REMOVAL OF CHLORIDE FROM RARE EARTH WASTEWATER INDUSTRIAL USING BIOREMEDIATION HYBRID WITH ELECTROCOAGULATION SYSTEM

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2018

ACKNOWLEDGEMENT

Alhamdulillah, finally we manage to complete this research “STUDY THE IPTIMUM PARAMETER IN CHLORIDE REMOVAL FROM RARE EARTH WASTEWATER INDUSTRIAL USING BIOREMEDIATION HYBRID WITH ELECTROCOAGULATION SYSTEM”. I am so blessed to Allah as He gave me tremendous courage, strength and spirit while facing all the obstacles while completing this research. Firstly, I would like to thank my parents, my sibling, wife and my family. I pray and wish all of them always in a good health and been cherished by Allah immortality, AMIN.

I am also indebted to my research members, which contributed an ideas and times in completing this research. They offered me his wisdom and expertise throughout the course of the project in order for me to understand concept of the research. I am grateful as they always put in a lot of effort in order to help me in any way they could to ensure this research can be done smoothly. Thank you for your support and brilliant ideas.

To my students, Faten Ahada, Mariah, Tahfiz and Asmaa’ Asilah, whose directly and indirectly contributed for this research. I appreciate all of your time in completing this research. Thank you very much.

ABSTRACT

Chloride (Cl^-) is a major anion found in all natural waters. It occurs naturally and is also a relatively minor contaminant. Currently, a larger amount chloride (Cl^-) in wastewater was generated from rare earth industrial. Chloride is non-toxic to humans, however, it can bring harmful to some plants and aquatic. There is needed for treatment to remove chloride from wastewater before discharged to river or water bodies. Furthermore, chloride is also a very corrosive agent, and elevated levels pose a threat to infrastructure, such as road beds, bridges, and industrial pipes. The effect caused by these hazardous pollutants and growing concerns to environmental issues led to remove chloride concentration from rare earth wastewater by using bioremediation hybrid with electrocoagulation system. The application of yeast in the wastewater treatment has potential in the treatment and reuse of wastes containing solids and high concentrations of salt, fat and antibiotics. However, Electrocoagulation is a novel method in wastewater treatment especially in chloride removal and this emerging technology combines the functions and advantage of conventional methods such as coagulation, flotation, and electrochemistry in water and wastewater treatment. The treated rare earth wastewater was tested for its chloride (Cl^-) concentration to determine the percentage of reduction by measured using spectrophotometer. Results shows S. cerivisiae cells grew and adapted well under condition 10 g/L NaCl in suitable nutrient medium. Yeast was able to growth in standard (10 hr), synthetic chloride (6 hr) and actual wastewater (6 hr) with OD increased from 0.8 to 2.4, 0.8 to 1.2 and 0.4 to 0.6 respectively. Besides that, the optimum yeast able to growth in standard pH 6 at first 9 hours with OD increased from 1.1 to 2.1. Thus, the samples directly treat by using electrocoagulation system. The result shows ferum plate able to remove chloride concentration which is 75.0 % removal at 5 minute and 2 Ampere. The information obtained from this study is useful for scale up purpose in the rare earth industry that choose bioremediation hybrid with electrocoagulation system method to remove chloride concentration from rare earth wastewater.

ABSTRAK

Klorida (Cl^-) adalah anion utama yang terdapat di semua perairan semulajadi. Ia berlaku secara semulajadi dan juga merupakan bahan pencemar kecil. Pada masa kini, sejumlah besar klorida (Cl) dalam air sisa dihasilkan dari industri nadir bumi. Klorida tidak memberi kesan toksik kepada manusia, tetapi ia boleh mendatangkan bahaya kepada beberapa tumbuhan dan akuatik. Terdapat rawatan yang diperlukan untuk membuang klorida daripada air sisa sebelum dilepaskan ke sungai atau perairan. Tambahan pula, klorida juga merupakan agen yang sangat menghakis, dan berpotensi tinggi dalam menimbulkan ancaman kepada infrastruktur, seperti besi jalan, jambatan, dan paip perindustrian. Kesan yang disebabkan oleh bahan pencemar berbahaya ini dan kebimbangan yang semakin meningkat terhadap isu-isu alam sekitar dalam penyingkiran kepekatan klorida daripada air sisa nadir bumi dengan menggunakan bioremediasi hibrid dengan sistem electrocoagulation. Aplikasi yis dalam rawatan air sisa mempunyai potensi dalam rawatan dan penggunaan semula bahan buangan yang mengandungi pepejal dan kepekatan garam, lemak dan antibiotik yang tinggi. Walau bagaimanapun, Electrocoagulation adalah kaedah baru dalam rawatan air sisa terutamanya dalam penyingkiran klorida dan teknologi baru ini menggabungkan fungsi dan kelebihan kaedah konvensional seperti pembekuan, pengapungan, dan elektrokimia dalam rawatan air dan air sisa. Air sisa nadir bumi yang telah dirawat, kepekatan klorida (Cl^-) akan diuji untuk menentukan peratusan pengurangan dengan menggunakan spektrofotometer. Keputusan menunjukkan sel *S. cerivisiae* tumbuh dan hidup dengan baik dalam keadaan 10 g/L NaCl dalam medium nutrien yang sesuai. Yis boleh membesar dalam standard (10 jam), sintetik klorida (6 jam) dan air sisa sebenar (6 jam) dengan OD meningkat dari 0.8 hingga 2.4, 0.8 hingga 1.2 dan 0.4 hingga 0.6, masing-masing. Selain itu, optimum kemampuan pertumbuhan yis dalam standard pH 6 pada awal 9 jam dengan OD meningkat daripada 1.1 hingga 2.1. Oleh itu, sampel terus langsung dirawat dengan menggunakan sistem electrocoagulation. keputusan menunjukkan plat ferum dapat mengyingkirkan kepekatan klorida iaitu 75.0% penyingkiran dalam masa 5 minit dan 2 Ampere. Maklumat yang diperoleh dari kajian

