# SYNTHESIS AND CHARACTERIZATION OF BIOCERS AS HIGH-PERFORMANCE BIOSORBENTS FOR DYE REMOVAL PROCESS

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To my supportive husband, Hafiz, my beautiful children, Husna, Ayra, Adam, and my beloved parents, Wan Mohd Zawawi and Sarah.

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# ABSTRACT

The immobilization of the biological species such as prokaryotic and eukaryotic cell systems (e.g. bacteria, animal and plant cells, and fungi) and cell-derived subunits (e.g. proteins and enzymes) within inorganic oxide matrices as a class of nanocomposite materials especially through sol-gel technique attracts increasing interests for biocatalysis, bioremediation and structured material templates applications. These interests are justified due to the unique approach to explore the richness of the biological structures for technical uses. In this study, Biocers (biologically modified ceramics) of *Trametes versicolor* (TV) embedded in silica matrices was synthesized according to the sol-gel method using tetraethyl orthosilicate (TEOS) as a precursor. The synthesized materials namely free silica (SS), hybrid silica with PVA (hSS), TV Biocers (TVB), hybrid TV Biocers with PVA (hTVB), acclimatized TV Biocers (TVB/AC) and PVA-hybrid acclimatized TV Biocers (hTVB/AC) were characterized using scanning electron microscope, transmission electron microscope, Fourier transform infrared spectroscopy, nitrogen adsorption-desorption measurement and laccase enzyme catalytic activity assay. The performance of the TV Biocers as biosorbents was evaluated using dyes as model emerging organic micropollutants carried out in batch (i.e. shake flask) and continuous (packed-bed) systems. It was observed that the dye removal performance, $\eta$ (mmol/g) of the TV Biocers for methylene blue (MB) and malachite green (MG) respectively was 7.400 and 5.569 mmol/g which was 18 and 128 % higher than the SS and free TV cells. These results demonstrated that the TV Biocers can offer better dye removal performance through a combination of adsorption and biodegradation processes. The optimization experiment was carried out using MG due to its higher removal performance (adsorption and biodegradation) than MB. The dye removal performance by hTVB was found maximum when operated at pH 6, temperature of 30 $^{o}$C, 150 rpm of agitation speed, and a biocers/dye ratio of 30 % (w/v) with MG at concentration of 0.5mM. The hTVB was also able to remove MB, methyl orange, and reactive red. The acclimatized TV Biocers (i.e. TVB/AC and hTVB/AC) was studied for dye removal performance in batch and packed-bed process. The highest dye removal by the hTVB/AC was at concentration of 0.2 mM, temperature of 30 $^{o}$C, and pH 7 for batch process and fastest at concentration of 0.05 mM, bed height of 1 cm and flow rate of 3.0 mL/min for continuous process. The biosorption thermodynamics and kinetics as well as biodegradation kinetic studies were conducted for all biocers (i.e. SS, hSS, TVB, hTVB, TVB/AC and hTVB/AC) for both batch and continuous processes. High correlation coefficients favour Langmuir isotherm and Elovich model for batch and continuous process for biosorption. Meanwhile, the biodegradation fitted the Haldane model well for batch and continuous process. This is the first reported study on the immobilization of the TV cells in silica matrices for removal of emerging organic micropollutants.

