Potential of *Epipremnum aureum* in Reduction of COD in Industrial Wastewater

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DECLARATION

I declare that this thesis entitled "Potential of *Epipremnum aureum* in Reduction of COD in Industrial Wastewater" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : ______________________________

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ABSTRACT

Wastewater from industrial, agricultural, and urban activities is usually channelled directly into the streams. High chemical oxygen demand (COD) level in water indicated a vast amount oxidizable matter that consume a lot of dissolved oxygen in water. This results in adverse impacts on both aquatic ecosystems and human health. Wastewater from the fish cracker industry typically has high organic content and high COD value. In this study, *Epipremnum aureum (E.aureum)* was chosen in phytoremediation treatment because it is abundance and economical. This study emphasized on the optimum absorption parameters such as initial concentration of wastewater, retention time for treatment, initial COD concentration of wastewater and number of plants used in one treatment that affecting removal of COD in the wastewater. The wastewater's quality value was analysed and determined according to EQA standard thus considered as polluted. From the result, it was found that the highest percentage removal was 82.3 % with retention time of 10 days, initial wastewater sample with pH 6, initial COD concentration of wastewater sample of 75 % and treatment with 2 plants. Based on the study, *E.aureum* is proved to be a good COD removal plant.

Potensi *Epipremnum aureum* Dalam Pengurangan COD Dalam Air Sisa Industri

ABSTRAK

Air sisa dari aktiviti perindustrian, pertanian dan bandar biasanya disalurkan terus ke sungai. Kepekatan permintaan oksigen kimia (COD) yang tinggi di dalam air menunjukkan sejumlah besar jirim yang boleh dioksidasi mengunakan banyak oksigen terlarut di dalam air. Ini memberi kesan yang buruk kepada kedua-dua ekosistem akuatik dan kesihatan manusia. Air sisa dari industri keropok ikan biasanya mempunyai kandungan organik yang tinggi dan kepekatan COD yang tinggi. Dalam kajian ini, *Epipremnum aureum* (*E.aureum)* dipilih sebagai rawatan phytoremediation kerana ia senang didapati dan ekonomik. Kajian ini memberi penekanan kepada parameter penyerapan optimum seperti kepekatan awal air kumbahan, masa pengekalan untuk rawatan, kepekatan COD awal air kumbahan dan bilangan tumbuhan yang digunakan dalam satu rawatan yang mempengaruhi penyingkiran COD dalam air sisa. Nilai kualiti air sisa dianalisis dan ditentukan mengikut Standard EQA yang dianggap sebagai tercemar. Hasilnya, didapati bahawa penyingkiran peratusan tertinggi adalah 82.3% dengan masa pengekalan 10 hari, sampel air buangan awal dengan pH 6, kepekatan awal COD sampel air sisa sebanyak 75 % dan rawatan dengan 2 tumbuhan. Berdasarkan kajian, *E.aureum* telah terbukti sebagai penyingkir COD yang baik.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BOD	Biochemical Oxygen Demand
cm	Centimeter
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
EQA	External Quality Assessment
HCl	Hydrochloric Acid
L	Liter
mL	Milliliter
mg/L	Milligram/liter
SME	Small and medium Enterprise
NaOH	Sodium Hydroxide
SD	Standard Deviation
TNT	Trinitrotoluene
TDS	Total Dissolved Solids
WQI	Water Quality Index
WWF	World Wide Fund

LIST OF SYMBOLS

=	Equals
-	Minus
%	Percentage
° C	Temperature (degree Celsius)

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Chemical Oxygen Demand (COD) is a chemical method for measuring the amount of reductive substances in water samples that need to be oxidized. In the study of the properties of river pollution and industrial wastewater, as well as in the operation and management of wastewater treatment plants, it is an important and rapid determination of organic pollution parameters, often expressed as symbolic COD. High COD level indicates that there is a greater amount of oxidizable organic material in the sample, which cause dissolved oxygen (DO) levels to decrease. A reduction in DO level will lead to anaerobic conditions, which is dangerous to higher aquatic life (Zheng et al., 2008). Hence, COD should be reduced and phytoremediation is one of the methods.

Phytoremediation is one of the methods used in treatment of contaminated soils, sludges, sediments, surface water and groundwater by plants to transfer, contain or convert pollutants to ensure environment safety (Salt et al., 2008). The medium of phytoremediation are usually soil and water bodies that are contaminated by heavy metals, inorganic matter, radioactive elements or organic matter. Through several phytoremediation actions of plants, the pollutants can be purified (Ali et al., 2013).

According to Black (2005), phytoremediation is a very potential and very high effective method in treatment of contaminant because through absorption, evaporation, root filtration, degradation and stabilization of plants, the pollutants in the soil or water can be purified. It has a lot of advantages for example, it has low cost, and can be said that totally no damage to the environment because it does not has secondary pollution. Since 1990s, phytoremediation has become a frontier topic in controlling environmental pollution.

Epipremnum aureum (E. aureum) can be used in phytoremediation and it is a plant that belongs to the genus Kirin leaf, large evergreen vine, growing in the tropics, often climbing in the rainforest rocks and tree trunks, well root developed and can be hydroponic cultivation. Because of the tenacious vitality, *E. aureum* can grow well whether it is planted in pot or hydroponics grown by just a few stalks. Therefore, *E. aureum* has the ability in impurity adsorption (Kim et al., 2014).

1.2 Problem Statement

Rivers contaminated by sewage and sediments contain high levels of organic pollutants, and become breeding grounds for harmful bacteria and viruses that may cause outbreaks of water borne diseases for example cholera, typhoid and hepatitis A that are harmful to human. Contaminants such as fertilizers, pesticides, herbicides and heavy metals will cause health hazards to aquatic life and when human beings consume the contaminated seafood, chronic long-term illness or even serious, death in human will occur.

The measure of amount of oxygen needed in the water during the decomposition of organic matter such as carbon, hydrogen, oxygen, nitrogen, phosphorus and sulphur or the oxidation of inorganic chemicals such as ammonia and nitrite called Chemical Oxygen Demand (COD). Large amount of reducing substances mainly organic pollutants will cause high chemical oxygen demand in water (Buchanan, 2003).

Phytoremediation can be used in COD reduction (Chandekar & Godboley, 2015). The aquatic plants are used to remove the contaminants from sample wastewater. The pollutant removal depends upon influent waste water sample concentration, number of plants used for each treatment and initial pH of the waste water sample. Nowadays, phytoremediation technology has been widely applied as a brilliant technique for waste water treatment, pollution control and environmental improvement by many countries. The phytoremediation technology started because phytoremediation technique is economically and not only suitable in the developed countries but also in developing countries which have low operation and maintenance cost with better efficiency than any other treatment methods.

In this study, the potential of *E. aureum* in treatment of industrial wastewater was investigated with the characteristics, strong hydroponic ability and pollution resistance in reducing the COD of the waste water.

To test the potential of *E. aureum* in treatment of industrial wastewater, the hydroponics ability and pollution resistance of the plants to the waste water and the ability of the plants in reducing rate of COD according to several parameters such as the number of plants used, the initial concentration of water samples and pH of the water samples were studied as the independent variables.

1.3 Expected Outcomes

The different initial concentration of the sample waste water will influence the COD percentage removal or removal efficiency of COD by the plant. The number of plant used in treatment of waste water sample will increase the percentage removal of COD. The optimum initial pH of the waste water will cause the most efficient of phytoremediation process because the pH is optimum for plant growth.

At the same time, the hydroponic ability of plants shows that the plants have the ability for treatment of the waste water without any medium to growth such as soil. The number of leaves, plant length and fresh weight of *E. aureum* shows the hydroponic ability and pollution resistance of the plants to the waste water. The increase of fresh weight or the growth rate indicates that the plants have the pollution resistance to the waste water sample. Therefore, growth status indicates the purification effect of the plants toward contaminated medium.

1.4 Objectives

i. To determine the parameter affecting COD reduction using *E. aureum.*

ii. To determine the pollution resistance and hydroponics activity of *E. aureum*.

