REMOVAL OF HEAVY METAL USING SEQUENCING BATCH REACTOR (SBR)

MUHAMMAD SYAFIQ BIN MOHD SHAFEI

BACHELOR OF CHEMICAL ENGINEERING
UNIVERSITI MALAYSIA PAHANG

REMOVAL OF HEAVY METAL USING SEQUENCING BATCH REACTOR (SBR)

MUHAMMAD SYAFIQ BIN MOHD SHAFEI

Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering

Faculty of Chemical & Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

JUNE 2017

UNIVERSITI MALAYSIA PAHANG

DECLARATION OF THESIS AND COPY RIGHT

Author’s Full Name :

Date of Birth :

Title

Academic Session :

I declared that this thesis is classified as:

☐ **CONFIDENTIAL** (Contains confidential information under the Official Secret Act 1972)*

☐ **RESTRICTED** (Contains restriction information as specified by the organization where research was done)*

■ **OPEN ACCESS** I agree that my thesis to be published as online open access (Full text)

I acknowledge that University Malaysia Pahang reserve the right as follows:

1. The Thesis is the Property of University Malaysia Pahang.
2. The Library of University Malaysia Pahang has right to make copies for the purpose of research only.
3. The Library has the right to make copies of the thesis for academic exchange.

Certified By:

(Student’s Signature)

(Supervisor’s Signature)

New IC /Passport Number
Date:

Name of Supervisor
Date:

NOTES : *If the thesis is CONFIDENTIAL or RESTRICTICTED, please attach with the letter from the organization with period and reasons for confidentiality or restriction

SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering.

Signature	:
Name of main supervisor	: DR. ZULKIFLY BIN JEMAAT
Position	: SENIOR LECTURER
Date	: 21 JUNE 2017

STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree

Signature :
Name : MUHAMMAD SYAFIQ BIN MOHD SHAFEI
ID Number : KE13036
Date : 21 JUNE 2017

Dedicated to my mother and father.

ACKNOWLEDGEMENT

Alhamdulillah to Allah s.w.t for all His blessings and knowledge upon me to be given the opportunity to finish this project successfully. Without Him, there is no way I am able to go this far. Next, I would like to express my special appreciation and thanks to my supervisor, Dr. Zulkifly Bin Jemaat, who have been a brilliant mentor for me. I would like to thank you for your never-ending support during my tenure as research student under your guidance, for giving insightful comments and suggestions of which without it, my research path would be a difficult one. Your advice on my research has been valuable.

A special thanks to my mother and father. Words cannot express how grateful I am to them for the love and support throughout these years. Your prayer for me was what sustained me thus far, truly.

I am also indebted to the Ministry of Higher Education and Universiti Malaysia Pahang for funding my study.

I would also like to thank all of my friends who supported me in writing, and motivate me to strive towards my goal. I am sincerely grateful to the staffs of Chemical Engineering and Natural Resources Faculty who helped me in many ways and made my stay in UMP pleasant and unforgettable.

Sincerely, thank you very much for all the prayer, support and guidance.

ABSTRACT

Commonly, the removal of heavy metals from wastewater is by using physical and chemical methods. Due to high operating cost of those methods, the search for cost effective and environmental friendly methods is essential. One of the attractive methods is by using biological approach. In highlight to that, this study is conducted with the main objective is to investigate and evaluate the feasibility of removing heavy metals (Zinc and Copper) in wastewater by using indigenous microorganisms in a sequencing batch reactor (SBR). Such microorganisms could be possibly found in activated sludge from palm oil mill pond in which utilized as inoculum in this study. Prior to the experimental work, SBR was inoculated with activated sludge. Then, SBR was fed with synthetic wastewater containing Zinc and Copper at certain concentrations. The presence of the metals was determined by using Atomic Absorption Spectrometry (AAS). In addition, the variation of pH between 2 and 9 was experimented to evaluate the efficiency of the removal in response to the pH changes. As a result, the range of pH 6.0 – 8.0, shows the most efficient removal for Copper was up to 95% and Zinc 97% by using SBR inoculated with activated sludge originated from palm oil mill pond. This suggests that biological method could be utilized to remove heavy metals from wastewaters. In addition, pH plays an important role when dealing with microorganism. A correct condition is necessary for the microorganism to fully optimized its activity.

ABSTRAK

Kebiasaannya, penyingkiran logam-logam berat daripada air sisa ialah dengan menggunakan kaedah fizikal dan kimia. Disebabkan kos operasi yang tinggi, pencarian untuk kaedah yang lebih jimat dan mesra alam adalah diutamakan. Salah satu kaedah yang menarik perhatian ialah dengan pendekatan biologi. Oleh yang demikian, kajian ini dijalankan adalah untuk menyiasat dan menilai kebolehlaksanaan penyingkiran logam-logam berat (tembaga dan zink) di dalam air sisa dengan menggunakan mikroorganisma yang diproses di dalam *Sequencing Batch Reactor (SBR).* Mikroorganisma tersebut mungkin boleh didapati di dalam enapcemar diaktifkan yang diperolehi daripada kolam minyak sawit di mana ia digunakan sebagai inokulum dalam kajian ini. Sebelum eksperimen dijalankan, SBR diinokulatkan dengan enapcemar diaktifkan. Kemudian, SBR dimasukkan dengan air sisa sintetik yang mengandungi tembaga dan zink pada kepekatan tertentu. Kehadiran logam-logam tersebut ditentukan oleh *Atomic Absorption Spectrometry (AAS)*. Selain itu, perbezaan antara pH 2.0 dan 9.0 juga diaplikasikan untuk menilai tahap kecekapan penyingkiran logam-logam tersebut terhadap perubahan pH tersebut. Keputusannya, lingkungan pH 6.0 – 8.0 dipilih, kerana kecekapannya untuk menyingkirkan sehingga 95% logam tembaga dan 97% untuk logam zink dengan melalui proses di dalam *Sequencing Batch Reactor (SBR)* diinokulatkan dengan enapcemar diaktifkan dari kolam minyak sawit. Ini mencadangkan bahawa kaedah biologi boleh digunakan untuk penyingiran logam-logam daripada air sisa. Secara keseluruhannya, pH memainkan peranan yang penting apabila melibatkan dengan mikroorganisma. Satu situasi yang tepat diperlukan untuk mikroorganisma berada dalam keadaaan yang aktif sepenuhnya.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
AGWSP	Attached-Growth Waste Stabilization Ponds
BOD	Biochemical Oxygen Demand
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
DOE	Department of Environmental
FAS	Fixed Activated Sludge
FBE	Fluidized Bed Electrochemical Reactor
$HAuCl_4$	Chloroauric Acid
HCL	Hydrochloric Acid
K_2PdCl_4	Potassium Tetrachloropalladate
MCL	Maximum Contaminant Level
MLSS	Mixed Liquor Suspended Solids
NaOH	Sodium Hydroxide
PBBR	Packed Bed Biofilm Reactor
PCB	Printed Circuit Board
SBR	Sequencing Batch Reactor
TS	Total Solid
TSS	Total Suspended Solids
VSS	Volatile Suspended Solids
WSP	Waste Stabilization Pond

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

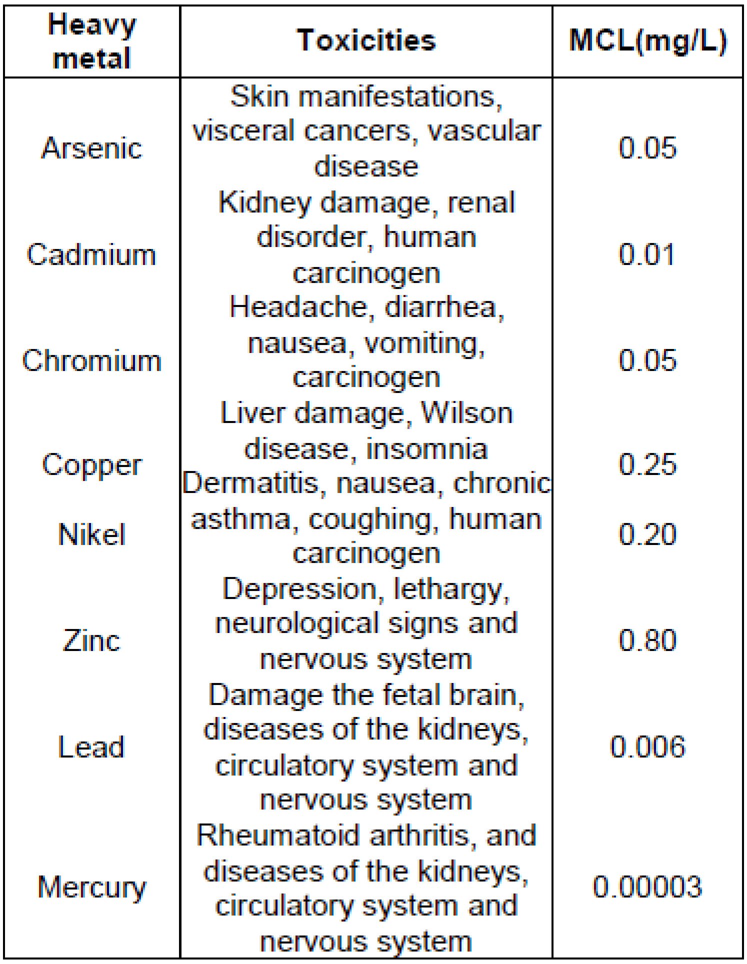
According to Dhokpande and Kaware (2013), it was a very serious problem concerning the presence of heavy metals such as Copper, Silver, Zinc, Cadmium, Gold, Mercury, Lead, Chromium, Iron, Nickel, Tin, Arsenic, Selenium, Molybdenum, Cobalt, Manganese, and Aluminium in many wastewaters and also industrial effluents. These heavy metals carry toxic and carcinogenic agents in which these agents posed a deadly threat to human and nature especially when these metals are exposed to the water which is the main source of every living creature. Some of the toxic metals are Zinc, Copper and nickel in which they have a high toxicity level either in low concentration or high concentration. **Table 1.1** shows the maximum contaminant level limits of several heavy metals in industrial effluents.

Since before, the removal of heavy metals has been going on quite successfully conducted by some researchers and industries worldwide. Variety of methods have been proposed by these researchers to remove the heavy metals in many different kind of wastes. The methods range from chemically involved methods, mechanical methods and biological methods. Each and every method has its own specialty. For instance, the pyrometallurgical method is one of the mechanical method used to remove metals in large scale industries. This method applied a very high heat treatment at above 1200℃ and includes smelting and roasting of the waste to recover the precious metals (Liew, 2008). Aside from that, the efficiency of this process is not that high because of the high temperature treatment which can damage the metals.

The chemical methods involve using specific chemical solution as to remove these heavy metals. An example in one of the experiment run by Sheng and Etsell (2007), a medium, aqua regia is used to dissolve particular precious metals which are gold and also some platinum group metals. According to them also, aqua regia is a mixture of three parts concentrated hydrochloric acid and one parts nitric acid. Ammen

(1984) also stated that aqua regia is very flexible, easy and has low capital requirement which is the reason to be selected as the recovery medium for gold. However, a method has been proposed which is more environmentally safe and also economically possible. The use of microorganisms in metals recovery process has been the main technology in biological methods this past few years. In Willner et al. (2015), the method of bioleaching has been conducted to recover various kind of metals in a lot of wastes with the involvement of different groups of microorganisms. For example, *Penicillum sp.* and *Aspergillus niger* are some of the microorganisms used in bioleaching method. The demand for this green technology application has been increasing a lot lately.

Table 1.1: The MCL (Maximum Contaminant Level) standards for the most hazardous heavy metals (*Babel and Kurniawan, 2003*)



Heavy metal	Toxicities	MCL(mg/L)
Arsenic	Skin manifestations, visceral cancers, vascular disease	0.05
Cadmium	Kidney damage, renal disorder, human carcinogen	0.01
Chromium	Headache, diarrhea, nausea, vomiting, carcinogen	0.05
Copper	Liver damage, Wilson disease, insomnia	0.25
Nikel	Dermatitis, nausea, chronic asthma, coughing, human carcinogen	0.20
Zinc	Depression, lethargy, neurological signs and nervous system	0.80
Lead	Damage the fetal brain, diseases of the kidneys, circulatory system and nervous system	0.006
Mercury	Rheumatoid arthritis, and diseases of the kidneys, circulatory system and nervous system	0.00003

1.2 Motivation

Heavy metals removal from wastewaters nowadays was very costly and has become the subject of considerable interest due to the strict legislation and law (Dhokpande and Kaware, 2013). However, recent studies have proved the efficiency of biological methods which emphasize the cost effectiveness and environmental friendly. Other available methods are considered to be cost consuming and not environmental friendly such as pyrometallurgy and hydrometallurgy. The usage of microorganisms such as algae, fungi, bacteria actinomycetes might be a relevant medium to be used to remove these metals due to the ability and properties of some of these microorganisms which able to bind and concentrate metal ions even in inactive state (Dobson and Burgess, 2007). Even so, this method is still not applied in the large industrial scale because more work need to be done in order to better understand the feasibility, mechanisms, and technology know-how of the methods.

