have capability to degrade lignin, several wood components, and many recalcitrant compounds. They have been proved being a suitable organism for textile effluent treatment and dye removal. Extracellular enzymes of fungal mycelia are an additional advantage comparing of single cell organism in decolorizing of dye. These fungal enzymes are also valuable in tolerating high toxic concentrations of pollutants (Kaushik & Malik, 2009).

Mechanisms reaction of fungi to dye can be classified into biodegradation, biosorption and bioaccumulation. Biodegradation is the biological process depending on energy and the breakdown of dye into various byproducts involves the action of various enzymes. Biosorption do not involve metabolic energy or transport but a binding of dye molecule to the biomass either dead or living. While, bioaccumulation is pollutants accumulation by actively growing cells as its metabolism (Z Aksu & Donmez, 2005; Tobin, White, & Gadd, 1994).

Some studies investigated white rot fungi with their lignin degrading enzymes to decolorize various textile dyes (Bhatti, Akram, & Asgher, 2008; Champagne & Ramsay, 2005; Cripps, Bumpus, & Aust, 1990). Lignin degrading enzymes likes Lignin peroxidases (LiP), Mn peroxidases (MnP) and laccases are secreted when fungal's growth in limited nutrient, either carbon or nitrogen sources (Cameron, Timofeevski, & Aust, 2000; Kirk & Farrell, 1987; Leonowicz et al., 2001). The first dye decolorization using *Phanerochaete chrysosporium* was reported by Tien and Kirk (1983). Further, the dye decolorization by new species was evaluated by others (Asgher et al., 2008; Levin, Papinutti, & Forchiassin, 2004; Mendonca, Jara, Gonzalez, Elissetche, & Freer, 2008; Robinson & Nigam, 2008; Santos, Neto, Tavares, & Costa, 2004). Twenty nine species of white rot fungi were capable of dye decolorization (Wesenberg, et al., 2003). Dye decolorization capabilities vary with species of the fungal or enzyme (Chagas & Durrant, 2001; Nyanhongo et al., 2002).

Manganese peroxidase (MnP) and laccase were the main enzymes detected in *Trametes versicolor* and the involvement of each enzyme to dye decolorization depended on the dye (Champagne & Ramsay, 2005; Swamy & Ramsay, 1999). MnP was more efficient than laccase in decolourizing the azo dye Acid red 27 compared an anthraquinone dye Remazol Brilliant Blue R. On the other hand, laccase was efficient in decolorizing of anthraquinone dye.

Several studies which investigated WRF as bioremediation agents to treat textile wastewater have been reviewed. Those studies have shown the potential of white-rot fungi to treat real wastewater from the textile industry, but most of them have been performed with some preconditioning of wastewater (dilution, pH adjustment, sterilisation, addition of nutrients). Therefore, nowadays the application of such fungi at industrial scale is still a technical challenge (Couto, 2013). However, the technology would be developed to dissolve of the problems and the application of WRF in industrial scale could be implementated even in a small scale.

2.4.1 Factors Influencing Dye Decolorization

2.4.1.1 Effect of Initial Dye Concentration

Dye removal may be influenced by initial dye concentration through a combination factors like toxicity at higher concentration for fungi, ability of enzyme to degrade the substrate at very low concentration and the time period required to reach the maximum decolorization. Decolorization of Reactive Blue 25 by *Aspergillus ochraceus* was reported by Parshetti *et al.*, (2007). They took more periods when dye concentration was increase, where 100 mg/l concentration needed 20 days and 400 mg/l need 40 days to be decolorized. Kapdan *et al.*,(2000) reported that decolorization of Everzol Turqoise Blue G by *Coriolus versicolor* was nearly 100% at concentrations of 100 to 500 mg/l for 3 to 5 days and mostly 80% at concentrations of 700 to 1200 mg/l for 9 days. From those studies, it indicates that

decolorization efficiency was affected by the time period and initial dye concentration. The dye class may influence to dye decolorization. Phthalocyanin dyes are comparatively easier to remove than azo and anthraquinone dyes. Anthraquinone were more resistant towards degradation due to their fused aromatic structures, which remain coloured for long periods of time.

2.4.1.2 Effect of Initial pH

Several studies have investigated the effect of initial pH for dye decolorization by fungi. Some decolorization results were better under acidic condition, and others were better at a neutral or slightly alkaline pH. In Table 2.2, the different type of dye and fungi was studied to observe the effect of pH in decolorization at wide range (3-11).

Table 2.2 Effect of pH in Dye Decolorization

Type of dye	Fungi	pH	Decolorization result/efficiency	References
Everzol Turqoise Blue G	*Coriolus versicolor*	4.5	99%	(Kapdan *et al*., (2000)
		6 and 7	50%	
Reactive Blue 25	*Aspergillus ochraceus*	3	87%	Parshetti *et al*., (2007)
		5	100%	
		7	81%	
		9	70%	
Astrazon Blue FGRL, Astrazon Red FBL, Astrazon Black FDL	*Funalia trogii*	6-11	Good removal	Yesilada *et al*., (2003)
Solar Golden Yellow R	*Schyzophyllum commune*	4.5	73%	Asgher *et al*., (2008)
		5	59%	
		6	8%	
Cotton Blue	*Penicillium ochrochloron*	3	80%	Shedbalkar *et al*., (2008)
		5	83%	
		7	93%	
		9	88%	
		11	80%	

From those studies, the optimum pH was various which may depend on fungus and dye being treated.

2.4.2 Bioreactor Modes of Dye Removal

There are many studies investigating dye removal in bioreactors. White rot fungi have been used to decompose several recalcitrant dyes in different reactor configurations, including fixed film bioreactors, packed bed reactors, rotating biological contactors, and pulsed flow reactors as shown by Table 2.3. Generally the operations were performed in batch, semibatch or continuous mode and attached growth reactors were mostly used to decolorize dye using WRF. For the operating parameters, HRT was in range 24 hr until 6 days and temperature's average was 25^0C with pH 4.5-6.4. Only few of fluidized bed bioreactor study used to decolorize dye using WRF. Zhang *et al*.,(1998) studied decolorization of cotton bleaching effluent with unidentified WRF. They found MnP as the enzyme detected in that study with retention time for 3 days and the decolorization efficiency was 75 to 80%.

Table 2.3 Bioreactors of Dye Decolorization Using White Rot Fungi

Type of reactor	Fungi	Dye	Detected Enzyme	Support media	Operating parameters	Efficiency	Reference
Pulse bed bioreactor	*P. chrysosporium*	Polymeric dye (Poly R-478)	MnP	Polyurethane foam (1.8 gr)	Hydraulic retention time: 24 hr Temperature: 25^{o}C	65 to 80%	Mielgo *et al*., (2002)
Rotating Biological contactor	*Coriolus versicolor*	Turqoise Blue G			Hydraulic retention time: 48 hr Temperature: 28^{o}C pH:4.5 to 5	80%	Kapdan *et al.*, (2002)
Fluidized bed bioreactor	Unidentified white rot fungi	Cotton bleaching effluent	MnP		Retention time: 3 days Temperature: 25^{o}C	75 to 80%	Zhang *et al.*, (1998)
Two phase bioreactor	*Coriolus versicolor f. antarcticus*	Malachite green	MnP, Laccase		First phase: RT 48 min, temperature 28^{o}C, pH: 6 Second phase: retention time 120 min, temperature 50^{o}C, pH 6.4	50% 82%	Diorio *et al*., (2008)
Packed bed bioreactor	*Irpex lacteus*	Remazol brilliant blue r	MnP, Laccase	Polyurethane foam Pine wood	Retention time 6 days, temperature 25^{o}C	85.8% 100%	Kasinath *et al.*, (2003)

2.5 Statistical Analysis of Experimental Data

2.5.1 Regression Analysis

Regression analysis is an evaluation of one or more independent variables relationship x_1, x_2, x_3..., x_k to a single continuous dependent variable y (Kleinbaum, Kupper, & Muller, 2008). Application of regression analysis include to:

- Determine which of several independent variables are important and which are not for describing or predicting a dependent variable;
- Determine the best mathematical model for describing the relationship between a dependent variable and one or more independent variables;
- Assess the interactive effects of two or more independent variables with regard to dependent variable.

Regression methods are frequently used to analyze data from unplanned experiments and very useful for designing experiments where something has gone wrong (Montgomery, 2001). A linear regression model is commonly used to describe the relationship of dependent and independent variable.

The linear regression model with more than one independent variable is called by multi-linear regression model, which is described by the equation (2.1):

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + ... + \beta_m x_m + \varepsilon \qquad (2.1)$$

where y is response variable

x_1, x_2, $x_{3,.....,}x_m$ are independent/regressor/predictor variable;

β_i= 0, 1, 2,…m are the coefficients for the i^{th} power x;

ε is random error.

In some situations, the influence of an independent on a dependent variable is not linear (curvilinear). It means a linear function does not fit the experimental data properly. One of the approaches for evaluating curvilinear is polynomial regression. A higher order polynomial regression model is described by equation (2.2):

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i<j} \beta_{ij} x_i x_j + \varepsilon \qquad (2.2)$$

Although the relationship between *y* and *x* is not linear, the polynomial model is still considered to be a linear model. This is often called model fitting.

2.5.2 Response Surface Methodology

Response Surface Methodology (RSM) is a collection of mathematical and statistical techniques useful for developing, improving and optimizing processes and can be used to evaluate the relative significance of several affecting factors even in the presence of complex interactions (Myers & Montgomery, 2002). This method is employed after a "vital few" controllable factors are identified and the goal is to find the factor settings that optimize the response. By careful design and analysis of experiments, it seeks to relate a response (output variable) to the levels of a number of predictors (G. E. P. Box & Drapper, 2007). These are the advantages that can be achieved by screening experiment of RSM:

- Eliminate insignificant variables for further investigation;
- Determine optimal settings of many discrete variables;
- Identify a small number of important variables for further investigation.

The application of experimental design and response surface methodology (RSM) in textile effluent treatment process can result in improved decolorization,

reduced process variability, time and overall costs. Additionally, the factors that influence the experiments are identified; optimized and possible synergic or antagonistic interactions that may exist between factors can be evaluated (G. Box, Hunter, & Hunter, 1978). RSM has been extensively applied on biotechnological problems namely optimization of medium composition (A. Tavares, Coelho, Agapito, Coutinho, & Xavier, 2006), fermentations and food processes (Yann, Didier, & Daniel, 2005), etc.

Some studies are using RSM to optimize dye decolorization. Srinivasan et al, (2008) studied decolorization of Reactive Orange-16 and Reactive Red-35 by *Trametes versicolor*. They employed full factorial central composite for experimental design and optimized the result by Response Surface Methodology (RSM). The effect of dye concentration, glucose and ammonium chloride on decolorization was studied. They reported that concentrations of glucose and dye have main effect for dye decolorization. From optimization, maximum decolorization was obtained up to 90.7%. The other study was employed Box-Behnken design to decolorize azo dye Disperse Yellow-211 by bacterial strain *Bacillus subtilis* (Sharma, Singh, & Dilbaghi, 2009). RSM was employed to study operating variables: temperature, pH, and initial dye concentration. The optimum values of variables were found 100 mg/l of initial dye concentration, pH 7, and 32.5 ^{o}C of temperature. Maximum decolorization reached up to 80%. Application of RSM was also used by Tavares et al. (2009) which combined pH, temperature and enzyme concentration to optimize dye decolorization by commercial laccase. From those studies, it was concluded that RSM is a suitable approach in determining optimum variables for achieving maximum decolorization.

2.6 Reaction Kinetic Model

A mathematical modeling can be used for kinetic of reaction studies. The most important thing to do is to find the accurate model to estimate the reaction rate close to

the experimental data. There are many studies on dye decolorization by fungi, microbe or its enzymes have been published, but a few studies discuss about the reaction rate of dye decolorization. Michaelis-Menten kinetic model is usually used for enzyme kinetic model. This model was used to simulate several reactive dye decolorizations by commercial laccase in batch reactor (Cristovao et al., 2008). A simulation of Michaelis-Menten was also employed to decolorize Acid Violet 7 by *Trametes versicolor* pellets and Direct Black 38 by *Cardiobacterium huminis* in batch cultures, since the increasing of decolorization rate within dye concentration (Bafana, Devi, Krishnamurthi, & Chakrabarti, 2007; F. Zhang & Yu, 2000). Ong et al (2007) used first order kinetic model to analyze decolorization of Methylene blue by biological granular activated carbon packed column. While, the other study used the Freundlich and Langmuir adsorption models to simulate decolorization of Remazol Black B by *Rhizopus arrhizus* by mathematical description of biosorption equilibrium (Zumriye Aksu & Tezer, 2000).

CHAPTER III

MATERIALS AND METHODS

3.1 Dye

Reactive Remazol Brilliant Blue R (RBBR) was purchased from Sigma Aldrich, Inc. This dye is also known as Cavalite Brilliant Blue R, C.I. Reactive Blue 19, C.I. Reactive Blue 19 disodium salt, Reactive Blue 19, or Remalan Brilliant Blue R (Wang, Hung, Lo, & Yapijakis, 2004). The empirical formula is $C_{22}H_{16}N_2Na_2O_{11}S_3$ and the molecular weight is 626.54. The RBBR was classified as Vinyl Sulfone Reactive dye. Chemical structure of RBBR is presented in Figure 3.1.