# ABSTRAK

Immobilisasi spesies biologi seperti sistem sel prokariot dan eukariot (contohnya sel bakteria, sel haiwan, sel tumbuhan, dan kulat) dan subunit sel yang diperolehi (contohnya protein dan enzim) dalam matriks oksida bahan bukan organik sebagai bahan nano terutamanya melalui teknik sol-gel semakin menarik minat untuk dijadikan aplikasi biomangkin, biopemulihan dan templat bahan terstruktur. Ini adalah wajar kerana pendekatannya yang unik dalam meneroka kekayaan struktur biologi bagi kegunaan teknikal. Dalam kajian ini, Biocers (seramik terubahsuai secara biologi) daripada *Trametes versicolor* (TV) tertanam dalam matriks silika telah disintesis mengikut kaedah sol-gel menggunakan tetraetilortosilikat (TEOS) sebagai prapenanda. Bahan-bahan yang disintesis iaitu silika bebas (SS), silika hibrid dengan PVA (hSS), Biocers TV (TVB), Biocers TV hybrid (hTVB), Biocers TV tersuai diri (TVB/AC) dan Biocers TV hibrid-tersuai diri (hTVB/AC) telah dicirikan menggunakan mikroskop imbasan elektron, mikroskop penghantaran elektron, spektroskopi trasformasi inframerah Fourier, penjerapan nyah-jerapan nitrogen dan aktiviti pemangkin cerakin pengukuran enzim lakase. Prestasi TV Biocers sebagai bio-penjerap dinilai menggunakan pencelup sebagai model bahan pencemar mikro organik yang dijalankan dalam sistem kelompok (kelalang goncang) dan berterusan (lapisan terpadat). Diperhatikan bahawa prestasi penyingkiran pencelup, $\eta$ (mmol / g) Biocers TV untuk MB dan MG masing-masing adalah 7,400 dan 5,569 mmol / g di mana 18 % dan 128% lebih tinggi daripada SS dan sel TV bebas. Keputusan ini menunjukkan bahawa Biocers TV boleh menawarkan prestasi penyingkiran pencelup yang lebih baik melalui gabungan proses penjerapan dan biodegradasi. Eksperimen pengoptimuman dijalankan menggunakan MG kerana prestasi penyingkirannya (penjerapan dan biodegradasi) yang tinggi berbanding MB. Penyingkiran pewarna oleh hTVB ditemui maksimum pada pH 6, suhu 30 $^{o}$C, kelajuan pengadukan 150 rpm, dan nisbah biocer/pencelup sebanyak 30% (w/v) pada kepekatan MG 0.5 mM. hTVB juga dapat menyingkirkan MB, metil jingga dan reaktif merah. TV menyesuai diri (TVB/AC dan hTVB/AC) telah diuji untuk prestasi penyingkiran pencelup dalam proses kelompok dan berterusan. Penyingkiran pencelup oleh hTVB / AC adalah tertinggi pada kepekatan 0.2 mM, suhu 30 $^{o}$C dan pH 7 untuk proses kelompok dan terpantas pada kepekatan 0.05 mM, ketinggian lapisan 1 cm dan kadar aliran 3.0 ml/min untuk proses berterusan. Termodinamik dan kinetik proses penjerapan dan kajian kinetik biodegradasi dilakukan untuk kesemua biocer (SS, hSS, TVB, hTVB, TVB/AC dan hTVB/AC) bagi kedua-dua proses kelompok dan berterusan. Pekali korelasi yang tertinggi memihak kepada model garis sesuhu Langmuir dan model kinetik Elovich untuk proses kelompok dan berterusan bagi penjerapan. Sementara itu, biodegradasi pula padan dengan model Haldane bagi proses kelompok dan berterusan. Ini adalah kajian yang pertama dilaporkan mengenai immobilisasi sel TV dalam matriks silika bagi penyingkiran bahan pencemar mikro organik.

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| | | |
|---|---|---|
| AB | - | Adam-Bohart |
| ABTS | - | Azino-bis (3-ethylbenzoline-6-sulfonic acid) diammonium |
| ATCC | - | American Tissue Culture Collection |
| AU | - | Activity Unit |
| BET | - | Brunauer-Emmet-Teller |
| BJH | - | Barrett-Joyner-Helenda |
| C | - | Carbon |
| DMP | - | Dimethoxyphenol |
| EDCs | - | Endocrine Disrupting Compounds |
| FTIR | - | Fourier Transform Infrared Spectroscopy |
| $H_2O$ | - | Water |
| HCl | - | Hydrochloric Acid |
| hSS | - | Hybrid silica with PVA |
| hTVB | - | Hybrid TV Biocers with PVA |
| hTVB/AC | - | Hybrid acclimatized (in dye) TV Biocers with PVA |
| LiP | - | Lignin Peroxidase |
| MB | - | Methylene blue |
| MG | - | Malachite green |
| MO | - | Methyl orange |
| MnP | - | Manganese Peroxidase |
| N | - | Nitrogen |
| NaCl | - | Sodium Chloride |
| NAD | - | Nitrogen Adsorption/ Desorption |
| NaOH | - | Sodium Hydroxide |
| O | - | Oxygen |
| OTC | - | Oxytetracycline |
| PAHs | - | Polyaromatic Hydrocarbons |
| PCBs | - | Polychlorinated Biphenyls |
| PCPs | - | Personal Care Products |
| PFAs | - | Polyfluorinated Alkylated Substances |
| PFO | - | Pseudo-first order |

