SIMULTANEOUS REMOVAL OF COLOUR, ORGANIC COMPOUNDS AND NUTRIENTS FROM SYNTHETIC WASTEWATER CONTAINING AZO DYE USING AEROBIC - ANAEROBIC BAFFLED CONSTRUCTED WETLAND REACTOR

by

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LIST OF ABBREVIATIONS

ABCW	Aerobic - Anaerobic Baffled Constructed Wetland
AR27	Acid Red 27
AO7	Acid Orange 7
B	Bottom region
C	Compartment
CAC	Codex Alimentarius Commission
$CaCl_2 \cdot 2H_2O$	Calcium Chloride Dihydrate
CH_3COONa	Sodium Acetate
C_6H_5COONa	Sodium Benzoate
CH_2O	Formaldehyde
COD	Chemical Oxygen Demand
CW	Constructed Wetland
CO_2	Carbon dioxide
DCIP	Dichloroindophenol
DNA	Deoxyribonucleic Acid
DNRA	Dissimilatory Nitrate Reduction to Ammonia
DO	Dissolved oxygen
DOE	Department of Environment
FTIR	Fourier Transform Infrared Spectrophotometer
FWS	Free Water Surface Flow
GCMS	Gas Chromatography Mass Spectrometry
HF	Horizontal Flow
HFCW	Horizontal Subsurface Constructed Wetland
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic Retention Time

H_2O	Water
$K_2HPO_4 \cdot 3H_2O$	Dipotassium Hydrogen Phosphate Trihydrate
MnP	Manganese Peroxidase
$MgCl_2 \cdot 6H_2O$	Magnesium Chloride hexahydrate
M	Middle region
N_2	Nitrogen
NaCl	Sodium Chloride
NADH	Nicotinamide Adenine Dinucleotide
NBR	Nitrile Butyl Rubber
NH_3	Ammonia
NH_4^+	Ammonium
NH_4NO_3	Ammonium Nitrate
NIST	National Institute of Standards and Technology
NO_3^-	Nitrate
NO_2^-	Nitrogen Dioxide
NR	Natural Rubber
O_2	Oxygen
OH^-	Hydroxide
ORP	Oxidation Reduction Potential
PU	Polyurethane
PVC	Polyvinyl Chloride
SF	Surface Flow
SSF	Subsurface Flow
T	Top region
T-N	Total Nitrogen
TOC	Total Organic Carbon

T-P	Total Phosphorous
TSS	Total Suspended Solid
UFCW	Up-flow Constructed Wetland
UV	Ultraviolet
UV-Vis	Ultraviolet Visible Spectroscopy
VF	Vertical Flow
VFCW	Vertical Flow Constructed Wetland
1-AN-4-S	1-aminenaphthylene-4-sulfonic acid
1-AN-2-H-3, 6-DS	1-aminenaphthylene-2-hydroxy-3, 6-disulfonic acid

LIST OF SYMBOLS

%	Percentage
ml	Milliliter
L	Liter
Mg/L	Milligrams per liter
cm	Centimeter
d	Day
˚C	Degree Celsius
cm^2	Centimeter Square
cm^{-1}	Wavelength Per Unit Distance (Centimeter Inverse)
g	Gram
m/z	Mass to charge ratio
$g/O_2/m^2/d$	Grams per oxygen per meter square per day
h	hour
Kg	Kilogram
m	Meter
mm	Millimeter
µm	Micrometer
°C/min	Degree Celsius per minute
mV	Millivolt
nm	Nanometer
µL	Microliter
mL/min	Milliliter per minute
min	Minute
M	Molar

Penyingkiran Serentak Warna, Sebatian Organik, dan Nutrien dari Air Sisa Sintetik yang Mengandungi Dye Azo Menggunakan Reaktor Paya Tiruan Sesekat Aerobik - Anaerobik