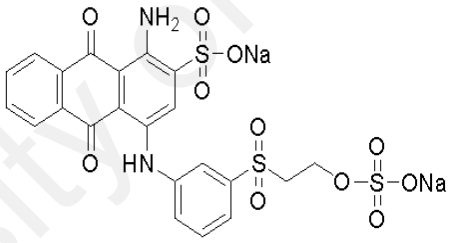


Figure 3.1 Chemical Structure of RBBR

3.2 Culture Growth Medium

Trametes sp. culture was obtained from the Institute of Biological Sciences University of Malaya. The white rot fungus was cultivated on GYMP (glucose yeast malt peptone) medium containing the nutrient substances as shown in Table 3.1 and incubated at 28^0C for 7 days.

Table 3.1 GYMP Medium Formulation

Components	g L^{-1}
Glucose	20
$MgSO_4.{}_7H_2O$	0.5
K_2HPO_4	1
KH_2PO_4	0.46
yeast extract	2
Malt extract	2
Peptone	2
NH_4Cl	0.1
Agar	18

3.3 Fungal Pellet Preparation

Fungal pellets were grown in 100 ml liquid medium in 250 ml flask. The liquid medium contained GYMP where the composition is similar to Table 3.1 with the exclusion of solidifying agar. This medium was aseptically inoculated with 5.0 ml mycelium suspension from actively growing culture on agar plate. Subsequently, this culture was incubated on an orbital shaker (160 rpm) at 28^0 C for 5 days.

3.4 Standard Calibration Preparation

A standard calibration was prepared by measuring the absorbance of aqueous RBBR at 590 nm using dye concentrations ranging from 10 to 60 ppm with three replicates. The relationship between concentration and absorbance is shown in Figure 3-2.

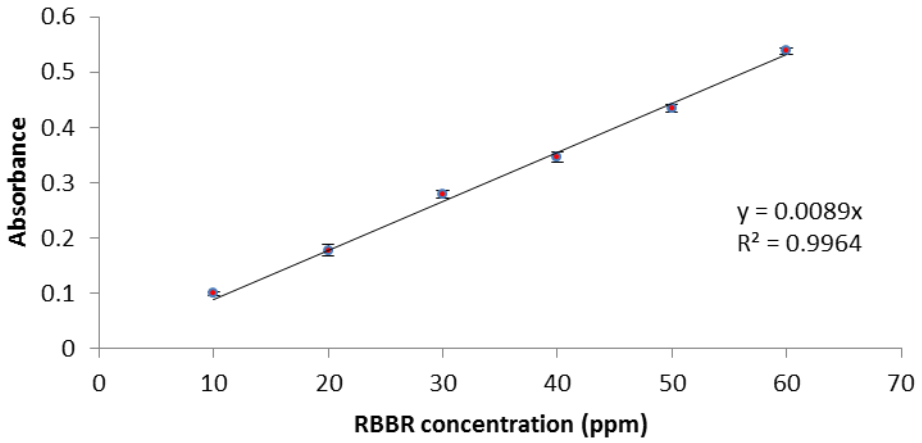


Figure 3.2 Standard Calibration of Absorbance at 590 nm to Determine Concentration of Aqueous RBBR

An equation generated by this calibration is *y= 0.008x,* where *y* is the absorbance and *x* is the concentration of RBBR. The correlation of coefficient for the relationship is R^2 = 0.996. This equation was used to calculate the initial dye concentration before treatment and its residual concentration following the decolorization process.

3.5 Verification of Biological Decolorization

Control experiments were performed to verify that decolorization process was *via* biological route instead of simple adsorption. These experiments were carried out in Erlenmeyer flask containing 100 ml aqueous RBBR and 3.47 g heat killed pellet. Concentration of the RBBR in solution was 100 ppm. After shaking at 160 rpm for two days, the concentration of the dye was measured spectrophotometrically at 590 nm. The results showed that the color was adsorbed up to 98% by heat killed pellet (Fig.3a). No further decolorization of the adsorbed dye was observed with time. However, when living pellets were used, very little dye was adsorbed by the biomass with time (Fig.3b). This indicated that living biomass possess active mechanism to exclude the dye from being adsorbed. Furthermore, the color intensity of the dye solution was decreased progressively with time when living biomass was used. This supported the hypothesis that RBBR decolorization was via biological mechanism.

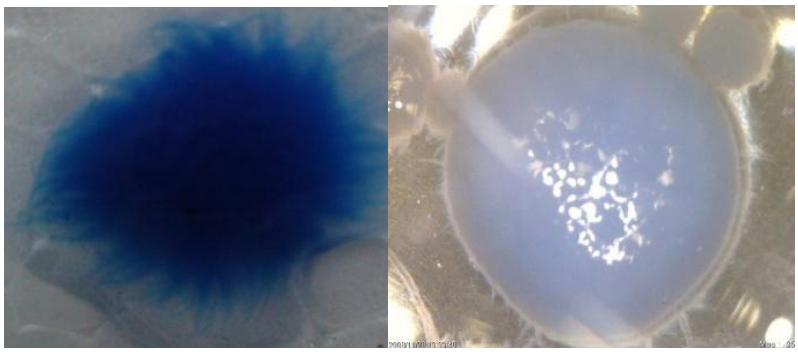


Figure 3.3. The difference routes of dye removal by:
a.heat-killed pellet, b. living pellet

3.6 Statistical Optimization of RBBR Decolorization in Aqueous Batch

Statistical optimization of RBBR decolorization used in aqueous batch is RSM. The first step in RSM is to find a suitable approximation for the true functional relationship between *y* and independent variables *x*. A low order polynomial in some region of the independent variables is usually employed. If the response is well modeled by a linear function of the independent variables, then the approximating functions is the first-order model as described by equation (2.1). If there is curvature in the system, then a polynomial of higher order must be used, such as the second-order model, which is described by equation (2.2) (Montgomery, 2001).

Designs for fitting response surfaces are called response surface design. One of the commonly used and efficient designs in response surface modelling is Box–Behnken design. The Box-Behnken design is an independent quadratic design in which it does not contain an embedded factorial or fractional factorial design. In this experimental design, the treatment combinations are at the midpoints of edges of the design space and the centre. These designs are rotatable (or nearly rotatable) and require three levels of each factor. Figure 3.3 provides a graphical sketch of the experimental layout of Box-Behnken design with three factors.

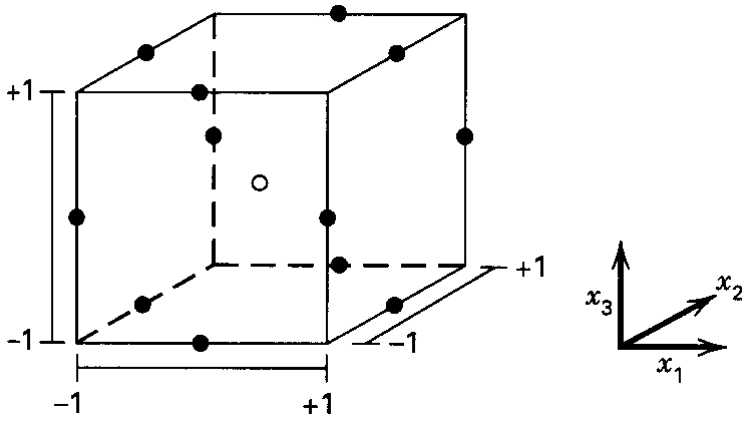


Figure 3.4 Box Behnken Design with Three Factors

The advantages of Box-Behnken design include:

- It uses only three levels of each factor;
- It is near rotatable design;
- It needs fewer number of experimental runs than does a central composite design when the factor (k) = 3 or 4; however, when $k \geq 5$, this advantages disappears.

In this study, design at three factors (initial dye concentration, pH, and mass of pellet) with three replicates was performed. These factors were selected because they were hypothesized to affect decolorization process. The levels of the factors are presented in Table 3.2 and the total experiments number for this decolorization process is shown in Table 3.3.

Table 3.2 Experimental Factors and Its Levels for Three-Level Box Behnken Design

Coded factor	Factors	Coded level		
		-1	0	+1
x_1	Initial dye concentration (ppm)	50	65	80
x_2	pH	4	5	6
x_3	Mass of pellet (gr/100 ml)	4	5	6

The studies were conducted in 100 ml of aqueous RBBR and the pH of solution was adjusted to the desired value from the initial pH 5.6 using 0.01 M HCl and 0.01 M NaOH. The pH adjustment by base and/or acid addition did not affect the color intensity of the dye solution. The flasks were shaken at 160 rpm for 2 days.

Table 3.3 Experimental Runs of RBBR Decolorization By *Trametes* sp.

Run	Factors			Run	Factors		
	x_1	x_2	x_3		x_1	x_2	x_3
1	0	+1	+1	**17**	-1	0	-1
2	+1	0	+1	**18**	-1	+1	0
3	-1	0	-1	**19**	0	0	0
4	-1	-1	0	**20**	+1	+1	0
5	-1	0	+1	**21**	0	-1	-1
6	0	0	0	**22**	+1	+1	0
7	+1	0	+1	**23**	-1	+1	0
8	0	+1	-1	**17**	-1	0	-1
9	+1	+1	0	**18**	-1	+1	0
10	0	-1	-1	**19**	0	0	0
11	0	+1	-1	**20**	+1	+1	0
12	0	0	0	**21**	0	-1	-1
13	+1	0	-1	**22**	+1	+1	0
14	-1	0	+1	**23**	-1	+1	0
15	+1	0	-1	**24**	0	+1	+1
16	0	+1	-1	**25**	-1	-1	0

Table 3.2 (continued)

Run	Factors			Run	Factors		
	x_1	x_2	x_3		x_1	x_2	x_3
26	0	-1	-1	**36**	-1	0	-1
27	0	0	0	**37**	-1	-1	0
28	0	0	0	**38**	0	-1	+1
29	0	+1	+1	**39**	0	0	0
30	+1	-1	0	**40**	0	0	0
31	0	-1	+1	**41**	0	0	0
32	0	0	0	**42**	0	-1	+1
33	-1	0	+1	**43**	+1	-1	0
34	-1	+1	0	**44**	+1	0	-1
35	+1	0	+1	**45**	+1	-1	0

The experimental design and analysis of variance (ANOVA) were performed using Minitab® Release 14.12.0 statistical software (Minitab Inc.). A second order polynomial regression model was used to approximate the response (Eq. 3.1).

$$\eta = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (3.1)$$

where:

η is the response (decolorization efficiency);

X_1, X_2, X_3 are coded levels of the independent factors.

β_i are the regression coefficients, β_0 the constant term; β_1, β_2, β_3 the coefficients for linear effects; β_{11}, β_{22}, β_{33} the coefficients for quadratic effects, and β_{12}, β_{13}, β_{23} the coefficient for interaction effects.

3.7 Kinetic study of decolorization in batch shake flasks and continuous reactor

3.7.1 Rate constant

Kinetic is concerned with the rates of reactions by which the reactants are converted into the products. The rate of reaction at given temperature is usually expressed as the changes in concentration respect to time, *dC/dt*. The rate equation for a reaction is the differential equation.

The generalized equation for the rate is

$$-\frac{dC}{dt} = kC^n \qquad (3.2)$$

k = rate constant

C = concentration of reactant, mass per unit volume

n = exponential power

Using the data from batch experiments, the coefficients can be determined using integration methods, which are summarized in Table 3.4.

Table 3.4 Methods to Determine Reaction Rate Constant

Reaction	Exponential power,n	Integrated form	Grapycally method
Zero order $r_c = \frac{dC}{dt} = k$	0	$C - C_0 = -kt$	Plotting C versus t
First order $r_c = \frac{dC}{dt} = kC$	1	$\ln \frac{C}{C_0} = kt$	Plotting $-\ln (C/C_0)$ versus t
Second order $r_c = \frac{dC}{dt} = kC^2$	2	$\frac{1}{C} - \frac{1}{C_0} = kt$	Plotting 1/C versus t

(Tchobanoglous, Burton, & Stensel, 2003)

The rate constant (*k*) was examined using zero order, first order, and second order kinetic models. To obtain the rate constant, the concentration of dye data was collected every 30 minutes until equilibrium. Reaction was performed in a closed system and constant volume (100 ml of aqueous RBBR) with the optimum variables' settings, which was obtained from the previous optimization studies.

3.7.2 Kinetic Model in Reactor

Reactors may be classified according to their mode of operation as continuous flow and batch reactors. A continuous flow reactor has a continuous stream of reactants entering and continuous stream of products leaving. However, a batch reactor does not have continuous stream. The reactants are added, the reaction occurs, and then the products are discharged.

3.7.2.1 Batch Reactor

The derivation of material balance equation for a batch reactor is written as follows:

[Accumulation] = [input] – [decrease due to reaction] – [output]

$$\frac{dC}{dt}V = QC_0 - QC + r_c V \qquad (3.3)$$

Because Q=0, the resulting equation for a batch reactor is

$$\frac{dC}{dt} = r_c \qquad (3.4)$$

The point is that when flow is not occuring, the concentration per unit volume is changing according to the applicable rate expression. On the other hand, when flow is occurring, the concentration in the reactor is also being modified by the inflow and outflow from the reactor. If the rate of reaction is defined as first-order (i.e, r_c = - kC), integration of model is

$$\int_{Co}^{C} \frac{dC}{C} = -k\int_{o}^{t} dt = kt \qquad (3.5)$$

$$\ln C - \ln C_0 = -kt - 0$$

$$ln\frac{C}{C_0} = -kt$$

The resulting expression is

$$\frac{C}{C_0} = e^{-kt} \qquad (3.6)$$

3.7.2.2 Continuous Reactor

Continuous flow reactor may be classified as plug flow, dispersed plug flow, and completely mixed reactor. In a plug flow reactor, the elements of the fluid that entered the reactor at the time flow through it with the same velocity and leave at the same time. The travel time of the fluid is equal to the theoretical detention time and there is no longitudinal mixing (Reynolds, 1982).