ini berguna untuk tujuan skala di industri nadir bumi yang memilih kaedah bioremediasi hibrid dengan sistem elektrokoagulasi untuk mengyingkirkan kepekatan klorida daripada air sisa nadir bumi.

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Cl	Chloride
Hr	Hour
Min	Minutes
s	Second
OD	Optical Density
nm	nanometer
μL	micro liters
mL	mili liter
μ	micro (1.0×10^{-6})
g	gram
kg	kilogram
ppm	part per million
ABS	Absorbance
COD	Chemical Oxygen Demand
DOE	Department of Environment
EC	Electrocoagulation
LAMP	Lynas Advanced Materials Plant
MAREC	Malaysian Rare Earth Corporation
RRE	Rare Earth Elements
TSS	Total Suspended Solid
YEPD	Yeast, Peptone, & Dextrose

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

In Malaysia, there were two rare earths processing plants namely Asian Rare Earth (ARE) and the Malaysian Rare Earth Corporation Plant (MAREC) in Perak, which were operated until 1992 and were subsequently closed due to problems pertaining to disposal of large amount of radioactive waste. Recently, Lynas Advanced Materials Plant (LAMP) has become one of the largest rare earths processing plant in the world producing rare earth elements (REE) in Gebeng, Pahang (Kuan, et al., 2016). Rare Earth Elements (RRE) are often used in industry for the production of glass additives, fluorescent materials, catalyst, ceramics, lighters, superconductors, magnets or condenser (Andrès, et al., 2003). The use of rare earth elements in various technologies continues to grow despite some alternatives being found for particular uses. Hence, the wastewater produced from the separation process of rare-earth elements usually contains high concentrations of chloride. It is known that substantial quantities of chloride in water can induce problems related to the toxicity to aquatic organisms.

Chlorides are the natural substances which are found in the water bodies in varying amounts. However, their concentrations are significantly low. Chlorides are present in all potable water supplies and in sewage, usually as a metallic salt. However in the industrial, domestic and agricultural wastewaters that are generated from the human society may contain large amount of chlorides, which can cause significant disruption in the ecological balance. High chloride concentrations in water are not known to have toxic effects on humans, although large amounts may act corrosively on metal pipes and may be harmful to plant life. Chloride in water may be considerably increased by treatment processes in which chlorine or chloride is use. Waste water containing chloride ion which is discharged from landfill is high concentrated saline solution, and is has caused the corrosion of waste pipe, the scale block, agriculture wreck of crops by damage from salt.

1.2 Motivation and Problem Statement

The quality of water resources becomes worse day by day due to the continuous addition of undesirable chemicals in water. The presence of chloride in water resulting from the industrial activities is also another concern (Rusyniak et al., 2010). Wastewater is very hard to treat due to the chloride compound are typically very complex and non-biodegradable. Basically, these effluents has high chloride concentration which are produced from mining, mineral processing, metallurgical operations, and paint manufacturing (Aeisyah et al, 2014). It is seriously harmful to natural aquatic environment when wastewater containing high chloride concentration is released without prior pre-treatment.