| | | |
|---|---|---|
| PSO | - | Pseudo-second order |
| RR | - | Reactive red |
| SEM | - | Scanning Electron Microscope |
| $SiO_2$ | - | Silica Oxide |
| Si-OH | - | Silanol group |
| SOLAC | - | Sol-gel Laccase |
| SS | - | Free silica |
| STP | - | Standard Temperature Pressure |
| TEA | - | Triethylamine |
| TEM | - | Transmission Electron Microsscope |
| TEOS | - | Tetraethoxysilane |
| TV | - | *Trametes versicolor* |
| TVB/AC | - | Acclimatized (in dye) TV Biocers |
| TVB | - | TV Biocers |
| UTM | - | Universiti Teknologi Malaysia |
| UV | - | Ultraviolet |
| WRF | - | White Rot Fungi |
| YN | - | Yoon-Nelson |

# LIST OF SYMBOLS

| | | |
|---|---|---|
| μ | - | micro |
| $A_t$ | - | Temkin constant (L/g) |
| $\beta_L$ | - | External mass transfer coefficient in liquid phase (cm/s), |
| $b_t$ | - | Temkin isotherm constant (J/mol) |
| $C_{AO}$ | - | Initial concentration of biosorbents (mmol/ml) |
| $C_A$ | - | Concentration of biosorbents (mmol/ml) |
| $C_e$ | - | Concentration at equilibrium (mmol/L) |
| $C_t$ | - | Concentration at time t (mmol/L) |
| $C_0$ | - | Initial concentration (mmol/L) |
| $D_{eff}$ | - | Effective liquid film diffusion coefficient (cm/h) |
| $D_f$ | - | Film diffusion coefficient (m2/h) |
| $D_p$ | - | Particles diameter (nm) |
| F | - | Ratio of the amount of dye adsorbed at time t |
| F | - | Flow rates |
| g | - | gram |
| $k_1$ | - | rate constant of the pseudo-first order kinetic model |
| $k_2$ | - | rate constant of the pseudo-second order kinetic model |
| $k_a$ | - | the sorption equilibrium constant |
| $K_F$ | - | Freundlich constant related to the biosorption capacity (L/mol) |
| $K_L$ | - | Langmuir constant related to the biosorption capacity (L/mol) |
| $K_{id}$ | - | Intraparticle diffusion constant |
| $N_T$ | - | External mass transfer |
| τ | - | Time required to achieve 50% breakthrough |
| η | - | Dye removal performance |
| $q_{exp}$ | - | Experimental data biosorption capacity |
| $q_{cal}$ | - | Model calculated biosorption capacity |
| $Q_e$ | - | Equilibrium biosorption capacity (mmol/g), |
| $Q_{max}$ | - | Maximum biosorption capacity |
| $Q_t$ | - | Biosorption capacity at time t |
| r | - | radial position |
| R | - | Universal gas constant (8.314 J/mol.K) |