1.3 Problem Statement

In mining industry for instance, the effluent discharge usually contains arsenic, lead, Copper, Zinc and other heavy metals which holds a very high toxicity level (Celebi and Özdemir, 2014). However, according to standard B of Environmental Quality (Industrial Effluents) Regulation 2009 set by Department of Environmental (DOE), the amount of certain heavy metals for instance Copper and Zinc allowed is not to exceed 1.0 mg/L and 2.0 mg/L respectively, in the industrial effluents discharge. The industries currently applied many types of treatments such as in mechanical and chemical methods to remove the heavy metals. However, those approaches have a few major drawbacks especially in costing, efficiency, environmental impacts and maintenance. Taking pyrometallurgical method for example, it is one of the physical method that still active nowadays and uses a very high heat treatment to recover the metals. Though, the heavy impacts it emits to the environmental become the main disadvantages of this method (Liew, 2008). Instead, one of the most applied chemical method is chemical precipitation using lime as the agent. Despite that, this method requires a large amount of chemicals if want to reduce to an acceptable level of discharge. It also has slow metal precipitation, poor settling, the aggregation of metal precipitates and long term environmental impacts of sludge disposal (Aziz et al., 2008). As an alternative, the application of biological methods has proven to be more effective recently. Such example of biological methods is trickling filter, bio-filter and bacteria and microorganism application. Therefore, this particular project is to conduct an experimental research to investigate the method for heavy metal removal from readily synthetic waste by working with natural microorganisms. This project is also expected to be an eco-friendly method and has a low investment cost.

1.4 Objective

The following is the objective of this research:

1) To study the feasibility of heavy metals removal using sequencing batch reactor (SBR) inoculated with activated sludge originated from palm oil mill.

1.5 Scope of Study

The following are the scopes of this research:

1) To evaluate the heavy metal (Copper and Zinc) removal from synthetically develop waste solution with designated formulation in a sequencing batch reactor.
2) To determine the concentration of heavy metal (Copper and Zinc) extracted from the waste using Atomic Absorption Spectrometry (AAS).
3) To determine the optimum pH for heavy metal (Copper and Zinc) removal.
4) To evaluate the physical and chemical characteristics of activated sludge during the removal period by conducting analysis of Total Suspended Solid (TSS), Volatile Suspended Solid (VSS) and Chemical Oxygen Demand (COD).

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

There are various methods for the removal of heavy metals from wastewater. This section will elaborate and explain some of the methods and reactors that are frequently applied to recover or remove heavy metals in wastewater. The methods are ranging from physical, chemical and biological categories.

2.2 Metal Recovery Related Study

The recovery of metals is widely experimented with the application of many types of methods. According to Jacobsen (2005), two significant methods which are pyrometallurgy and hydrometallurgy have been used extensively by industries and companies as a cycling techniques to recover precious metals from wastewater. **Table 2.1** compares the features of both the metallurgy methods. The hydrometallurgical method includes adsorption by ion exchange resin, solvent extraction and reduction of precious metals precipitate by reagents (Das, 2010). There are also other methods discussed in this part.

Table 2.1: Comparison between pyrometallurgy and hydrometallurgy (*Liew, 2008*)

Parameter	**Pyrometallurgy**	**Hydrometallurgy**
Gas emission	High, varies from CO2, greenhouse gaseous, volatile organic carbon	Low (room temperature) and easy to treat
Dioxin potential	High	No dioxins

Dust potential	High, during material handling and transport	Low, dissolve in solution or taken care off by pollution control equipment
Economics	Huge investment capital and low job creation	High job creation as processes involved are labour intensive
Social acceptance	Low, due to high environmental liabilities	High, cleaner environment with highly effective and mature pollution control methodology
Energy	High (up to 1200°C)	Low (room temperature)
Recovering rate	Low (only fraction of metals), useful non-metals are incinerated and impossible to recover	High recovery, clean separation of material types enable individual effective recovery
Final residue	High. (slag and dusts), potential metal trapped to reduce recovery effectiveness	Low. Only mixed plastics, which could be recycled into engineered plastic
Conditions of work place	Hard conditions around furnaces	Clean condition

2.2.1 Pyrometallurgical Method

A mechanical method applied to recover precious metals in waste. Based on Zaid Ahmad (2015), pyrometallurgical used very high temperatures for thermal treatment of waste that can change physically and chemically. This process includes calcining, roasting, smelting and refining. Calcining involves thermal decomposition of the materials while roasting is a combination of reactions such as solid-gas phases, oxidation, reduction, chlorination, sulfation and pyro-hydrolysis. Other than calcining, smelting also is a process encompassing of thermal reactions. The last process is there to remove the impurities.

One of the pyrometallurgical process implemented is Noranda process as reported by Veldhuizen and Sippel (1994) and shown in **Figure 2.1**, the materials that entered the reactor was immersed with a 1250℃ of molten metal bath. While at it, the materials were churned by a mixture of supercharged air (up to 39% oxygen). Later, the materials will pass through an agitated oxidation zone which will convert impurities into oxides that will become fixed in a silica-based slag. The slag was cooled and milled to recover more metals. Copper matte which containing precious metals was removed and transferred to the converters. The upgrading of the converters has improved the process purity into 99.1%. The other 0.9% contain precious metals along with other recoverable metals.

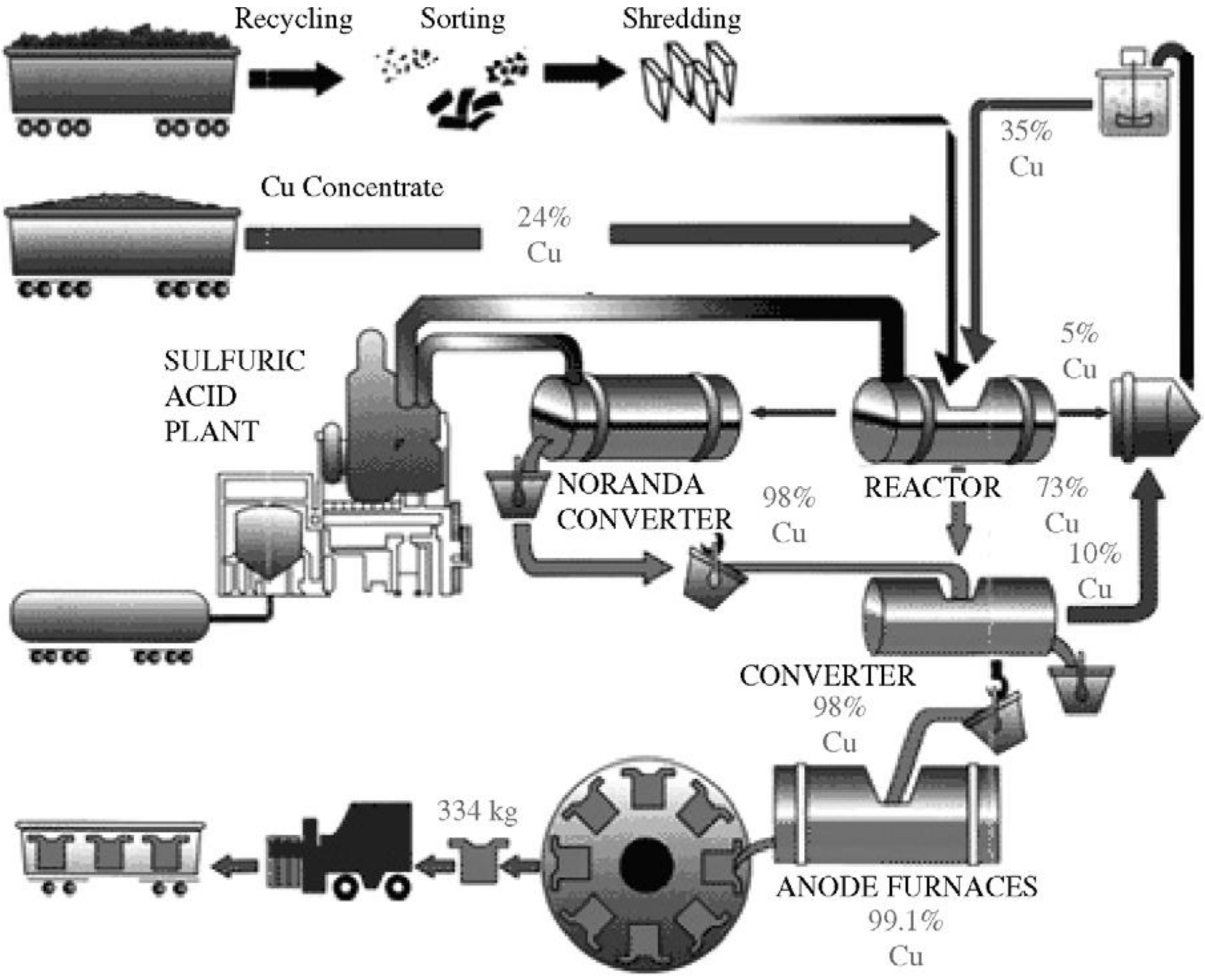


Figure 2.1: Schematic diagram of Noranda process (*Cui and Zhang, 2008*)

As in Makinen et al. (1988), the precious metals are recovered from iron bearing slags which are created in nickel and Copper smelting process. In this experiment, the slags are treated while in reducing conditions in the range of temperatures 1100℃ - 1400℃. Later, the slags will be reduced more by sulphidization and after that, in the form of molten slags, they are cooled under controlled conditions. The precious metals which have been crushed and grinded, become concentrated into iron-based metal phase. Thus, they can later be separated by using magnetic separation.

.

2.2.2 Hydrometallurgical Method

According to Liew (2008), hydrometallurgy is sometimes called as leaching, is involved in the selective dissolution of metals from waste and the usage of aqueous chemicals in low temperature to recover metals. The recovered metals are extracted using electrolysis from the solution. Moreover, this method is used more frequently than pyrometallurgy method because of the precision and cost effective. One of the process of hydrometallurgy is ion exchange resin.

2.2.2.1 Recovery Using Ion Exchanged Resin

Gomes et al. (2001) stated that ion exchange resins have become a major technology in water treatment process even at water drinking level. Particularly, ion exchange resins are used to create water with a standard quality required by many industrial processes. They are practiced in processing water polluted influent to the specific level where it is considered safe and healthy. In addition, ion exchange resins can also be implemented to non-metallic anions and water soluble ionic organic compounds.

Experiment done by Els et al. (1997) reported that pure precious metal salt containing palladium, K_2PdCl_4, was used in a single metal synthetic solution to be extracted by ion exchange resins. Amberlite IRA 900 was a commercially available anion resin was used to absorb the palladium. The resin was first washed with deionized water to remove any foreign ions. Then it was screened to determine the particle size used in this experiment. The resin was soaked in 2M caustic solution for about 10 days. Exposure to the hydroxide during the time in caustic solution will forced all the contaminant ions out of the resin beads. After that exposure, the caustic solution will be poured off. The resin was again washed in a column with deionized water and converted back into the chloride form by feeding 2M HCl. The pH of the resin was 7 for the final rinse with deionized water. In addition, all instruments and containers need to be rinsed with chronic acid and then re-rinsed with deionized water. A required amount of resin was measured out into plastic measuring container and compacted until the bed depth stabilized. The precious metal solutions of known concentration was exposed to various quantity of resin, 10% to 200%. After being sealed in a plastic bottles, they were rolled on a bottle roller for approximately 36 hours. The precious metal concentrations were determined using atomic absorption spectrophotometry. The rate of palladium chloride absorption onto the resin was discovered.

2.2.3 Chemical Precipitation

Ku and Jung (2001) stated that chemical precipitation is effective and one of the most applied process in industry. The reasons are chemical precipitation is relatively simple and inexpensive to operate. In this process, the chemicals will react with the heavy metal ions present, to form insoluble precipitates. That insoluble precipitates are later separated by sedimentation and filtration. Two conventional chemical precipitation processes that are quite known, hydroxide precipitation and sulphide precipitation. **Table 2.2** summarize some of the studies that have applied chemical precipitation methods for heavy metals recovery.

