DECOLOURISATION AND COD REDUCTION OF METHYLENE BLUE AND DYE WASTEWATER USING *Sphingomonas paucimobilis*

CHE NORAINI BINTI CHE HASNAM

UNIVERSITI SAINS MALAYSIA

2013

DECOLOURISATION AND COD REDUCTION OF METHYLENE BLUE AND DYE WASTEWATER USING *Sphingomonas paucimobilis*

by

CHE NORAINI BINTI CHE HASNAM

Thesis submitted in fulfillment of the requirements for the degree of
Master of Science

JULY 2013

ACKNOWLEDGEMENT

The research and writing of this dissertation could not be performed without the help of many persons who I am thankful for their support and continuous assistance. First of all, I would like to extend my sincere appreciation to the following people for the contribution towards this thesis.

I would like to express my deepest gratitude for all the support, advices, and confidence to my supervisor, Prof Madya Dr Norhashimah Morad, my co-supervisors, Prof Madya Dr Norli Ismail and Prof Teng Tjoon Tow for all their support, enthusiasm and encouragement. Not to forget to postdoctoral fellow Dr Chimezie Jason Ogugbue for his valuable advice, guidance, and opinion during this research.

I also highly appreciate the lab assistants, Mr Sadali Othman, Mr Ravi, Mr Mohd Fadzli Ghazali and all the lab assistants for their technical assistance and help. Thanks to all my colleagues for the academic and social stimuli, for all their help and guidance during this period of work. I would also like to express my heartfelt thanks to Penfabric, Penang for his providing me the industrial textile effluents.

Last but not least, to my father and mother, Mr Che Hasnam Hassan and Mdm Zaliha Ismail and all my family members for their continued love, support and putting up with me throughout my research. Financial assistance over the years from the Institute of Postgraduate Studies is gratefully acknowledged. This work was funded also by Postgraduate Research Grant Scheme with grant number 1001/PTEKIND/834088. Thank you very much to all that contributed to this period of my life.

CHE NORAINI BINTI CHE HASNAM
JULY 2013

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ASBR	Anaerobic sequencing batch reactor
ATP	Adenosine-5’-triphosphate
BOD	Biochemical oxygen demand
BOD_5	Five-day biochemical oxygen demand
cBOD	Carbonaceous biochemical oxygen demand
CI	Colour index
COD	Chemical oxygen demand
CSTR	Continuous stirred tank reactor
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
EPS	Extracellular polymeric substance/exopolysaccharide
F/M	Food-microorganism ratio
FTIR	Fourier transform infrared
HMDS	Hexamethyldisilazane
HRT	Hydraulic retention time
IC	Internal circulation
MB	Methylene blue
MBR	Membrane-coupled bioreactor
MCRT	Mean cell residence time
MLD	Mega liter per day
MLVSS	Mixed liquor volatile suspended solids

MPN	Most probable number
NADH	Nicotinamide adenine dinucleotide
OD	Optical density
PGA	Polyglutamic acid agar
SEM	Scanning electron microscopy
SLR	Sludge loading rate
SRT	Sludge retention time
SS	Suspended solid
TEM	Transmission electron microscopy
UASB	Upflow anaerobic sludge blanket

LIST OF SYMBOLS

k	Specific substrate utilisation rate (day^{-1})
K_d	Endogenous decay coefficient (day^{-1})
K_I	Substrate inhibition constant
K_s	Saturation constant (mg/L)
Q	Flowrate in treatment system
q	Specific substrate utilisation rate
r_g	Bacterial growth mass
S	Initial substrate concentration (mg/L)
S_e	Substrate concentration in reactor at given time (mg BOD/L)
S_o	Concentration of growth-limiting substrate in media
s	Seconds
T	Time
V	Volume of the reactor
X	Biomass in reactor (mg/L)
X_m	Stationary population size (bacteria do not grow)
X_v	Mixed liquor volatile suspended solids (mg/L)
Y	Yield coefficient
y_{obs}	Observed yield coefficient
λ	Wavelength/lag time
µm	Micrometer

μ	Specific growth rate (day^{-1})
μ_m	Maximum growth rate (day^{-1})
Θ	Hydraulic retention time (day)
Θ'	Mean cell residence time or the sludge age (day)
Θ_c	Critical retention time (day)

LIST OF PUBLICATIONS AND SEMINARS

Symposium

1. Che Noraini, C. H., Norhashimah, M., Norli, I., and Teng, T., T. (2011). Determination of Biological Kinetic Parameters For Textile Dye Wastewater by *Sphingomonas paucimobilis*, 2nd Symposium of USM Fellowship, Nov 2011, Vistana Hotel, Penang, Malaysia.

Conference

1. Che Noraini, C. H., Norhashimah, M., Norli, I., Teng, T. T., and Ogugbue, C. J. (2011). A Study of Cellular Morphological Changes During Biodegradation of Methylene Blue Dye by *Sphingomonas paucimobilis* Using Field Emission Scanning Electron Microscopy. International Congress of the Malaysian Society for Microbiology, 8-11 December 2011, Bayview Beach Resort, Penang, Malaysia.

Journal

1. Che Noraini, C. H., Morad, N., Norli, I., Teng, T. T., and Ogugbue, C. J. (2012). Methylene Blue Degradation by *Sphingomonas paucimobilis* under Aerobic Conditions.*Water Air Soil Pollution*. 223 (8), 5131-5142.