ABSTRAK

Tesis ini membentangkan pendekatan yang lebih baik untuk penyingkiran warna, sebatian organik dan nutrien secara serentak dari air sisa yang mengandungi pewarna azo dengan menggunakan reaktor paya tiruan yang mempuyai zon aerobik dan anaerobik berganda. Paya tiruan konvensional kekurangan rawatan aerobik dan anaerobik yang merupakan aspek penting dalam rawatan pewarna azo, nutrien dan sebatian organik. Reaktor paya tiruan aerobik dan anaerobik yang beroperasi secara berjujukan menguntungkan dari segi ekonomi untuk merawat air sisa yang mengandungi pewarna azo kerana gabungan proses ini berupaya untuk menguraikan sebatian organik, menyahwarna, menitrifikasi secara aerobik dan mendenitrifikasi secara anaerobik nutrien dalam air sisa. *Acid Red 27* (AR27) dipilih sebagai bahan pencemar model kerana penggunaannya yang luas dan berbahaya yang boleh menyebabkan kesan buruk pada manusia dan haiwan selain klasifikasinya sebagai pengganggu endokrin. Bahagian pertama kajian ini melibatkan penyiasatan prestasi sistem ini dari segi penyingkiran bahan nitrogen dan organik. Bahagian kedua terdiri daripada pengenalan AR27 ke dalam sistem dan kesan AR27 ke atas prestasi sistem. Bahagian akhir kajian ini membincangkan analisis terperinci tentang penyahwarnaan lengkap, mineralisasi AR27 dan laluan degradasi yang dicadangkan berdasarkan analisis tersebut. Aerobik - Anaerobik constructed wetland (ABCW) terdiri daripada 5 sesekat menegak, memastikan aliran ke atas dan ke bawah dalam sistem, yang menyediakan laluan rawatan yang lebih lama melalui media paya tiruan. Reaktor ABCW ditanam dengan *P.australis*, di mana masa penahanan hidraulik ditetapkan selama 1 hari dan dibekal air sisa sintetik yang mengandungi AR27. Pengudaraan dibekalkan untuk mengawal zon aerobik dan anaerobik dalam sistem untuk membolehkan pencemar menjalani sesiri rawatan aerobik dan anaerobik. Prestasi ABCW dinilai dari segi penyingkiran COD, NH_4^+, NO_3^-, penyahwarnaan dan mineralisasi. Keadaan aerobik dan anaerobik dipantau melalui profil ORP sementara mineralisasi sebatian organik dan laluan degradasi dicadangkan berdasarkan beberapa analisis terperinci. Reaktor ABCW menunjukkan prestasi yang baik dalam penyingkiran serentak sebatian organik dan nutrien, berdasarkan penyingkiran COD yang berkisar antara 88 hingga 98%; penyingkiran NH_4^+ antara 93 hingga 98%; pencapaian 100% penyahwarnaan dan mineralisasi lengkap metabolit AR27. Prestasi ABCW ini disebabkan oleh laluan panjang yang dilalui oleh air sisa sekaligus meningkatkan masa hubungan pencemar dengan mikrob dan rizom. Kesan sinergistik kombinasi rizom, mikrob dan pengudaraan tambahan juga memainkan peranan penting dalam prestasi tinggi reaktor ABCW.

Simultaneous Removal of Colour, Organic Compounds, and Nutrients from Synthetic Wastewater Containing Azo Dye Using Aerobic-Anaerobic Baffled Constructed Wetland Reactor

ABSTRACT

This thesis presents an improved approach on the simultaneous removal of colour, organic compounds, and nutrients from wastewater containing azo dye by using multiple zoned aerobic and anaerobic baffled constructed wetland reactors. Conventional constructed wetlands lack the provision of sequential aerobic and anaerobic treatment which is essential in the treatment of azo dyes, nutrients and organic compounds. An aerobic and anaerobic constructed wetland reactor is operationally and economically advantageous to adopt in the treatment of wastewater containing azo dye since the combination of these processes has the capability to mineralize organic compounds, decolourize colour from dye, aerobically nitrify and anaerobically denitrify nutrients in the wastewater. Acid Red 27 (AR27) was chosen as the model pollutant due to the wide usage and harmful nature which may cause adverse effects in human and animals besides being classified as an endocrine disruptor. The first part of this study involves the investigation of the performance of this system for nitrogen and organic compounds removal. The second part comprises of the introduction of AR27 into the system and the effects of AR27 on the performance of the system. Final part of the study revolved around the detailed analysis of the complete decolourization and mineralization of AR27. Besides that, a degradation pathway was proposed based on that analysis. The Aerobic - Anaerobic Baffled Constructed Wetland Reactors (ABCW) comprises of 5 vertical baffles that ensures an upward and downward flow within the system, which provides a longer treatment pathway through the wetland media. The ABCW reactor were planted with *P.australis*, where the hydraulic retention time was set to 1 day and fed with synthetic wastewater containing AR27. Intermediate aeration was supplied to control the aerobic and anaerobic zones within the system to enable the pollutants to pass through a series of sequential aerobic and anaerobic treatment. Performance of the ABCW was evaluated in terms of COD, NH_4^+, NO_3^- removal, decolourization and mineralization. Aerobic and anaerobic conditions were monitored through the ORP profiles while mineralization of organic compounds and proposed degradation pathway was conducted by several detailed analyses. The ABCW reactors demonstrated good performance in the simultaneous removal of colour, organic compounds, and nutrients, as COD removal ranged from 88 to 98%; NH_4^+ removal ranged from 93 to 98%; achieved 100% colour removal and complete mineralization of AR27 metabolites. This performance of the ABCW could be attributed to the long pathway travelled by the wastewater hence increasing contact time of pollutants with microbes and rhizomes. The synergistic effect of the combination of rhizomes, microbes and supplementary aeration also played an important role in the high performance of the ABCW reactor.