Table 2.2: Heavy metal removal using chemical precipitation method

Species	**Initial metal concentration**	**Precipitant**	**Optimum pH**	**Removal efficiency, (%)**	**Reference**
Cr^{3+}	53.63 mg/L	CaO and MgO	8.0	>99	Guo et al., 2006
Cu^{2+}, Zn^{2+}, Pb^{2+}	0.018, 1.34, 2.3 mM	H_2S	3.0	100, >94, >92	Alvarez et al., 2007
CuEDTA	25, 50, 100 mg/L	1,3,5-hexahydrotriazinedithiocarbamate	3.0	99.0, 99.3, 99.6	Fu et al., 2007
Hg^{2+}	65.6, 188 µg/L	1,3-benzenediamidoethanethiolate	4.7 and 6.4	>99.9	Blue et al., 2008
Cu^{2+}, Zn^{2+}, Cr^{3+}, Pb^{2+}	100 mg/L	CaO	7-11	99.37-99.6	Chen et al., 2009
Zn^{2+}	32 mg/L	CaO	9-10	99-99.3	Gosh et al., 2011
Fe^{2+}, Zn^{2+}, Ni^{2+}	13.79, 1.65, 2.0 mg/L	Alum solution	8, 10, 10	89.58, 92.3, 94.22	Ibigbami et al., 2016

2.2.3.1 Hydroxide Precipitation

Huisman et al. (2006) stated that hydroxide precipitation is the most extensively used chemical precipitation technique. This is due to its simplicity, low cost and ease of pH control. Fu and Wang (2011) also added that the solubility of the various metal hydroxides are minimized in the pH range of 8.0-11.0. Based on the low cost and ease of handling features, lime is more preferred choice compared with other hydroxides in industry (Baltpurvins et al., 1997).

2.2.3.2 Sulphide Precipitation

One of the advantage of using sulphide is that the solubility of the metal sulphide precipitates is dramatically lower than hydroxides. It is also not amphoteric. Therefore, this process can achieve higher degree of metal removal over a broad pH range than hydroxide precipitation. In addition, metal sulphide sludge shows better thickening and dewatering characteristics than metal hydroxide sludge (Fu and Wang, 2011).

2.2.3.3 Chemical Precipitation Combines with Other Method

Reported by Gonzalez-Munoz et al. (2006) that sulphide precipitation is to reuse and recover heavy metal ions and applied nanofiltration as the second step. Surprisingly, the outcome of the experiment is sulphide precipitation has successfully reduced the metal content while nanofiltration produced solutions which capable to directly reused in the plant.

In Ghosh et al. (2011), electro-Fenton process and chemical precipitation (lime precipitation), are used to treat rayon industry wastewater. The processes have reduced approximately 88% of COD and 99% to 99.3% of Zinc removal is accomplished. The pH is in the range of pH 9 to 10.

According to Papadopoulos et al. (2004), ion exchange process is used individually and combines with chemical precipitation to remove nickel from wastewater. With the combination of both treatments, higher removal of nickel at 94.2% to 98.3% is obtained.

2.2.4 Biosorption

Latest development of recovery expertise has shown the beneficial and advantages of biosorption method. Das (2010) said that biosorption is a promising technology for precious metals recovery with the application of microorganisms in the process. Also in Mao et al. (2009), the biosorption method is a metabolism independent process which happen in the cell wall of the microorganism and the mechanism responsible for the uptake of the metals may not be the same by depending on the bacteria type used. Biosorption is consider to be a physico-chemical process. It includes mechanisms such as absorption, adsorption, ion exchange, surface complexation and precipitation (Gadd, 2009)

Research done by Mane et al. (2011) was to remove a heavy metal named selenium, Se, by using pretreated algal biomass, *Spirogyra sp* and *Nostoc*. The biomass was treated with chemical and later used it for the removal of selenium. It was shown in this experiment that the algal can remove a significant amount of selenium when treated physically or chemically compared with the usage of live algae. Also, pretreat the biomass with NaOH indicated an increase on biosorption of selenium by almost in comparison with living biomass. In Wang and Chen (2009) stated that the adsorption capacity of a biomass can be increased by modifying the biomass physically or chemically.

2.3 Biological Method Used for the Recovery of Heavy Metal

Biological method is a very cost effective and eco-friendly process in which it can also be used in situ. With a proper design it can also reduce or eliminate industrial process operations while applicable to be integrated with many systems (Tewari et al., 2005). In **Table 2.3** shows a list of biological methods practice in industries to recover heavy metals.

Table 2.3: Biological methods used by various researches for heavy metals removal

Biological Method
By bacteria and microorganism
Activated sludge process
Bio-filter
Anaerobic digestion
Stabilization pond

2.3.1 By Bacteria and Microorganism

Based on Ilhan et al. (2004), chromium, lead and Copper ions were removed from industrial wastewater by using *Staphylococcus saprophytics*. The research was done to study the effect of varying the pH, temperature and initial concentration on the removal. Later, the optimum pH for the three metals were obtained respectively; 2, 3.5 and 4.5 and also their concentration at that particular pH; 88.66 mg/L, 100 mg/L and 44.94 mg/L. As a conclusion, *Staphylococcus saprophytics* was compatible with mainly lead and chromium based on the result. One of the research conducted by Antunes et al., (2001) stated the usage of *Azolla fliculoides* in the process to extract gold from aqueous solution. The organism was used as the biosorbent instead by considering the cost with other absorbents. The biomass and gold concentration were first adjusted by referring to the respective experiment. The samples were continuously agitated at 200 rpm particularly at room temperature. Then the aliquots were recovered and filtered using cellulose-acetate filters. By using atomic absorption spectrophotometry, the filtrate was analysed for the presence of gold. The gold concentration was discovered rather fast within the first 20 minutes. In this study also indicated the effect of some parameters on the recovery process.

According to Hosea et al., (1986), gold is bound to *Chlorella vulgaris*, an algal species, from aqueous solution. The process was conducted under various condition to suggest its potential as gold recovery method. Gold standards were prepared by dissolving gold powder in aqua regia and then diluted. Standardized $HAuCl_4$ stock solution was used in this experiment. Thiourea (99 + %) was also used and solutions were prepared daily. The algae cells were washed prior to use to minimized the effects of extracellular biomolecules. The cells were then suspended in 0.01 M sulphuric acid. The suspended cells were agitated for a few minutes, centrifuged and later decanted in which this was done twice. pH was adjusted by adding sodium hydroxide or sulphuric acid while the stirring process occur. *Chlorella vulgaris* was suspended to the solution of $HAuCl_4$ while continuously agitated for 15 minutes. Let the mixed solution be centrifuged and analysed for the amount of gold bounded in the algae cells. The concentration of gold was analysed using atomic absorption spectrophotometer with an air-acetylene flame. There was a relative amount of ionic and atomic algal-bound gold present and it was increasingly with time.

2.3.2 Activated Sludge Process

In Hasani et al. (2010), a fixed activated sludge system is used in the operation for the treatment of wastewater pertaining heavy metals compounds; chromium, lead and nickel. This research is conducted with fixed activated sludge (FAS) system. FAS system is a composition of activated sludge system and trickling filter in which media is used to form biofilms in the aeration tank as to enhance the treatment performance. In addition, synthetic solution is prepared prior to the experiment as to provide a wastewater with a COD of 550-600 mg/L. It is shown that, the experiment is conducted by varying the concentration inlet of the heavy metals compounds at 1, 5, 10, 50 and 100 mg/L and later, will produce a different removal efficiency values in the FAS. The increasing of heavy metals concentration has caused the decreasing of COD and MLSS removal efficiency at the beginning of period. However, they start to re-increase the removal efficiency by microorganism which acclimation to changes. In the result, it stated that the COD removal efficiency in the FAS is about 96% and the acclimation period for microorganism is short.

2.3.3 Bio-Filter

Based on Srivastava and Majumder (2008), bio-filters are one of the latest and most favourable development in biological processes in the treatment of heavy metals contaminated wastewater. In this process, microorganism is fixed to a porous medium to breakdown the pollutants which present in the wastewater. The microorganism will grow in a bio-film on the surface of the medium, or suspended in the water phase surrounding the medium particles. Also, the filter bed medium comprises of relatively inert substances. They ensure that the surface attachment area is large and with additional nutrient supply. The overall effectiveness of a bio-filter is mostly controlled by the properties and characteristics of the support medium in which includes porosity, degree of compaction, water retention capabilities and the ability to host microbial populations.

Søgaard et al. (2000) stated that, a research of iron removal through sand filters of three different fresh water plants in Denmark. The water is two biotic and one abiotic. The experiment compared the rate of precipitation of iron in biotic and abiotic conditions. The microorganism used in this study is *Gallionella ferruginea.* It is reported that the biotic iron precipitation is 60 times faster than abiotic precipitation. Also, the biotic sludge is 7-9 times denser than abiotic.

2.3.4 Anaerobic Digestion

In Tyagi et al. (1988), metals are removed by chemical treatment and microbial leaching processes from anaerobically digested sludge. Acid treatment process indicated the chemical treatment, while in the microbial process, *Thiobacillus thiooxidans* and *Thiobacillus ferooxidans* are used and they are autotroph. The study proved that when the sludge solid concentration and pH decrease, the removal of metals increase. The metals that are removed in this experiment are Zinc, lead, Copper and cadmium.

2.3.5 Stabilization Pond

According to Polprasert and Charnpratheep (1989), an experiment is conducted on attached-growth waste stabilization ponds (AGWSP) to investigate the performance of AGWSP units that receives cadmium and chromium shock loadings. The study shows that AGWSP units have less concentration of the applied heavy metals presents in the effluents compared with the effluents in waste stabilization pond (WSP) units which without attached-growth media. The attached-growth media is actually serves as a habitat for the growth of bacteria and algae and therefore, increase the total potential pond biomass. The efficient performance of AGWSP units is because of the adsorption of the applied heavy metals on the attached-growth media.

2.4 Reactor Used for Heavy Metal Recovery

In Schultz et al. (2005) reported the usage of fluidized bed electrochemical reactor (FBE), as shown in **Figure 2.2**, to recover heavy metals from industrial wastewater. Metallic particles are use as cathode in this reactor. As the cathode is a bed of particles and contain a high degree of agitation within the bed, the concentration of polarization is reduced and the possibility to electrowin metals down to parts-per-million concentrations without loss of current efficiency increases. Thus, making the FBE unique for their capacity and operability in many fields of electrochemical technology (Kazdobin et al., 2000). Aside from that, a very good applications are expected especially in extraction metallurgy (Kaminari et al., 2005).

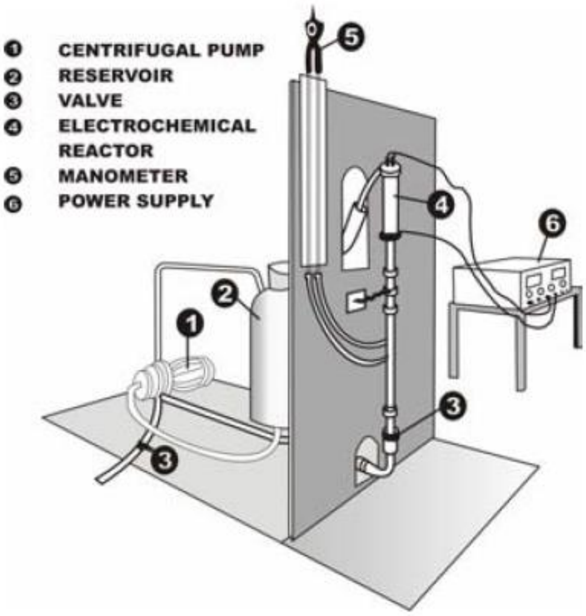


Figure 2.2: Schematic diagram of FEB unit *(Schultz et al., 2005)*

Azizi et al. (2016) reported the usage of modified packed bed biofilm reactor (PBBR) in wastewater industries. This study investigates the efficiency of PBBR in heavy metals removal. PBBR is actually based on attached-growth technology where a biofilm is created on the supporting media. Therefore, the amount of biomass concentration is of vital importance for removing heavy metals from the system. PBBR also focusing on biosorption process to remove heavy metals. **Figure 2.3** shows the schematic diagram of PBBR processing diagram to treat wastewater.