PENYAHWARNAAN DAN PENURUNAN COD METILENA BIRU DAN AIR SISA PEWARNA MENGGUNAKAN *Sphingomonas paucimobilis*

ABSTRAK

Penyahwarnaan mikrob dan degradasi ialah salah satu proses alternatif yang mesra alam dan kos berdaya saing dalam rawatan air sisa pewarna. Dalam kajian ini, penyahwarnaan dan penyeleraian metilena biru (MB) oleh bakteria *Sphingomonas paucimobilis* yang diasingkan daripada air sisa industri telah dikaji.Keputusan eksperimen dinilai berdasarkanpengurangan permintaan oksigen kimia (COD) dan penyahwarnaan air sisa pewarna menggunakan *S. paucimobilis*.Keputusan yang diperoleh mendapati bahawa kepekatan COD berkurang iaitusebanyak 92.99% dan mencapai 85% dalam penyahwarnaanmaing-masing pada keadaan optimum pH 9.0 dan pada saiz inokulum 185-mL dalam HRT pengeraman 5 hari. Sampel yang diambil dari kelalang yang mengandungi air sisa pewarna yang telah dinyahkan menggunakan *S. paucimobilis*pada pH 9.0 dianalisis menggunakan FTIR menyatakan mekanisma penyahwarnaan warna adalah disebabkan biodegradasi. Di samping itu, analisis terhadap sel *S. paucimobilis* dalam air sisa pewarna menggunakan mikroskop elektron pengimbasan dan mikroskop elektron penghantaran menunjukkan penghasilan rembesen EPS oleh sel tersebut dimana EPS tersebut bertindak sebagai mekanisma pertahanan fisiologi untuk memastikan penyerapan terkawal molekul pewarna ke dalam struktur sel. Eksperimen telah dilakukan di bawah keadaan mantap untuk menentukan pekali kinetik seperti hasil pertumbuhan (Y), pereputan biojisim tertentu (K_d), penggunaan substrat spesifik maksimum (K), ketepuan berterusan bagi substrat (K_s), kadar maksimum pertumbuhan spesifik (μ_{max}), dan masa pengekalan kritikal (θ_c). Penilaian koeffisiendinilai dengan menggunakan persamaan Monod.Pekali bio-kinetik Y, K_d, k, Ks, dan μ_{max} yang telah didapati masing-masing ialah 1.879, 0.013 per hari, 0.540 per hari, 4214.7 mg/L dan 1.015 per hari. Hasil keputusan nilai pekali bio-kinetik yg diperoleh dapat membantu dalam menyediakan alat yang berkesan bagi meningkatkan dan mengoptimumkan prestasi sistem loji rawatan yang berurusan dengan air sisa pewarna walaupun pada kepekatan pewarna yang tinggi menggunakan*S. paucimobilis*.

DECOLOURISATION AND COD REDUCTION OF METHYLENE BLUE AND DYE WASTEWATER USING *Sphingomonas paucimobilis*

ABSTRACT

Microbial decolourisation and degradation is an environmentally friendly and cost competitive alternative process in the treatment of dye wastewater. In this study, the decolourisation and degradation of Methylene blue (MB) by a *Sphingomonas paucimobilis* strain isolated from industrial wastewater was investigated. Experimental results were assessed in terms of chemical oxygen demand (COD) reduction and decolourisation of dye wastewater using *S. paucimobilis.* It was found to be capable in reducing the concentration of COD at 92.99 % and achieving 85 % in decolourisation at optimal conditions of pH 9.0 and at inoculums size of 185-mL within incubation HRT of 5 days. Analysis of samples extracted from decolourised culture flasks at pH 9.0 using Fourier transform infrared (FTIR) spectroscopy confirmed that the mechanism of colour removal was due to biodegradation. In addition, scanning and transmission electron microscopy revealed the secretion of exopolysaccharides (EPS) by *S. paucimobilis* cells on exposure to dye that showed a probable physiological defence mechanism to ensure controlled diffusion of dye molecules into cellular structures. The experiment were performed under steady state conditions to determine the kinetic coefficients such as growth yield (Y), specific biomass decay (K_d), maximum specific substrate utilisation (K), saturation constant for substrate (K_s), maximum specific biomass growth rate (μ_{max}), and critical retention time (θ_c). The kinetic coefficients were evaluated using Monod equation. The bio-kinetic coefficients Y, Kd, k, Ks, and μ_{max}were found to be 1.879, 0.013 per day, 0.540 per day, 4214.7 mg/L and 1.015 per day, respectively. These results were found to serve as a useful tool that can be used for improving and optimising the

system performance of treatment plants dealing with dye wastewater by using *S. paucimobilis* even at high concentration of dye.

CHAPTER ONE
INTRODUCTION

1.1 Introduction

Dyes are an important class of water pollutants which usually have a synthetic origin and complex aromatic water-soluble molecular structures having potential application in various industries such as textile, paint, cosmetic, carpet, leather, paper and plastic among others that will make them more difficult to biodegrade (Aksu and Tezer, 2005).The recalcitrant nature of modern synthetic dyes has led to the imposition of strict environmental regulations. From an environmental point of view, the removal of synthetic dyes is of great concern, since some dyes and their degradation products may be carcinogenic, mutagenic and toxic and, consequently, their treatment cannot depend on biodegradation alone (Myslak and Bolt, 1998).Dyes display a wide variety and thus do possess very different chemical and physical properties. Out of many contaminants presents in wastewater, such as acids, bases, toxic organic and inorganic dissolved solids, and colours, colour is considered the most undesirable and is mainly caused by dyes and are generally considered to be highly toxic to the aquatic biota (Gupta et al., 2005). Colour removal, especially from textile wastewaters, has been a great challenge over the last decades, and recently there is no single and economically attractive treatment that can effectively decolourise dyes, and new technologies for wastewater decolourisation are especially needed (Naima et al., 2007).Removal of colour from dye bearing wastewater is a complex problem becauseeven less than 1 mg/L of the dye produces obvious colouration and it is difficult to effectively treat using conventional methods (Kumar et al., 2006). The decolourisation of dyes is an essential aspect of wastewater treatment before discharge. Dyes may significantly affect photosynthetic activity in aquatic life and reduce light penetration as a result of the colouration of the water in streams (Ozturk and Abdullah, 2006). Hence, decolourisation of dye effluent via the removal of dyes has become an important aspect of textile wastewater treatment (Kannan and Sundaram, 2001).