1.5 Scope of Study

In this study, COD removal rate of the waste water was determined by evaluating the initial and final COD reading of the waste water after the treatment with *E. aureum*. The hydroponic ability and pollution resistance of *E. aureum* were determined by recording and observing the number of leaves, plant length of each plant before and after the technique of phytoremediation by *E. aureum*. By calculating the plant length and number of leaves of each plant, it indicates the hydroponic ability and pollution resistance of *E. aureum.* The parameters affecting the COD removal rate were studied such as the initial concentration of the waste water, initial pH of the waste water and the number of plants used in each treatment.

1.6 Significance of Study

Nowadays with the population growth, social development and industrial progress, water pollution has attracted worldwide attention. The higher the amount of chemical oxygen demand obtained, the more serious the degree of contamination of water bodies, and the source of these organic pollution is likely to be organic fertilizers, chemical plants and pesticides. If not treated in a timely manner, these organic matters are likely to be deposited at the bottom of the river as the sediments. After several years, there will be lasting damage to water bodies and the ecological system in the river will be completely destroyed after the constant death of aquatic organisms. In this process, if people feed on these creatures in the water, they will inhale a lot of harmful substances which will deposit in the body, as the main causes of cancer, deformities, mutations and so on. Besides, if people use contaminated water in agriculture, then the crops will also be affected, after going through the food chain, human will ingest large number of harmful substances (Mazumder, 2005).

Aquatic plants with high purification effect and certain economic value are mostly use in phytoremediation. The use of *E. aureum* that is abundant and affordable can help reducing the COD concentration of the wastewater by absorbs the pollutants as the nutrient to grow. It also helps clean up the air pollutants and as the developed suitable plant that can be used in treating wastewater and water. Besides, this can help reduce the cost in cleaning the pollutants in the water compared to other methods. Hence, *E. aureum* is a new plant to be used in phytoremediation in wastewater treatment compared to other existing plants. Usually in the phytoremediation of water, aquatic plants were chosen, *E. aureum* is a terrestrial plant that can be planted in aquatic environment due to the hydroponic ability. Therefore *E. aureum* can be easily plant and use in water treatment process complying with the principle of waste minimization and cleaner production.

CHAPTER 2

LITERATURE REVIEW

2.1 Phytoremediation

Phytoremediation is a process that uses plants to transfer, remove or convert contaminants from medium such as soil and water to make them environmentally friendly (Tangahu et al., 2011). The common target of phytoremediation are usually soil or water bodies that contaminated by pollutants. Phytoremediation is a potential technique in treatment of pollutant especially in developing environment. It has the advantages of low input, cause no damage to the soil and aqua ecological environment because it does not produce secondary pollution (Pilon, 2005).

2.1.1 Rhizosphere Biodegradation

Rhizosphere biodegradation, also referred to as phytostimulation which means decompose of organic contaminants presented in the soil with enhanced microbial activity in the rhizosphere or plant root zone (Wang et al., 2011). First and foremost, compounds such as enzymes, organic acids, sugars, carbohydrates and phenolics that exuded by the roots enrich indigenous microbe populations. Secondly, oxygen that is brought by the root systems of the plants to the rhizosphere will ensure aerobic transformations. Thirdly, the fine-root biomass of the plants will increase the availability of organic carbon. Next, presence of mycorrhizae fungi within the rhizosphere helps breakdown organic pollutants (Abhilash et al., 2009). All these microbial activity will reduce the COD content of the groundwater due to reducing of the organic matters. There are several enzyme systems in sediments and soils which are dehalogenase, nitroreductase, peroxidase, laccase and nitrilase (Anderson et al., 2010).

2.1.2 Phytostabilization

Phytostabilization refers to the establishment of the plant that cover on the surface of the contaminated sites to convert pollutants in the soil into relatively harmless substances (Bolan et al., 2011). The process includes transpiration and root growth of the plants to immobilize contaminants by reducing leaching, prevent erosion and creating aerobic environment in the rhizosphere. Phytostabilization can be enhanced by the use of soil improvers that immobilize metals to combine with plants that are tolerant to high levels of contaminants and low-fertility soils (Cheraghi et al., 2011). Phytostabilization protect contaminated soil from erosion and reduce soil leakage to prevent metal contaminants from leaching and strengthen the fixation of contaminants in the soil by accumulation of metal roots and precipitation or root surface holding (Mendez & Maier, 2007).

2.1.3 Phytoaccumulation

Phytoaccumulation or also known as phytoextraction is a type of phytoremediation which uses plants or algae to remove contaminants from soils, sediments or water into harvestable plant biomass (Kamal et al., 2004). The plant will accumulate the contaminants into the roots and above ground shoot or leaves. The plant capable of growing in soils with high concentration of metal, absorbing metal through their root or the action that take larger than normal amount of contaminants from the soil are called hyperaccumulation (Tang & Angela, 2019). In this research, the phytoaccumulation ability of *Epipremnum aureum* (*E. aureum*) will accumulate the oxidizable pollutants within the plant.

2.1.4 Rhizofiltration

With the strong absorption of plant roots, precipitates metal or organic absorbed and concentrated. According to Dushenkov et al. (2015), plant roots can adsorb a large amount of lead, chromium and other metals from sewage. It can also be used for the treatment of radioactive pollutants, hydrophobic organic pollutants such as trinitrotoluene TNT as well as reducing the COD. The medium required for root filtration is water-based. Therefore, root filtration is an important way for phytoremediation of water bodies, shallow lakes and wetland systems (Rawat et al., 2012). The selected plants are also dominated by aquatic plants.

2.1.5 Phytovolatilization

Phytovolatilization is a process of releasing volatile compounds or metabolic products into the atmosphere by the transpiration or photosynthesis of plants. Hydroxyl is an oxidant formed in the photochemical cycle, and many organic compounds in the underground environment can quickly react with hydroxyl group when they enter the atmosphere (Limmer, & Burken, 2016). Nitrate reductase and gum oxidase in plants can decompose drug waste and bind into new plant tissues or organic fragments, becoming an integral part of organic matter to achieve the goal of detoxification.

2.1.6 Phytodegradation

In this process, plants actually metabolize and destroy contaminants within plant tissues. Phytodegradation or phytotransformation, is the degradation of pollutants taken by plant within the metabolic processes, the enzymes produced by the plants also breakdown the contaminants that surrounding the roots or in rhizosphere. Newman & Reynolds (2004) claimed that complex organic pollutants are first degraded into simpler molecules then incorporated into the plant tissues as nutrient to help the plant to grow faster. Plants that contain enzymes will catalyse and accelerate chemical reactions hence reduce the treatment time of contaminant by the plants.

2.2 Statistical Analysis of COD Removal

The term "statistics" originated from the investigation of national conditions and was originally intended for national sentiment. In general, statistics include three meanings: statistical work, statistics, and statistical science (Assen, Aert & Wicherts, 2015). The relationship between statistical work, statistical data, and statistical science is: the result of statistical work is statistical data, and the basis of statistical data and statistical science is statistical work. Statistical science is both a theoretical summary of statistical work experience and a guide to statistical work (Mead, 2017). Each science has its own establishment, development and objective conditions. Statistical science is a marginal discipline that combines statistical work experience, socio-economic theory, and econometric methods.

2.2.1 Standard Error Bar

Error bars are commonly used for statistical or scientific data to show potential errors or uncertainty relative to each data marker in the series. The error line can use standard deviation (average deviation) or standard error (Krzywinski, & Altman, 2013). Generally, these two are used. If the difference between the two errors has the error line, the standard deviation (std. deviation) and the standard error (std.error) can be used.

Error bars can communicate how spread the data are around the mean value. If there is small SD bar, it indicate that the graph has low spread and data are clumped around the mean whereas for larger SD bar the graph has larger spread and the data are more variable from the mean (Connell & Khaicair, 2014). Next, error bars indicate the reliability of the mean value as a representative number for the data set. In other words, how accurately the mean value represents the data (small SD bar = more reliable, larger SD bar = less reliable).