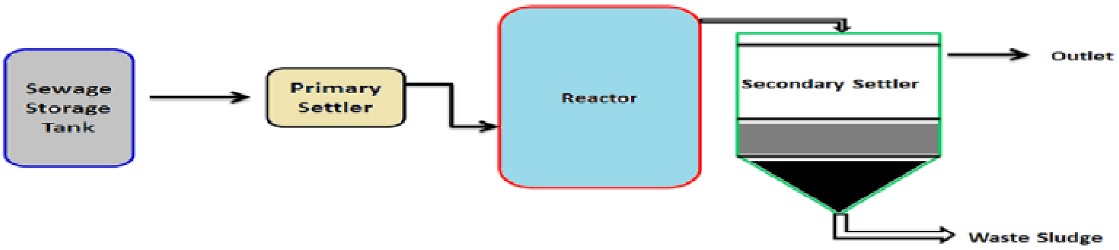


Figure 2.3: Schematic diagram of PBBR *(Azizi et al., 2016)*

2.5 Sequencing Batch Reactor (SBR)

Historically, sequencing batch reactors (SBR) have been around since 1920s according to *Sequencing Batch Reactor Design and Operational Considerations (2005)* and currently, they have been used all over the world to treat both municipal and industrial wastewaters. SBR is also used in areas that have low or varying flow patterns. With the development of technology particularly in aeration devices and computer control systems, the usage of SBR have become a practical choice rather than the conventional activated sludge system. In **Table 2.4** summarize some of the industries or sectors that have involve in the usage of SBR in their wastewater treatments.

Table 2.4: Industry/Sector that practices SBR as wastewater treatment alternatives *(Sequencing Batch Reactor Design and Operational Considerations, 2005)*

Industry/Sector
Municipalities
Resorts
Casinos
Dairy
Pulp and paper
Tanneries
Textiles

According to Vigneswaran (2009), conventional activate sludge systems are space oriented in which the wastewater will flows from one tank into the next on a continuous basis and essentially all tanks have a fixed liquid volume. However, SBR is a time oriented process and system as the flow, energy input and tank volume varying based on some predetermined, periodic operating strategy. In accordance to Irvine et al. (1979), it is best to define SBR as a time oriented, batch process which fall under the category of an unsteady-state activated sludge system.

2.5.1 Practical Use of SBR

Particularly in limited amount of space, SBR is very suitable as the treatment only takes place in a single basin rather than multiple basins. This will indirectly save a lot of space. Less than 10 mg/L of total suspended solid values can be achieved consistently through the usage of decanters in which eliminate the need for clarifier.

The treatment cycle in SBR can be adjusted to suit the specific systems and processes such as to undergo aerobic, anaerobic and anoxic conditions. The purpose of these adjustments are to achieve biological nutrient removal which include nitrification, denitrification and phosphorus removal. Less than 5 mg/L of biochemical oxygen demand (BOD) levels can be attained consistently, while less than 5 mg/L of total nitrogen limits can be accomplished through aerobic conversion of ammonia to nitrates (nitrification) and anoxic conversion of nitrates to nitrogen gas (denitrification) within the similar tank. Also, less than 2 mg/L of phosphorus limits can be achieved over the combination of biological treatment (anaerobic phosphorus-absorbing organisms) and chemical agents (aluminium or iron slats) within the vessel and treatment cycle.

In addition, older wastewater treatment facilities are able to be retrofitted or added to an SBR as the basins are already present. With the cost effective advantage offered by SBR, and the strict permits in wastewater discharging, the need for reconsidering the design process has become very important (*Sequencing Batch Reactor Design and Operational Considerations, 2005)*.

2.5.2 SBR Technology in Wastewater Treatment

Theoretically, SBR system is a set of tanks which operates on a fill and draw basis. In the beginning of the process, the tank will be filled in the discrete period of time and then operated as a batch reactor. Later, the mixed liquor is allowed to settled for some time and the clarified supernatant is drawn from the tank. Obviously, the difference between SBR and conventional continuous flow activated sludge system is SBR conveys functions such as equalization, aeration and sedimentation in a time rather in a space sequence.

The advantage of the time oriented SBR is its flexibility of operation. This is because, the total time in the SBR is used to establish the size of the system. It can also be related to the total volume of a conventional continuous flow facility. Therefore, it can be said that the fraction of time dedicated to a specific function in the SBR is similar and equivalent to some corresponding tank in a space oriented system. In addition, the relative tank volume in conventional activated sludge system is specific and fixed, and also cannot be shared or redistributed as easily as in SBR.

The result of the flexibility of SBR associated with working in time rather than in space, indicated that it can be simulated and operated either as a labour intensive, low energy, high sludge yield systems or as an energy intensive, low labour, low sludge yielding system for essentially the same physical plant. **Table 2.5** describes more on the advantages and disadvantages of SBR.

Table 2.5: Advantage and disadvantage of SBR (*EPA, 1999*)

Advantage	**Disadvantage**
Equalization, primary clarification (in most cases), biological treatment and secondary clarification can be achieved in a single reactor vessel	Higher level of sophistication is required (compared to conventional systems), especially for larger systems, of timing units and controls
Operating flexibility and control	Higher level of maintenance (compared to conventional systems) associated with more sophisticated controls, automated

	switches and automated valves
Minimal footprint	Potential of discharging floating or settled sludge during the draw or decant phase with some SBR configuration
Potential capital cost savings by eliminating clarifiers and other equipment	Potential plugging of aeration devices during selected operating cycles, depending on the aeration system used by manufacturer
	Potential requirement for equalization after the SBR, depending on the downstream processes

2.5.3 Major Phases of SBR Operation Cycle

SBR combine all its processes and treatment steps into a single basin or tank, while conventional facilities depend on multiple basins. An operating cycle of a SBR consists of five unique phases which referred to as; Fill, React, Settle, Draw and Idle phases as shown in **Figure 2.4** (Vigneswaran, 2009). These steps are able and can be altered depending on the operational applications. In **Table 2.6** shows the explanation and description on each phase in a cycle of SBR.

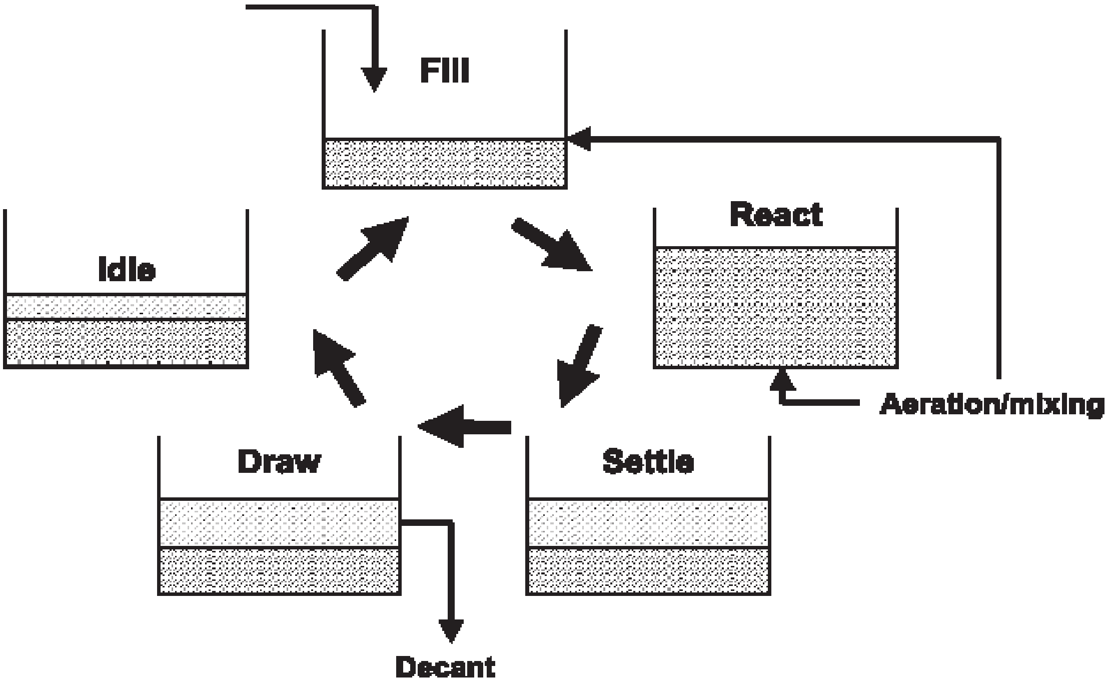


Figure 2.4: SBR cycle diagram *(source: http://www.biodos.org/)*

Table 2.6: SBR phases (*Sequencing Batch Reactor Design and Operational Considerations, 2005)*

Fill		
In this phase, the basin receives influent wastewater which contain food to the microbes in the activated sludge. Theoretically, creating an environment for biochemical reactions to occur. Mixing and aeration can be varied throughout the fill phase in order to create three different scenarios:		
Static Fill	**Mixed Fill**	**Aerated Fill**
There is no mixing or aeration while the influent wastewater is filling the basin or tank. Static fill is applied during the initial start-up phase of a facility, at plants which do not require to nitrify or	Under this scenario, mechanical mixers are active, however, the aerators remain off. A uniform blend of influent wastewater and biomass is created by the mixing action. Anoxic condition	In aerated fill condition, the aerators as well as the mixing units are both active. The contents of the tank are aerated as to convert the anoxic or anaerobic condition to aerobic. No particular

denitrify, and during low-flow periods to save power. This is because the mixers and aerators remain off in which create a scenario of energy-savings components.	occurred since there is no aeration in which promotes denitrification. However, anaerobic condition can also happen throughout the mixed-fill phase. While in anaerobic condition, the biomass will undergo a release of phosphorus and later, reabsorbed by the biomass when aerobic condition is established. (Phosphorus release will not occur in anoxic condition)	adjustment needed to reduce organics and achieve nitrification. However, it is necessary to switch off the oxygen to promote anoxic conditions to achieve denitrification. By regulating the on and off of the oxygen during this phase, with the blowers, oxic and anoxic conditions are created in which allowing for nitrification and denitrification. Throughout this phase, dissolved oxygen (DO) need to be always observed as to prevent it to go over 0.2 mg/L.
React		
During this particular phase, no wastewater will enter the basin or tank and the mechanical mixing and aeration units are on. The rates of organic removal increases dramatically as there are no additional volume and organic loadings. Furthermore, most of carbonaceous BOD removal happens in this phase. In addition, extra nitrification occurs by allowing the mixing and aeration to continue. Most of the denitrification takes place in mixed fill phase. The phosphorus released during mixed fill phase and plus some additional phosphorus is taken up in the react phase.		
Settle		
Activated sludge is allowed to settle while under inactive conditions as there are no flow enters the basin and no aeration and mixing takes place. The activated sludge unconditionally forms flocculent mass while settling. The sludge mass is called		

sludge blanket. This phase is the most critical part of the cycle because, if the solids do not settle quickly, some sludge can be drawn off during the following decant phase in which will degrade effluent quality.
Draw/Decant
A decanter is used to remove the clear supernatant effluent. When the settle phase is completed, a signal is sent to the decanter for the opening of the effluent-discharge valve. There are currently floating and fixed-arm decanters. Floating decanters maintain the inlet orifice slightly below the water surface. It is to minimize the removal of solids in the effluent removed in the decant phase. Floating decanters also offer the operator a flexibility to vary fill and draw volumes. Meanwhile, fixed-arm decanters are less expensive. They can also be designed to allow the operator to lower or raise the level of the decanter.
Idle
A small amount of activated sludge at the bottom of the basin is pumped out which is a process called wasting. The time varies according to the influent flow rate and operating strategy.

2.5.4 Heavy Metal Treatment Using SBR

Chen and Huang (2014) have reported that, SBR is used to recover heavy metals from printed circuit board (PCB) wastewater sludge. In this research, two important parameters are introduced to SBR, sludge solid content and cycle time, to investigate the effects towards the recovery process. Two SBR are applied in this experiment, R1 and R2. In addition, this experiment is operated for 4 cycle times for both of the SBR. **Table 2.7** summarize the parameters used in the process.

Table 2.7: SBR parameter for R1 and R2 *(Chen and Huang, 2014)*

Parameter	**R1**	**R2**
Initial sludge total solid (TS), % (w/v)	0.5, 2.0	0.5, 1.0, 3.0, 5.0
Cycle, day	20	10
Feeding, h	0.5	0.5
Aeration, h	478	238
Sludge settling, h	1	1
Decanting, h	0.5	0.5
Agitation, rpm	200	200
Air flow rate, L/min	4.0	4.0
Temperature, °C	55	55

2.6 Effect of pH on Metal Ion Formation/Precipitation in Solution

pH according to Helmenstine (2017) is a measure of hydrogen ion concentration in a solution. It is also known as a measure of acidity or alkalinity of a solution. An aqueous solution with 25°C and pH less than seven are acidic, while those with pH greater than seven are basic or alkaline. A "neutral" is when the pH is at 7.0 because of the equal concentration of H_3O^+ and OH^- as in pure water.