A wide range of methods has been developed for the removal of synthetic dyes from waters and wastewaters to decrease their impact on the environment. The technologies or methods involve adsorptionon inorganic or organic matrices/various sorbents (Chen and Zhao, 2009), decomposition by oxidation, photodegradation, decolourisation by photocatalysis, and/or by oxidation processes, microbiological or enzymatic decomposition (Forgacs et al., 2004).Naturally, the removal of dyes is currently based on a wide variety of physicochemical procedures, which include flocculation combined with flotation, membrane filtration, electrokinetic coagulation, ozonation, oxidation, precipitation, ion exchange and adsorption (Tan et al., 2009). Considering technology limitation, economic restriction and generation of secondary pollution using physical and chemical treatment, biological treatment processes are frequently used to treat dye wastewater. The ability of microorganisms to carry out dye decolourisation has received much attention (Banat et al., 1996; Pearce et al., 2003). Microbial decolourisation and degradation of dyes is seen as a cost effective method for removing these pollutants from the environment. Thus, biological systems have to be designed that work under such conditions and still effectively not only decolourise but preferably completely degrade dyestuff. In addition, the use of whole bacterial cells was used for the reduction of water-soluble dyes present in textile dyeing wastewater.

Microbial biotransformation reactions are the key techniques for the biological removal of dyes. A number of microorganisms have been found to be able to decolourise and to degrade a variety of textile dyes including bacteria, fungi, and yeasts (Banat et al., 1996; Martins et al., 1999). Besides pure cultures, mixed microbial populations have also been employed for dye decolourisation. Hence, interest is now focused on the bacteria, which can perform high rate decolourisation (Asgher et al., 2008). Microorganisms for dye decolourisation may be obtained simply by isolation of existing dye degrading cultures from environmental samples (e.g. textile effluents), by adaptation of promising strains to conditions present in textile effluents or by construction of suitable organisms employing genetic methods (Daneshvar et al., 2007).

The purpose of determining the biokinetic coefficients is to obtain information on the rate of cell growth and consumption of substrate, which enables process engineers to determine the volume of the reactor and understand the process control through system simulation (Al-Malack, 2006). Biokinetic modelling applied to wastewater treatment aims at predicting the performance of the microbiological system and its response to modification of operation parameters and consequently at optimising the biodegradation processes and treatment efficiency.

The Monod equation has been widely applied to describe microbial growth, but it has no mechanistic basis and is purely empirical. In the research history of microbial growth, the Monod equation is one of the most used models (Bagley and Brodkorb, 1999). Monod kinetics had been used as a basic tool analysing system performance of biological wastewater treatment. This model is expressed in the simplest form showing a functional relationship between the specific growth rate and substrate utilisation rate (Reardon et al., 2002).

Experimental data obtained in a laboratory investigation were used to determine the relevant kinetic coefficients involving in the resulting model. The values of kinetic coefficients are to be estimated by means of regression analysis of experimental data generated from a lab-scale and/ or pilot-scale experimental setups operated at various hydraulic hydraulic retention times (HRTs) (Metcalf and Eddy, 2003). The batch kinetics tests were independently conducted to determine biokinetic parameters used as input in the model.Moreover, it helps to understand the metabolic performance of the microorganisms when fed with the substrate and other components in synthetic dye effluent in a batch reactor system. Although there are many parameters characterising the microorganisms and substrates, the half saturation constant (K_s) and maximum specific growth rate (μ_{max}) are most important parameters for understanding biodegradability, affinity and compatibility of substrates to microorganisms.

This study aims to investigate the potential of *Sphingomonaspaucimobilis* for the treatment of dye wastewater. *S. paucimobilis* was chosen because of the ability in removal colours of dye (Sanayei et al., 2009; Ayed et al., 2009). In addition, correlation of kinetic properties with dye concentration and other rate-dependent environmental parameters (pH and inoculums size) were characterised using FTIR, SEM and TEM. Hence, the aim of this study was to provide quantitative information on the biodegradability of the dye wastewater.

1.2 Problem statement

The generation of large amount of coloured effluent by textile industries constitutes one of the largest contributions to water pollution globally. Many dyes and their breakdown products are toxic and have been shown to be carcinogens and mutagens. However, the treatment approaches are often expensive, time consuming and cause secondary pollution problems. Therefore, an effective approach in wastewater treatment technology using microbes should be developed in the removal of colour in wastewater.

Although there have been many studies on the effectiveness of dye wastewater treatment using bacteria, the relationship of factors i.e, hydraulic retention time (HRT), pH and dosage of bacteria have not been widely researched (Rajamohan and Karthikeyan, 2006). Therefore, in this research these factors are considered and optimised.Another factor that is not widely reported is the mechanism of removal. Although reports on percentage of COD reduction and colour removal are extensive, publications on possible mechanisms of dye removal are almost nonexistent (Balu and Radha, 2009; Bell et al., 2001).

For better understanding of microbial kinetics in the treatment of dye wastewater, it is necessary to evaluate the kinetic coefficients for the batch reactor system. The literature information about the kinetics of decolourisation and the environmental factors affecting the decolourisation rates on dye wastewater is relatively scarceespecially using Monod equation(Amar et al., 2008).Thus, the results of the study could provide a scientific and engineering basis to design a new reactor systemin order to gain an understanding of the tools for predicting design parameters in treating dye wastewater generated from industrial manufacturing.