A "significant difference" means that the results or data gained are most likely not due to sampling error or chance. In any experiment or observation that involves sampling from a population, there is always will have observed effect occurred due to sampling error alone (Correll, & Gleicher, 2013). But if result is "significant," then the investigator may conclude that the observed effect actually reflects the characteristics of the population rather than just sampling error or chance. The standard deviation error bars on a graph can be used to get a sense for whether or not a difference is significant by look for overlap between the standard deviation bars.

When standard deviation error bars overlap to each other with great amount, these indicate that the difference is not statistically significant. A statistical test must perform to draw a conclusion. When standard deviation error bars overlap to each other by small potion, it's a clue that the difference is probably not statistically significant. A statistical test must be performed to draw a conclusion. When standard deviation error bars do not overlap, the difference may be significant, but not confirmed. Therefore, must perform a statistical test to draw a conclusion (Drikakis, & Inok, 2010).

2.3 Water Quality

Water quality is a tool to describe condition of the water for example, the physical (such as chromaticity, turbidity, stench, etc.) of water bodies, the properties of chemistry (inorganic and organic matter) and the characteristics and composition of organisms (bacteria, microorganisms, plankton, benthic organisms). In order to evaluate the quality of water bodies, a series of water quality parameters and water quality standards are specified.

In Malaysia, National Water Quality Standards provides the standards of river water parameters grouped into different classes of different quality levels. Environment Quality (Industrial Effluents) Regulations 2009 stated the acceptable conditions for discharge of industrial effluent for mixed effluent of Standards A and B (Table 2.1). Standard A and B represent the discharge upstream and discharge downstream of raw water intake respectively. The common parameters to identify the standard of effluent discharge are Temperature, pH Value, BOD_5, COD, Oil and Grease, Suspended Solid and Ammonical Nitrogen shown in Table 2.2 (Al-Mamun & Zainuddin, 2013).

Table 2.1 Acceptable conditions for discharge of industrial effluent containing Chemical Oxygen Demand (COD) for specific trade or industry sector

Trade/Industry	Unit	Standard A	Standard B
(a) Pulp and paper industry			
(i) Pulp mill	mg/L	80	350
(ii) Paper mill (recycled)	mg/L	80	250
(iii) Pulp and paper mill	mg/L	80	300
(b) Textile industry	mg/L	80	250
(c) Fermentation and distillery industry	mg/L	400	400
(d) Other industries	mg/L	80	200

(Source: Taha et al., 2011a)

Table 2.2 National Water Quality Standards for Malaysia

PARAMETER	UNIT	CLASS					
		I	IIA	IIB	III	IV	V
Ammoniacal Nitrogen	mg/l	0.1	0.3	0.3	0.9	2.7	> 2.7
Biochemical Oxygen Demand	mg/l	1	3	3	6	12	> 12
Chemical Oxygen Demand	mg/l	10	25	25	50	100	> 100
Dissolved Oxygen	mg/l	7	5 – 7	5 – 7	3 – 5	< 3	< 1
pH	–	6.5 – 8.5	6 – 9	6 – 9	5 – 9	5 – 9	–
Total Dissolved Solid	mg/l	500	1000	–	–	4000	–
Temperature	°C	–	Normal + 2 °C	–	Normal + 2 °C	–	–

(Source: Taha et al., 2011b)

Notes :
− : Undefined

Chemical oxygen demand (COD) is a measure of the capacity of water to consume oxygen during the oxidation of organic material and inorganic chemicals (ammonia and nitrite), into carbon dioxide and water (Yang et al., 2009). COD indirectly measures the amount of organic matter in sample. Higher COD levels translate into greater amount of oxidizable organic material in the sample, which will reduce dissolved oxygen levels (Hajali, 2016). As gauge of organic matter in water, COD is more advantageous than biochemical oxygen demand (BOD) because BOD relies on microorganism to break down the organic matter in sample which will take course over the period of typically 5 days. Whereas, it is only a matter of hours for COD test to complete, therefore speeding up the time for water treatment if the water is tested to be under standards.

2.4 Characterization of Potential in Reduction of COD in Industrial Wastewater by *Epipremnum aureum (E. aureum)*

Chemical Oxygen Demand (COD) analysis method is used to analyse the COD removal by calculate the percentage removal. For the analytical method, the number of leaves and plant length of each plant is observed regularly to test for the hydroponic ability and pollution resistance of plant to the waste water (Gleba et al., 2009). The water sample is taken for COD analysis. The detection items are COD analysis by potassium dichromate method (including spectrophotometry). *Epipremnum aureum* absorbs benzene, trichloroethylene, formaldehyde and so on in the air hence it is ideal for placement in newly renovated rooms. Besides, *E. aureum* can convert formaldehyde into substances such as sugar or amino acids in metabolism, break down benzene emitted by copiers and printers. In addition to its high ornamental value, Bringslimark et al. (2007) had found that a basin of *E. aureum* in a room with 8 to 10 m^2 is equivalent to an air purifier that effectively absorbs harmful gases such as formaldehyde, benzene and trichloroethylene in the air.

2.4.1 Hydroponic Ability of *E. aureum*

The hydroponic domestication of plants refers to the process of plants adapting to the aquatic environment by short-term scientific domestication, improvement and cultivation of plants making the plants adapt to the terrestrial environment in short period (Erro et al., 2010). It is mainly include the transform of plants from soil to the water. In this experiment, the whole plant was taken out from the pot with soil, the root system was washed and inserted directly in the water. The light had to be sufficient. After the domestication, the original soil roots of plants will rot and the new roots will grow after the plant had adapted to the aquatic environment (Sardare, & Admane, 2013). The hydroponic ability of the plants show that the ability of the plants to grow in aqua environment. Hydroponics is the ability that does not need soil and is to allow the plants roots to directly contact with the solution, while also enable accessibility of oxygen for proper growth of the plants. The hydroponic ability of the plants show that the plants are suitable to plant directly to the contaminated water without any medium, the contaminant can be the nutrient for plants growth. Plants will grow bigger and faster due to easier obtain of nutrients hence increase the rate of COD removal (Tripp, 2014).

2.4.2 Pollution Resistance of *E. aureum*

Some leaves experience equivalent pollutant absorption but can vary in resistance ability. In this sense *E. aureum* is considered a species resistant to environmental pollution. Next, *E. aureum* is potentially bioindicators for specific soil contaminants such as Copper, Chromium and Zinc (Cruz et al., 2018).

According to Safronova et al. (2011), the increase of quantity of leaves and plant stem length indicate that the plant has the ability to grow in polluted medium therefore suitable to be used in phytoremediation to remove contaminant especially in reducing of COD concentration.

2.5 Factor Affecting the Removal of COD by *E. aureum*

2.5.1 Initial pH of the Waste Water Sample

There are many organic acids, inorganic acids, alkalis and salts in the soil. The different contents of various substances make the soil show different acidity and alkalinity. Soil acidity and alkalinity can be expressed by pH value. Traditionally, soil with pH values ranging from 6.5 to 7.5 is called neutral soil. The optimum pH of the *E. aureum* is between 6 and 7 as the suitable pH for all plants (Reyes et al., 2010). Bacterial activity that releases enzymes to breakdown contaminants is particularly affected by pH, because bacteria operate best in the pH range of 5.5 to 7.0. In the pH range 5.5 to 6.5, plant nutrients are most available to plants. Therefore, *E. aureum* is suitable to grow in pH 5 to 6.

2.5.2 Concentration of the Waste Water Sample

The concentration of the waste water indicate that the concentration or amount of the pollutants in the waste water. The higher the initial concentration of the waste water, the lower the growth rate of the plant due to the high amount of pollutants in the waste water that exceed the capacity of the plants can withstand. The higher the initial concentration of the waste water, the higher the amount of organic pollutant, the higher the COD rate, the lower the efficiency of the plant in removing COD by phytoremediation hence more time needed to treat the water (Singh et al., 2012).