| | | |
|---|---|---|
| $R_L$ | - | Dimensionless constant separation factor |
| $r^2$ | - | Linear regression coefficient for isotherm and kinetic models |
| T | - | Absolute temperature (298 K) |
| U | - | Laccase activity |
| $\Delta G^0$ | - | Gibb free energy (kJ/mol) |
| $\Delta H^0$ | - | Enthalpy change (kJ/mol) |
| $\Delta S^0$ | - | Entropy change (kJ/mol) |
| $V_{pore}$ | - | Pore volume (cm3/g) |
| 1/n | - | Biosorption intensity of the biosorbents |
| $\delta$ | - | The thickness of the film layer (cm) |
| $\varphi$ | - | Associate parameter of water (2.60) |
| $M_B$ | - | Molecular weight of water (18 g/mol) |
| $\mu_f$ | - | Viscosity of water (0.89) |
| $V_A$ | - | liquid molar volume at normal boiling temperature |
| mL | - | milliliters |
| mg | - | milligrams |
| L | - | Liters |
| $^oC$ | - | Degree Celcius |
| cm | - | centimeters |
| % | - | percents |
| $x^2$ | - | Linear regression coefficient for isotherm and kinetic models |
| $\varepsilon$ | - | Absorptivity of ABTS |
| Z | - | Bed height |

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# CHAPTER 1

# INTRODUCTION

## 1.1 Background of Research

Dye is a coloured substance that has an affinity to the substrate to which it is being applied. The dye is generally applied in an aqueous solution, and may require a mordant to improve the fastness of the dye on the fibre (Booth *et al.*, 2000). There are quite a few ways to classify dyes. For example, they may be classify based on charge characteristics, such as cationic, anionic and non-ionic dyes. The organic dyes can also be classified according to their chemical structure into groups such as azo compounds, anthraquinones, triarylmethanes and phthalocyanines. Dye can also be classified according to their solubility and chemical properties, for instance acid dyes, disperse dyes, direct dyes, reactive dyes, mordant dyes, vat dyes and sulphur dyes. The use of different dyes is dependent on the characteristics of the fibre, the specific colour to be applied and the desired outcome required on the fibre.

Over the past century, dyes play a significant role in the economy of many countries. Dyes have been widely used in many industries such as textiles, foods, plastics, leathers and pulps. Dyes are one of the most abundant organic pollutants that are produced from various industries and can be found in our wastewater treatment. The release of coloured wastewater from fast growing industries such as textile, paper and plastic may present eco-toxic hazard and may eventually lead to a high environmental impact if not treated effectively b eforehand. For instance, malachite green is a most commonly used for dyeing of cotton, silk, paper, leather and also in manufacturing of paints and printing inks. Most of the dyes, including malachite green, are toxic and must be removed before discharge into receiving streams (Srivastava *et al.,* 2004). These important sectors use large amounts of process water and produce great amounts of polluted discharge. Water is used for cleaning the raw material and for many flushing steps during the whole production.

Produced wastewater should be cleaned from fat, oil, colour and other chemicals, which were used during the several production steps.

The discharge of coloured effluents from various industries without decolourization procedure may cause serious problems in the receiving environments. Depending on the exposure time and dye concentration, dyes may have acute and/or chronic effects on exposed organisms. Dyes may absorb and reflect sunlight that entering the water, affecting the growth of organisms thus interferes with the food chain. Both public and authorities are very aware with a slight abnormal colouration of surface waters (Shah and Patel, 2014). Moreover, the presence of even a small fraction of dyes in water is highly visible due to high colour or tinctorial value of dyes. This will have an effect on the aesthetic value of the water resources. Common waste treatment procedure unable to remove dye due to their complex aromatic structure that is resistant to light, biological activity, ozone and other degradative environments (Joshi *et al.*, 2004). The possible long-term effects of a few dyes and dye degradation products are becoming of increasing concern. Some dye effluents are found mutagenic, carcinogenic and/or allergenic effects (Alves de Lima *et al.*, 2007). The highest rates of toxicity were found amongst basic and diazo dyes (Yahagi *et al.*, 1975). Furthermore, dyes have also an effect on photosynthetic activity in aquatic life by reducing light penetration (Lavanya, 2014) and may also be toxic to certain forms of aquatic life due to presence of metals and chlorides in them (Khayatzadeh and Abbasi, 2010). Dyes have also been known to interfere with certain municipal wastewater treatment operations such as UV disinfection (Ramakrishna and Viraraghavan, 1996).