1.3 Objectives of the study

Theobjectives of this research are as follow:

a) To determine the optimum hydraulic retention times (HRTs), pH and dosage of bacteria for decolourisation and COD reduction of dye wastewater using *Sphingomonaspaucimobilis*.

b) To determine the mechanisms of microbial decolourisation in dye wastewater.

c) To determine the kinetic coefficients of biodegradation on dye wastewater using Monod equation model.

1.4 Significance of the study

The treatment of dye wastewater using microorganisms for the removal of synthetic dyes offers considerable advantages which is relatively inexpensive, and because of the running costs are low and the end products of complete mineralisation are not toxic (Chen et al., 1999).The approach however, suffers from a number of difficulties in data interpretation and rigidity of optimisation processes. The use of pH and dosage of bacteria provides a basis on colour removal. With the emergence of kinetic concepts, the process of designing biological wastewater treatment for dye effluent to achieve optimal treatment results can be made by optimising the kinetic parameter such as HRT.

Secondly, this study also demonstrates the application of biological treatment in treating a typical high strength industrial dye wastewater. Finally, information on the biokinetic parameters is important for better understanding of the performance of dye treatment by biological method. This study is significant to gain better understanding of how to use kinetic approach to operate and design the biological system process applied in the treatment of industrial dye wastewater. Another significant contribution of this study is that the applicability of the treatment process can be tested using the kinetic data obtained in this research.

1.5 Limitations of the study

The study only focused on lab-scale experimentsthat emphasise on the kinetic parameters analysis of bench scale studies.The results of the study are applicable only to the condition prevailing in this study. Applicability to the actual field conditions needs further investigation, and require up scaling of model to pilot-scale.The temperature in this study is assumed to be constant, i.e. at room temperature. Daily fluctuations of temperature are considered negligible.

1.6 Outline/ Organisation of the thesis

This thesis consists of five chapters. The first chapter (Introduction)presents some general overview on dye wastewater, its treatments and introduction of bio-kinetic study. In this chapter, problem statements, significance of this research work, and the objectives are presented.

Chapter Two (Literature review) describes the literature studies or a review of available literature on this topicincludinghistory of dye pollution, classification of dyes based on types and characteristics and model kinetics.

Chapter Three (Materials and method) describes in detail the materials and methods that are used in the research work. The methodology is explained in detail for better understanding. In addition, the statistical analysis such as analysis of variance (ANOVA) and factorial design are also described.

Chapter Four (Results and discussion) depicts the research findings. The results are explained, discussed and supported with previous research work done by other researchers.

Chapter five (Conclusions and recommendations) give conclusion and recommendations from the current study. The conclusions are based on the results obtained towards the objective of this study. This is followed by the recommendations, suggestions and improvement for the future studies in this related field.

CHAPTER TWO
LITERATURE REVIEW

2.1 History of dye pollution

Dye wastewater arises as a direct result of theproduction of the dye and also as a consequence of itsuse in many industrial activities such as in the food, cosmetics, craft mills, tannery, textile, leather, pulp, paper mill, plastics, wool, jute, leather, ceramics and other industries (e.g., cotton and dyeing silk) (Kuhad et al., 2004).Many natural dyes have been known for a long time. Dyes are ionic and aromatic organic compounds with delocalised electron system (Pointing, 2001). The presence of ionising groups alters absorption and provides bonding affinity. There are morethan 100 000 commercially available dyes with over 7×10^5 tons of dyes produced annually worldwide (Robinson, 2001). In India, an average textile mill producing 60×10^4 m of fabric per day is likely to discharge approximately 1.5 MLD of effluent (COINDS, 1999). Effluent discharge from textile and dye stuff industries to neighbouring water bodies and wastewater treatment systems is currently causing significant health. The concentration of dye in the effluent is determined by the dye exhaustion properties, i.e. the proportion of dye that is fibre substantive and ranges from 95-98 % for acid, basic and disperse dyes, though 60 to 80 % for reactive dyes and 40-60 % for the balance of dyes (Carliell, 1993; Buckley, 1992). Dye effluent is tough to be degraded because of the presence of dyes, which have complex aromatic molecular structure and synthetic origin and because of the colour in dye effluent is highly visible that will discourage the downstream use of wastewater and affects aesthetics, water transparency and gas solubility in water even in small amount, and especially because many dyes are carcinogenic and mutagenic like benzidine, dye effluent treatment becomes inevitable. Dyes degradation led to aromatic amines, which are known to be toxic (Chen et al., 2003). However, even if dyes are not toxic, the toxicity of their by-products has to be considered. Coloured industrial effluent is the most obvious indicator of water pollution (Wong and Yu, 1999). Discharge of this coloured effluents into rivers and lakes results into reduced dissolved oxygen concentration, thus creating anoxic conditions that are lethal to resident organisms. They can be removed from wastewater because dyes and pigments are visible pollutant even at low concentrations (Celekli et al., 2010). Many dyes or their metabolites have effects on aquatic life and humans (Gong et al., 2007).

Dyes of different structures are often used in the textile processing industry, and, therefore, the effluents from the industry are markedly variable in composition (Bulc and Ojstrsek, 2008). The wastewater from an industry may be organic or inorganic in nature or a combination of both. In most cases, it contains toxic ingredients (Srinivasan and Viraraghavan, 2010). The direct effect of wastewater pollution is to deplete, through the excessive organic load, the dissolved oxygen (DO) content of receiving waters to the point that the stream becomes incapable of exercising the self-purification processes. The deoxygenation may be high enough to destroy practically all fish and other aquatic life. Plants and algae kinds of photosynthetic organisms produce oxygen when there is a sufficient light source. During times of insufficient light, these same organisms consume oxygen, resulting in the depletion of DO levels. Biochemical oxygen demand (BOD) is the consumption of DO caused by microorganisms as they decompose organic material by chemical oxidation. BOD related to microorganism is called carbonaceous biochemical oxygen demand (cBOD) and the source material for cBOD is organic matter. cBOD results when oxygen is consumed by microorganisms in converting material into new cells (Lee and Jones-Lee, 2003). Hence, the presence of dyes in wastewaters is one of the major environmental problems. Actually, they can be removed from wastewater because dyes and pigments are visible pollutant even at low concentrations. The treatment of textile wastewater is of great concern because of their toxicity as well as aesthetic problems being created.