The material balance is

[Accumulation] = [input] – [decrease due to reaction] – [output]

For steady state, the accumulation term is zero, thus, the material balance become

[input] = [output] + [decrease due to reaction]

$$QC = Q(C - dC) + r\,dV$$
$$QdC = r\,dV$$

Substituting $r = -kC$

$$QdC = -kC\,dV$$

Rearranging and setting the limit for integration gives

$$\int_{Co}^{Ct} \frac{dC}{C} = -\frac{k}{Q}\int_{0}^{v} dV \qquad (3.7)$$

where C_o= reactant concentration entering the reactor (mg/L)

C_t = reactant concentration leaving the reactor (mg/L)

Q = volumetric flow rate (L/hr)

V = volume of the reactor (L)

The integration of above equation is

$$\ln C_t - \ln C_o = -k\frac{V}{Q} \qquad (3.8)$$

Since the detention time of reaction θ = V / Q, equation become

$$\frac{C_t}{C_0} = e^{-k\theta} \qquad (3.9)$$

In mixed flow reactor, the fluid element upon entering is immediately dispersed throughout the reactor volume. The reactor contents are uniform and are identical with effluent streams. Completely mixed reactor basins are usually circular or square tanks. For the case of the completely mixed reactor, the material balance is

[Accumulation] = [input] – [decrease due to reaction] – [output]

$$\frac{dC}{dt} = \frac{Q}{V}C_0 - kC - \frac{Q}{V}C \qquad (3.10)$$

Since the accumulation term d*C*/d*t* = 0 for steady state and θ = V/Q, equation (2.11) become

$$0 = \frac{C_0}{\theta} - kC - \frac{C}{\theta} \qquad (3.11)$$

Re-arranging gives

$$kC = \frac{C_0 - C}{\theta}$$

$$C = \frac{C_0}{k\theta + 1} \qquad (3.12)$$

To generate a kinetic model in continuous fluidized bed, a material balance of continuous reactor was employed. The performance of reactor was observed by examining the experimental data to plug flow and mixed flow models. The rate constant was achieved from batch study and the constant value will be used to calculate the

model output concentration from reactor. To determine a real concentration profile in the reactor, concentration of dye was calculated every 60 minutes at differential height of column in steady state condition.

3.8 Fluidized Bed Biological Reactor (FBBR) Studies

The FBBR was constructed as a Perspex cylinder with a working volume of 1 L. The reactor column had an internal diameter 5 cm and maximum height 54 cm. The system was equipped with feeding tank, peristaltic pump, aerator, diffuser, over flow and reservoirs. A mesh was placed at the upper of column to prevent the fungal pellets from exiting the column. The synthetic dye solution was pumped from the bottom of the column and filled overall volume height of the column. The air was injected from the base of the column to disperse the fungal pellet and to provide aeration. Figure 3.4 illustrates the schematic of the FBBR process.

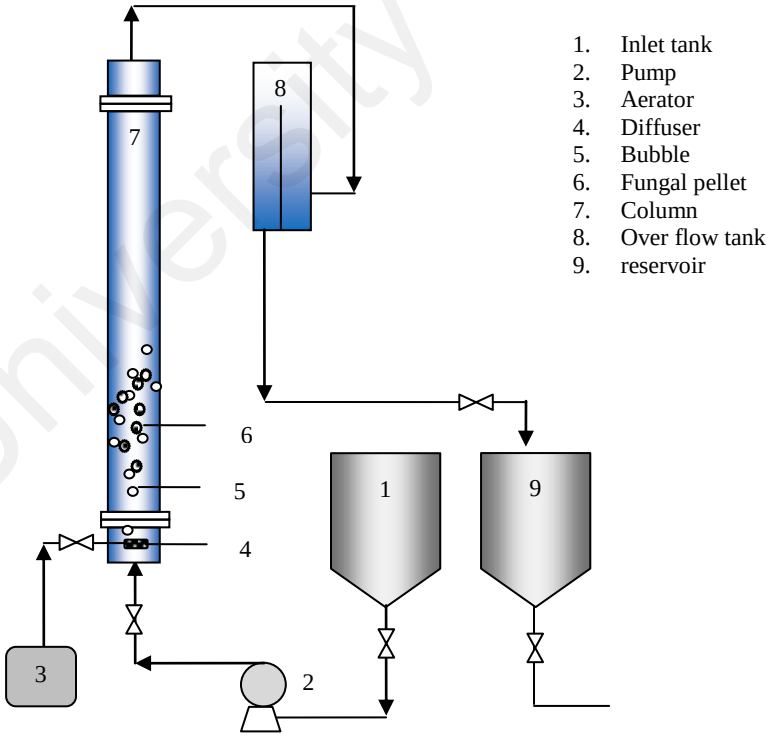
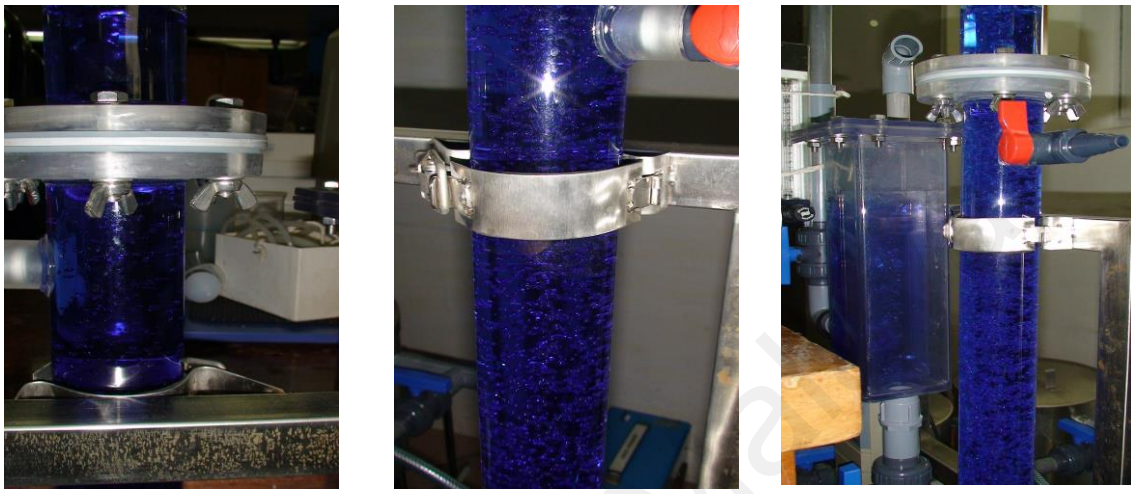


Figure 3.5 Fluidized Bed Process Schema

These experiments examined the effects of hydraulic retention time (HRT) of the RBBR solution (6, 12, and 24 hr) and air-flow rate (0.2, 0.6, and 1.2 l min^{-1}) in three replicates. The process is depicted in Figure 3.5 at three different airflow rates. The total runs for RBBR decolorization in the FBBR studies is shown in Table 3.5.



(a) (b) (c)

Figure 3.6 Air flow rate for RBBR decolorization; (a) 0.2 l min^{-1}, (b) 0.6 l min^{-1}, (c) 1.2 l min^{-1}

The system was operated continuously for 3 days. The percentage of RBBR decolorization was calculated every 12 hr. The effects of both variables were determined using statistical analysis and employed the second order polynomial model.

Table 3.5 Experimental Runs of RBBR Decolorization in FBBR

Runs	Factors		Runs	Factors	
	x_1	x_2		x_1	x_2
1	1.2	24	**15**	0.6	24
2	1.2	6	**16**	0.2	6
3	0.6	24	**17**	0.6	6
4	1.2	12	**18**	0.2	12
5	0.2	12	**19**	0.6	12
6	0.6	6	**20**	1.2	6
7	0.2	24	**21**	0.6	6
8	0.6	12	**22**	1.2	24
9	0.2	6	**23**	1.2	12
10	0.6	12	**24**	0.2	6
11	1.2	24	**25**	0.6	24
12	1.2	12	**26**	0.2	12
13	0.2	24	**27**	0.2	24
14	1.2	6			

x_1= air flow rate (l min^{-1}), x_2= HRT (hr)

3.9 Analytical Procedure

The samples were withdrawn and centrifuged for 15 minute at 7000 rpm. The supernatant of aqueous RBBR was analyzed at a visible spectrum of 590 nm using a UV-Vis spectrophotometer (Jasco, Japan) to measure the absorbance. A standard calibration of absorbance versus concentration was prepared to calculate the residual concentration of the dye.

This concentration values were used to calculate decolorization efficiency as shown by equation (3.13).

$$Decolorization(\%) = \frac{(C_i - C_f)}{C_i} x100\% \qquad (3.13)$$

where C_i is the initial concentration of dye (ppm) and C_f is the final concentration of dye (ppm).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Aqueous Batch Studies

4.1.1 Effect of selected variables towards dye decolorization

From the response surface design, 45 replicated runs were generated. The results of runs for three selected variables for RBBR decolorization are shown in Table 4.1.

Table 4.1 Runs of RBBR Decolorization Result

Run	Factors			% Decolorization	
	X_1	X_2	X_3	Actual	Predicted
1	0	+1	+1	27.96	29.19
2	+1	0	+1	20.40	21.75
3	-1	0	-1	59.20	61.78
4	-1	-1	0	72.11	63.42
5	-1	0	+1	47.45	61.78
6	0	0	0	29.61	29.22
7	+1	0	+1	18.76	21.75
8	0	+1	-1	28.59	29.19
9	+1	+1	0	21.87	21.72
10	0	-1	-1	22.89	30.86
11	0	+1	-1	33.30	29.19
12	0	0	0	30.90	29.22
13	+1	0	-1	26.51	21.75
14	-1	0	+1	55.94	61.78
15	+1	0	-1	27.47	21.75
16	0	+1	-1	24.36	29.19
17	-1	0	-1	55.98	61.78
18	-1	+1	0	71.66	61.75

Table 4.1 continued

Run	Factors			% Decolorization	
	X_1	X_2	X_3	Actual	Predicted
19	0	0	0	30.55	29.22
20	+1	+1	0	20.22	21.72
21	0	-1	-1	28.98	30.86
22	+1	+1	0	22.76	21.72
23	-1	+1	0	59.57	61.75
24	0	+1	+1	25.26	29.19
25	-1	-1	0	68.73	63.42
26	0	-1	-1	23.47	30.86
27	0	0	0	30.22	29.22
28	0	0	0	30.32	29.22
29	0	+1	+1	28.66	29.19
30	+1	-1	0	20.95	23.39
31	0	-1	+1	34.84	30.86
32	0	0	0	32.26	29.22
33	-1	0	+1	53.83	61.78
34	-1	+1	0	61.43	61.75
35	+1	0	+1	16.81	21.75
36	-1	0	-1	77.91	61.78
37	-1	-1	0	62.44	63.42
38	0	-1	+1	35.77	30.86
39	0	0	0	30.80	29.22
40	0	0	0	31.18	29.22
41	0	0	0	30.79	29.22
42	0	-1	+1	32.65	30.86
43	+1	-1	0	22.03	23.39
44	+1	0	-1	27.29	21.75
45	+1	-1	0	20.82	23.39

X_1 = Initial dye concentration (ppm); X_2 = pH; X_3 = mass of pellets (gram)

The dye decolorization percentage varied within the range of 16.81% to 77.91%. Using a full quadratic regression model, only initial dye concentration was found to be significant ($p < 0.05$) for the main factors, where the decolorization percentage

decreased with the increase in initial dye concentration (Fig. 4.1). The main effects of pH (p = 0.459) and mass of pellets (p = 0.152) were found to be insignificant on dye decolorization within their experimental ranges tested (Fig.4.2).

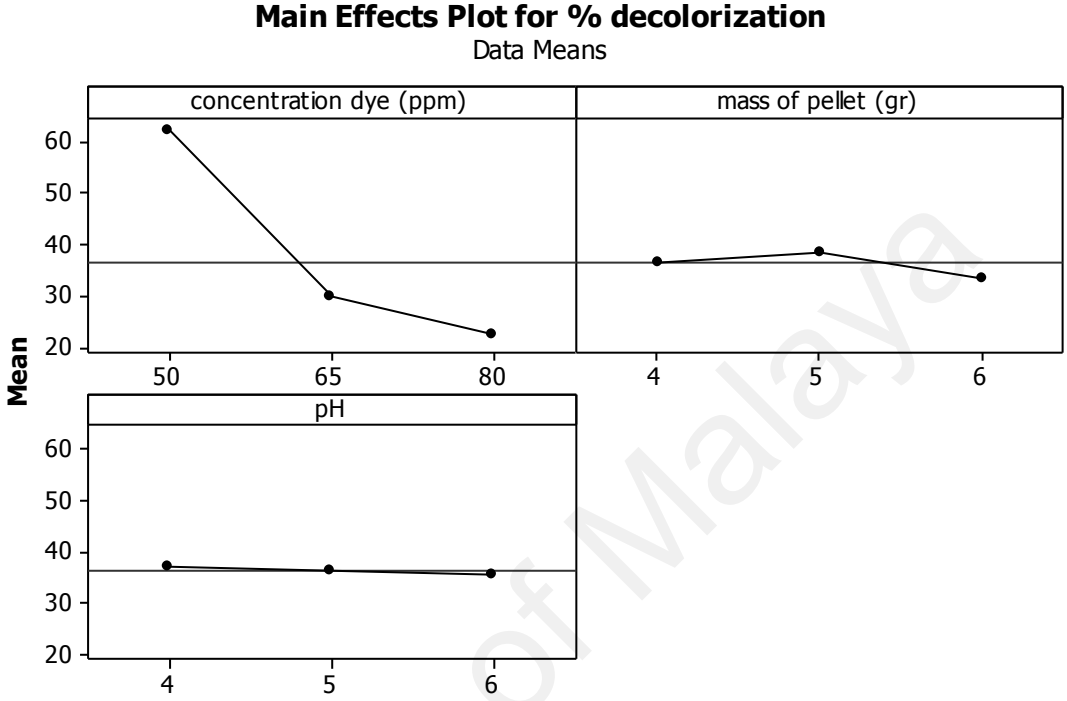


Figure 4.1 Main Effect Plot of Each Variable

Meanwhile, for the squared effect only initial dye concentration was found to be significant to the decolorization percentage ($p < 0.05$). The squared effects of pH (p = 0.623) and mass of pellets (p = 0.127) were insignificant on the decolorization of dye. The interaction effects among all the variables tested were found to be insignificant at 5% confidence interval.