CHAPTER 1: INTRODUCTION

1.1 Background

Lately, urbanization practices, unforeseen industrialization, rapid increase in population and standards of living has caused a rise in wastewater production from several anthropogenic activities globally. Developed industrialized countries are able to treat their wastewater accordingly however, it might be economically challenging for developing or underdeveloped countries to treat these wastewater discharge using conventional wastewater treatment methods which might affect the quality of the environment and adversely impact the community due to waterborne diseases (Pereyra, 2015).

Most conventional wastewater treatments are costly for these countries and might produce toxic end products which may require further treatment, therefore a more cost effective, environmental friendly, reliable and practical wastewater treatment alternative is an alternative solution (Korkusuz, Demirer, & Beklioglu, 2002). This approach is also a better alternative for developed countries that are facing rapid informal urban growth where over population and inappropriately constructed cities may be a cause of concern as this phenomenon may promote spread of infectious diseases and decrease the amount of green spaces within the city. Therefore, an appropriate solution for these problems is to utilize natural wastewater treatment systems in an environmental friendly way where the benefits of natural processes are utilized without external chemical sources (Pereyra, 2015).

Constructed wetlands (CW) are wastewater treatment systems composed of treatment cells in a built and partially controlled environment which is designed to provide wastewater treatment (Omprakash & Sied, 2014). The system may deal with a variety of pollutant load with high concentration and flow rate; and is based on physical, chemical and biological processes (Brix, Koottatep, & Laugesen, 2007; Sindilariu, Brinker, & Reiter, 2009). The function of a CW is mainly to treat a variety of liquid wastes such as storm water, sewage and wastewater. In other words, CWs are engineered marshes that replicate natural processes to cleanse water. Over the years, CWs have been used in many countries to treat landfill leachate, municipal wastewater, agricultural, aquaculture and industrial wastewater (Vymazal & Kropfelova, 2011).

There are many benefits of the usage of CW in treatment of wastewater. Besides being economically feasible to construct and operate, it is also easy to maintain. CWs are dependable, effective and environmental friendly wastewater treatment. Moreover, CWs are able to function in both large and small volumes of water with varying pollutant levels. The effluent, once treated by the wetland is clean and can be reused for other useful purposes. In fact, CW treatment process by itself can integrate beneficial uses. It is common knowledge that CWs are aesthetically pleasing and is able to accommodate wildlife besides being a recreational area for human (Wallace & Knight, 2006; Brix et al., 2007; Sindilariu et al., 2009; Tanner, Sukias, Headley, Yates, & Stott, 2012). Beaumont, Texas for instance has CWs for treating municipal sewage that also functions as city parks (Moulton & Jacob, 2000). An aerobic and anaerobic constructed wetland reactor is operationally and economically advantageous to adopt in the treatment of high strength industrial wastewaters since it couples the benefit of anaerobic with aerobic digestion.

Three main components in a constructed wetland are the impermeable layer, the wetland media and the emergent plant. Some of the material that have been used as wetland media include gravel, raw rice husk, coconut fibre, crushed pine bark and others (Tee, Lim, Seng, & Nawi, 2012). The common macrophytes used in constructed wetlands are *P.australis*, *T. latifolia*, and Bulrush. These macrophytes had a positive effect in most studies where removal of organics is discussed (Kaseva, 2004; Akratos & Tsihrintzis, 2007).