2.1.1 Classification of dye

Dyes are a group of complex organic materials which enter the environment due to various processes. Dyes can be classified according to either chemical structure (chemical classification) or by usage (colouristic classification) and on their chemical constitution or on the basis of application to fibers.Certain groups of dyes with their specific chemical character and the methods of dyeing are dependent on each other. There are 9 classes of dye that acting as substrate to bacteria that is applied in this research that is acid dye, azo dye, direct dye, basic dye, disperse dye, mordant dye, reactive dye, sulphur dye, vat dye (Al-Momani et al., 2002; Xu et al., 2004).

2.1.2 Type of dyes

There are many structural varieties of dyes that fall into either the cationic, non-ionic or anionic type. Anionic dyes are the direct, acid and reactive dyes. Brightly coloured, water-soluble reactive and acid dyes are the most problematic, as they tend to pass through conventional treatment systems unaffected (Willmott et al., 1998). Non-ionic dyes refer to disperse dyes because they do not ionise in an aqueous medium. Concern rises, as many dyes are made from carcinogens such as benzidine and other aromatic compounds (Bae and Freeman, 2007; Christie, 2007). Anthraquinone-based dyes are most resistant to degradation due to their fused aromatic ring structure. Table 2.1 shows classification of dyes.

Table 2.1Dye classification (Teng and Low, 2012)

Type of Dye	**Characteristics**	**Substrates/ Type of fibres**	**Method of Application**
Acid	Most acid dyes are azo (yellow to red, or a broader range colours in case of metal complex azo dyes), anthraquinone or triarylmethane (blue and green) compounds. When in solution are negatively charged (anionic compounds); bind to the cationic NH_3^+- groups present in fibres	Nylon, wool, polyamide, silk, modified acryl, paper, inks, and leather	Usually from neutral to acidic dye baths. The adjective “acid” refers to the ph in acid dyebaths rather to the presence of acid groups (sulphonate, carboxyl) in the molecular structure of these dyes
Azoic and Ingrain	Insoluble products of a reaction between a coupling component and a diazotised aromatic amine that occurs in the fibre. Characterized by the presence of one or more azo groups (-N N-)	Cotton, viscose, rayon, cellulose acetate, and polyester	Fibre impregnated with coupling component and treated with stabilized diazonium salt
Basic	Cationic compounds that bind to the acid groups of the fibre, usually syhthetic fibres like modified polyacryl	Synthetic fibres, paper, polyacrylonitrilemodified nylon, polyester, and inks	Applied from acidic dye baths
Direct	Large molecules bound by Van der Waals forces to the	Cellulose fibres, cotton, rayon, paper, leather, nylon, and viscose	Applied from neutral or slight alkaline baths containing additional

	fibre. Mostly azo dyes with more than one azo bond or phthalocyanine, stilbene or oxazine compounds		electrolyte
Disperse	Scarcely soluble dyes that penetrate the fibre through fibres (cellulose acetate, polyester, polyamide, acryl, etc.) swelling	Polyester, polyamide, acetate, acrylic, and plastics	Fine aqueous dispersions applied by high temperature, pressure. or lower temperature carrier methods
Fluorescence brighteners	Mask the yellowish tint of natural fibres	Soaps and detergents, all fibres, oils, paints, and plastics	From solution, dispersion, or suspension in a mass
Food, drug and cosmetic	Non- toxic and not used as textile dyes. Azo, anthraquinone, carotenoid, and triarylmethane	Foods, drugs, and cosmetics	
Metal complex	Strong complexes of one metal ion (usually chromium, copper, cobalt or nickel) and one or two dye molecules (acid or reactive dyes)	Silk, wool, and polyamide	
Mordant	Require the addition of a chemical that combines with the dye and the fibre, like tannic acid, alum, chrome alum, and other salts of aluminium, copper, chromium, iron, potassium, and tin	Wool, leather, silk, paper, modified cellulose fibres and anodised aluminium	Applied in conjunction with chelating Cr salts
Natural	Obtained mainly from plants	Food, cotton, wool, silk, polyester, polyamide, and polyacrylonitrile	Applied as mordant, vat, solvent, or direct and acid dyes
Pigment	Insoluble, non-ionic compounds or insoluble salts that	Paints, inks, plastics, and textiles	Printing on the fibre with resin binder or dispersion in the mass

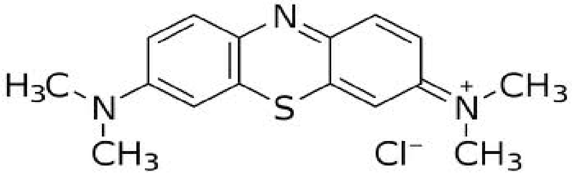
	retain their crystalline or particulate structure throughout their application		
Reactive	A heterocyclic aromatic ring substituted with chloride or fluoride, e.g. dichlorotriazine. Form covalent bonds with OH-, NH-, or SH- groups. High wet fastness profiles, ease of application, brilliant colours, variety of their colour shades, and minimal energy consumption. The most common of reactive dyes are azo dyes	Cotton, wool, silk, and nylon	Reactive site on dye reacts with functional group on fibre to bind dye covalently under influence of heat and pH (alkaline)
Solvent	Non ionic dyes that dissolve the substrate to which they bind	Plastics, gasoline, varnish, lacquer, stains, inks, oils, waxes, and fats	Dissolution in the substrate
Sulphur	Complex polymeric aromatics with heterocyclic S-containing rings	Cellulose fibres, cotton, rayon, and viscose	Aromatic substrate vetted with sodium sulphide and re-oxidized to insoluble sulphur-containing products on fibre
Vat	Insoluble coloured dyes which on reduction give soluble colourless forms (leuco form) with affinity for the fibre; on exposure to air are reoxidised	Cellulose fibres, cotton, rayon, viscose, and wool	Water insoluble dyes solubilised by reducing with sodium hydrosulfite, then exhausted on fibre and re- oxidised
Oxidation bases	Aniline black and indeterminate structures	Hair, fur, and cotton	Aromatic amines and phenols oxidised on the substrate