Due to these possible drawbacks of dyes, many practices for wastewater decolourization treatment had been applied including physical, chemical and biological treatment. Several techniques for dye removal have been developed, and some have been widely employed. However, the conventional single methods are not efficient enough, thus, there is always a need for designing new materials that can adsorb and degrade dye with high removal performance capacity and selectivity as well as cost efficient.

## 1.2 Problem Statement

Immobilization is an attractive way for biological components physiological capabilities enhancement. Biocomponents such as enzymes or cells are connected to a surface by self-adhesion or chemical bonding or entrapped in the interstices of fibrous or porous materials or physically linked within or by solid or porous matrices. This may lead to increased resistance on changes in environment such as pH or temperature. However, an efficient system to avoid the problem of fixing biocomponents or biomolecules firmly without altering their original conformations and activities is still challenging for the utilization of biochemical functions of active biocatalyst. A recent trend in the field of bio-engineered materials is the research of nanocomposites, where bioactive compounds are embedded or immobilized contained by inorganic nanostructured oxide matrices (Soltmann *et al.*, 2003).

Mixing a ceramic-like oxide matrix with biological system ("biocers") presents several returns. The main advantage of biocers is the mutual influence of the mechanical, chemical, thermal, and photochemical stability of the inorganic host matrix with the high variability of the sol-gel method (e.g., chemical modifications, tailored porosity) and the extensive range of sol-gel derived materials (e.g., coatings, granules, shaped-bulk products) enhance the technical applications of immobilization (Soltmann and Böttcher, 2008). Silica-derived ceramic matrices employment can ensure the viability of the encapsulated living cells. Silica is toxicologically and biologically inert and usually not a food source of microorganism and thus makes it suitable to be incorporated with biological components such as cell. It was demonstrated that biocers is practical to be used in many applications such as biosensors, bioremediation, structured material templates, at least at laboratory scale (Flickinger *et al.*, 2009). Moreover, the Biocers that employ sol gel matrix protects the biocatalyst and prevents cell lysis, thus lead to activity and stability preservation and improvement. It has been reported that the fine porosity of sol-gel allows nutrients to reach the cell and by-products to escape so that the physiological capabilities of the cells can be enhanced (Guan *et al.*, 2008). The favourable surface/ mass ration, being inertness, mild operative conditions and mechanical strength or organically modified sol-gel materials are always a purpose why sol-gel method is selected. On the contrary to organic polymeric matrices, silica matrices are inert and more resistant to microbial attack and contamination. Furthermore, the sol-gel process involving mild reaction conditions, because the chemical reactivity of precursor compounds accomplishes the free energy requirement leading to Si-O-Si network as a stable reaction product (Carturan *et al.,* 2004).

Despite all benefits of using biocers in several applications, there is no report of synthesis of biocers comprised of *Trametes versicolor* fungus using sol-gel method. Many studies of biocers using one of the most extensively used enzyme; laccase is widely reported (Mohidem and Mat, 2009a, 2009b, 2010a, 2010b, and 2011; Zhang *et al.,* 2013; Galliker *et al.*, 2010; Manna and Amutha, 2017). However, these studies only used the derived sub-unit components from the fungus (enzyme), not the fungus itself. The usage of fungus as whole is suggested as to give more benefits rather than used a single enzyme. The loss of biological activity of an enzyme during immobilization or while it is in use is possible and thus using whole cell can counter this problem. The immobilized cells possess regeneration capability and are particularly suitable for multiple enzymatic reactions. The advantage of using cell instead of enzyme include the need to purify is not needed, the cell system is less sensitive to operating conditions changes such as pH, and higher loading support could also be investigated. Using fungus, particularly gives several advantages especially based on its principal mechanism that comprise of biosorption, bioaccumulation and biodegradation. The three mechanism are hugely beneficial as to give extra credits to ceramic that solely make use of biosorption only.