There are various types of constructed wetlands used to treat wastewater. Free water surface (FWS) and subsurface flow (SSF) are the common types of constructed wetlands. SSF can be further categorized depending on the flow direction into vertical flow (VF) and horizontal flow (HF). Despite the various designs and benefits, CWs still possess certain shortcomings, such as insufficiency of both aerobic and anaerobic zones in a single system, that could be addressed (Tee et al., 2012).

Azo dyes are commonly utilized in several industries such as in textile dying, paper printing, colour photography, pharmaceuticals and cosmetics (Elisangela et al., 2009). The utilization of azo dye in numerous industries, brand azo dye as the most frequently used dyes, therefore the largest class of dyes that are discharged into the environment (Saratale R, Saratale G, Kalyani, Chang, & Govindwar, 2009). There are more than 100,000 commercially available dyes with approximately 7×10^7 tons or more of dyestuff produced annually globally (Anjaneya, Souche, Santosh, & Karegoudar, 2011). Approximately 30,000 to 150,000 tons of dye discharge into receiving waters are led by industrial dying processes which may be carcinogenic and toxic to living beings (Anjaneya et al., 2013).

This study evaluates the performance of the Aerobic - Anaerobic Baffled Constructed Wetland (ABCW) reactor in terms of removing colour, organic compounds and nutrients in synthetic wastewater. Gravel is used as the wetland media in this research as it provides support for the plant and attached growth biomass. The ABCW reactor provided multiple aerobic, anaerobic and anoxic conditions for wastewater treatment enhancement. These conditions were achieved by adding vertical baffles along the width of the reactor to provide the upward and downward flow and by providing supplementary aeration to control the respective zones.

1.2 Problem Statement

Wastewater from industries such as the textile industry contains xenobiotics such as azo dyes which do not degrade under natural environmental conditions due to its complex structure. In Malaysia, textile finishing wastewater constitutes for 22% of the total volume of industrial wastewater produced owing to the batik industry. In 2008, Malaysia was the fifteenth largest manufacturer of textile fibre and ninth largest in Asian regions. The release of dyes into the environment during textile fibre dyeing and finishing processes is a main source of water pollution (Pang & Abdullah, 2013). Among all the states in Malaysia, Johor has been most affected by the textile industry, followed by Pulau Pinang and Selangor with the percentage of water pollution sources at 22.8, 22.6 and 15.6% respectively (Muyibi, Ambali & Eissa, 2008). These statistics correlates to the number of textile finishing plants located at the afore mentioned states indicating that the wastewater from textile industry has been adversely affecting water bodies.

Azo dyes are tough to degrade due to its complex structure and synthetic nature. Basically, sequential anaerobic and aerobic processes are required for complete degradation of azo dyes (Sponza & Isik, 2005). This requirement is because of the need for aerobic processes to mineralize aromatic amines residues resulting from anaerobic decolourization which resists anaerobic degradation. Aerobic processes are favourable for removing organic matter and nitrification but are poor in decolourization and denitrification whereas anaerobic processes prove to be efficient for decolourization and denitrification (Libra, Borchert, Vigelahn, & Storm, 2004; Vymazal & Kropfelova, 2011).

Several methods have been used in the treatment of textile effluents in order to achieve decolourization. However, these methods are not feasible due to complexity of components, maintenance cost, power demand, high investments and other disadvantages (Mbuligwe, 2005). Hence, there is a need for a more feasible treatment alternative. CWs have been used for various types of wastewater treatment and the results have been promising. Nevertheless, there is limited literature on the treatment of azo dyes using CWs to study the feasibility of CW in treating azo dyes (Bulc & Ojstrsek, 2008).

With regard to CWs, the standard types are vertical flow (VF) and horizontal flow (HF) CWs. HFCWs have insufficient aerobic zones while VFCWs have insufficient anaerobic zones. Field measurements of these wetlands have demonstrated inadequate oxygenation of the macrophyte rhizosphere which attributed to incomplete nitrification leading to limited nitrogen removal in HFCWs, while VFCWs possess greater oxygen transport capacity that causes efficient NH_4-N removal but limited denitrification (Vymazal, 2007; Tuncsiper, 2009). Hence, the use of one of these CWs for complete azo dye and nutrient removal would be inefficient due to their incapability to provide both aerobic and anaerobic conditions simultaneously. Usually, these types of CWs are combined to provide a high removal efficiency particularly for nitrogen removal (Vymazal & Kropfelova, 2011). The combination of these CWs may achieve higher treatment efficiencies, however there are limitations to these hybrid systems. The limitations include a necessity for a relatively large land area and a recycling system for the wastewater to go through a series of oxidation and reduction condition repeatedly.