2.1.2.1 Basic dye

Basic dyes fallwithin the class of polymethine dyes and are known ascationic polymethines because of ionisation in solutionand the presence of amino groups or alkylamino groupsas the dye auxochromes. They possess an overall positivecharge and are therefore widely used to bind tonegatively charged substrates. It has high brilliance and intensity of colours and is highly visible even in a low concentration. Most basic dyes are diarylmethane, triarylmethane, anthraquinone or azo compounds. Basic dyes represent approximately 5 % of all dyes listed in the Colour Index (Van der Zee, 2002).

Methylene blue (MB) also called Basic Blue 9 is one of example of a basic, cationic and dark green dye that forms a deep blue solution in water. The chemical formula of MB is $C_{16}H_{18}N_3SCl.3H_2O$ (3,7-bis(dimethylamine)-phanazathionium chloride; tetramethylthionine chloride) and the molecular weight is 319.89 g/mol with the aromatic moiety planner having the dimethyl amino groups attached to the aromatic unit (Ong et al., 2005). It is usually used as an antidote in cyanide poisoning, as a bacteriological stain, as a mild antiseptic agent in clinical therapy, as a colourant and a chemical indicator. MB is the most commonly used substance for dying cotton, wood and silk (Muthuraman et al., 2009). Methylene blue is not regarded as acutely toxic, but it can have various effects. On inhalation, it can give rise to short periods of rapid or difficult breathing, while ingestion through the mouth produces a burning sensation and may cause nausea, vomiting, diarrhea, and gastritis. A large amount creates abdominal and chest pain, severe headache, profuse sweating, mental confusion, painful maturation, and methemoglobinemia-like syndromes (Bhattacharyya et al., 2005). Therefore, the treatment of effluent containing such dye is of interest due to its harmful impacts on receiving waters. Characteristics of MB dye are shown in Table 2.2.

Table 2.2 Characteristics of methylene blue dye (MB) (Ehrampoush et al., 2010)

Chemical structure	
Type of dye	Cationic
Symbol/abbreviation	MB
Colour index	52015
Molecular formula	$C_{16}H_{18}N_3SCl$
Molecular weight (g/mol)	319.85
Maximum absorption wavelength (nm)	640
Chromopore	Thiazine group

2.2 Treatment of dye

Presence of colour in dye bearing effluents is one of the complex environmental problems. A wide range of methods has been developed for the removal of synthetic dyes from waters and wastewater to decrease their impact on the environment. Several factors that determine the technical and economic feasibility of each single dye removal technique include dye type and concentration, wastewater composition, operation cost (energy and material), environmental fate and handling costs of generated waste products. Many treatment processes have been applied for the removal of dyes from wastewater such as: photocatalytic degradation (Sohrabi and Ghavami, 2008; Sleiman et al., 2007), sonochemical degradation (Abbasi and Asl, 2008), micellar enhanced ultrafiltration (Zaghbani et al., 2008), cation exchange membranes (Wu et al., 2008), electrochemical degradation (Fan et al., 2008), adsorption/precipitation (Zhu et al., 2007), integrated chemical-biological degradation (Sudarjanto et al., 2006), integrated ion(III) photoassisted-biological treatment (Sarria et al., 2003), solar photo-Fenton and biological processes (Garcia-Montano et al., 2008), Fenton-biological treatment scheme (Lodha and Chaudhari, 2007), and adsorption on activated carbon (Hameed and Daud, 2008; Wu and Tseng, 2008). In general, each technique has its limitations. Removal of dye in wastewater has been made by physical, physico-chemical, biological and/or chemical processes. Biological and chemical methods involve the destruction of the dye molecule, whilst physical methods usually transfer the pollutant to another phase. In contrast, bacterial decolourisation is normally faster.

The removal of colour from wastewater is relatively more important than the removal of soluble colourless organic substances, which usually contribute the major fraction of biochemical oxygen demand. Currently various chemical, physical and biological treatment methods are used to remove colours. Lately, removal of dyes from effluents is by physico-chemical means. Because of some disadvantages such as excess dosage of chemicals, difficulty, high energy, chemical cost and disposal problems (production of sludge or potential toxic byproducts and ineffective removal of crominance, most of the chemical and physical methods for treating dye wastewater are not widely applied in the textile industries. There is a need to find alternative treatments that are effective in removing dyes from large volumes of effluents and low in cost, such as biological or combination systems. The promising and ecofriendly microbial decolourisation and detoxicification is a cost competitive alternative compared to the physical and chemical methods. Figure 2.1 shows the flow chart of wastewater treatment plant of Penfabric Mill 3 in Penang.