The formation or precipitation of metal ions can clearly be affected by pH as reported in Albrecht et al., (2011). However, the understanding regarding this information is currently very limited despite many industrial and laboratorial processes being conducted under variable conditions.

In Albrecht et al., (2011), it is stated that Cu^{2+} and Zn^{2+} ions increase in acidic conditions, while in alkaline medium, solid $Cu(OH)_2$ or $Zn(OH)_2$ colloidal particles are formed. **Figure 2.5** and **Figure 2.6** display the diagram of Copper and Zinc concentration against a variation of pH.

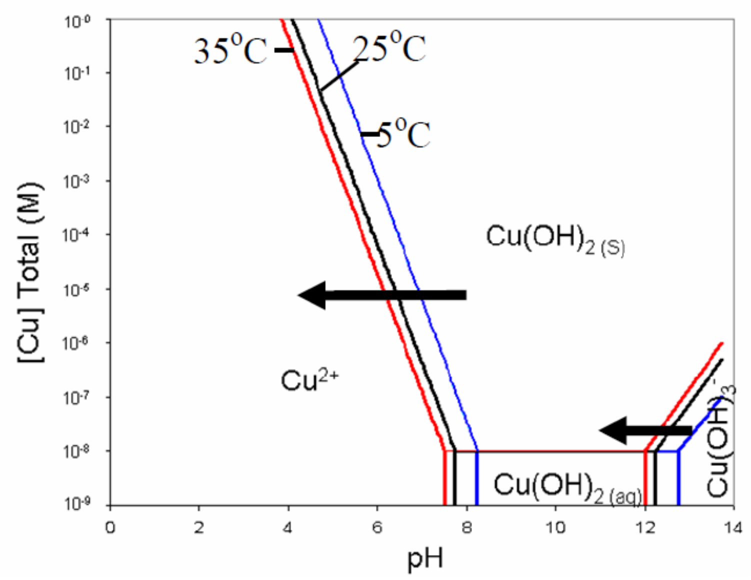


Figure 2.5: Diagram of Copper concentration against pH (*Albrecht et al., 2011*)

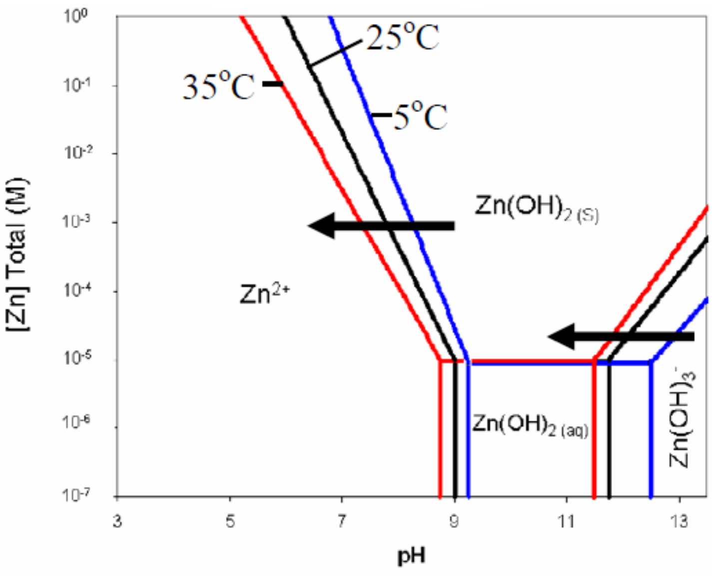


Figure 2.6: Diagram of Zinc concentration against pH (*Albrecht et al., 2011*)

As shown in both **Figure 2.5** and **2.6**, concentration and temperature can determine the state of the metals Copper and Zinc at different pH either in ion, solid or aqueous. At 25°C, with low Copper concentration and ow pH values up to 7.5, the Cu^{2+} ion is the dominant species. Then at higher pH values up to pH 12.3, $Cu(OH)_2$ is the dominant species. Above that pH, $Cu(OH)_3^-$ ion is formed. The case is almost similar with metal Zinc but at a different range of pH as shown in **Figure 2.6**.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This section discusses about all the materials and methods involve in this study. There is also a deliberation pertaining the equipment used in this experiments including the analysis required.

3.2 Overall Workflow

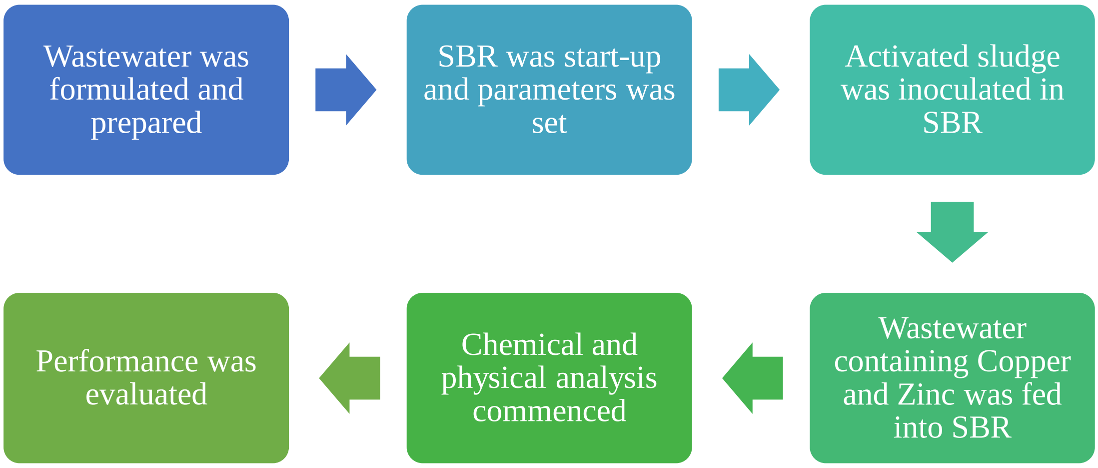


Figure 3.1: The flow diagram of the removal process

Based on **Figure 3.1**, the synthetic wastewater was first needed to be formulated and prepared. It was according to Martín-Hernández et al. (2009) with specific amount required to formulate the wastewater. Then, the SBR settings and parameters were input before starting up the experiment and later, activated sludge was inoculated into the SBR. The SBR cycle was set at 24 hours as referred in **Table 3.1**. A certain range of pH of the synthetic wastewater feeding was manipulated in this study to observe the influence of pH for Copper and Zinc removal. The data is displayed as in **Table 3.2**.

To determine the concentrations of metals consumed in the sludge, Atomic Absorption Spectrometry (AAS) was used by referring to *Multi-Elemental Determination of Metals by Atomic Absorption Spectroscopy*. The Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) were measured by adopting on *Standard Operating Procedure for: Total Suspended Solids Determination Using Vacuum Filtration and Oven Drying* (Jacobs, 2010). The pH was determined to ascertain the efficiency of heavy metals removal. It was done according to *Test Method for the Determination of pH Value of Water or Soil by pH Meter, Geotechnical Engineering Bureau (2015).* Chemical Oxygen Demand (COD) was also measured by following *HACH* written procedure.

Table 3.1: SBR cycle configuration applied in this study

Process	**Period**
Influent feeding	4 minutes
Aeration	23 hours 45 minutes
Settling	10 minutes
Effluent withdrawal	2 minutes
Total cycle	**24 hours**

Table 3.2: pH of the synthetic wastewater feeding during the experimental period

Day	**pH**
1 - 36	6.0 – 8.0
37 – 48	2.0 – 3.0
49 – 64	6.0 – 8.0
65 – 71	9.0 – 10.0
>72	6.0 – 8.0

Figure 3.2: SBR system employed in this study

3.3 Material

3.3.1 Inoculum

Activated sludge from palm oil mill plant in Lepar was utilised as inoculum in this study.

3.3.2 Synthetic Wastewater

Synthetic wastewater was formulated based on Martín-Hernández et al. (2009). The compositions of the chemical used is tabulated in **Table 3.3** below;

Table 3.3: List of chemicals used for synthetic wastewater formulation (*Martín-Hernández et al., 2009*)

Chemical name	Concentration, mg/L
Monopotassium phosphate	41
Calcium chloride	88
Urea	12
Ammonium chloride	106
Yeast extract	2
Sodium chloride	176
Magnesium sulphate hexahydrate	198
Iron (II) sulphate	4
Zinc sulphate heptahydrate	4
Copper (II) sulphate pentahydrate	2
Boric acid	0.02

3.3.3 List of Equipment

a) Sequencing Batch Reactor (SBR)
b) Atomic Absorption Spectrometry (AAS)
c) pH meter
d) COD Digestion Reactor/Spectrophotometer, HACH DR/2400 @ DR/2800
e) Oven
f) Furnace

3.4 Method/Tool

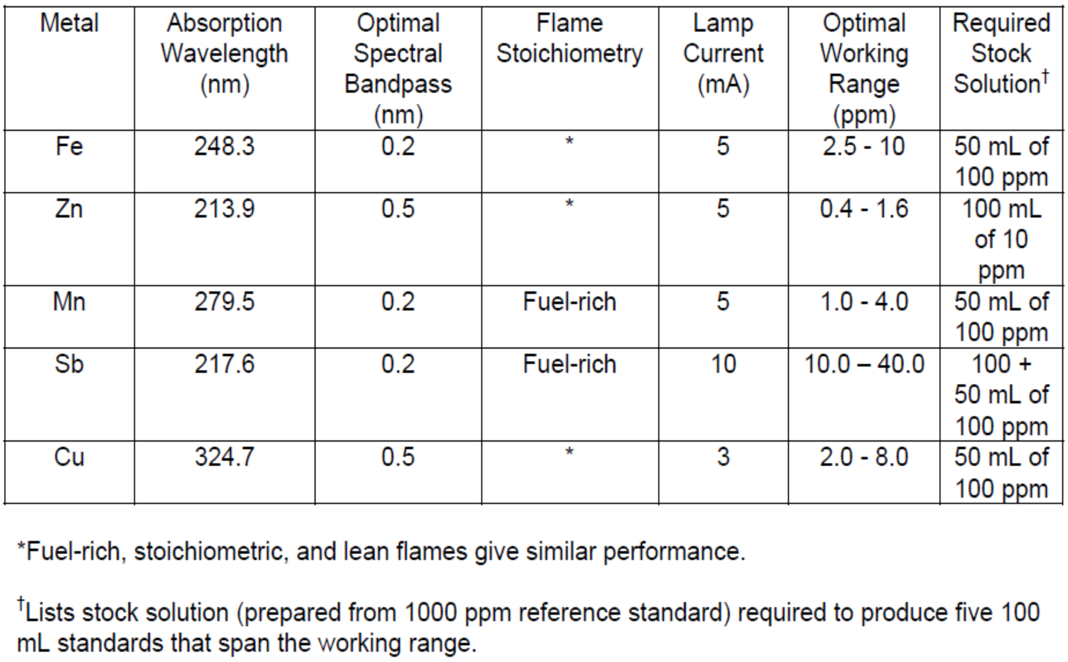
3.4.1 Metal Concentration Determination

The concentration of dissolved metals was measured by using Atomic Absorption Spectrometry (AAS). The method was referred from *Multi-Elemental Determination of Metals by Atomic Absorption Spectroscopy* and the procedure is showed in **Table 3.4**.

Table 3.4: Procedure for using AAS to measure metals concentrations (*Multi-Elemental Determination of Metals by Atomic Absorption Spectroscopy*)

Step	Procedure/Description
1	The sample was digested by dissolving approximately 0.10 g (weigh accurately) in a mixture of 25 mL of concentrated nitric acid and 10 mL of concentrated sulphuric acid in a beaker (done this operation in fume hood).
2	The sample was heated (a watch glass was put over the beaker) in order to get all the material to dissolved.
3	Once dissolved, the mixture was added slowly to ~50 mL of distilled water in a 100 mL volumetric flask. The beaker was rinsed several times to ensure that all material was transferred. Diluted to the mark with distilled water.
4	Standard curve was prepared by referring to the wanted metals. Referred **Figure 3.3**.
5	Using the provided 1000 ppm reference AAS standards, five calibration solutions were prepared that span the working ranges in **Figure 3.3** for the elements to be studied. 100 mL volumetric flasks were used for these standards and all dilutions were performed with volumetric pipettes. The solvent for all dilutions were 2% (v/v)

	nitric acid.
6	The instrument was zero while aspirating 2% (v/v) nitric acid into the flame. Each standard was aspirated into the flame and the absorbance was recorded; repeated three times.
7	With identical instrument settings as for preparing the calibration in Step 1 to 3, the sample was aspirated and the absorbance was recorded for each metal. The measurement was repeated three times. (Note that serial dilutions may be required to obtain an absorbance reading within the working range for each metal).
8	The exact concentrations of the calibration standards were calculated.