Sometimes, immobilization draws issues like mass transfer limitations due to changes in structure. Thus, the incorporation of additive to the biocers was proposed to overcome this issue. Additive able to protect the cell by preparing a shield-like between the protein and its environment. Additives are believed to provide additional sites for hydrogen bonding with the enzyme surface, decreasing dehydration and provides barrier for enzymes from unfolding by covering the interface (Villalonga *et al.,* 2000). The addition of additive process is simple, fast and economic for enhancement of enzyme and cell stability. The immobilized cell activity can also be enhanced in the presence of additive. Several chemical reagents used as an additive include surfactants, polyhydric alcohols, methyl esters, and metal ions. Some additive for examples polyvinyl alcohol (PVA) and polyethylene glycol (PEG) were proven to protects enzymes from denaturing effects without affecting their reaction rates (Soares *et al.*, 2002). The PVA and PEG have higher molecular weight, thus able to cover a large superficial area. This will help to improve the cell activity due to conformational and structural changes with the decrease in surface coverage (Wehtje *et al.*, 1993). In general, the addition of additive during immobilization may improve operational stability (Zhang *et al.*, 2014).

The synthesized biocers is studied for removal of dye as model emerging organic pollutants. Although selected dye stimulates biological reactions at low concentrations, most of dye are considered toxic to microorganisms at moderate concentrations and can cause inhibitory effects on the biological processes. Therefore, it is important that microorganisms were first acclimatized to dye before immobilization and used for further treatment process. The acclimatization of fungi in dye may influence the further removal dye studies. During acclimatization, the fungi was adjusted to a gradual change it is environment to allow them to a maintain performance and adhering changes across a range of environmental conditions. The interference from the functional group from the dye pollutant that may altered the nutrient dependency of the fungi thus affect the metabolic capacity and behavior of the fungus (Rajeswari *et al.*, 2013).

## 1.3 Objectives and Scopes of Study

The objectives and scopes of this research are:

i. To synthesize and characterize the TV Biocers

The *Trametes versicolor* (TV) strain ATCC 42530 was obtained from the American Type Culture Collection. The free silica (SS) and TV Biocers (TVB) will be prepared using tetraethyl orthosilicate (TEOS) as a silica precursor using sol-gel method. Sol is a stable suspension of colloidal solid particles in a liquid while gel is a porous, three-dimensional and continuous solid network surrounding a continuous liquid phase. The sol-gel process involves conversion of monomers into colloidal solution (sol) that acts as the precursor for an integrated network (gel) and comprises of five steps namely hydrolysis, condensation, gelation, ageing and drying. Hydrolysis occurs when TEOS and water are mixed and produces an intermediate silanols (Si-OH). In the subsequent step, condensation takes place to form bridging oxygen or a siloxane group Si-O-Si. As the sol aggregates the viscosity will increase until a gel is formed. Biological component was added at pre-gelation process. The sol-gel transition (gelation) is reached when a continuous network is formed and as the viscosity rapidly increase, the solvent is trapped inside the gel (ageing). The drying process was then taking place in order to remove remaining liquid (solvent) phase. During this step, a significant amount of shrinkage and densitification occur. The porosity of the gel was strongly determined by the rate of which the solvent can be removed.

The hybrid free silica (hSS) and hybrid TV Biocers (hTVB) were prepared using TEOS as a silica precursor and additive to further improve the catalytic activity and stability of sol-gel silica. The effect of additives types and loadings was investigated. Two different additives (PVA and PEG) were introduced at pre-gelation process in order to further protect the cell and enzyme from aggregation and unfolding effects. The TV cell was also acclimatized in dye before immobilized in sol-gel silica (TVB/AC). The dye used for acclimatization was the same as the dye tested for dye removal performance, which is malachite green (MG). The MG concentration was increased gradually from 10 mg/L until 50 mg/L, each for 7 days of incubation period. After each time interval, the TV cells were harvested and used as inoculation for subsequent dye concentration. The procedures were similar to the cell entrapment in objective number 1, except that TV cell was mixed with additives at prior gelation.