2.5.3 Contact Duration of *E. aureum* with Wastewater

The contact duration of *E. aureum* with wastewater means the duration of treatment of waste water sample by the *E. aureum* (Wolcott et al., 2016). Based on previous study, the removal efficiency of COD can reach about 30 % to 40 % by using *E. aureum* within 1 week and the efficiency will be increase as the contact duration of *E. aureum* with wastewater increase (Chen et al., 2011).

2.5.4 Number of *E. aureum* Used

The number of *E. aureum* used to be contact with wastewater for phytoremediation purpose to reduce COD concentration of the waste water can affect the efficiency of the COD reduction. The more the number of plants used, the more efficient the removal of COD. The more the plant used in the treatment, the higher the transpiration intensity, absorption and transport ability of pollutants increase (Lin et al., 2003).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials and Wastewater Sampling

Wastewater sample was collected from a local small and medium enterprise (SME) that process fish cracker which is located in Tumpat, Kelantan due to the high content of COD in the water. The shop and its processing materials were pictured in Appendix A. *Epipremnum aureum* (*E. aureum*) was brought from Taiping, Perak. Besides, Sodium hydroxide (NaOH), Sulphuric acid (H_2SO_4), distilled water, 1 L beakers, 50 mL beakers, volumetric flasks, droppers, YSI multiparameter and COD kit were used in this study.

3.2 Methods

3.2.1 Industrial Wastewater Characterization

Industrial wastewater was collected from fish crackers company with HDPE sampling bottle to conduct the analysis ex-situ. The collected waste water was undergone the COD analysis to determine the concentration of COD in the sample collected. Wastewater in-situ analysis was carried out when taking the sample in fish cracker producer Kedai Pak Su Do. The parameter of temperature, total dissolved solid, pH, salinity and dissolved oxygen (DO) were analysed using the YSI Multiparameter.

3.2.2 Study of the Hydroponic Ability

The whole plant of *E. aureum* was removed from the pot and the soil on the root was washed with water then inserted directly in the beaker filled with tap water. The water inside the beaker was changed often and rotten roots were removed for one week and the plants adapted to the aquatic. The number of leaves and plant length were measured.

3.2.3 Study of the Pollution Resistance of *E. aureum*

The plants that are adapted to the water based environment were removed to the beaker filled with 500 ml of waste water sample. Step 2 was repeated by changing the 500 ml of waste water sample to distilled water as the control. The number of leaves and plant length were measured.

3.2.4 Effect of initial pH of the Waste Water to Reduce COD

The concentration of the waste water was fixed by adding 50 ml of waste water and 450 ml of distilled water make up 10 % concentration of 500 ml water sample to be treated in a beaker. The initial pH of the water samples were adjusted to pH 5, 6, 7 and 8 by H_2SO_4 and NaOH in 4 different beakers. A blank model was prepared by adding 500 ml of distilled water in a beaker without adjust the pH as the blank. One *E. aureum* was planted in the beakers filled with waste water with pH 5, 6, 7, 8 and blank respectively for 1 week. All the plants were ensured with 30 cm long and same quantity of leaves by cut down extra stem and leaves. Number of leaves and plant length were measured in day 1, 4 and 7 to ensure the accuracy of the COD removal of the plants.

3.2.5 Effect of the Initial Concentration of the Waste Water for Plants to Reduce COD

The concentration of the waste water was vary by adding 50 ml, 125 ml, 250 ml, 375 ml of waste water and add up to 500 ml by distilled water in beaker to obtain 10 %, 25 %, 50 % and 75 % concentration of water sample. 500 ml of waste water sample was added in a beaker without adding distilled water. Then, a blank model was prepared by adding 500 ml of distilled water in a beaker without adjust the pH as the blank. The pH of the waste water was adjusted to optimum pH which was pH 6 by H_2SO_4 and NaOH except for blank. One *E. aureum* was planted in the waste water with concentration 10 %, 25 %, 50 %, 75 %, 100 % and blank respectively for 1 week. All the plants were ensured with 30 cm long and same number of leaves. The initial quantity of leaves and plant length were measured in day 1, 4 and 7.

3.2.6 Effect of Number of Plants in Reducing COD

The optimum concentration of the waste water was set with 75 % and added up to 500 ml by distilled water in two beakers. The waste water was adjusted to optimum pH 6 by H_2SO_4 and NaOH. Two blank models were prepared by adding 500 ml of distilled water in a beaker without adjust the pH as the blank. One *E. aureum* was planted in 1 beaker with waste water and 1 in blank. Step 3 was repeated with 2 *E. aureum* and all 4 beakers leaved for 1 week. All the plants were ensured with 30 cm long and same number of leaves. The plants were observed in day 1, 4 and 7.

3.2.7 Effect of Hydraulic Retention Time of Treatment of Wastewater by the Plant and COD Reducing

The optimum concentration of the waste water was set to be 75 % of concentration and added up to 500 ml by distilled water. The waste water was adjusted to pH 6 by H_2SO_4 and NaOH. A blank model was prepared by adding 500 ml of distilled water in a beaker without adjust the pH as the blank. Due to the result that was almost the same among 1 plant treatment and two plants treatment in last test, both number of *E. aureum* which were 1 plant and 2 plants were planted in the waste water with 75 % concentration of waste water and blank for 2 weeks respectively. The plants were ensured with 30 cm long and same number of leaves. The quantity of leaves and length of plant were measured.

3.2.8 Control experiment for COD removal

For the analysis of parameter pH affecting the COD concentration of the waste water, the beakers with the amount similar as the variable sets was prepared to test whether the surrounding will reduce the COD concentration of waste water after several days expose to the surrounding. The concentration of the waste water was fixed by adding 50 ml of waste water and 450 ml of distilled water make up 10% concentration of 500 ml water sample to be treated in a beaker. The pH of the waste water was adjusted to pH 5, 6, 7 and 8 by H_2SO_4 and NaOH in 4 different beakers. A blank model will be prepared by adding 500 ml of distilled water in a beaker without adjust the pH. *Epipremnum aureum* was not planted in these beakers which filled with waste water with pH 5, 6, 7, 8 and blank respectively for 1 week as the control set. Number of leaves and plant length was measured and COD analysis of the waste water was carried out in day 1, 4 and 7 to ensure the accuracy of the COD removal of the plants. The method was repeated as a control for the COD removal for the other parameters by repeating the methods used in analyse the effect of each parameters to COD removal. The only difference in this test was no *E. aureum* was planted in the beakers.

3.2.9 Statistical Analysis of COD Removal

In this test, the bar graph of the effect of various parameters versus average COD removal percentage (%) was plotted by using Eq. (3.1):

$$\% \text{ removal efficiency} = \frac{C_0 - C_t}{C_0} \times 100\% \quad (3.1)$$

Where,

C_0 = the concentration of pollutants in the water at the beginning of the test;

C_t = concentration of pollutants in the water body on day t

Standard error bar were plotted and analysed by determine the standard deviation and mean of the sample to determine the accuracy of the data.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Water Quality of Waste Water Using YSI Multiparameter

Table 4.1 showed the results for each parameter through in-situ and ex-situ analysis of wastewater collected for first and second time. The readings of COD for first water sample and second water sample exhibited the largest difference from the set EQA standard for industrial effluent in appendix B, with 916 mg/L of COD for first water sample and 1792 mg/L for second water sample which had far exceeded the effluent standard B. The differences between first water sample and second water sample was large due to different date of sampling. The first sampling was on 2 May 2019 which the producer did not operate while second sampling was on 13 September 2019 which the producer operated. Therefore, the water sample in second sampling was fresh discharged hence higher COD concentration. COD decreases with increasing time because the bacteria in the water will grow and degrade the organic matters in water sample (Mohammed et al., 2014). Also, the values of DO and salinity of wastewater were recorded at well above their respective standard B.