Metal	Absorption Wavelength (nm)	Optimal Spectral Bandpass (nm)	Flame Stoichiometry	Lamp Current (mA)	Optimal Working Range (ppm)	Required Stock Solution†
Fe	248.3	0.2	*	5	2.5 - 10	50 mL of 100 ppm
Zn	213.9	0.5	*	5	0.4 - 1.6	100 mL of 10 ppm
Mn	279.5	0.2	Fuel-rich	5	1.0 - 4.0	50 mL of 100 ppm
Sb	217.6	0.2	Fuel-rich	10	10.0 – 40.0	100 + 50 mL of 100 ppm
Cu	324.7	0.5	*	3	2.0 - 8.0	50 mL of 100 ppm

*Fuel-rich, stoichiometric, and lean flames give similar performance.

†Lists stock solution (prepared from 1000 ppm reference standard) required to produce five 100 mL standards that span the working range.

Figure 3.3: Information for several metals that can be measured quantitatively with AAS and atomization by an air-acetylene flame (*Multi-Elemental Determination of Metals by Atomic Absorption Spectroscopy*)

3.4.2 TSS and VSS Analysis

The procedure to determine the Total Suspended Solid (TSS) was referred from *Standard Operating Procedure for: Total Suspended Solids Determination Using Vacuum Filtration and Oven Drying* (Jacobs, 2010). **Table 3.5** explains the steps required to conduct the analysis.

Table 3.5: Procedure for TSS analysis (*Jacobs, 2010*)

Step	Procedure/Description
1	Filters were washed and dried for this analysis prior to use by rinsing the filter with deionized water three times while vacuum was applied through the vacuum filtration assembly. Then placed in a drying oven with a temperature of 110℃ to 115℃ for a minimum of one hour.
2	The weight of the pre-washed and prepped filter was recorded.
3	The sample collection time, pH and temperature recorded.
4	Filtering apparatus were assembled by placing the filter paper in a cleaned Buchner funnel and suction began.
5	The filter was wet with a small volume of reagent-grade water to seat it.
6	The sample container was shook, stirred or mix in order to obtain a more uniform (preferable homogenous) aliquot for analysis.
7	Immediately after homogenizing the sample, the required aliquot was collected in a clean graduated cylinder. The sample aliquot volume was recorded.

8	The volume was slowly poured onto the seated glass microfiber filter until the entire aliquot had been filtered. If residue lingers in the cylinder, the cylinder was rinsed with deionized water and this rinse water was filtered.
9	After filtering the sample aliquot, the Buchner funnel was washed clean of any residual sample with deionized water, allowing completely drainage between washings and suction was continued until filtration was complete.
10	Filter from filtration apparatus was carefully removed using forceps, tweezers or tongs, and transferred to an aluminium or glass weighing dish as support.
11	Filters were dried for a minimum of 1 hour at 110°C to 115°C in drying oven.
12	After drying was complete, the filters were reweighed and the dry filter and residue weight were recorded.
13	The following calculation was used to determine TSS; $TSS\left(\frac{mg}{L}\right) = \frac{(Residue + filter)(mg) - Filter\ (mg)}{Sample\ filtered\ (mL)} \times 1000(\frac{mL}{L})$

3.4.3 pH Measurement

pH was also determined in this experiment. This parameter is important to determine the efficiency of the heavy metal removal at different pH.The method was based on *Test Method for the Determination of pH Value of Water or Soil by pH Meter, Geotechnical Engineering Bureau (2015)* and has been explained further in **Table 3.6**.

Table 3.6: Method for using pH meter on soil (*Geotechnical Engineering Bureau, 2015*)

Step	Procedure/Description
1	The material was separated on the ¼ in. (6.3 mm) sieve. Only the minus ¼ in. (6.3 mm) material was to be used for testing.
2	30±0.1 g of soil was weighed and placed into the glass beaker.
3	30±0.1 g of distilled water was added to the soil sample. Stirred to obtain a soil slurry and then covered with watch glass.
4	The sample stood for a minimum of one hour, was stirred every 10 to 15 minutes. This was to allow the pH of the soil slurry to stabilize.
5	After one hour, the temperature of the sample was stabilized. The temperature of the sample was measured and the temperature controller of the pH meter was adjusted to that of the sample temperature. This adjustment was done just prior to testing.
6	The pH meter was standardized by means of the standard solutions provided.
7	Immediately before immersing the electrode into the sample, the sample was

	stirred well with a glass rod. The electrode was placed into the soil slurry solution and gently turned beaker to make good contact between the solution and the electrode. Do not place electrode into the soil; only into the soil slurry solution.
8	The electrode was required an immersion 30 seconds or longer in the sample before reading to allow the meter to stabilize. If the meter has an auto read system, it will automatically signal when stabilized.
9	The pH value was read and recorded to the nearest tenth of a whole number. If the pH meter reads to the hundredth place, a round off rule was applied as followed: If the hundredth place digit was less than 5, leave the tenth place digit as was. If it was greater than 5, round the tenth place digit up one unit. If the hun-dredth place digit equals 5, round the tenth place digit to the nearest even number.
10	The electrode was rinsed well with distilled water, then dabbed lightly with tissues to remove any film formed on the electrode. Caution: Do not wipe the electrodes as this may result in polarization of the electrode and consequent slow response.

3.4.4 Chemical Oxygen Demand (COD) Analysis

Chemical oxygen demand (COD) was used as a measure of oxygen requirement of a sample that was susceptible to oxidation by strong chemical oxidant. The mg/L COD results were defined as the mg of O_2 consumed per liter of sample under conditions of this procedure. The method to analyze COD is as **Table 3.7**.

Table 3.7: Method for COD analysis (*HACH*)

Step	**Procedure/Description**
1	The COD Reactor was turned on. Preheated to 150°C. The safety shield was placed in front of the reactor.
2	The caps from two COD Digestion Reagent Vials were removed. *Be sure to use vials for the appropriate range.
3	Held one vial at a 45-degree angle. A clean volumetric pipette was used to add 2.00 mL of sample to the vial. This was the prepared sample.
4	Held a second vial at a 45-degree angle. A clean volumetric pipette was used to add 2.00 mL de-ionized water to the vial. This was **the blank**.
5	The vials were capped tightly. Rinsed them with de-ionized water and wiped with a clean paper towel.
6	The vials were held by the cap over a sink. Gently inverted several times to mix. The vials were placed in the preheated COD Reactor. *The sample vials will become very hot during mixing.
7	The vials were heated for two hours.

8	The reactor off was turned off. Waited about 20 minutes for the vials to cool to 120°C or less.
9	Each vial was inverted several times while still warm. The vials were placed into a rack and cooled to room temperature.
10	**Hach Programs** was touched. Program **430 COD LR** (Low Range) was selected. **Start** was touched.
11	The outside of the vials were cleaned with a damp towel followed by a dry one to remove fingerprints or other marks.
12	The 16-mm adapter was installed. The blank was placed into the adapter. **Zero** was touched. The display will show: **0 mg/L COD**.
13	When the timer beeps, the sample vial was placed into the adapter. **Read** was touched. Results will appear in mg/L COD.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Introduction

All the data and findings during the experiment are discussed in this section. The results are presented in graph for easier understanding.

4.2 Heavy Metals Concentration Analysis

4.2.1 Copper Concentration Analysis

The experiment was conducted for almost 70 days in the SBR inoculated with activated sludge. In **Figure 4.1**, the removal efficiency of Copper showed a rather high percentage of removal, 80% to 95%, during Day 1 until Day 19, at pH 6 - 8. From day 20 until day-50, the pH of the feeding was changed to between 2 and 3. During this period, the result indicates the percentage removal was dropped significantly to, 0.1% from 60%. From Day 50 onwards, the percentage inconsistently fluctuated at the range of 13% to 84%. During this time, the pH was manipulated twice in the period of Day 49 – 64 (pH 6 – 8) and Day 65 – 71 (pH 9 – 10). Later after Day 72, the pH was reverted back to 6 – 8. The changes of pH can also be referred in **Figure 4.3** and **Table 3.2**.

One of the possible reason for the decreasing and inconsistent removal efficiency was because of a problem occur during Day 19, to the SBR. The problem has caused some of the activated sludge inside the SBR washed out from the reactor during the effluent discharge, can be referred in **Figure 4.5**, which led to the decreasing of microorganism inside the SBR. This has led to the low consumption of Copper which can be proven with the low removal efficiency trend lines in the graphs. Since there was very little biomass in the reactor, the reactor was re-inoculated at day-42. After that day, the consumptions or removal was gradually increased, although there were still some inconsistencies in the pattern.

To further explained the result, on the effect of pH manipulation towards the removal of Copper. As stated in Albrecht et al., (2011), with the Copper concentration at 1.3×10^{-3}M, differences of pH can affect the state of Copper whether in ion, solid or aqueous. In this case, the abnormally high influent and effluent concentrations during Day 37 – Day 48 and low removal efficiency for Copper was because of the decreasing of pH from range 6.0 – 8.0, to pH 2.0 – 3.0, shown in **Figure 4.3** and **Table 3.2**. As the pH was decreased to 2.0 – 3.0, the state of Copper was mostly in ion, Cu^{2+}, though explained the high concentration of Copper found in the influent during that time. However, the high concentration of effluent might be due to the microorganism in the activated sludge cannot cope with the heavy acidic condition of the influent, thus reduce the removal efficiency of Copper.

The sudden change of pH whether 2.0 – 30 or 9.0 – 10.0, can affect the ability of microorganism to consume metals as they probably were more to the neutral environment (Thompson, 2017). They might need more time to adjust towards the new environment if other pH was applied.

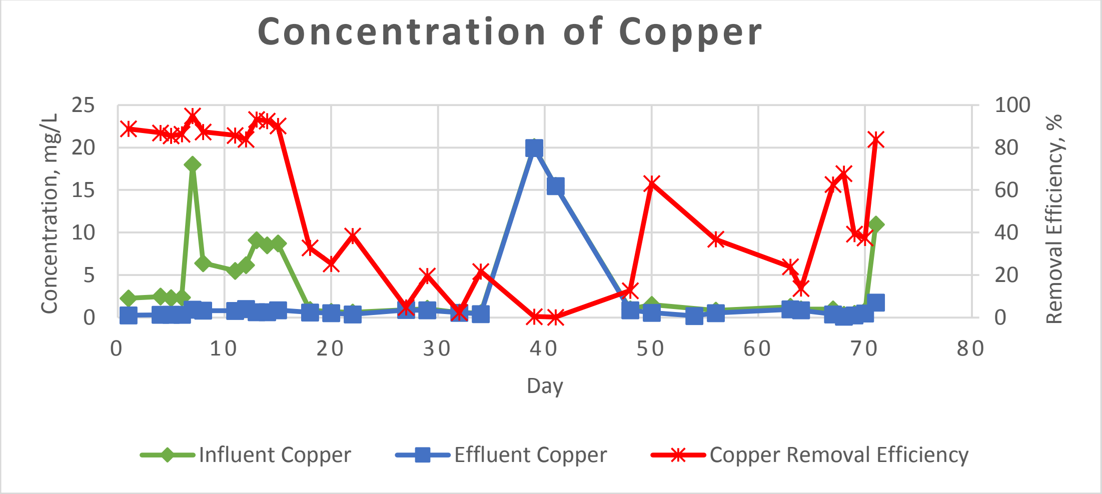


Figure 4.1: Result of Copper concentration in effluent

4.2.2 Zinc Concentration Analysis

In **Figure 4.2**, the removal efficiency of Zinc indicates a similar trend with Copper removal during Day 1 until Day 19, which was around 69% to 97% removal efficiency at pH 6 – 8. Until Day 50, the pH was changed to 2 – 3, and showed the removal was decreased from 60% to 1%. After Day 50 onwards, the pH was manipulated as stated in part 4.2.1 above. The percentage during these time was 13% to 87% removal.

Similar situation has occurred during Day 19, as mentioned in part 4.2.1, where a problem emerged to the SBR. This situation has led to some sludge was washed out during the effluent discharged. The effect of Zinc concentration at different pH was studied by Albrecht et al., (2011) where the state of Zinc; ion, solid or aqueous, depends on the pH and concentration used. As in this experiment, at $5.5 \times 10^{-4}\mathrm{M}$ of Zinc concentration and pH 2.0 – 3.0, most of the Zinc were in ion state, which explained the high concentration found in the effluent during that pH period.