The synthesized SS, hSS, TVB, hTVB, and TVB/AC were characterized by a scanning electron microscope (SEM), transmission electron microscope (TEM), Fourier transform infrared (FTIR) spectrophotometer, nitrogen adsorption/ desorption (NAD analyzer and cell mass catalytic activity measurement for further understanding of physical and biological properties of synthesized TV Biocers. The cell mass catalytic activity was determined by using 2,2'-azino-bis (3-ethylbenzoline-6-sulfonic acid) diammonium salt (ABTS) as a substrate. A comparison study of free and immobilized cell catalytic activity was also conducted.

ii. To evaluate the batch dye removal performance of TV Biocers

The performance of SS, hSS, TVB, hTVB and TVB/AC were carried out in Erlenmeyer flask as batch reactor using malachite green (MG) dyes as emerging organic micropollutants model. Dye removal performance was investigated at various initial pH, temperature, initial dye concentrations, agitation speed, substrate/ medium ratio, and other type of dye such as reactive red (reactive-azo dye), methylene blue (cationic heteropolyaromatic dye) and methyl orange (anionic-azo dye). The removal of dye was studied in terms of biosorption and degradation. Removal of dyes was analyzed using adsorption isotherm (Langmuir, Freundlich and Temkin) and kinetic (pseudo-first order, pseudo-second order, Elovich and diffusion models) for biosorption process and biodegradation kinetic model namely Haldane, Aiba and Edward kinetic model for biodegradation process.

iii. To evaluate the continuous dye removal performance of TV Biocers

The continuous dye removal performance was evaluated using fixed-bed adsorber. The performance was described through the concept of breakthrough concentration curves. The effect of bed height, flow rate and initial dye concentration was investigated. The cumulative biosorption data was analyzed using isotherm and kinetic models. The existing isotherm models such as Langmuir, Freundlich and Temkin isotherm models were used to analyse biosorption isotherm data. The pseudo-first order, pseudo-second order and Elovich kinetic models were used for the biosorption kinetic data analysis. The performance of the fixed-bed adsorber was analyzed using Thomas, Adam-Bohart and Yoon-Nelson models. The dye removal data were also analyzed using existing biodegradation kinetic model namely Haldane, Aiba and Edward kinetic model.

## 1.4 Thesis Outline

This thesis contains five chapters. Chapter 1 presents research introduction, problem background, objectives and scopes of this study, thesis outline and chapter summary. Literature reviews on micropollutants, biodegradation, immobilization, biocers, white-rot fungi *Trametes versicolor* and sol-gel technology are presented in Chapter 2. Chapter 3 discusses about research methodology which comprises research materials and experimental procedures including TV Biocers preparation and characterization and biodegradation testing evaluation. The results and discussions of TV Biocers synthesis and characterizations, evaluation of dye removal process and performances were presented in Chapter 4. Chapter 5 presents the conclusions of the study and recommendations for future work. This is followed by the list of references cited in the thesis.

## 1.5 Summary

Colour has always played a significant part in our lives, including presenting different cultures of human being all over the world, influencing the clothes we wear and the furnishings that we used. However, the existence of dye in environment especially in water can cause impacts in human health as well as to the environment. This motivated research and development scholars all over the world to explore new method to minimize the dye environmental impacts. The removal of dye through various physical, chemical and biological processes has been applied in removing dye. However, the effectiveness, pros and cons are varies depending on the types of dye, cost, procedures and environment condition. Combining physical and biological process shows promising result in removing dye. In this study, *Trametes versicolor* (known as laccase enzyme producing fungi) cells are immobilized in silica using sol-gel method to remove dye at environmentally relevant concentrations.

The immobilization of *Trametes versicolor* in inorganic matrices (biocers) using sol-gel method incorporated with additive has so far not been reported. Growth condition, cell morphology and physiology, physical and chemical properties, enzyme activity and chemical stability of cell immobilization in biocer was studied. The dye removal performance by TV Biocers has also not been reported which it will thus be the subject of the present studies.

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