Table 4.1 Water quality of fish cracker's wastewater

Physio-chemical Parameter	1^{st} water sample	2^{nd} water sample	Mean	EQA Standard B
DO (%)	11.6	4	7.8	-
pH	4.88	5.45	5.17	5.5-9.0
COD (mg/L)	916	1792	1354	200
TDS (mg/L)	903.5	491.9	697.7	-

Table 4.1 (Continued)

Temperature (˚C)	28.52	30.14	58.66	40

Notes :
− : Undefined

From the values of the parameter obtained, the values indicated serious polluted of the industrial effluent by the fish cracker industry as the pH value and COD reading of the effluent had exceeded the EQA Standard B. The COD reading of the effluent of the fish cracker industry showed that the waste water was in classified as Class V which exceeds 100 mg/L (Table 4.2).

Table 4.2 National Water Quality Standards for Malaysia

PARAMETER	UNIT	CLASS					
		I	IIA	IIB	III	IV	V
Ammoniacal Nitrogen	mg/L	0.1	0.3	0.3	0.9	2.7	> 2.7
Biochemical Oxygen Demand	mg/L	1	3	3	6	12	> 12
Chemical Oxygen Demand	mg/L	10	25	25	50	100	> 100
Dissolved Oxygen	mg/L	7	5 – 7	5 – 7	3 – 5	< 3	< 1
pH	–	6.5 – 8.5	6 – 9	6 – 9	5 – 9	5 – 9	–
Total Dissolved Solid	mg/L	500	1000	–	–	4000	–
Temperature	°C	–	Normal + 2 °C	–	Normal + 2 °C	–	–

(Source: Taha et al., 2011c)

Notes :
− : Undefined

From Table 4.2, the water that classified in Class V has the water characteristic that more severe than Class IV which is irrigation. Therefore, the fish cracker's wastewater can certainly pollute the water and critically harming the ecosystem if it is being channelled directly into the irrigation system without treatment. The mean COD concentration among first sample and second sample was higher than 100 mg/L that was 1354 mg/L. According to Ching & Redzwan (2017), the fish processing sector contributes serious organic pollution loads and high salinity, the rate and efficiency of COD removal decrease significantly with the increase in salt content above 20 g/L. For both water samples, the salinity had reached 999.99 g/L based on YSI multiparameter. Therefore, the COD concentration of the wastewater was high.

4.2 Hydroponic Ability of the *Epipremnum aureum* (*E. aureum*)Plant

From Figure 4.1, there were new roots that grew by the plant after 7 days grew in the distilled water indicated that the plant was suitable to plant in water based medium. From Figure 4.2, there were some new shoots that grew by the plant after 14 days indicated that the plant had the ability to grow and reproductive in water based medium although it took longer time. Therefore the plant had the hydroponic ability.

Figure 4.1: The hydroponic roots of the *E. aureum* grew after transfer from soil to water.

Figure 4.2: The new shoots grew after the plant grew in water.

4.3 Study of the Pollution Resistance of *E. aureum*

From Figure 4.3, there were new shoots that grew by the plant after 14 days grew in the sample water with 50 % concentration indicated that the plant is suitable to plant in polluted water or industrial effluent. From Figure 4.3, there were some shoots that were rotten but there was also new shoots grew by the plants although it took longer time (two weeks). Therefore the plant had the pollution resistance (Min et al., 2014).

Figure 4.3 New shoots grew by *E. aureum* in sample water with 50 % of concentration after 14 days

4.4 COD Reduction Efficiency Analysis

4.4.1 COD Removal Percentage Based on Different pH of Water Sample

Figure 4.4 indicated that the effect of different pH of water sample which were pH 5, 6, 7, 8 to the average COD removal percentage within duration of 7 days with initial concentration of wastewater which was 10 %. For pH 5, the COD concentration of water sample without plant (control) had reached 9 % of removal in day 4 and increased to 27.49 % in day 7 compared to initial COD concentration. While for two water samples with 1 plant, the average COD concentration reached 51.62 % of removal in day 4 and increased to 87.29 % in day 7 compared to initial COD concentration.

For pH 6, the COD concentration of water sample without plant (control) had reached 13.93 % of removal in day 4 and increased to 25.41 % in day 7 compared to initial COD concentration. While for two water samples with 1 plant, the average COD concentration reached 53.88 % of removal in day 4 and increased to 89.72 % in day 7 compared to initial COD concentration.

For pH 7, the COD concentration of water sample without plant (control) had reached 11.11 % of removal in day 4 and increased to 58.33 % in day 7 compared to initial COD concentration. While for two water samples with 1 plant, the average COD concentration reached 53.22 % of removal in day 4 and increased to 80.01 % in day 7 compared to initial COD concentration.

For pH 8, the COD concentration of water sample without plant (control) had reached 8.15 % of removal in day 4 and increased to 46.74 % in day 7 compared to initial COD concentration. While for two water samples with 1 plant, the average COD concentration reached 64.72 % of removal in day 4 and increased to 79.62 % in day 7 compared to initial COD concentration.

The results presented in Table 4.4 show that the best pH for the reduction of COD concentration was found to be pH 6. It is believed that the pH affects the reduction of COD from the wastewater sample by affect the metabolism of the plant for nutrient uptake. Figure 4.4 indicated that the average COD removal of water sample with plant increased from pH 5 to pH 6, after reached the highest removal that is 89.72 % in pH 6, the average removal of COD decreased from pH 7 and pH 8 because *E. aureum* can perform better in remediation action with optimum pH of water surrounding. Although pH 5 has high removal of COD, but compared to pH 6 which is nearer to neutral, pH 6 is more optimum pH for reaching well phytoremediation process by *E. aureum* (Hung & Xie, 2009). Therefore, *E. aureum* is suitable to be planted in slightly acidic medium.

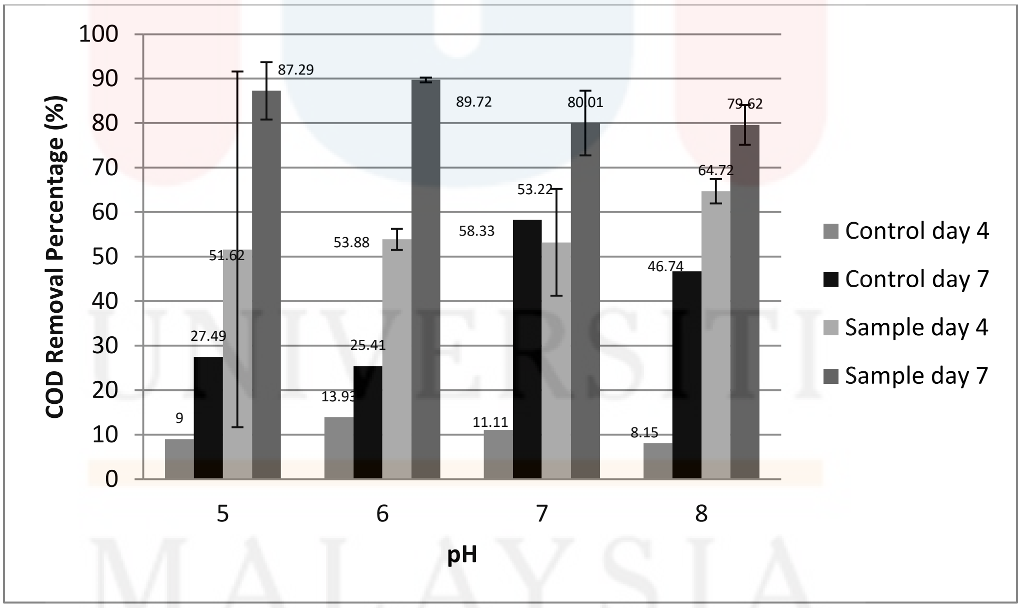

Figure 4.4 Graph showing the effect of pH of water sample to the average COD removal percentage (%)

From the graph, the final COD removal percentage of water sample in pH 7 and pH 8 increased from initial to day 4 then decreased during day 7. During day 4, The COD removal of water sample with pH 8 reached the highest percentage due to the uneven of size of the plant used but when time goes on, the efficiency of the plant no longer maintain due to the unsuitable pH of water (Zhu et al., 2017). The standard error bar of pH 6 show that the duplicate data had high accuracy therefore the gap of the standard error bar was small (Renne et al., 2011). The pH 6 had the highest COD removal percentage and as the only one pH that gained high removal of COD over the treatment period of 7 days because the bottom of standard deviation error bars of pH 6 did not overlap among others shown that the difference of COD removal for all pH may be significant. Therefore pH 6 is the most suitable for *E. aureum* planting at the same time reaching high removal rate of COD removal.