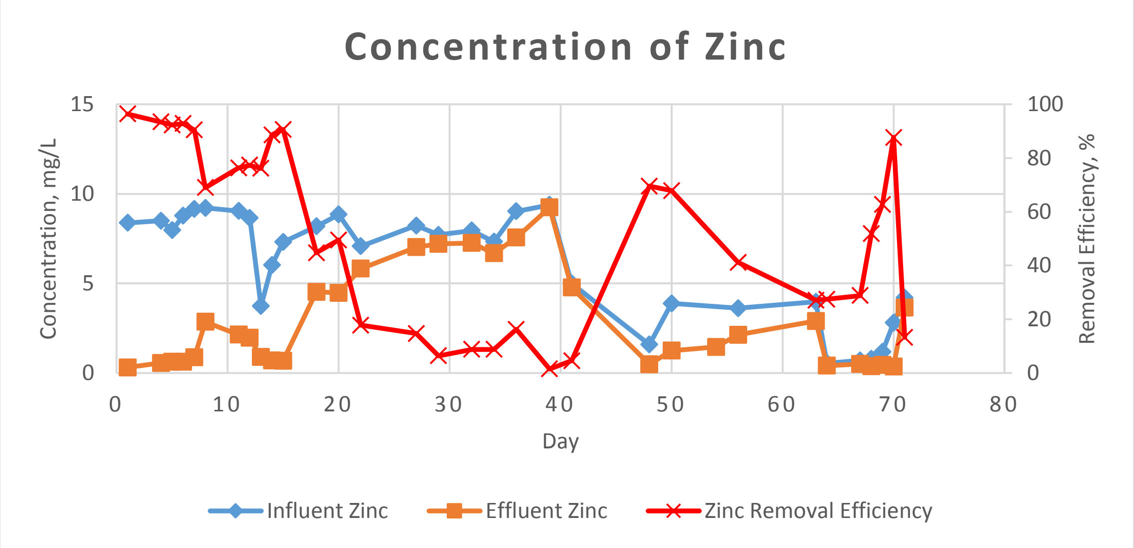


Figure 4.2: Result of Zinc concentration in effluent

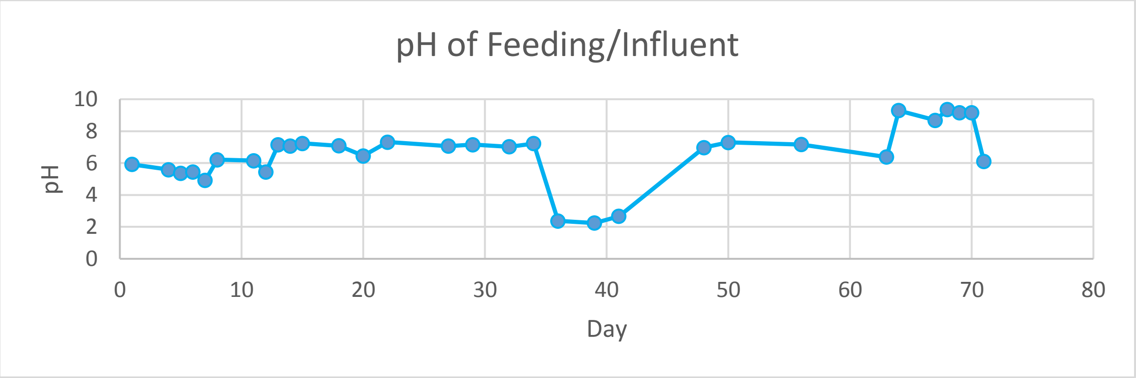


Figure 4.3: Result of pH in influent

4.3 Chemical Oxygen Demand (COD)

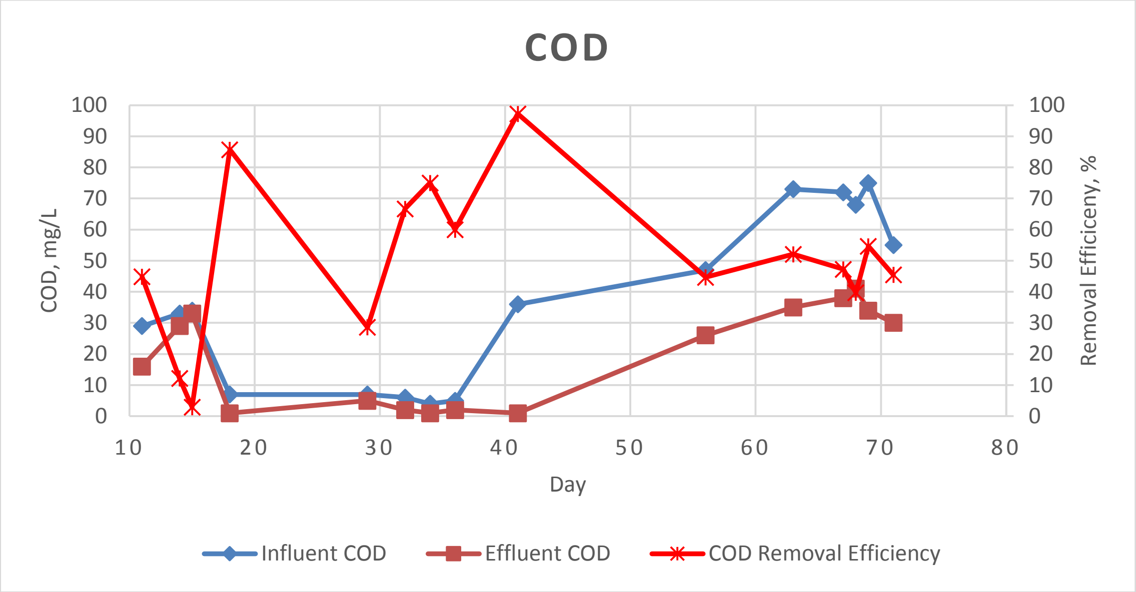


Figure 4.4: Result of COD in the effluent

Based on **Figure 4.4**, the Chemical Oxygen Demand (COD) of the influent and effluent of SBR was analyzed. The result showed that the influent always had higher COD values compared to the effluent and also had a high removal efficiency. The influent which had been treated in SBR and later became effluent had decreasing COD values indicated that the treatment was rather successful. Furthermore, according to standard B of Environmental Quality (Industrial Effluents) Regulation 2009 set by Department of Environmental (DOE), the lowest COD value allowed is 200 mg/L which already been complied in this experiment. The highest removal efficiency was during Day 42 with about 97% of removal occur and the lowest reached was 2% at Day 15.

4.4 Total Suspended Solid (TSS) and Volatile Suspended Solid (VSS)

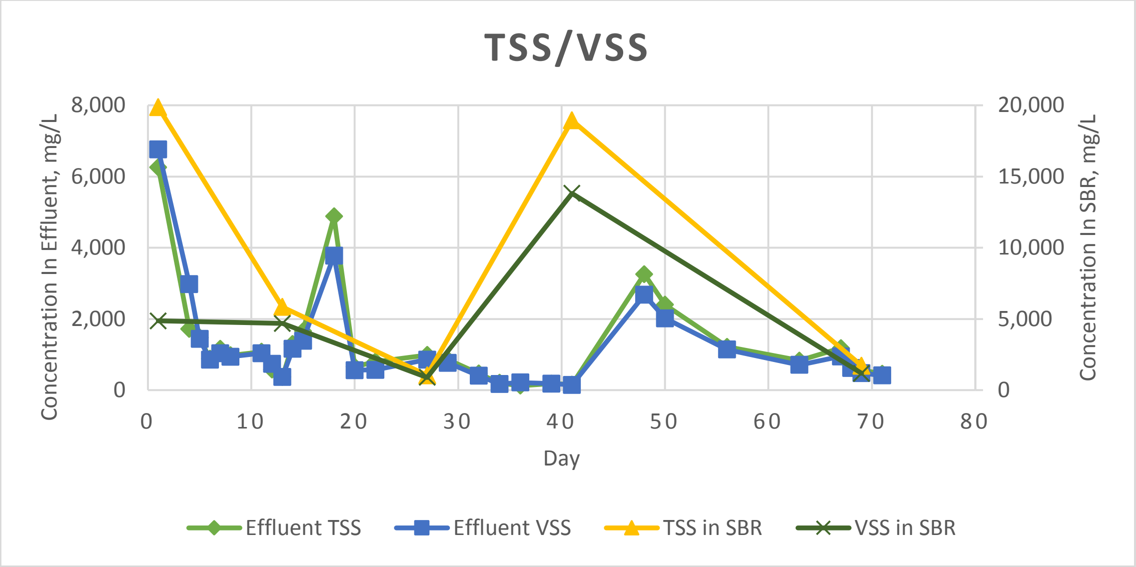


Figure 4.5: Result of TSS/VSS in the effluent and SBR

Figure 4.5 shows the concentration of solids in the effluent discharge and also in the SBR. Though the concentration of solid in the SBR was only analysed gradually unlike the solids in the effluent. The results indicated that the Total Suspended Solid (TSS) and Volatile Suspended Solid (VSS) in the effluent and SBR gradually decreasing. This experiment needed about 50 days for the solids concentration to comply with the standard B of Environmental Quality (Industrial Effluents) Regulation 2009 set by Department of Environmental (DOE), which is 100 mg/L.

However, during Day 19, the disruption of SBR's malfunction had led to some amount of activated sludge in the SBR to be washed out. Thus, reducing the concentration of solids inside the SBR. In Day 42, the activated sludge was re-inoculated again as the amount has been reduced in which a re-inoculation was necessary so that the experiment can be continued.

4.5 Adsorption Test

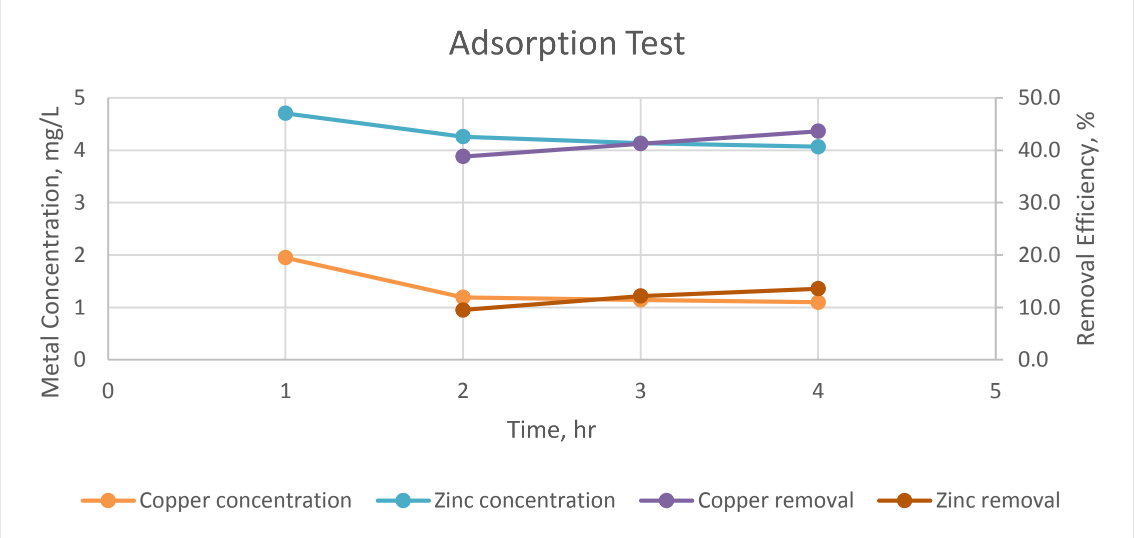


Figure 4.6: Result of adsorption test

A simple experiment toconfirm the hypothesis of which was the agent (microorganism or activated sludge) that consumed or adsorbed the heavy metals in this experiment, a simple adsorption test was done. The sludge was first treated with 5 mM of hydrogen peroxide to eliminate the microorganism in it. Then, four samples were analysed after period of 1 hour. The result gained is shown in **Figure 4.6**.

Initially, the concentration of Copper and Zinc were 1.949 mg/L and 4.705 mg/L respectively, and gradually decreasing after each sample analysing at each hour. Moreover, the removal of both metals constantly increase at each hour, indicating the decreasing of metals concentration in the sludge. At the last hour, the removal of Copper was 43.6% and Zinc 13.6% compared to the first 1 hour, Copper 38.8% and Zinc 9.5%.