The lowest decolorization was observed at maximum initial concentration dye (pH 5 and 6 g pellets), while the highest decolorization was obtained when low initial dye concentration was used (pH 5 and 4 g pellets). This significant difference in the observed decolorization efficiency at different initial dye concentrations clearly showed that high initial RBBR concentration (80 ppm) might be detrimental to the biological decolorization activity of the fungus.

In order to evaluate the possible interaction(s) that may exist between the factors, and to optimize the factors' level to get the maximum decolorization, a quadratic model regression was performed using the collected data set. With the percentage of decolorization as process response, it is shown that the full quadratic polynomial model used was not a good fit to the regression data, where the *p* value for lack of fit was significant ($p < 0.000$) (Table 4.2).

Table 4.2 ANOVA for Data Fitting Using Full Quadratic Regression Model

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	11619.1	11619.1	1291.01	46.89	0.000
Linear	3	9690.1	9690.1	3230.04	117.31	0.000
Square	3	1821.5	1821.5	607.15	22.05	0.000
Interaction	3	107.5	107.5	35.85	1.30	0.289
Residual Error	35	963.7	963.7	27.53		
Lack of fit	3	421.6	421.6	140.53	8.30	0.000
Pure error	32	542.1	542.1	16.94		
Total	44	12582.8				

Note: **DF** degree of freedom; **Seq SS** sequential sum of squares; **Adj SS** adjusted sum of squares; **Adj MS** adjusted mean of squares; ***F***-statistics; ***P***-value.

It is suspected that in this particular instance, too many regressors in the full quadratic model resulted in a collinearity effect, a situation where the independent variables have highly correlated and the effects of each variable cannot be separately estimated. When there are too many regressors, this model is said to be "over-fit." An alternative scheme in choosing between competing multiple regression models was employed in the form of a best subset regression in order to rationally select the variables for model building. This approach will make use of Mallows' C_p statistic. If P regressors are selected from a set of $K > P$, C_p is defined as

$$C_p = \frac{\sum_{i=1}^{N}(Y_i - Y_{pi})^2}{S^2} - N + 2P \qquad (4.1)$$

where Y_{pi} is the predicted value of i^{th} observation of Y from the P regressors; S^2 is the residual mean square after regression on the complete set of K regressors; and N is the sample size. The C_p statistic can be used in selecting a reduced model as long as S^2 is non-zero. It helps to strike an important balance with the number of predictors in the model. A model with too many predictors can be relatively imprecise while one with too few can produce biased estimates. A Mallows' C_p value that is close to the number of predictors plus the constant indicates that the model is relatively precise and unbiased in estimating the true regression coefficients and predicting future responses.

Table 4.3 Best Subsets Regression

Variables	R^2	R^2 (adjusted)	Mallows' C_p	S	Initial dye concentration	Mass of pellets	pH
1	76.4	75.9	1.1	8.3086	x		
1	0.5	0.0	136.5	17.066		x	
2	76.9	78.5	2.2	8.3230	x	x	
2	76.5	75.4	2.8	8.3832	x		x
3	77	75.3	4	8.3996	x	x	x

From Table 4.3, it is suggested that a reduced model with the two terms "initial dye concentration" and "pH" is relatively precise and unbiased because its' Mallows' C_p (2.8) is closest to the number of predictors plus the constant (3). The Mallows' C_p should be examined in conjunction with other statistics included in the best subsets output such as R^2, adjusted R^2, and S. A good model should have high R^2 and adjusted R^2, small S, and Mallows' C_p close to the number of variables plus the constant contained in the model, where in this case $R^2 = 76.5$; adjusted $R^2 = 75.4$; $S = 8.3832$ and $C_p = 2.8$.

A new and reduced polynomial model was formulated based on the main (linear) and squared effects of initial dye concentration and pH variables. ANOVA results from this model are presented in Table 4.4.

Table 4.4 ANOVA for data fitting using reduced regression model

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	4	11385.3	11385.31	2846.33	95.08	0.000
Linear	2	9631.1	9631.14	4815.57	160.85	0.000
Square	2	1754.2	1754.18	877.09	29.30	0.000
Residual Error	40	1197.5	1197.50	29.94		
Lack of fit	4	201.2	201.26	50.29	1.82	0.147
Pure error	36	996.3	996.34	27.68		
Total	44	12582.8				

Note: **DF** degree of freedom; **Seq SS** sequential sum of squares; **Adj SS** adjusted sum of squares; **Adj MS** adjusted mean of squares; ***F***-statistics; ***P***-value.

It showed that the alternative model is significant with a high *F*-statistic (95.08) (or p = 0.000), and most importantly insignificant for the lack of fit to the data (p = 0.147). The regression coefficients, *t*- and *p*- values for all the linear and squared effects of the variables are given in Table 4.5.

Table 4.5 Regression Coefficients for Reduced Model

Terms	Coefficients	SE coefficients	*t*-value	*p*-value
Constant	29.22	1.518	19.255	0.000
dye concentration	-20.015	1.117	-17.921	0.000
pH	-0.835	1.117	-0.748	0.459
dye concentration*dye concentration	12.5467	1.639	7.655	0.000
pH*pH	0.8117	1.639	0.495	0.623

Note: **SE** standard error

A reduced polynomial was generated relating the response to the linear and squared regression model i.e:

$$\eta = 29.22 - 20.015X_1 - 0.835X_2 + 12.5467X_1^2 + 0.8117X_2^2 \qquad (4.2)$$

The coefficient of correlation R^2 for the above equation when fitted to the experimental data was 90.48%. Therefore, this equation can be used for predicting response at any combination of three variables within the experimental range tested. The closer the values of R^2 to 1, better the correlation between the experimental and predicted values (Montgomery 2005). The predicted responses value using reduce polynomial equation (4.2) are shown in Table 4.1.

4.1.2 Analysis of residuals normality

The residual is the difference between the predicted response values and the observed values. Residuals were the best estimate of errors. To check the errors graphically, the residual plots can be used (Fig. 4.2).

Figure 4.2 presented the difference residual plots. These are the explanation for each graph:

- Normal probability plot was used to specify whether the data were normally distributed, or if the response were influenced by other variables or outliers exist in the data. The normal probability plot of the residuals should roughly follow a straight line. From this experimental result, the residuals appeared follow the straight line, which showed that no evidence of non-normality, skewness, outliers, or the influence of unidentified variables;

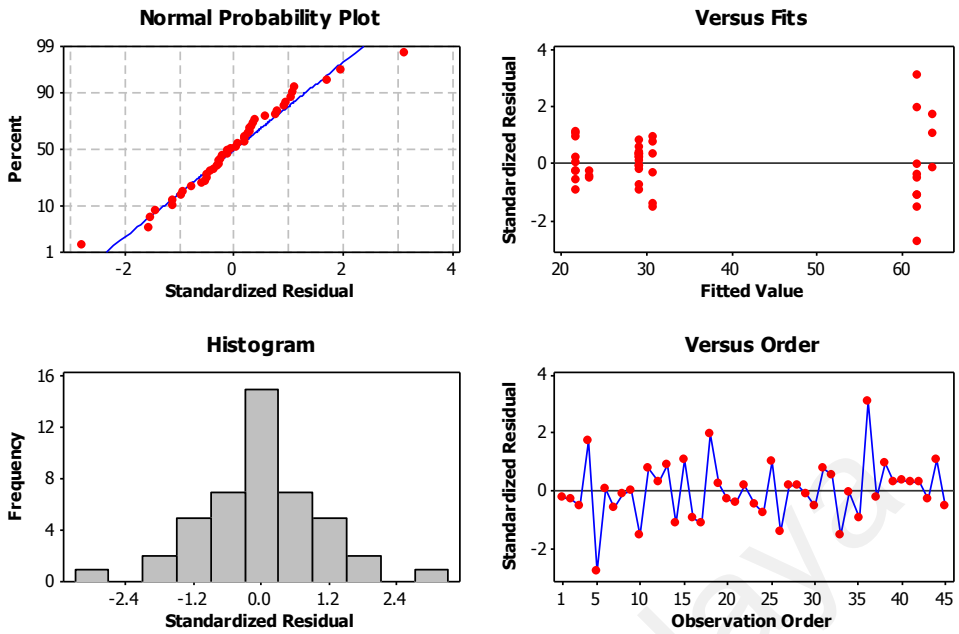


Figure 4.2 Residual Plot for % Decolorization

- Residuals versus fitted values indicate whether the variance is constant, or a nonlinear relationship exists, or outliers exist in the data. The residuals should be scattered randomly about zero. Based on this plot, the residuals appeared to be randomly scattered about zero, with majority of the data point lied within -2 to +2 standardized range. Hence, there was no evidence of non-constant variance, missing terms, outliers, or influential points exist;
- The histogram indicates if the data were skewed or outliers exist in the data. The histogram of the residuals should be bell-shaped, which exactly shown by the histrogram in Fig 4.2; therefore it could be stated that the data showed the behaviour of normal distribution;
- Residuals versus the order of the data indicated whether there were systematic effects in the data due to time or data collection order. The residuals in the plot should fluctuate in a random pattern around the centerline. Hence, there was no evidence that the error terms were correlated with one another i.e. the indication

of systematic errors. The variation in the data can be confidently attributed to random error.

4.1.3 Optimization of variables level

Response Optimizer function of the Minitab® software was used to find the optimum values of key variables. The solution of equation (4.2) aimed to achieve in a minimum of 65% in dye decolorization.

Table 4.6 Optimization values of variables

	Variables		Composite desirability	Predicted value
	pH	Initial concentration (ppm)		
Global solution	4	50	0.96	63.4%
Conditioned value	5.6	50	0.90	61.6%

Based on this, a global solution of 50 ppm initial concentration of dye at pH 4 was obtained from the initial conditions of: 50 ppm initial dye concentration, 4 g pellets mass and at pH 4 dye solution. The maximum percentage of decolorization predicted by the model was at 63.4%, with composite desirability value of 0.96. This means that it is possible to achieve the stated target of decolorization percentage 96 times out of 100 runs.

For a practical purpose of working with a less acidic dye solution, the value of the pH was arbitrarily set at 5.6 (basic pH of RBBR). The adjustment in the value of pH was proven to be insignificant to the degree of decolorization by the fungus as shown by the simulation results where percentage of decolorization was predicted at 61.6%, with composite desirability value of 0.90 (Table 4.6). This percentage is very close to the earlier predicted percentage of 63.4%. Subsequent verification experiments confirmed the optimization results where the decolorization percentage was at $67.9 \pm 5.4\%$.

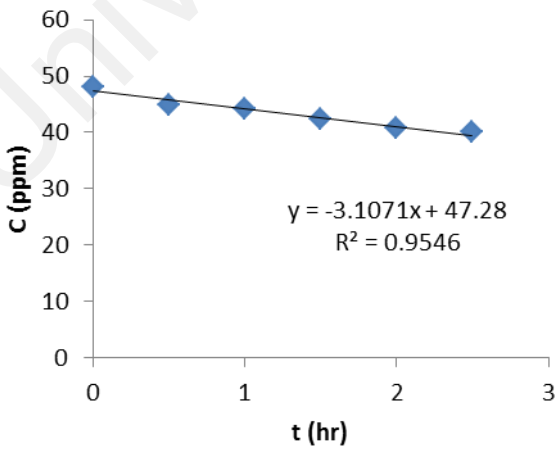
4.1.4 Kinetic study in batch flask

In this study, the RBBR solution was set at optimum value achieved in previous study i.e at 5.6 of pH, 50 ppm of initial dye concentration and 4 gr of pellets. Samples of RBBR were collected every 30 minutes for residual dye concentration analysis as shown in Table 4.7.