4.4.2 COD Removal Percentage Based on Different Concentration of Water Sample

Figure 4.5 indicated that the effect of different concentration of water sample which were 10 %, 25 %, 50 %, 75 % and 100 % to the average COD removal percentage within duration of 7 days with different initial average concentration of COD. For concentration 10 %, the COD concentration of water sample without plant (control) had reached 25.27 % of removal in day 4 and increased to 53.30 % in day 7 compared to initial COD concentration. While for two water samples with 1 plant, the average COD concentration reached 54.47 % of removal in day 4 and increased to 87.97 % in day 7 compared to initial COD concentration.

For 25 % concentration of sample water, the COD concentration of water sample without plant (control) had reached 24.19 % of removal in day 4 and increased to 26.22 % in day 7 compared to initial COD concentration. While for two water samples with 1 plant, the average COD concentration reached 55.53 % of removal in day 4 and increased to 88.88 % in day 7 compared to initial COD concentration.

For 50 % concentration of sample water, the COD concentration of water sample without plant (control) had reached 14.78 % of removal in day 4 and increased to 22.61 % in day 7 compared to initial COD concentration. While for two water samples with 1 plant, the average COD concentration reached 54.71 % of removal in day 4 and increased to 90.20 % in day 7 compared to initial COD concentration.

For 75 % concentration of sample water, the COD concentration of water sample without plant (control) reached 11.17 % of removal in day 4 and increased to 11.56 % in day 7 compared to initial COD concentration. While for two water samples with 1 plant, the average COD concentration reached 56.62 % of removal in day 4 and increased to 91.57 % in day 7 compared to initial COD concentration.

For 100 % concentration of sample water, the COD concentration of water sample without plant (control) had reached 10.21 % of removal in day 4 and increased to 11.17 % in day 7 compared to initial COD concentration. While for two water samples with 1 plant, the average COD concentration reached 44.50 % of removal in day 4 and increased to 73.43 % in day 7 compared to initial COD concentration.

Figure 4.5 indicated that the average COD removal of water sample with plant increased from concentration 10 % to 75 %, after reached the highest removal that was 91.57 % in 75 % concentration of water sample, the average removal of COD decreased to 73.43 % for 100 % concentration of sample water because *E. aureum* can perform better in remediation action with optimum concentration of water sample. As the concentration of the water sample increased, the organic compound increased hence more nutrients for plant to grow and carry out phytoremediation. While when 100 % concentration of water sample is used, the plant cannot adapt to the water sample with too high of pollutants hence the COD removal percentage decreased (El-Hady, & Shanan, 2010). The study of Wang (2017), the increase of salt content in sewage, the plant height, above ground and underground biomass, total biomass, leaf length, leaf width and leaf area of the plants decreased. As a result, the root-shoot ratio was significantly increased, and the degree of inhibition above ground was greater than that of the root system, indicating that the sensitivity of the root system to saline wastewater was lower than that of stems and leaves. Therefore, the roots of *E. aureum* cannot adapt to the wastewater with 100 % concentration as increase of concentration of wastewater increase salt concentration as well.

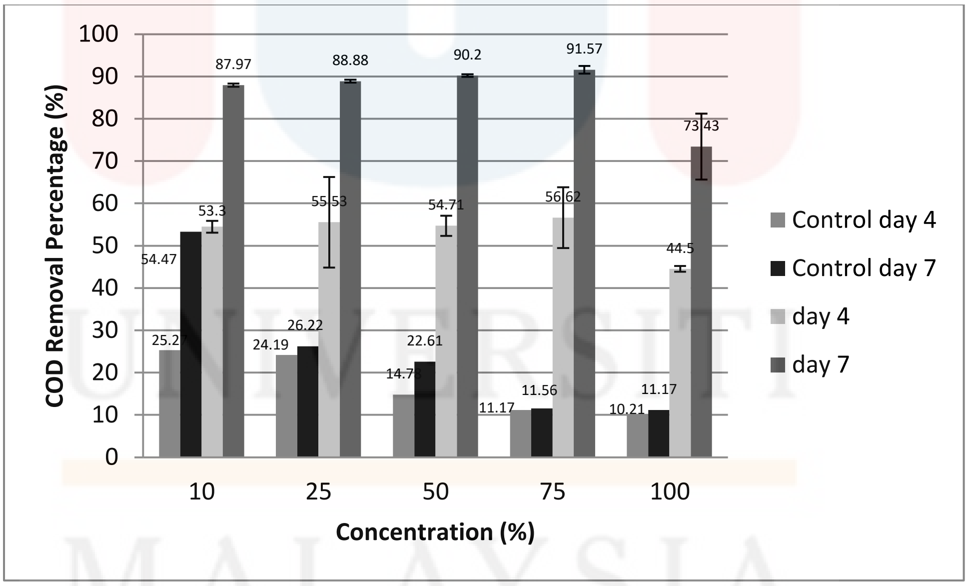

Figure 4.5 Graph showing the effect of concentration of water sample to the average COD removal percentage (%)

Figure 4.5 shown that 75 % concentration of sample water was still as the most suitable concentration in order to reach the highest COD removal rate as it still reached 80.01 % of final removal in day 7 after considered the control set data. From the graph, the final COD removal percentage of 10 % concentration of water sample showed big different with the others due to the high COD removal of the control set. This may due to the position of the beaker placed that caused uneven evaporation rate in the laboratory.

COD concentration of the wastewater was efficiently reduced in 75 % concentration of water sample and as the only one pH that will gain high removal of COD over the treatment period of 7 days. Although the standard deviation error bars of 75 % concentration almost overlap with 50 % concentration but they actually did not overlap among others therefore shown that the difference may be significant but in low rate.

From the graph, 50 % concentration of water sample had high probability in reaching the highest COD removal rate too. However, for the industrial and commercial factor in effluent treatment, 75 % of concentration was preferred because more waste water can be treated at one time (Jagtap et al., 2014).

4.4.3 COD Removal Percentage Based on Different Number of Plant Used in Water Sample

Figure 4.6 indicated that the effect of different number of plants in 1 beaker with 75 % concentration of water sample which were 1 plant and 2 plants to the average COD removal percentage within duration of 7 days with different initial average concentration of COD. The COD concentration of water sample without plant (control) had reached 9.16 % of removal in day 4 and increased to 14.55 % in day 7 compared to initial COD concentration. While for two water samples with 1 plant, the average COD concentration had reached 53.98 % of removal in day 4 and increased to 91.53 % in day 7 compared to initial COD concentration.

The data of COD removal percentage of water sample without plant (control) for sample set with 1 plant had repeated for sample set with 2 plants which was 9.16 % of removal in day 4 and 14.55 % in day 7 compared to initial COD concentration. While for two water samples with 2 plants, the average COD concentration reached 62.78 % of removal in day 4 and increased to 95.78 % in day 7 compared to initial COD concentration.

Figure 4.6 indicated that the beaker with 2 plants filled with 75 % concentration of sample water reached the higher average COD removal percentage. More *E. aureum* plant in the same medium, the more efficient the COD removal of the water sample because the phytoremediation rate was doubled. From the study of Saleh (2012), added of plant instead of first batch, an increase uptake was recorded for following second addition with 96.7 % as compared to the result obtained in this study that is 95.78 %. Therefore, with adding of number of plants will cause COD removal percentage that is higher than 95 % which is very satisfy.