Based on this result, adsorption by activated sludge to reduce the heavy metals concentration is possible. In relation to the results that were obtained in this study, biological removal and adsorption could be the processes involved during the treatment. It can be observed that about 10% and 40% for Zinc and Copper, respectively.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion, the results have shown that the removal of Copper and Zinc metals by using SBR inoculated with activated sludge is feasible and achievable. The range of pH, 6.0 – 8.0 is chosen as the best condition for the influent feeding which can reach up to 95% for Copper and 97% for Zinc removal. COD analysis also showed a positive result with 97% as the highest COD removal efficiency and 2% as the lowest. Solids concentration also displayed a positive result in which more time was required to gradually decrease the concentration. In addition, the adsorption test has shown the relation of the performance of activated sludge to adsorb Copper and Zinc. In all, the objective of this experiment is successfully achieved.

5.2 Recommendation

To improve this study, it is first recommended to run this experiment in a very long period of time rather than a month or two. After all, the usage of SBR itself need more time because time is the main operation of SBR. Next is, with the no time limitation, the pH manipulation can also be applied with great consideration so that the microorganism can slowly and consistently adapt to the changing pH (if applied). With that, there might be a better result after carefully consider the microorganism condition.

REFERENCES

Albrecht, T. W. J., Addai-Mensah, J., & Fornasiero, D. (2011). Effect of pH, concentration and temperature on Copper and Zinc hydroxide formation/precipitation in solution. *Chemeca 2011: Engineering a Better World: Sydney Hilton Hotel, NSW, Australia, 18-21 September 2011*, 2100.

Ammen, C.W. (1984) *Recovery and Refining of Precious Metals*. Van Nostrand Reinhold, Toronto, Canada.

Antunes, A. P. M., Watkins, G. M., & Duncan, J. R. (2001). Batch studies on the removal of gold (III) from aqueous solution by *Azolla filiculoides*. *Biotechnology letters*, *23*(4), 249-251.

Azizi, S., Kamika, I., & Tekere, M. (2016). Evaluation of Heavy Metal Removal from Wastewater in a Modified Packed Bed Biofilm Reactor. *PloS one*, *11*(5), e0155462.

Babel, S., Kurniawan, T.A., (2003). Various treatment technologies to remove arsenic and mercury from contaminated groundwater: an overview. In: Proceedings of the First International Symposium on Southeast Asian Water Environment, Bangkok, Thailand, 24‑25 October, pp. 433‑440.

Babel, S., & Kurniawan, T. A. (2004). Cr (VI) removal from synthetic wastewater using coconut shell charcoal and commercial activated carbon modified with oxidizing agents and/or chitosan. Chemosphere, 54(7), 951-967.

Baltpurvins, K. A., Burns, R. C., Lawrance, G. A., & Stuart, A. D. (1997). Effect of electrolyte composition on Zinc hydroxide precipitation by lime. *Water Research*, *31*(5), 973-980.

Barakat, M. A. (2011). New trends in removing heavy metals from industrial wastewater. Arabian Journal of Chemistry, 4(4), 361-377.

Celebi, A., & Özdemir, S. (2014). Mining wastewater management and its effects on groundwater and ecosystems. *Water Science and Technology*, *70*(9), 1481-1487.

Chen, S. Y., & Huang, Q. Y. (2014). Heavy metals recovery from printed circuit board industry wastewater sludge by thermophilic bioleaching process. *Journal of Chemical Technology and Biotechnology*, *89*(1), 158-164.

Cui, J., & Zhang, L. (2008). Metallurgical recovery of metals from electronic waste: A review. Journal of hazardous materials, 158(2), 228-256.

Das, N. (2010). Recovery of precious metals through biosorption—a review. *Hydrometallurgy*, *103*(1), 180-189.

Dhokpande, S. R., & Kaware, J. P. (2013). Biological methods for heavy metal removal—A review. *International Journal of Engineering Science and Innovative Technology*, *2*(5), 304-309.

Dobson, R.S., Burgess, J.E., 2007. Biological treatment of precious metal refinery wastewater: a review. Minerals Engineering 20, 519–532.

Els, E. R., Lorenzen, L., & Aldrich, C. (1997). The recovery of palladium with the use of ion exchange resins. Minerals engineering, 10(10), 1177-1181.

EPA. (1999). Wastewater Technology Fact Sheet. Sequencing Batch Reactors. United States.

Fu, F., & Wang, Q. (2011). Removal of heavy metal ions from wastewaters: a review. *Journal of environmental management*, *92*(3), 407-418.

Gadd, G. M. (2009). Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. *Journal of Chemical Technology and Biotechnology, 84*(1), 13-28.

Geotechnical Engineering Bureau. (2015). Geotechnical test method: test method for determination of pH value of water or soil by pH meter. GTM-24. State of New York. Department of Transportation.

Ghosh, P., Samanta, A. N., & Ray, S. (2011). Reduction of COD and removal of Zn 2+ from rayon industry wastewater by combined electro-Fenton treatment and chemical precipitation. *Desalination*, *266*(1), 213-217.

Gomes, C. P., Almeida, M. F., & Loureiro, J. M. (2001). Gold recovery with ion exchange used resins. Separation and Purification Technology, 24(1), 35-57.

Gonzalez-Munoz, M. J., Rodriguez, M. A., Luque, S., & Alvarez, J. R. (2006). Recovery of heavy metals from metal industry waste waters by chemical precipitation and nanofiltration. *Desalination*, *200*(1), 742-744.

Hasani, A., Hosseinzadeh, I., & Torabifar, B. (2010). Investigation of Using Fixed Activated Sludge System for Removing Heavy Metals (Cr, Ni and Pb) from Industrial Wastewater.

Helmenstine, A. M. (2017). pH Definition in Chemistry. Retrieved May 20, 2017 from https://www.thoughtco.com

Hosea, M., Greene, B., Mcpherson, R., Henzl, M., Alexander, M. D., & Darnall, D. W. (1986). Accumulation of elemental gold on the alga Chlorella vulgaris. *Inorganica Chimica Acta*, *123*(3), 161-165.

Huisman, J. L., Schouten, G., & Schultz, C. (2006). Biologically produced sulphide for purification of process streams, effluent treatment and recovery of metals in the metal and mining industry. *Hydrometallurgy*, *83*(1), 106-113.

Ibigbami, T.B., Dawodu, F.A., & Akinyeye, O.J. (2016). Removal of Heavy Metals from Pharmaceutical Industrial Wastewater Effluent by Combination of Adsorption and Chemical Precipitation Methods. *American Journal of Applied Chemistry*. Vol. 4, No. 1, 2016, pp. 24-32.

Igwe, J., & Abia, A. A. (2006). A bioseparation process for removing heavy metals from waste water using biosorbents. *African Journal of Biotechnology*, *5*(11).

Ilhan, S., Nourbakhsh, M. N., Kiliçarslan, S., & Ozdag, H. (2004). Removal of chromium, lead and Copper ions from industrial waste waters by Staphylococcus saprophyticus. *Turkish Electronic Journal of Biotechnology*,*2*, 50-57.

Irvine, R. L., Fox, T. P., & Richter, R. O. (1977). Investigation of fill and batch periods of sequencing batch biological reactors. *Water Research*, *11*(8), 713-717.

Irvine, R. L., Miller, G., & Bhamrah, A. S. (1979). Sequencing batch treatment of wastewaters in rural areas. *Journal (Water Pollution Control Federation)*, 244-254.

Jacobs Engineering Group, Inc. (2010. Standard operating procedure for: total suspended solids determination using vacuum filtration and oven drying. TVA-KIF-SOP-49. *Tennessee Valley Authority*.

Jacobsen, R.T., (2005). Removing contaminants from spent catalysts. Chemical Engineering Progress 101, 20–23.

Kaminari, N. M. S., Ponte, M. J. J. S., Ponte, H. A., & Neto, A. C. (2005). Study of the operational parameters involved in designing a particle bed reactor for the removal of lead from industrial wastewater—central composite design methodology. *Chemical Engineering Journal*, *105*(3), 111-115.

Kazdobin, K., Shvab, N., & Tsapakh, S. (2000). Scaling-up of fluidized-bed electrochemical reactors. *Chemical Engineering Journal*, *79*(3), 203-209.

Ku, Y., & Jung, I. L. (2001). Photocatalytic reduction of Cr (VI) in aqueous solutions by UV irradiation with the presence of titanium dioxide. *Water Research*, *35*(1), 135-142.

Kurniawan, T. A., Chan, G. Y., Lo, W. H., & Babel, S. (2006). Physico–chemical treatment techniques for wastewater laden with heavy metals. *Chemical engineering journal*, *118*(1), 83-98.

Liew, F.C. (2008). Pyrometallurgy Versus Hydrometallurgy. Singapore, *TES-AMM. Engineering Department.*

Makinen, J. K., Murden, K. B., & Saari, H. P. (1988). U.S. Patent No. 4,717,419. Washington, DC: U.S. Patent and Trademark Office.

Mane, P. C., Bhosle, A. B., Jangam, C. M., & Vishwakarma, C. V. (2011). Bioadsorption of selenium by pretreated algal biomass. *Advances in applied science research*, *2*(2), 202-207.

Mao, J., Won, S. W., Vijayaraghavan, K., & Yun, Y. S. (2009). Surface modification of Corynebacterium glutamicum for enhanced Reactive Red 4 biosorption. *Bioresource technology*, *100*(3), 1463-1466.

Martín-Hernández, M., Carrera, J., Pérez, J., & Suárez-Ojeda, M. E. (2009). Enrichment of a K-strategist microbial population able to biodegrade p-nitrophenol in a sequencing batch reactor. *Water research*, *43*(15), 3871-3883.

Papadopoulos, A., Fatta, D., Parperis, K., Mentzis, A., Haralambous, K. J., & Loizidou, M. (2004). Nickel uptake from a wastewater stream produced in a metal finishing industry by combination of ion-exchange and precipitation methods. *Separation and Purification Technology, 39*(3), 181-188.

Polprasert, C., & Charnpratheep, K. (1989). Heavy metal removal in attached-growth waste stabilization ponds. *Water Research*, *23*(5), 625-631.

Schultz, D. R., Ponte, M. J. J. D. S., Kaminari, N. M. S., & Ponte, H. A. (2005). Heavy Metals Recovery from Industrial Wastewater Using Fluidized Bed Electrochemical Reactor. In *Proceedings of the COBEM 2005: 18 th International Congress of Mechanical Engineering*.

Sheng, P. P., & Etsell, T. H. (2007). Recovery of gold from computer circuit board scrap using aqua regia. *Waste management & research*, *25*(4), 380-383.

Søgaard, E. G., Medenwaldt, R., & Abraham-Peskir, J. V. (2000). Conditions and rates of biotic and abiotic iron precipitation in selected Danish freshwater plants and microscopic analysis of precipitate morphology. *Water Research*, *34*(10), 2675-2682.

Srivastava, N. K., & Majumder, C. B. (2008). Novel biofiltration methods for the treatment of heavy metals from industrial wastewater. *Journal of hazardous materials*, *151*(1), 1-8.

Tewari, N., Vasudevan, P., & Guha, B. K. (2005). Study on biosorption of Cr (VI) by Mucor hiemalis. *Biochemical Engineering Journal*, *23*(2), 185-192.

Thompson, N. (2017). Four Condition for Bacterial Growth. Retrieved May 20, 2017 from http://www.livestrong.com

Tyagi, R. D., Couillard, D., & Tran, F. (1988). Heavy metals removal from anaerobically digested sludge by chemical and microbiological methods. *Environmental Pollution*, *50*(4), 295-316.

Veldhuizen, H., & Sippel, B. (1994). Mining discarded electronics. Industry and Environment, 17(3), 7-11.

Vigneswaran, S., Sundaravadivel, M., & Chaudhary, D. S. (2009). Sequencing Batch Reactors: Principles, Design/Operation and Case Studies. *Waste Water Treatment Technologies-Volume II*, 24.

Wang, J., & Chen, C. (2009). Biosorbents for heavy metals removal and their future. *Biotechnology advances*, *27*(2), 195-226.

Water, N. E. I. (2005). Sequencing Batch Reactor Design and Operational Considerations.

Willner, J., Kadukova, J., Fornalczyk, A., & Saternus, M. (2015). Biohydrometallurgical methods for metals recovery from waste materials. *Metalurgija*, *54*(1), 255-258.

Zaid Ahmad, H., Khan, A., Raza, A., Noman, H. & Mushtaq, A. (2015). Final Report (CPT – II Mini Project). Extraction of Metals from Electronic Waste. Lahore. COMSATS Institute of Information Technology.