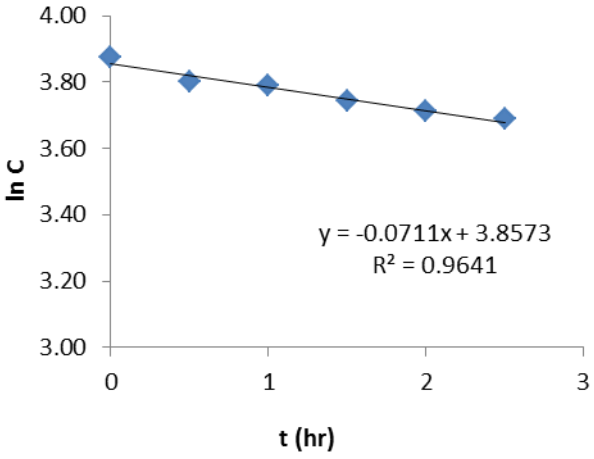
Table 4.7 Residual concentrations of RBBR as a function of time

Time (hr)	Concentration of RBBR (ppm)
0	48.17
0.5	44.83
1	44.17
1.5	42.29
2	40.88
2.5	40.04

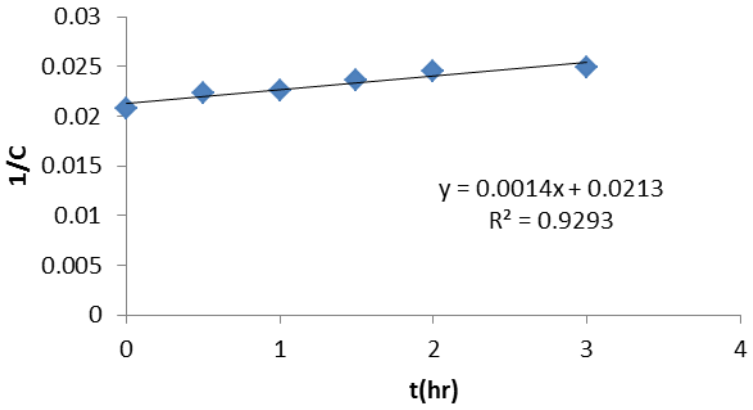
To find the kinetic constant of reaction (*k*), the experimental datas were tested with zero order, first order and second order model. The *k* value of each model was calculated from the slope of the residual concentration with time according to each respective model (Figure 4.3).



(a)



(b)



(c)

Figure 4.3 Plot of Residual Dye Concentration with Time; (a) zero order kinetic; (b) first order kinetic; (c) second order kinetic.

From Figure 4.3, it is clearly seen that the data fit each of model well. To select a better-fit model, the coefficient of correlation, R^2 value of each model must be calculated. A favorable model should be the model having R^2 value closest to 1. The k values of all model followed by their R^2 value are presented in Table 4.8.

Table 4.8 Apparent rate constant, *k* of RBBR decolorization

The model	k (hr^{-1})	R^2
Zero order reaction $C = -k.t + C_0$	3.107	0.954
First order $\ln C = -k.t + \ln C_0$	0.071	0.964
Second order $\frac{1}{C} = k.t + \frac{1}{C_0}$	0.001	0.929

where C: concentration of dye (ppm), C_0: initial concentration of dye (ppm), k: apparent rate constant (h^{-1}).

It is clear from Table 4.8 that all the models show good fit for the data, but the first order kinetic has R^2 value 0.964, higher than the other two kinetic models. Subsequently, this model was used to explain the kinetic of RBBR decolorization in a continuous fluidized bed biological reactor.

4.2 Decolorization Studies of RBBR in Continuous Fluidized Bed Biological Reactor

4.2.1 Graphical Analysis of Decolorization

A fluidized bed biological reactor containing *Trametes* sp. pellets was operated with optimized bio-chemical factors (mass of pellets, pH and initial concentration of RBBR) in continuous mode. Variations of hydraulic retention time (HRT) and gas flow rate were studied in order to come up with the operating conditions with maximum dye decolorization. There was no addition of co-substrate in this process; the decolorization depended solely on the capability of fungal pellets to effect the transformation of aqueous RBBR. Furthermore, the aeration and dispersion were supported by pressurized air supply. The retention time of liquid in the reactor was required to provide the contact time between pellets with the aqueous RBBR. The results of all the runs are shown in Figure 4.4.

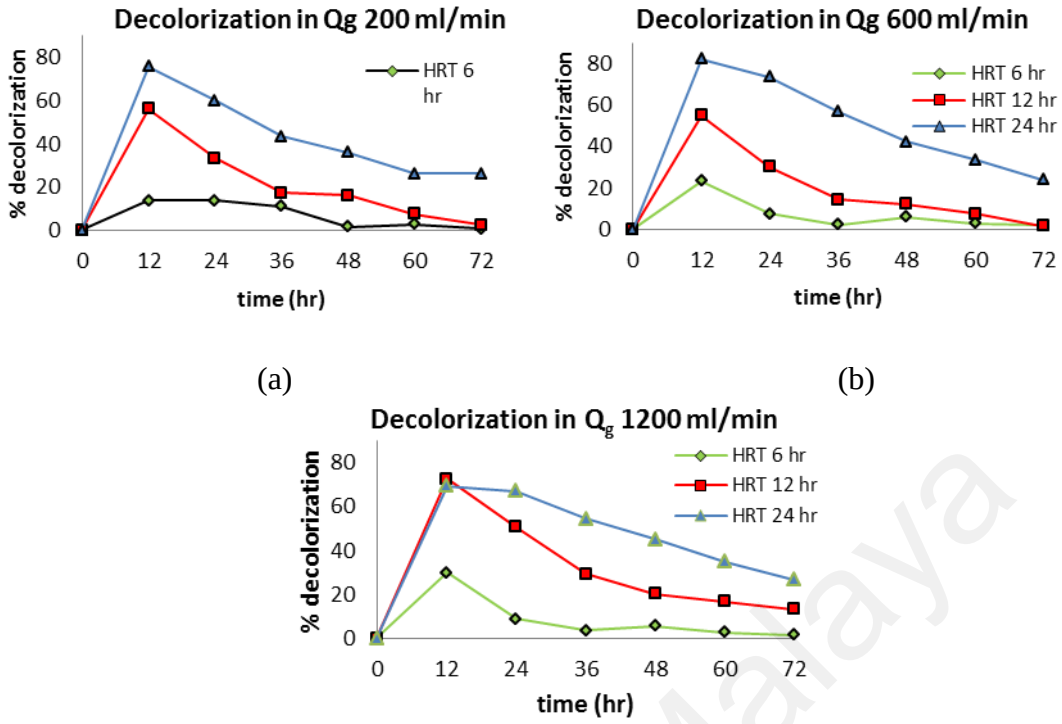


Figure 4.4 Decolorization of aqueous RBBR with different HRT and air flow rate (Q_g)

From all experiments, high decolorization rate was observed at 24 hr of HRT for all airflow rates tested. The highest removal efficiency was obtained at 12 hr after the process start-up. But the decolorization percentage decreased with time after it had increased. This shows that the decolorization capability of fungal pellets reduced with time, with continual flow of fresh dyestuff. However, dye decolorization of up to 26% could still be observed until 72 hr when HRT 24 hr was employed (Fig. 4.4). These results also imply that aqueous RBBR were not used as the carbon source. It is possible hypothesis that Trametes *sp* pellets could maintain their metabolism and hence the continuity of the decolorization reaction via co-substrate supplementation e.g. glucose. The pictures of decolorization result for HRT 24 hr are presented in Appendix A.

From Figure 4.4, the increase in residence times of aqueous RBBR in the reactor resulted in higher efficiency of color removal. It showed that more dye can be decolorized when the contact time between the pellets and the dye solution is longer, i.e.

sufficient time is required for the pellets to react with aqueous RBBR that passed through the column continuously. At 24 hr of HRT, comparisons between different airflow rates are presented in Figure 4.5.

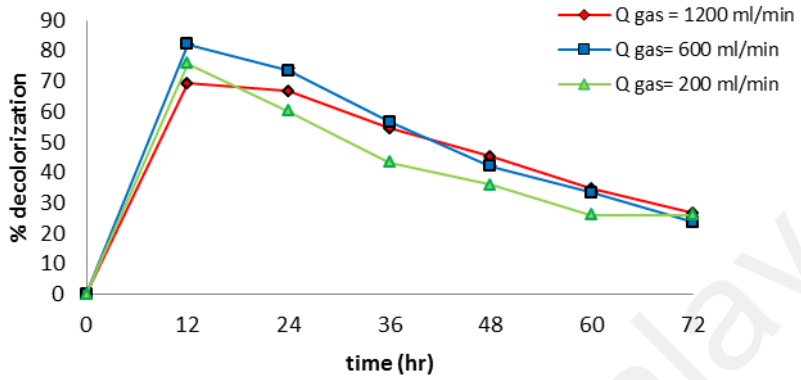


Figure 4.5 Decolorization of Aqueous RBBR with Different Air Flow Rates at 24 hr of HRT

From Figure 4.5, the different airflow rates (Q_g) on color removal has limited influence within the tested range 0.2 to 1.2 l min^{-1}. However, at the flow rate 0.6 l min^{-1} the highest dye decolorization efficiency was observed after 12 hr i.e. 82.2% of color removal.

4.2.2 Effect of HRT and Air Flow Rate on the RBBR Decolorization in FBBR

Experimental data consisted of two variables (HRT and air flow rate) with three levels and three replicates developed to investigate the influence of input variables on the experimental response. The experimental results were recorded at 12 hr after start-up, which had shown the highest percentage of decolorization previously. The results are shown in Table 4.9 followed by predicted results generated by regression analysis with second order polynomial model.

Table 4.9 Decolorization of aqueous RBRR in FBBR after 12 hr

No	X_1	X_2	% decolorization	
			Actual	Predicted
1	1.2	24	54.21	59.17
2	1.2	6	30.42	33.16
3	0.6	24	82.27	74.40
4	1.2	12	74.98	64.63
5	0.2	12	51.79	56.12
6	0.6	6	23.40	23.07
7	0.2	24	75.70	78.80
8	0.6	12	55.20	62.98
9	0.2	6	13.91	10.58
10	0.6	12	53.27	62.98
11	1.2	24	52.89	59.17
12	1.2	12	76.14	64.63
13	0.2	24	75.91	78.80
14	1.2	6	29.47	33.16
15	0.6	24	81.90	74.40
16	0.2	6	13.15	10.58
17	0.6	6	23.19	23.06
18	0.2	12	59.94	56.12
19	0.6	12	56.31	62.98
20	1.2	6	29.61	33.16
21	0.6	6	23.47	23.07
22	1.2	24	56.29	59.17
23	1.2	12	66.87	64.63
24	0.2	6	13.79	10.58
25	0.6	24	82.29	74.40
26	0.2	12	56.68	56.12
27	0.2	24	75.62	78.80

X_1= air flow rate (l/min), X_2= HRT (hr)

The aqueous RBBR decolorization varied within the range of 13.2% to 82.3%. The highest percentage decolorization of aqueous RBBR in this process was achieved at 24 hr of HRT and 0.6 l/min of air flow rate and the lowest percentage decolorization was at 6 hr of HRT and 0.2 l/min of air flow rate. Using a second order polynomial

model, both of air flow rate and HRT were found to be significant ($p < 0.05$) for the main factor. For the squared effect only HRT was found to be significant to the decolorization percentage ($p < 0.05$). The squared effect of airflow rate ($p = 0.193$) was insignificant on the decolorization of dye. The interaction effects among all the variables tested were found to be significant at 5% confidence interval. The estimated regression coefficients followed by the main, squared and interaction effects were given in Table 4.10.

Table 4.10 Regression analysis of decolorization of aqueous RBBR in FBBR

Predictor	Coefficients	SE coefficients	*t*-value	*p*-value
Constant	-68.54	8.89	-7.70	0.000
Air flow rate	56.78	16.53	3.44	0.002
HRT	13.76	1.15	11.91	0.000
Air flow rate*HRT	-2.34	0.39	-6.00	0.000
Air flow rate*air flow rate	-14.38	10.70	-1.34	0.193
HRT*HRT	-0.32	0.04	-8.78	0.000

Note: **SE** standard error

The second order polynomial regression model which related to the response and parameters i.e:

$$\eta = -68.54 + 56.78X_1 + 13.76X_2 - 14.38X_1^2 - 0.32X_2^2 - 2.34X_1X_2 \quad (4.3)$$

The regressions model equation obtained from experiments were tested for goodness of fit, R^2 was calculated to be 94.2%. The statistical significance of the ratio of mean square variation due to regressions and mean square residual error was tested using analysis of variance (ANOVA) and shown in Table 4.11.

Table 4.11 Analysis of variance for RBBR decolorization in FBBR

Source	DF	SS	MS	F	P
Regression	5	13417.9	2683.6	68.71	0.000
Residual error	21	820.2	39.1		
Total	26	14238.1			

Note: **DF** degree of freedom; **SS** sum of squares; **MS** adjusted mean of squares; ***F***-statistics; ***P***-value.

It showed that the polynomial regression model is significant with a high *F*-statistic (68.71) (or $p = 0.000$).