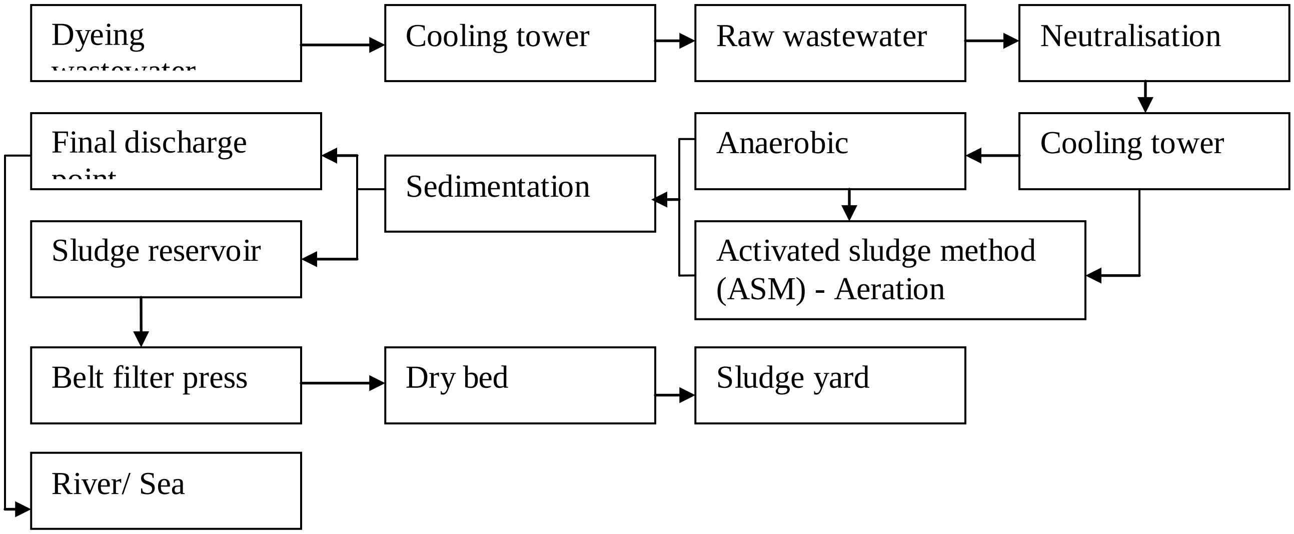


Figure 2.1 Flow chart of wastewater treatment plant of Penfabric Mill 3, Penang

2.2.1 Physical treatment

The coagulation process effectively decolourises insoluble dyes, such as disperse dyes, but does not work well for soluble dyes; it is also of doubtful value owing to the cost of treating the sludge and the increasing number of restrictions concerning their disposal.

Membrane separation technologies have been demonstrated to be a practical and competitive alternative for the removal of a variety of dyes from aqueous solutions because of their comparative ease of construction and process control, and feasible recovery of valuable dyes. Activated carbon is the most commonly used method for the treatment of dye bearing wastewaters. It is a well known material used in both gas and liquid phases, including medicinal use, gas storage, pollutant and odor removal, gas separation, and catalysis. Activated carbon, zeolite and diatomite have been used as efficient methods for adsorption. However, this method can be uneconomic due to high cost of activated carbon due to the use of non-renewable and relatively expensive starting materials such as coal (Martin et al., 2003).

2.2.2 Chemical treatment

Chemical destruction by oxidation with chlorine or ozone is effective, but the oxidants requirements are high and also expensive. Photochemical degradation in aqueous solution is likely to progress slowly because synthetic dyes are, in principle, designed to posses high stability to light. Another chemical method like surface absorption, chemical oxidation and advanced oxidation process are used. But, some of the disadvantages are high expenses related to consumed reactors and required energy (Tang, 2004).

Fenton's reagent, ozone, photochemical, sodium hypochlorite (NaOCl), electrolysis, ultrasound are other methods in physical treatment of dye. They may have potential for decolourisation. However, such technologies usually involve complicated procedures or are economically unfeasible (Pearce et al., 2003). Current treatment technologies for colour removal involving physical and chemical processes are presented in Table 2.3.

Table 2.3 Current treatment technologies for colour removal involving physical and/or chemical processes (Robinson et al., 2001)

Physical and/or chemical methods	**Advantages**	**Disadvantages**
Oxidation	Rapid process, applied in gaseous state: no alteration of volume	High energy costs, short half-life (20 min) and formation of by-products.
Adsorption	Good removal of a wide range of dyes	Absorbent requires regeneration or disposal, very expensive.
Membrane technologies	Removes all dye types	Concentrated sludge production.
Coagulation/flocculation	Economically feasible	High sludge production.

2.2.3 Biological treatment

The search for alternative and innovative wastewater treatment techniques has focussed on the use of biological materials. Biological processes have been used to treat a variety of municipal, industrial, and animal wastes. To date, the use of technologies based on microorganisms and/or microbial aggregates has provided a wide range of useful and promising wide range of useful and promising strategies to clean up many types of pollutants, such as cadmium, copper, lead and microcystins (Choi et al., 2009; Wu et al., 2010). The basic aim of biological treatment is to make the substrate present in the wastewater be utilised as a food source to the mixed microbial culture. Besides, the use of biomaterials for the treatments of wastewaters has many advantages over conventional treatments that are economic, nontoxic to environment and widespread compared to other treatment options thus lead to complete mineralisation of organic pollutants at low cost (Morias and Zamora, 2005). Microorganisms play a significant role in decomposition, ultimate mineralisation of biopolymers and xenobiotics like dyes and in carrying out this treatment (Varsha et al., 2011). Biological processes have potential to convert or degrade the pollutant into water, carbon dioxide and various salts of inorganic nature. The isolation of potent species and there by degradation is one of the interest in biological aspect of effluents treatment (Mohan et al., 2002) remove organics and colour of textile wastewater. Many microorganisms belonging to different taxonomic groups of bacteria; gram positive and gram negative (Chen et al., 2003; Moosvi et al., 2005), fungi (Zhang et al., 1999), actinomycetes (Zhou and Zimmermann, 1993; Khehra et al., 2005), yeast, algae (Dilek et al., 1999), dead bacteria, and plants (phytoremediation) have all been used for the purpose of decolourising dye-containing effluents (Aubert and Scgwitzguebel, 2004; Ghodake et al., 2009a; Kagalkar et al., 2009). Moreover, these are even capable of completely mineralising various types of dyes under certain environmental conditions.