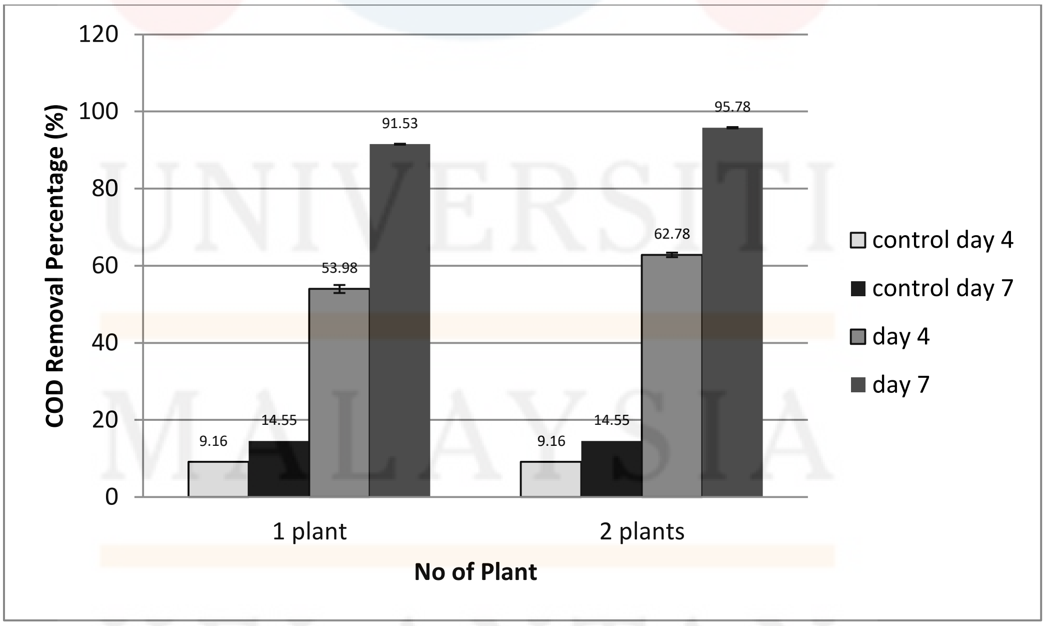

Figure 4.6 Graph showing the effect of number of plant in water sample with 75 % concentration to the average COD removal percentage (%)

The water sample with 75 % concentration treated by 2 plants had reached higher final COD removal percentage after subtract control data. The difference of the COD removal percentage among 1 plant treatment and 2 plants treatment was quite small that is 4.25 %. Therefore, for the industrial and commercial factor in effluent treatment, 1 plant treatment was preferred because more waste water can be treated at one time with less number of plant hence reduce the cost of planting the plant.

Figure 4.6 indicated that the 2 plants in treatment of 75 % concentration of water sample had the higher COD removal percentage compared to 1 plant treatment over the treatment period of 7 days because standard deviation error bars did not overlap among others shown that the difference may be significant.

4.4.4 COD Removal Percentage Based on Different Hydraulic Retention Time of Treatment in Water Sample

Due to the high similarity of COD removal result for 1 plant treatment and 2 plants treatment from previous experiment, the two types of treatment with different number of plants was investigated again together with various treatment duration. Figure 4.7 indicated that the effect of duration of treatment in 1 beaker with water sample with 75 % of concentration for both 1 plant and 2 plants which were 1 week to 2 weeks to the COD removal percentage (%) with different initial concentration of COD. The COD concentration of water sample without plant (control) had reached 9.16 % of removal in day 4, increased to 14.55 % in day 7, then to 17.12 % in day 10 and lastly to 17.47 % showing the efficiency of COD removal by the plants had decrease gradually.

While for two water samples with 1 plant, the average COD concentration reached 53.98 % of removal in day 4, increased to 91.53 % in day 7, then to 93.65 % in day 10. However the COD removal percentage has decreased to 85.47 % during day 14. The same results went to 2 plants treatment set as well. The average COD concentration of 2 plants treatment set had reached 62.78 % of removal in day 4, increased to 95.78 % in day 7, continuously increased to 99.42 % in day 10 and reach steep descend to 88.74 % during day 14. However in the overall, treatment with 2 plants had higher COD removal percentage compared to 1 plant treatment.

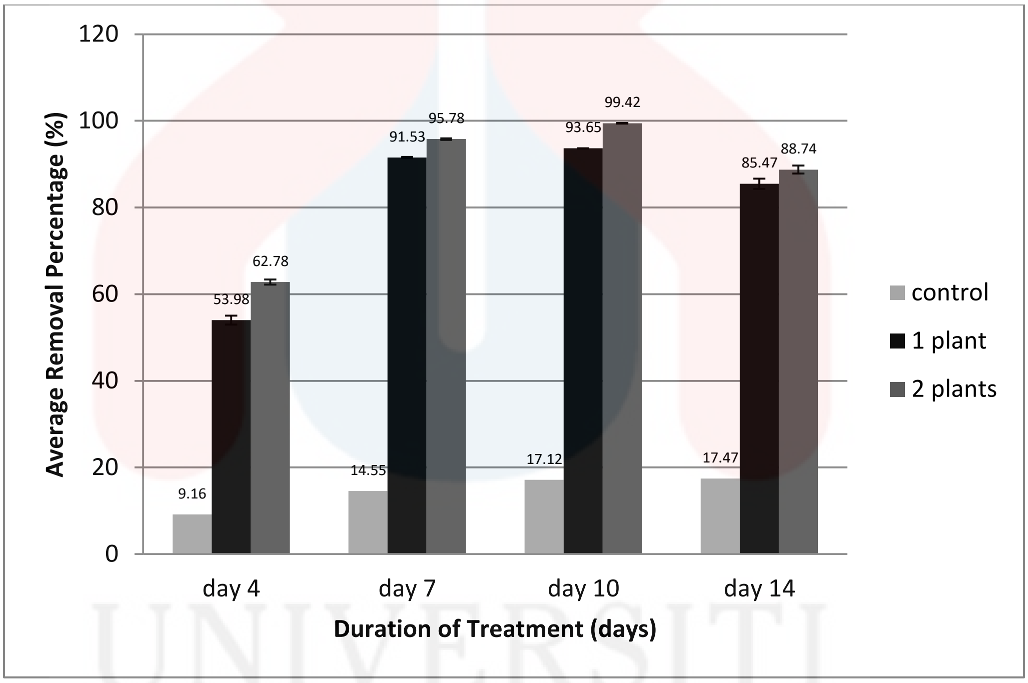

Figure 4.7 Graph showing the effect of number of plant and different hydraulic retention time in water sample with 75 % concentration to the average COD removal percentage (%)

For 2 plants treatment, although the COD removal percentage after including control set did not increasing obviously after day 7 treatment but it increased slightly in day 10 then only decreased in day 14 while for the 1 plant treatment, the COD removal percentage increased gradually but it decreased during day 10 continuously to day 14. This showed that the life time for *E. aureum* plant in the treatment of the sample water was only day 7 to day 10. After the duration, the plants will start to rot hence increase the COD concentration of the water sample. In this case, 2 plants were better than 1 plant treatment because when the plants started to rot, there will be more longer stem, more roots and more leaves to carry out phytoremediation (Rezania et al., 2015). Therefore, more *E. aureum* plant in the same medium, the more efficient the COD removal of the water sample because the phytoremediation rate was doubled.

The result of 1 plant treatment for both day 10 and day 7 had almost similar COD removal percentage due to their standard deviation error bars that almost overlapped shown that the difference may be low significant. 2 plants in treatment of 75 % concentration of water sample is most preferable among all the conditions because standard deviation error bars did not overlap among others shown that the difference may be significant.

If 1 plant treatment chose for the industrial and commercial factor, treatment duration with 7 days may be preferred because reduces time and cost in effluent treatment therefore more waste water can be treated at one time with less number of plant.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Statistical analyses with standard error bars were executed to optimize COD removal by *Epipremnum aureum (E. aureum)*. Phytoremediation reactions were carried out by the plant and the retention time, initial COD concentration, initial pH of water sample and the number of plants used in treatment were taken as its factors. Under statistical analysis, it was found out that *E. aureum* has the ability in reducing COD. The capability of COD removal by *E. aureum* was well explained with the graph. After analysis, it was found that the highest percentage removal was 82.3 % after including the control set with retention time of 10 days, initial wastewater sample with pH 6, initial COD concentration of wastewater sample with 75 % and treatment with 2 plants. Based on the data, *E. aureum* proved to be a good agent in removal on COD.