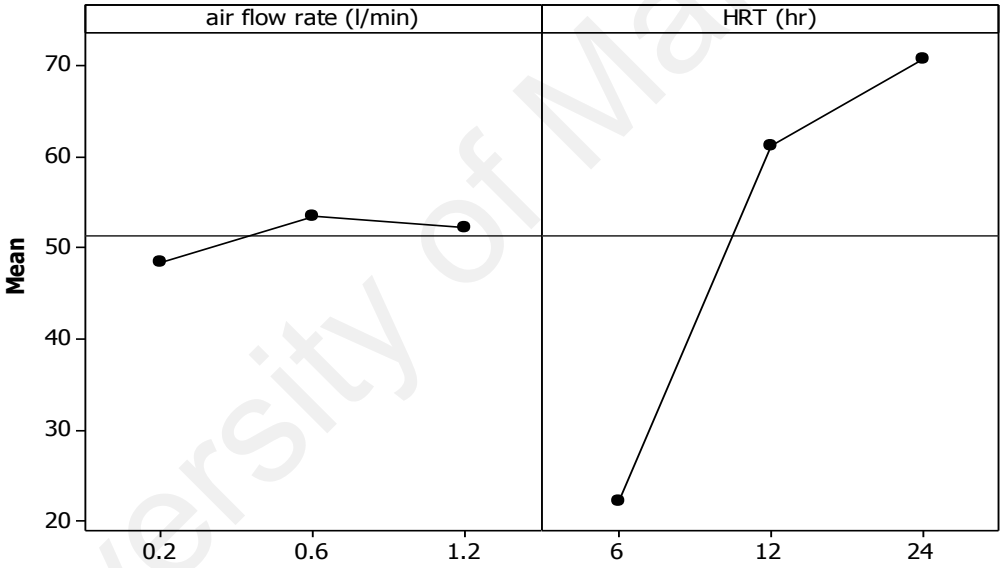


Figure 4.6 Main Effect Plot of RBBR Decolorization in FBBR

The decolorization efficiency obtained from the experimentation show variation depending on the influence of the variables. From Fig. 4.6, it is evident that the airflow rate had little effect within the range value tested since the change in the response was within a narrow range (48% to 52%). Meanwhile, the HRT had significant effect on the decolorization process with a relatively large change in the response (22% to 72%). When the HRT was increased from 6 to 24 hr, there was an increased in the RBBR

decolorization. From Fig 4.6, it was clearly observed that the maximum decolorization was found to be at 24 hr of HRT and 0.6 l/min of airflow rate.

The interactions plot is used to indicate the possible interactions between the two selected variables i.e. air flow rate and HRT to the decolorization process (Fig. 4.7). Intersection of the lines indicated the possible interaction(s) between any two chosen parameters on the response.

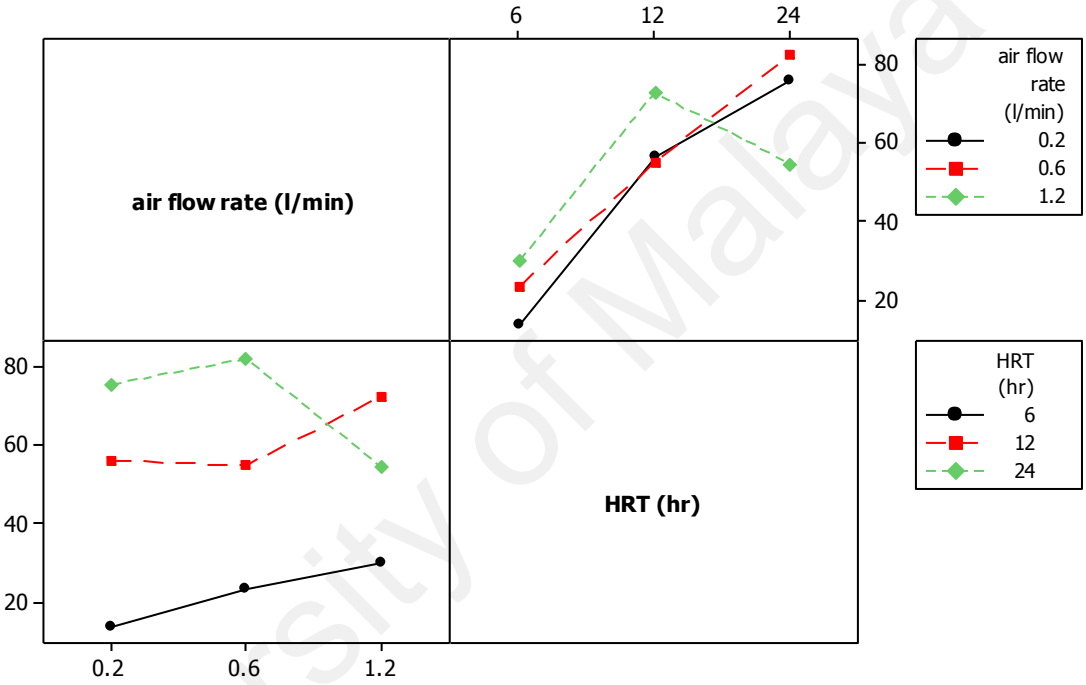


Figure 4.7 Interaction plot between air flowrate and HRT variables (Means represent the average decolorization percentage)

From Fig. 4.7, both panels show the relation between air flow rate and HRT:

- In row 1 panel, the black line (air flow rate= 0.2 l/min) and red line (air flow rate= 0.6 l/min) increased as HRT increased. While, the green line (air flow rate = 1.2 l/min) increased until HRT 12 hr and then decreased at 24 hr of HRT.
- In row 2 panel, the green line (HRT= 24 hr) increased until 0.6 l/min and decreased at 1.2 l/min of air flow rate. The red line (HRT= 12 hr) stayed the same rate until 0.6 l/min and increased at 1.2 l/min of air flow rate. The black line (HRT= 6 hr) increased as air flow rate increased.

Because HRT affected the response differently depending on the level of air flow rate, the two factors appeared to interact.

Contour plot and the three-dimensional surface plot show the relationship among HRT, airflow rate and the dependent variable i.e. percentage of decolorization. The primary utility of these plots is to obtain general trajectory of optimization's direction. From Figures 4.8 and 4.9, it shows that the percentage of decolorization increased as a function of increasing HRT values within the range of airflow rates tested, where the maximum value of decolorization could be as high as over 70%. From these plots, the trajectory of optimization's direction for dye decolorization pointed to HRT range between 16 to 24 hours and 0.2 to 1.2 l min^{-1} of airflow rate.

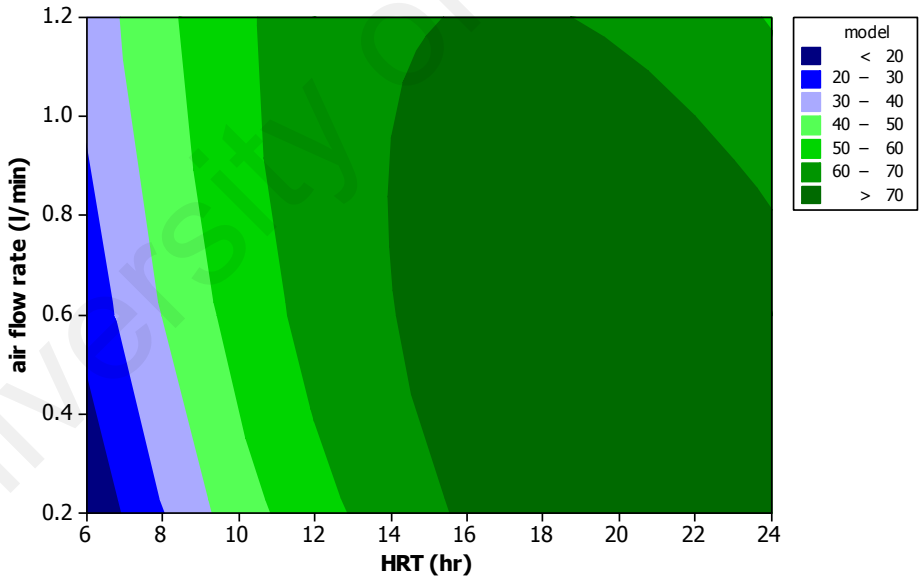


Figure 4.8 Contour Plots of the Effect of HRT and Air Flow Rate on RBBR Decolorization

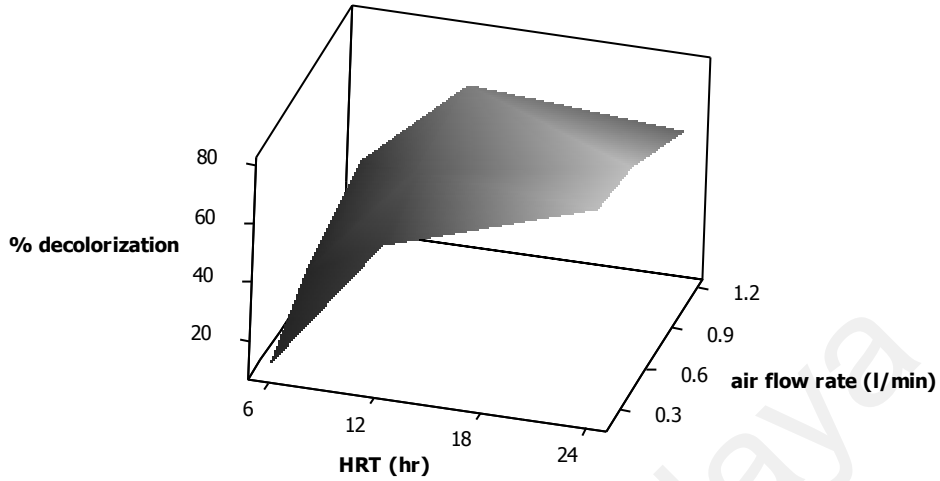


Figure 4.9 Three-dimensional surface plots for the effects of HRT and airflow rate on RBBR decolorization

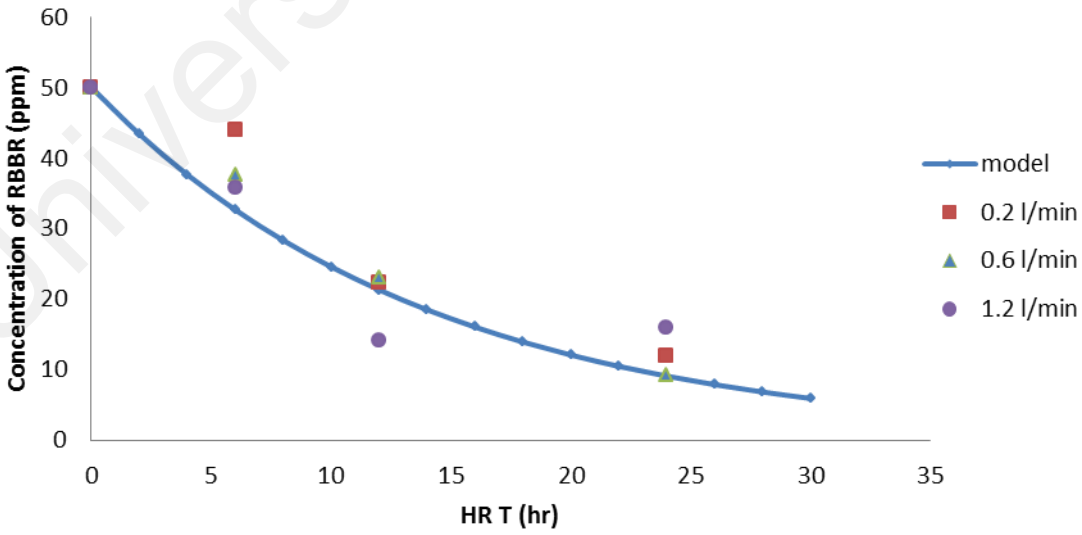
4.2.3 Kinetic study in continuous FBBR

Decolorization reaction in this study was evaluated in plug flow and mixed flow models to observe the performance of reactor in the decolorization of RBBR. The experimental data for kinetic study were outlet concentration collected at steady state condition for different of HRTs and each airflow rate as presented in Table 4.12, where inlet concentration of RBBR solution was set at 50 ppm.

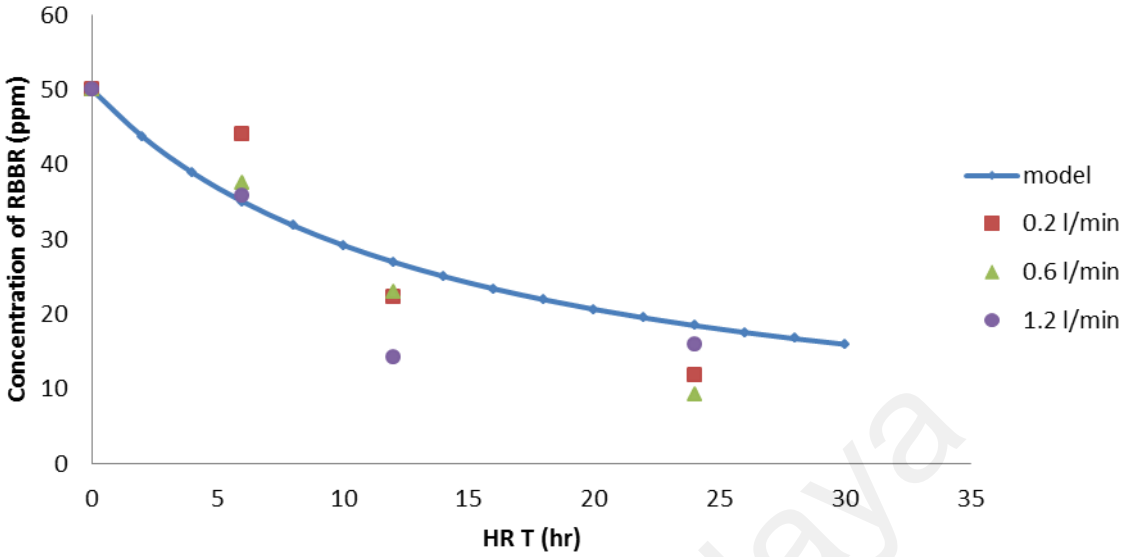
Table 4.12 Exit dye concentration in FBBR as a function of airflow rate and HRT

Airflow rate (l hr^{-1})	HRT (hr)	Outlet Concentration of RBBR (ppm)
0.2	6	44.06
	12	22.37
	24	11.89
0.6	6	37.56
	12	22.99
	24	9.24
1.2	6	35.78
	12	14.22
	24	15.96

Based on Eq. (3.9) for plug flow model, Eq. (3.12) for mixed flow model, and kinetic rate coefficient from batch study (0.071 hr^{-1}), exit concentration of aqueous RBBR could be predicted in different of HRT which is shown by Figure 4.10. Experimental result data were plotted to compare observation with prediction model.