The common biological treatment processes on dye decolourisation are classified as aerobic, anaerobic, and biological nutrient removal processes as well as sequential aerobic and anaerobic conditions. For biological treatment of the wastewater containing dyes, the microbial decolourisation and degradation of dyes has been of considerable interest. Many researchers have demonstrated partial or complete biodegradation of dyes by pure and mixed culture of bacteria, fungi, and algae either in their living or inactivated biomass to remove dyes from wastewaters. However, success of each process varied greatly depending upon both the structure of azo dye and the type of microbial culture being employed (Soares and Duran, 1998; Stolz, 2001; Chen et al., 2003; Asgher et al., 2006; Kalme et al., 2007; Revenker and Lele, 2007 and Mane et al., 2008). In recent years, much research is focused in the area of biodegradation of dyes because of the environmental problems.

2.2.4 Broad spectrum of technologies for dye wastewater treatment

Generally, the methodologies adopted for treatment of dye wastewater can be classified into three categories: physical, chemical and biological. These methods can be used either singularly or together in various combinations. Many treatment processes combine two or more treatment technologies to provide a better or more efficient treatment.Table 2.4 shows a tertiary treatment according to its category.

2.2.4.1 Physico-chemical processes

Physico-chemical processes of dye wastewater treatments include coagulation, adsorption, flocculation, electrochemical treatment (electrochemical destruction), in pair extraction, oxidation by chlorine, H_2O_2, ozone electrolysis (Ayed et al., 2009), chemical precipitation, sand filtration, membrane separation (hybrid coagulation membrane, hybrid adsorption membrane), ozonation, photochemical, electrokinetic coagulation (Robinson et al., 2001).

Table 2.4 Tertiary treatments (Teng & Low, 2012)

Category of treatment	**Type**
Physical	Adsorption, ion exchange, electronic coagulation, irradiation in oxidizing medium, filtration, reverse osmosis.
Chemical	Fenton’s reagent, sodium hypochlorite, photochemical oxidation, ozonation, cucurbituril, electrochemical destruction, electrolysis.
Biological	Adsorption on living/dead biomass, anaerobic treatments with single or mixed cultures of bacteria, biodegradation by white-rot fungi, enzymes.

2.2.4.2 Biological processes

Biological processes of dye wastewater treatments include activated sludge/fluidized biofilm/fixed film systems, simultaneous/sequential anaerobic-aerobic, fed-batch, sequencing batch or continuous feeding strategies, innovative approaches like fungi or algae. Table 2.5 shows previous work assessed the removal of MB dye using biotic and abiotic agents (Wafaa, 2006) by various type of dye removing agents.

Table 2.5 Type of treatment on MB dye based on its category of treatment

Category of treatment	**Type of treatment**	**References**
Physical	Adsorption technique using biosorbent *Ulva lactuca* and *Sargassum*	Turner et al., 2007; El-Sikaily et al., 2007; Tahir et al., 2008; Sari and Tauzin, 2008.
	Adsorption (agricultural wastes, industrial solid wastes, biomass, clays minerals, and zeolites).	Mohd. Rafatullah et al., 2010.
	Adsorption by using biosolid (waste sludge)	Sarioglu and Atay, 2006.
	Adsorption using tartaric acid-modified bagasse (TAMB)	Low et al., 2011.
	Hybrid adsorbent was prepared by pyrolysing a mixture ofcarbon and flyash	Ravikumar et al., 2005.
	Adsorption on commercial activated carbon (CAC) and activated carbon from bamboo dust, coconut shell, groundnut shell, rice husk, and straw	Kannan and Sundaram, 2001.
Chemical	Ultrasound (sono-sorption)	Entezari and Sharif Al-Hoseini, 2007.
	Hydrogen peroxide catalyzed by some supported alumina surfaces	Salem and El-Maazawi, 2000.
	Montmorillonite/$CoFe_2O_4$ composite with magnetic separation performance	Ai et al., 2011a.
	Solvothermal-synthesized graphene/magnetite composite	Ai et al., 2011b.

Biological	Use of loofa sponge as immobilizing matrix to entrap *Trichoderma viride* for the removal of MB from aqueous solution.	Saeed et al., 2009.
	Biosorption of MB by dead fungal biomass, *Aspergillus fumigates*	Abdallah and Taha, 2012.
	Biodegradation of MB by microbes in up-flow anaerobic sludge blanket (UASB)	Ong et al., 2005.

2.3 Bacteria and their roles

Bacteria can be classified in many ways. Bacteria is one of the microorganisms which are always used in biotechnological important beside the other microorganisms such as fungi, alga and actinomycetes due to their ability to produce ability extracellular polymer outside the cell wall surface. Bacteria that use organic compounds as their electron donor and their source of carbon for cell synthesis are called heterotrophic bacteria, or simply called heterotrophs. Since the removal and stabilisation of organic matter are the most important uses of biochemical operations, it follows that heterotrophic bacteria predominate in the systems.

Not all bacteria are beneficial can grow in biochemical operations: some are a nuisance. Bacteria can also be classified according to their function in biochemical operations. Many act as primary degraders and attack the organic compounds present in the wastewater, beginning their degradation. If an organic compound is one normally found in nature (biogenic), the primary degraders usually will completely metabolise it in an aerobic environment, converting it to carbon dioxide, water, and new biomass. Such ultimate destruction is called mineralisation and is the goal of most wastewater treatment systems.