5.2 Recommendation

There were also few recommendations were suggested in order to improve the phytoremediation efficiency. Firstly, the size of the leaves of *E. aureum* chose to run the test should be large and mature to increase photosynthesis and transpiration rate. Because when photosynthesis and transpiration rate increase, the plants will intake the pollutants in the wastewater more quickly. Following, the plants used in phytoremediation must be mature enough with sturdy stem and long roots to increase the absorption rate of pollutants in the wastewater.

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APPENDIX A

Figure A-1: In-situ analysis of the wastewater using YSI Multiparameter.

Figure A-2: Bucket of fish head that is not used in the processing of cracker.

Figure A-3: COD concentration of water sample after treatment with 14 days for sample 1

Figure A-4: Comparison among initial COD vials for distilled water and water sample with 100 %

APPENDIX B

Table B-4 Water Classes and Uses

CLASS	USES
Class I	Conservation of natural environment. Water Supply I – Practically no treatment necessary. Fishery I – Very sensitive aquatic species.
Class IIA	Water Supply II – Conventional treatment. Fishery II – Sensitive aquatic species
Class IIB	Recreational use body contact.
Class III	Water Supply III – Extensive treatment required. Fishery III – Common, of economic value and tolerant species ; livestock drinking.
Class IV	Irrigation
Class V	None of the above.

ENVIRONMENTAL QUALITY ACT, 1974
(Act 127)

Table B-5 COD concentration difference with parameter pH

ph		Control	sample 1	sample 2	average (sample)	standard deviation (sample)	standard error (sample)
5	day 1	211	184	197			
	day 4	192	37	151			
	day 7	153	15	34			
	difference day 1& 4	19	147	46			
	difference day 1 and 7	58	169	163			
	percentage removal day 4	9.004739	79.8913	23.35025	51.62078	39.98056	28.27053
	percentage removal day 7	27.48815	91.84783	82.74112	87.29447	6.439416	4.553355
6	day 1	244	225	182			
	day 4	210	100	87			
	day 7	182	24	18			
	difference day 1& 4	34	125	95			
	difference day 1 and 7	62	201	164			
	percentage removal day 4	13.93443	55.55556	52.1978	53.87668	2.37429	1.678877
	percentage removal day 7	25.40984	89.33333	90.10989	89.72161	0.549109	0.388278
7	day 1	108	175	199			
	day 4	96	67	110			
	day 7	45	26	50			
	difference day 1& 4	12	108	89			
	difference day 1 and 7	63	149	149			
	percentage removal day 4	11.11111	61.71429	44.72362	53.21895	12.01422	8.495334
	percentage removal day 7	58.33333	85.14286	74.87437	80.00861	7.260916	5.134243
8	day 1	184	204	180			
	day 4	169	68	67			
	day 7	98	48	31			
	difference day 1& 4	15	136	113			
	difference day 1 and 7	86	156	149			
	percentage removal day 4	8.152174	66.66667	62.77778	64.72222	2.74986	1.944444
	percentage removal day 7	46.73913	76.47059	82.77778	79.62418	4.459856	3.153595

Table B-6 COD concentration difference with parameter initial concentration of wastewater

Concentration of sample (%)		Control	sample 1	sample 2	average (sample)	standard deviation (sample)	standard error (sample)
10	day 1	182	119	187			
	day 4	136	53	87			
	day 7	85	14	23			
	difference day 1& 4	46	66	100			
	difference day 1 and 7	97	105	164			
	percentage removal day 4	25.27473	55.46218	53.47594	54.46906	1.40449	0.993125
	percentage removal day 7	53.2967	88.23529	87.70053	87.96791	0.378132	0.26738
25	day 1	492	282	413			
	day 4	373	104	215			
	day 7	363	32	45			
	difference day 1& 4	119	178	198			
	difference day 1 and 7	129	250	368			
	percentage removal day 4	24.18699	63.12057	47.94189	55.53123	10.73295	7.589339
	percentage removal day 7	26.21951	88.65248	89.10412	88.8783	0.319353	0.225817
50	day 1	920	658	752			
	day 4	784	309	328			
	day 7	712	66	72			
	difference day 1& 4	136	349	424			
	difference day 1 and 7	208	592	680			
	percentage removal day 4	14.78261	53.03951	56.38298	54.71125	2.364187	1.671733
	percentage removal day 7	22.6087	89.9696	90.42553	90.19757	0.322389	0.227964
75	day 1	1540	914	1077			
	day 4	1368	350	522			
	day 7	1362	71	98			
	difference day 1& 4	172	564	555			
	difference day 1 and 7	178	843	979			
	percentage removal day 4	11.16883	61.70678	51.53203	56.61941	7.194635	5.087375
	percentage removal day 7	11.55844	92.23195	90.90065	91.5663	0.94137	0.665649
100	day 1	1979	1197	1463			
	day 4	1777	670	805			
	day 7	1758	384	308			
	difference day 1& 4	202	527	658			
	difference day 1 and 7	221	813	1155			
	percentage removal day 4	10.20718	44.02673	44.97608	44.50141	0.671287	0.474672
	percentage removal day 7	11.16726	67.9198	78.94737	73.43358	7.797669	5.513784

Table B-7 COD concentration difference with parameter number of plant

Concentration of sample (%)		Control	sample 1	sample 2	average (sample)	standard deviation (sample)	standard error (sample)
1 plant	day 1	1168	1285	1217			
	day 4	1061	582	569			
	day 7	998	110	102			
	difference day 1& 4	107	703	648			
	difference day 1 and 7	170	1175	1115			
	percentage removal day 4	9.160959	54.70817	53.24569	53.97693	1.034133	0.731243
	percentage removal day 7	14.55479	91.43969	91.61873	91.52921	0.126605	0.089523
2 plant	day 1	1168	1103	1124			
	day 4	1061	406	423			
	day 7	998	45	49			
	difference day 1& 4	107	697	701			
	difference day 1 and 7	170	1058	1075			
	percentage removal day 4	9.160959	63.1913	62.36655	62.77892	0.583185	0.412374
	percentage removal day 7	14.55479	95.92022	95.64057	95.78039	0.197741	0.139824

Table B-8 COD concentration difference with parameter retention time

Concentration of sample (%)		Control	sample 1	sample 2	average (sample)	standard deviation (sample)	standard error (sample)
1 plant	day 1	1168	1285	1217			
	day 4	1061	582	569			
	day 7	998	110	102			
	day 10	968	82	77			
	day14	964	176	187			
	difference day 1& 4	107	703	648			
	difference day 1 and 7	170	1175	1115			
	difference day 1& 10	200	1203	1140			
	difference day 1& 14	204	1109	1030			
	percentage removal day 4	9.160959	54.70817	53.24569	53.97693	1.034133	0.731243
	percentage removal day 7	14.55479	91.43969	91.61873	91.52921	0.126605	0.089523
	percentage removal day 10	17.12329	93.61868	93.67297	93.64582	0.038388	0.027145
	percentage removal day 14	17.46575	86.3035	84.63435	85.46892	1.180271	0.834578
2 plant	day 1	1168	1103	1124			
	day 4	1061	406	423			
	day 7	998	45	49			
	day 10	968	6	7			
	day14	964	117	134			
	difference day 1& 4	107	697	701			
	difference day 1 and 7	170	1058	1075			
	difference day 1& 10	200	1097	1117			
	difference day 1& 14	204	986	990			
	percentage removal day 4	9.160959	63.1913	62.36655	62.77892	0.583185	0.412374
	percentage removal day 7	14.55479	95.92022	95.64057	95.78039	0.197741	0.139824
	percentage removal day 10	17.12329	99.45603	99.37722	99.41663	0.055723	0.039402
	percentage removal day 14	17.46575	89.39257	88.07829	88.73543	0.929332	0.657137