(a)



(b)

Figure 4.10 Comparing of experimental with prediction model (a) Plug flow, (b) Mixed flow

In Fig 4.10, the plug flow model shows a sharper curvature than the mixed flow model. This implies that for the same effluent concentration or the same degree of removal, the plug flow model requires shorter detention time. To determine which model explains the performance of FBBR, the residual sum of square between observation and prediction model was calculated. It is presented in Table 4.13.

Table 4.13 Residual sum of square of observation and prediction model

	Plug Flow	**Mixed Flow**
0.2 l/min	75.73	60.03
0.6 l/min	14.99	18.23
1.2 l/min	103.81	58.36

Residual sum of square (RSS) is the sum of square errors of prediction. The smallest RSS indicated a fit of model to the data.

$$RSS = \sum_{i=1}^{n} (y_i - \hat{y}_i)^2 \qquad (4.4)$$

y_i = the observed value

$\hat{y}_i$ = i^{th} fitted response value

The fitted response values for each experiment were obtained from linear regression model presented in Appendix B. From Table 4.13, the smallest RSS was achieved from the 0.6 l/min of air flow rate and it was categorized in plug flow model. This model (through material balance) indirectly demonstrated that the enzyme(s) might be responsible for RBBR decolorization due to these enzyme(s) is categorized as "biological decolorization activities agent".

CHAPTER V

CONCLUSIONS

1. The Aqueous RBBR decolorization by fungal pellets was successfully optimized using response surface methodology. Initial dye concentration was found to be a significant main factor to decolorization process comparing with mass of pellets and pH;
2. After optimization, maximum decolorization efficiency of 67.9 ± 5.4% was achieved when 50 ppm initial concentration of dye, 4 gram of pellet and pH 5.6 were used;
3. Kinetic of RBBR decolorization in batch flask with optimum values of variables was studied. The closest model to explain the kinetic of decolorization was the first order model with the kinetic constant (k) = -0.071 h^{-1} and R^2=0.964;
4. The optimized variables in batch flask study to gether with different hydraulic retention time (HRT) and air flow rate were operated in continuous fluidized bed biological reactor. The highest decolorization was achieved at 24 hr of HRT for all air flow rate tested;
5. In continuous reactor, fungal capability to decolorize aqueous RBBR reduced with time;
6. The effects of HRT and air flow rate in continuous reactor were studied by second order polynomial model. For the main and interaction factors, both of HRT and air flow rate were found to be significant on RBBR decolorization.

The maximum decolorization was obtained at 24 hr of HRT and 0.6 l/min of air flow rate;

7. Kinetic study in FBBR was studied by comparing plug flow and mixed flow model. Based on kinetic constants from batch study (0.071 hr^{-1}) and both of the models, the exit concentration of aqueous RBBR could be predicted. The plug flow model was chosen as the best fit model in FBBR process, particularly for 0.6 l/min of air flow rate.

BIBLIOGRAPHY

Aksu, Z., & Donmez, G. (2005). Combined effects of molasses sucrose and reactive dye on the growth and dye bioaccumulation properties of Candida tropicalis. *Process Biochemical, 40*, 2443–2454.

Aksu, Z., & Tezer, S. (2000). Equilibrium and kinetic modelling of biosorption of Remazol Black B by Rhizopus arrhizus in a batch system: effect of temperature. *Process Biochemistry, 36*, 431-439.

Alshamsi, F., Albadwawi, A., Alnuaimi, M., Rauf, M., & Ashraf, S. (2007). Comparative efficiencies of the degradation of Crystal Violet using UV/hydrogen peroxide and Fenton's reagent. *Dyes and pigments, 74(2)*, 283–287

Anjaneyulu, Y., Chary, N., & Raj, D. (2005). Decolourization of industrial effluent, available methods and emerging technologies: a review. *Review in Environmental Science and Biotechnology, 4*, 245-273.

Asgher, M., Batool, S., Bhatti, H., Noreen, R., Rahman, S., & Asad, M. (2008). Laccase mediated decolorization of vat dyes by Coriolus versicolor ibl-04. *Int Biodeterior Biodegrad, 62(4)*, 465–470.

Asgher, M., Kausara, S., Bhattia, N., Shah, H., & Ali, M. (2008). Optimization of medium for decolourization of Solar golden yellow R direct textile dye by Schizophyllum commune IBL-06. *Int Biodeterior Biodegrad, 61*, 189-193.

Aspland, J. R. (1997). *Textile dyeing and coloration*: American Association of Textile Chemists and Colorists.

Ay, F., Catalkaya, E., & Kargi, F. (2009). A statistical experiment design approach for advanced oxidation of direct red azo-dye by photo-fenton treatment. *Journal Hazard Material, 162(1)*, 230–236.

Bafana, A., Devi, S. S., Krishnamurthi, K., & Chakrabarti, T. (2007). Kinetics of decolourisation and biotransformation of direct black 38 by C. hominis and P. stutzeri. *Appl Microbiol Biotechnol, 74*, 1145-1152.

Banat, I., Nigam, P., Singh, D., & Marchant, R. (1996). Microbial decolorization of textile dye containing effluents: a review. *Bioresource Technology, 58*, 217-227.

Bank, W., Environment, U. N., & Organization, U. N. I. D. (1999). *Pollution Prevention and abatement handbook 1998; Toward Cleaner Production*. Washington D.C.

Barbusinski, K. (2005). The modified Fenton process for decolorization of dye wastewater. *Polish Journal of Environmental Studies, 14*(3), 281-285.

Bhatt, M., Patel, M., Rawal, B., Novotny, C., Molitoris, H. P., & Sasek, V. (2000). Biological decolorization of the synthetic dye RBBR in contaminated soil. *World Journal of Microbiology & Biotechnology, 16*, 195-198.

Bhatti, H., Akram, N., & Asgher, M. (2008). Optimization of culture conditions for enhanced decolorization of cibacron red fn-2bl by Schizophyllum commune ibl-6. *Applied Biochemical Biotechnology, 149*(3), 255-264.

Box, G., Hunter, W., & Hunter, J. (1978). *Statistics for experiments: An introduction to design, data analysis, and model building*: John Wiley & Sons, Inc.

Box, G. E. P., & Drapper, N. R. (2007). *Response surfaces, mixtures, and ridge analyses*. Canada: John Wiley and sons.

Cameron, M., Timofeevski, S., & Aust, S. (2000). Enzymology of phanerochaete chrysosporium with respect to the degradation of recalcitrant compounds and xenobiotics. *Applied Microbiology and Biotechnology, 54*(6), 751-758.

Cetin, D., & Donmez, G. (2006). Decolorization of reactive dyes by mixed cultures isolated from textile effluent under anaerobic conditions. *Enzyme and Microbial Technology, 38*, 926-930.

Chagas, E., & Durrant, L. (2001). Decolorization of azo dyes by Phanerochaete chrysosporium and Pleurotus sajorcaju. *Enzyme Microb Technology, 29*, 473-477.

Champagne, P., & Ramsay, J. (2005). Contribution of manganese peroxidase and laccase to dye decoloration by Trametes versicolor. *Applied Microbiology and Biotechnology, 69*(3), 276–285.

Christie, R. (2001). *Colour chemistry* (1st edn ed.). Cambridge (UK): Royal Society of Chemistry.

Couto, S. R. (2013). Treatment of Textile Wastewater by White-rot Fungi: Still a Far Away realty? *Textile and Light Industrial Science and Technology (TLIST), 2*(3).

Cripps, C., Bumpus, J., & Aust, S. (1990). Biodegradation of azo and heterocyclic dyes by Phanerochaete chrysosporium. *Appl Environ Microbiol, 56*(4), 1114-1118.

Cristovao, R. O., Tavares, A. P. M., Ribeiro, A. S., Loureiro, J. M., Boaventura, R. A. R., & Macedo, E. A. (2008). Kinetic modelling and simulation of laccase catalyzed degradation of reactive textile dyes. *Bioresource Technology, 99*, 4768–4774

Deveci, T., Unyayar, A., & Mazmanci, M. (2004). Production of Remazol Brilliant Blue R decolourising oxygenase from the culture filtrate of Funalia trogii ATCC 20080. *Journal of Molecular Catalyst B: Enzymatic, 30*, 25-32.

Diorio, L. A., Mercuri, A. A., Nahabedian, D. E., & Forchiassin, F. (2008). Development of a bioreactor system for the decolorization of dyes by Coriolus versicolor f. antarcticus. *Chemosphere, 72*, 150–156.

Doble, M., & Kruthiventy, A. (2007). *Green chemistry and process*: Academic Press.

Doble, M., & Kumar, A. (2005). *Biotreatment of industrial effluents*: Butterworth-Heinemann.

Dubrow, S., Boardman, G., & Michelsen, D. (1996). *Chemical pretreatment and aerobic anaerobic degradation of textile dye wastewater*: John Wiley & Sons.

Essadki, A., Bennajah, M., Gourich, B., Vial, C., Azzi, M., & Delmas, H. (2008). Electrocoagulation/ electroflotation in an external-loop air-lift reactor - application to the decolorization of textile dye wastewater: A case study. *Chemical Engineering Programme, 47*, 1211–1223.

Franciscon, E., Zille, A., Fantinatti-Garboggini, F., Silva, I. S., Cavaco-Paulo, A., & Durrant, L. R. (2009). Microaerophilic–aerobic sequential decolourization/biodegradation of textile azo dyes by a facultative Klebsiella sp. strain VN-31. *Process Biochemistry, 44*, 446–452.

Heinfling, A., Bergbauer, M., & Szewzyk, U. (1997). Biodegradation of azo and pthalocyanine dyes by Trametes versicolor and Bjerkandera adusta. *Applied Microbiology and Biotechnology, 48*, 261-266.

Hessel, C., Allegre, C., Maisseu, M., Charbit, F., & Moulin, P. (2007). A review Guidelines and legislation for dye house effluents. *Journal of Environmental Management, 83*, 171-180.

Hoign ́e, J. (Ed.) (1998) Chemistry of aqueous ozone and transformation of pollutants by ozonation and advanced oxidation processes (Vols. 5).

Kapdan, I., Kargia, F., McMullan, G., & Marchant, R. (2000). Effect of environmental conditions on biological decolorization of textile dyestuff by Coriolus versicolor in a rotating biological contactor. *Enzyme Microb Technology, 26*(5-6), 381-387.

Kapdan, I. K., & Kargi, F. (2002). Biological decolorization of textile dyestuff containing wastewater by Coriolus versicolor in a rotating biological contactor. *Enzyme and Microbial Technology, 30*, 195-199.

Karatas, M., Dursun, S., & Argun, M. E. (2009). Decolorization of reactive dyes under batch anaerobic condition by mixed microbial culture. *African Journal of Biotechnology, 8*(24), 6856-6862.

Kasinath, A., Novotny, C., Svobodova, K., Patel, K. C., & Sasek, V. (2003). Decolorization of synthetic dyes by Irpex lacteus in liquid cultures and packed-bed bioreactor. *Enzyme and Microbial Technology, 32*, 167–173.

Kaushik, P., & Malik, A. (2009). Fungal dye decolourization: Recent advances and future potential. *Environment International, 35*, 127–141.

Keharia, H., & Madamwar, D. (Eds.). (2004) Concise Encyclopedia of bioresource technology. New York: Food product press.

Khadhraoui, M., Trabelsi, H., Ksibi, M., Bouguerra, S., & Elleuch, B. (2009). Discoloration and detoxicification of a congo red dye solution by means of ozone treatment for a possible water reuse. *Journal Hazard Material, 161(2-3)*, 974–981.

Kilic, N., Nielson, J., Yuce, M., & Donmez, G. (2007). Characterization of a simple bacterial consortium for effective treatment of wastewaters with reactive dyes and Cr(VI). *Chemosphere, 67*, 826-831.

Kirk, T., & Farrell, R. (1987). Enzymatic "combustion": the microbial degradation of lignin. *Annual Rev Microbiology, 41*, 465–505.

Kleinbaum, D. G., Kupper, L. L., & Muller, K. E. (2008). *Applied regression analysis and other multivariable methods*: Thomson Learning Inc.

Kumar, K., Dastidara, M. G., & Sreekrishnan, T. R. (2009). Effect of process parameters on aerobic decolourization of reactive azo dye using mixed culture. *World Academy of Science, Engineering and Technology, 58*.

Kumar, M., Sridhar, T., Bhavani, K., & Dutta, P. (1998). Trends in colour removal in textile mill effluents. *Colourage, 40*, 25-34.

Leonowicz, A., Cho, N., Luterek, J., Wilkolazka, A., Wojtas-Wasilewska, M., Matuszewska, A., et al. (2001). Fungal laccase: properties and activity on lignin. *Journal Basic Microbiology, 41*(3-4), 185-227.

Levin, L., Papinutti, L., & Forchiassin, F. (2004). Evaluation of argentinean white rot fungi for their ability to produce lignin-modifying enzymes and decolorize industrial dyes. *Bioresources Technology, 94*(2), 169-176.

Lu, X., Yang, B., Chen, J., & Sun, R. (2009). Treatment of wastewater containing azo dye reactive brilliant red x-3b using sequential ozonation and upflow biological aerated filter process. *Journal Hazard Material, 161(1),* 241–245.

McKay, G. (1983). Adsorption of acidic and basic dyes onto activated carbon in fluidized beds. *Chemical Engineering Res. Div, 61*, 29-36.