Acid Red 27 (AR27) or more commonly known as amaranth dye is a naphthylazo, sulfonic acid based dye that is utilized to provide a red to purple colouration to cosmetics, medicines and food. AR27 is not permitted to be used in foods in the United States as it is not in the list of food additive standards of International Codex Alimentarius Commission (CAC) (Chen et al., 2015). Nonetheless, AR27 is still utilized as textile dyes for silk, wood, photography and as food dye for sweets, caviars and beverages. During the applying process of AR27 to phenol, paper, formaldehyde resins, and leather, excess dye would make its way into the wastewater (Karkmaz, Puzenat, Guillard, & Herrmann, 2004). The colour from this dye affects visibility, transparency, aesthetics and gas solubility in the water body. Besides that, the colour would also reduce light penetration into water that will affect the photosynthetic activity of aquatic plants (Vanhulle et al., 2007). Metabolic cleavage of azo linkage will lead to toxic by-products such aromatic amines that is mutagenic and carcinogenic which is a potential health hazard (Davies et al., 2009).

Removal of AR27 using constructed wetlands have not been reported by any researches and limited studies have been conducted on the removal of azo dyes using different types of constructed wetlands (Davies et al., 2009; Ong, Uchiyama, Inadama & Yamagiwa, 2009; Yadav, Jena, Acharya & Mishra, 2012). Therefore, this study led to the development of the Aerobic - Anaerobic Constructed Wetland reactor which incorporates multiple zones of aerobic, anoxic and anaerobic conditions within a single system which was achieved by adding vertical baffles in a horizontal CW. The vertical baffles would aid the upward and downward flow of wastewater through the system which would cause the pollutants to pass through a longer pathway and series of aerobic and anaerobic treatment hence, achieving higher treatment efficiencies in both nitrogen and azo dye removal. The concept behind this reactor is that the top region of the reactor would be in aerobic condition while the bottom region in anaerobic condition. This would allow the oxidized nitrogen in the top aerobic region through nitrification be denitrified in the bottom anaerobic region and the mineralization of aromatic compounds in the aerobic region resulting from the reduced azo bonds of AR27 in the anaerobic region.

Taking into account the above observation, the focus of this study is to evaluate the performance and effectiveness of the Aerobic - Anaerobic Baffled Constructed Wetland (ABCW) reactor in terms of organic compounds, nutrients and colour removal from wastewater containing AR27. Besides that, this study was also conducted to further explore the decolourization and degradation mechanism of AR27 and to propose a degradation pathway. Furthermore, the removal of AR27 in this type of system have not been explored before. Moreover, the simultaneous treatment of colour, organic compounds and nutrients in this type of system has not been reported by other studies. Besides that, the decolourization and mineralization of AR27 in an ABCW reactor has not been researched or studied till date. Therefore, this research aims to conduct this study and solve the problems that are currently faced.

1.3 Scope of Study

In light of the problems encountered with the utilization of CWs to treat colour, organic compounds and nutrients, this study aimed to incorporate both aerobic and anaerobic conditions as well as anoxic conditions into a single system by inserting vertical baffles in a horizontal constructed wetland which would allow an upward and downward flow. The idea behind this research is to ensure the bottom region of the reactor is in anaerobic condition while the top is in aerobic condition. Controlling of the particular zones is essential to make sure the pollutants would pass through a series of anaerobic and aerobic treatment to achieve higher removal efficiencies in both nitrogen and AR27 removal simultaneously. This system is equipped with supplementary aeration in order to control the respective aerobic, anoxic and anaerobic zones. To monitor and ensure the respective conditions within the reactor, oxidation reduction potential and dissolved oxygen are measured.

The performance and effectiveness of the Aerobic - Anaerobic Baffled Constructed Wetland (ABCW) reactor was evaluated in terms of organic compounds, nutrients (NO_3^-, NH_4^+) and colour removal from synthetic wastewater containing AR27 with the conventional macrophyte for phytoremediation in constructed wetlands which is *P.australis*. To ensure that the plants acclimatize well within the system and is not adversely affected by the presence of AR27 metabolites, plant growth is monitored in terms of number of stems and plant height. Besides that, this research was also conducted