McMullan, G., Meehan, C., Conneely, A., Kirby, N., Robinson, T., & Nigam, P. (2001). Microbial decolourisation and degradation of textile dyes. *Applied Microbiology and Biotechnology, 56*, 81-87.

Mendonca, R., Jara, J., Gonzalez, V., Elissetche, J., & Freer, J. (2008). Evaluation of the white-rot fungi ganoderma australe and ceriporiopsis subvermispora in biotechnological applications. *Journal Ind Microbiol Biotechnol, 35*(11), 1323-1330.

Merzouk, B., Gourich, B., Sekki, A., Madani, K., Vial, C., & Barkaoui, M. (2009). Studies on the decolorization of textile dye wastewater by continuous electrocoagulation process. *Chemical Engineering Journal, 149*, 2017-2214.

Mielgo, I., Moreira, M. T., Feijoo, G., & Lema, J. M. (2002). Biodegradation of a polymeric dye in a pulsed bed bioreactor by immobilised Phanerochaete chrysosporium. *Water Research, 36*, 1896–1901.

Montgomery, D. C. (2001). *Design and analysis of experiments* (Fifth ed.). New York: John Wiley & sons Inc.

Myers, R., & Montgomery, D. (2002). *Response Surface Methodology* (Second ed.): Wiley

Nakamura, T., Tokimoto, T., Tamura, T., Kawasaki, N., & Tanada, S. (2003). Decolorization of Acidic Dye by Charcoal from Coffee Grounds. *Journal of Health Science, 49(6),* 520-523.

Nyanhongo, G., Gomes, J., Gubitz, G., Zvauya, R., Read, J., & Steiner, W. (2002). Decolorization of textile dyes by laccases from a newly isolated strain of trametes modesta. *Water Research Elsevier, 36*(6), 1449-1456.

Ong, S.-A., Toorisaka, E., Hirata, M., & Hano, T. (2007). Treatment of methylene blue-containing wastewater using microorganisms supported on granular activated carbon under packed column operation. *Environ Chem Lett, 5*, 95-99.

Padmavathy, S., Sandhya, S., Swaminathan, K., Y. V. Subrahmanyam, Chakrabarti, T., & Kaul, S. N. (2003). Aerobic decolorization of reactive azo dyes in presence of various cosubstrates. *Chem. Biochem. Eng. Q., 17*(2), 147-151.

Parshetti, G., Kalme, S., & Gomare, S. (2007). Biodegradation of reactive blue-25 by Aspergillus ochraceus NCIM-1146. *Journal Biotechnology, 98*, 3638-3642.

Peralta-Zamora, P., Kunz, A., Moraes, S. G. d., Pelegrini, R., Molelro, P. d. C., Reyes, J., et al. (1999). Degradation of reactive dyes I. A comparative study of ozonation, enzymatic and photochemical processes. *Chemosphere, 38*(4), 835-885.

Reynolds, T. D. (1982). *Unit Operations and Process in Environmental Engineering*. California: Wadsworth, Inc.

Robinson, T., McMullan, G., Marchant, R., & Nigam, P. (2001). Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. *Bioresource Technology, 77 (3)*, 247-255.

Robinson, T., & Nigam, P. (2008). Remediation of textile dye waste water using a white-rot fungus Bjerkandera adusta through solid-state fermentation (ssf). *Applied Biochemical Biotechnology, 151*(2-3), 618-628.

Sandhya, S., Padmavathy, S., Swaminathan, K., Subrahmanyam, Y. V., & Kaul, S. N. (2005). Microaerophilic–aerobic sequential batch reactor for treatment of azo dyes containing simulated wastewater. *Process Biochemistry, 40*, 885–890.

Santos, A. D., Neto, J., Tavares, C., & Costa, S. d. (2004). Screening of filamentous fungi for the decolorization of a commercial reactive dye. *Journal Basic Microbiology, 44*(4), 288-295.

Sharma, P., Singh, L., & Dilbaghi, N. (2009). Optimization of process variables for decolorization of Disperse Yellow 211 by *Bacillus subtilis* using Box-Behnken design. *Journal of Hazardous Materials, 164*, 1024-1029.

Shedbalkar, U., Dhanve, R., & Jadhav, J. (2008). Biodegradation of triphenylmethane dye cotton blue by Penicillium ochrochloron MTCC 517. *Journal of Hazardous Materials, 157*, 472-479.

Slokar, Y., & Marechal, A. M. L. (1998). Methods of decoloration of textile wastewaters. *Dyes and pigments, 37(4)*, 335–356.

Smelcerovic, M., Dordevic, D., Novakovik, M., & Mizdrakovic, M. (2010). Decolorization of a textile vat dye by adsorption on waste ash. *Journal of the Serbian Chemical Society, 75 (6)*, 855-872.

Srinivasan, S. V., & Murthy, D. (2008). Statistical optimization for decolorization of textile dyes using *Trametes versicolor*. *Journal of Hazardous Materials*.

Sun, S., Li, C., Sun, J., Shi, S., Fan, M., & Zhou, Q. (2009). Decolorization of an azo dye Orange G in aqueous solution by Fenton oxidation process: Effect of system parameters and kinetic study. *Journal Hazard Material, 161(2-3)*, 1052–1057.

Supaka, N., Juntongjin, K., Damronglerd, S., Delia, M.-L., & Strehaiano, P. (2004). Microbial decolorization of reactive azo dyes in a sequential anaerobic–aerobic system. *Chemical Engineering Journal, 99*, 169–176.

Swamy, J., & Ramsay, J. (1999). The evaluation of white rot fungi in decolorization in the decolorization of textile dyes. *Enzyme and Microbial Technology, 24*, 130-137.

Tavares, A., Coelho, M., Agapito, Coutinho, & Xavier. (2006). Optimization and modeling of laccase production by Trametes versicolor in a bioreactor using statistical experimental design. *Applied Biochemical Biotechnology, 134*, 233-248.

Tavares, A. P. M., Cristovao, R. O., Loureiro, J. M., Boaventura, R. A. R., & Macedo, E. A. (2009). Application of statistical experimental methodology to optimize reactive dye decolourization by commercial laccase. *Journal of Hazardous Materials, 162*, 1255-1260.

Tchobanoglous, G., Burton, F. L., & Stensel, H. D. (Eds.). (2003). *Wastewater Engineering*. New York: McGraw Hill.

Thakur, I. (2006). *Environmental biotechnology; basic concepts and applications*: I. K International Pvt Ltd.

Tien, M., & Kirk, T. (1983). Lignin-degrading enzyme from the hymenomycete phanerochaete chrysosporium burds. *Science, 221*(4611), 661-663.

Tobin, J., White, C., & Gadd, G. (1994). Metal accumulation by fungi: applications in environment biotechnology. . *Journal Industrial Microbiology, 13*, 126-130.

Vandevivere, P., Bianchi, R., & Verstraete, W. (1998). Review: Treatment and reuse of wastewater from the textile wet-processing industry: Review of emerging technologies. *Journal Chemical Technology Biotechnology, 72(4).*

Venkat, M. S., Khrisna, M. S., & Karthikeyan, J. (2000). *Adsorption mechanism of acid-azo dye from aqueous solution on to coal/coal based sorbents and activated carbon*. Tirupathi, India: Student offset printers.

Vijayaraghavan, K., Won, S. W., & Yun, Y.-S. (2008). Treatment of Complex Remazol Dye Effluent using Sawdust and Coal-based Activated Carbons. *Journal of Hazardous Materials*.

Von, G. (2007). The basics of oxidants in water treatment. part b: ozone reactions. *Water Science Technology, 55*(12), 25–29.

Wang, L. K., Hung, Y.-T., Lo, H. H., & Yapijakis, C. (Eds.). (2004). *Handbook of industrial and hazardous wastes treatment* CRC Press.

Wesenberg, D., Kyriakides, I., & Agathos, S. (2003). White-rot fungi and their enzymes for the treatment of industrial dye effluents. *Biotechnol Adv, 22(1-2)*, 161-187.

Yann, D., Didier, B., & Daniel. (2005). Utilisation of the experimental designmethodology to reduce browning defects in hard cheeses technology. *Journal Food Eng, 68*, 481-490.

Yesilada, O., Asma, D., & Cing, S. (2003). Decolorization of textile dyes by fungal pellets. *Process Biochemistry, 38*, 933-938.

Young, L., & Yu, J. (2007). Ligninase catalysed decolorization of synthetic dyes. *Water Research Elsevier, 31*, 1187-1193.

Zhang, F.-m., Knapp, J. S., & Tapley, K. N. (1998). Decolourisation of cotton bleaching effluent in a continuous fluidized-bed bioreactor using wood rotting fungus. *Biotechnology Letters, 20*(8), 717-723.

Zhang, F., & Yu, J. (2000). Decolourisation of Acid Violet 7 with complex pellets of white rot fungus and activated carbon. *Bioprocess Engineering, 23*, 295-301.

Zidane, F., Drogui, P., Lekhlif, B., Bensaid, J., Blais, J., Belcadi, S., et al. (2008). Decolourization of dye-containing effluent using mineral coagulants produced by electrocoagulation. *Journal Hazard Material, 155(1-2)*, 153–163.

APPENDIX A

Result of FBR experiment with 24 of HRT after 12 hr

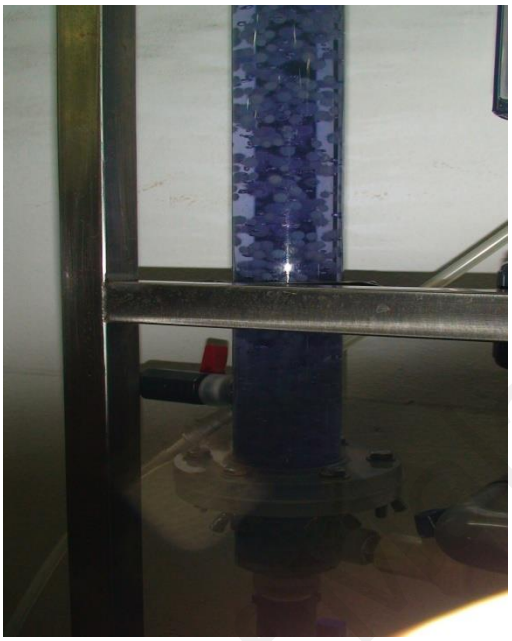


Figure 1. 0.2 l min^{-1} of air flow rate

Figure 2. 0.6 l min^{-1} of air flow rate

Figure 3. Comparison of initial dye concentration and result after 12 hr with 0.6 l min^{-1} of air flow rate

Figure 4. 1.2 l min^{-1} of air flow rate

APPENDIX B

Linear Regression Model of Plug Flow and Mixed Flow Data

A. Plug Flow Model

Table A.1 0.2 l min^{-1}

HRT	C observed (x)	C model (y)	fit y
0	50	50	44.89246787
6	44.05513	32.65581711	39.37889919
12	22.3701	21.32804782	19.26712179
24	11.8854	9.097712474	9.543088551
slope	0.927449831	-1.480023665	intercept
±	0.197922452	7.055136329	±
r2	0.916520216	6.153555605	s(y)
F	21.95789607	2	degrees of freedom
regression ss	831.463107	75.73249316	residual ss

Table A.2 0.6 l min^{-1}

HRT	C observed (x)	C model (y)	fit y
0	50	50	47.83747949
6	37.55623	32.65581711	35.69546768
12	22.98657	21.32804782	21.47911814
24	9.243703	9.097712474	8.069512091
slope	0.97575026	-0.950033516	intercept
±	0.089449974	3.008332695	±
r2	0.98346995	2.738246593	s(y)
F	118.9917681	2	degrees of freedom
regression ss	892.1996113	14.99598881	residual ss

Table A.3 1.2 l min^{-1}

HRT	C observed (x)	C model (y)	fit y
0	50	50	48.39475607
6	35.78477	32.65581711	34.77865085
12	14.21687	21.32804782	14.11976627
24	15.95893	9.097712474	15.78840422
slope	0.957853318	0.502090163	intercept
±	0.243465062	7.924176844	±
r2	0.885572736	7.204439954	s(y)
F	15.47835207	2	degrees of freedom
regression ss	803.3876901	103.8079101	residual ss

B. Mixed Flow Model

Table B.1 0.2 l min^{-1}

HRT	C observed (x)	C model (y)	fit y
0	50	50	45.25745374
6	44.05513	35.0631136	41.07156699
12	22.3701	26.99784017	25.8027588
24	11.8854	18.49112426	18.4202985

slope	0.704117457	10.05158087	intercept
±	0.176207919	6.281100872	±
r2	0.888688468	5.478434671	s(y)
F	15.96759031	2	degrees of freedom
regression ss	479.2392232	60.02649289	residual ss

Table B.2 0.6 l/min

HRT	C observed (x)	C model (y)	fit y
0	50	50	47.59106952
6	37.55623	35.0631136	38.31221635
12	22.98657	26.99784017	27.44816665
24	9.243703	18.49112426	17.20062552

slope	0.745662541	10.30794244	intercept
±	0.098618806	3.316693853	±
r2	0.966198861	3.018923292	s(y)
F	57.16960286	2	degrees of freedom
regression ss	521.0379204	18.22779569	residual ss

Table B.3 1.2 l/min

HRT	C observed (x)	C model (y)	fit y
0	50	50	48.20809642
6	35.78477	35.0631136	37.67341172
12	14.21687	26.99784017	21.68977823
24	15.95893	18.49112426	22.98079166

slope	0.741084365	11.15387815	intercept
±	0.182544471	5.941364461	±
r2	0.891783943	5.401722393	s(y)
F	16.48154563	2	degrees of freedom
regression ss	480.9085064	58.35720961	residual ss