Proper treatment of chloride removal is possible by using adsorption method. Adsorption has been proposed as an alternative treatment procedure. One of the fundamental requirements of adsorbent is its ability to be regenerated and reused over a number of adsorption cycles since this is both economical and environmentally friendly (Turhanen et al., 2015). One potential process in biological treatment such as halophilic bacteria, yeasts could be a reasonable approach for treatment of high salinity wastewater. The application of *Saccharomyces cerevisiae* or yeasts in the wastewater treatment has potential in the treatment and reuse of wastes containing solids and high concentrations of salt, fat and antibiotics. However, electrocoagulation is a novel method in wastewater treatment and this emerging technology combines the functions and advantages of conventional methods such as coagulation, flotation and electrochemistry in water and wastewater treatment. Thus, in this research aims to study the efficiency chloride removal by using bioremediation hybrid with electrocoagulation system from rare earth industrial wastewater. Besides that, this study also hoped to provide treatment alternatives and to widen the varieties for treatment of wastewater in the rare earth industry.

1.3 Objective

The following are the objectives of this research:

1. To study wastewater quality in rare earth industrial wastewater.
2. To study best parameter condition at pH 7, speed at 100 rpm and temperature at 37 °C for yeast growth.
3. To reduce the chloride concentration in rare earth industrial wastewater.
4. To determine the optimum operation parameters in chloride removal using yeast hybrid with electrocoagulation system.

1.4 Scopes of this research

The following are the scopes of this research:

i. To study wastewater quality of rare earth industrial based on preliminary testing by using pH, Chemical Oxygen Demand (COD), salinity, and Total Suspended Solid (TSS).
ii. To investigate best parameter condition for yeast growth study based on pH, speed, and temperature.
iii. To analyses chloride concentration in rare earth wastewater industrial by using yeast hybrid with electrocoagulation system.
iv. To analyses best operation parameters study based on time, voltage and current in chloride removal using yeast hybrid with electrocoagulation system.

1.5 Main contribution of this work

Based on the scope above, the research should be able to remove the chloride at higher percentage at optimum condition. It is also important to reduce the chloride level in the rare earth industrial wastewater.

1.6 Organization of this thesis

The general outline of this report is as follows:

Chapter 1 provides a background of the study. This chapter will give a brief explanation about rare earth wastewater treatment containing chloride that is harmful to aquatic and environment. *Saccharomyces cerevisiae* is one example of organism use in biological treatment hybrid with electrocoagulation process to reduce chloride concentration from rare earth wastewater. Next, the objectives and scope of this research are also mentioned here. Chapter 1 ends with the outline of this report.

Chapter 2 is literature review which first part explaining about the chloride in wastewater. This chapter covers the review of wastewater treatment method which are include physical and biological treatment. This chapter also presents general method treatment for chloride removal in wastewater via bioremediation process using yeast. The overview of the electrocoagulation process also states in this chapter, which are important in understanding their process that emerging technology combination. This chapter ends with the summary of the literature review.

Chapter 3 is explaining materials and methodology of the experiment. This chapter gives information about list of material, equipment and instruments used in this study. Methodology involves growth of chloride analysis, bioremediation treatment using yeast which is containing preparation and growth of yeast cell and also yeast in shake flask. Lastly, it is describe about electrocoagulation process. This chapter ends with the summary of materials and methods used.

This study continous with Chapter 4 presents the results and discussions. The subtopics that will be discussed on the characteristic rare earth wastewater, growth parameters for *Saccharomyces cerevisiae* and electrocoagulation for chloride removal Last but not least, Chapter 5 presents about the conclusion and recommendation of this study.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter describe the overview of the chloride in wastewater, wastewater treatment method and also bioremediation process using yeast. Besides that, this chapter also had an overview of the electrocoagulation process which is novel method in wastewater treatment and this emerging technology combination.

2.2 Chloride in Wastewater

In developing country, contamination in water sources because of industrialization such as textile, coal, food, leather, pharmaceutical, and dye industry, pesticides in agriculture, forestry and aquaculture drugs, municipal wastewater and global changes give huge impact to population (Zhou et al., 2015; Yagub et al., 2014). Water pollution is a great concern nowadays since water constitutes a basic necessity in life and used for living purpose. Chloride is one of contaminations in water that mostly pronounced than other pollutants especially when it is exposed to the ecosystem (Aeisyah, et al., 2014). The chloride commonly present in the wastewater is persistent and non-biodegradable in nature (Tripathi, & Rawat Ranjan, 2015). Chloride is categorized as a pollutant for many reasons. Chloride is necessary for water habitats to thrive, yet high levels of chloride can have negative effects on an ecosystem. Chloride may impact freshwater organisms and plants by altering reproduction rates, increasing species mortality, and changing the characteristics of the entire local ecosystem. Moreover, almost of chloride are highly toxic when their concentration exceeds their permissible limit in the ecosystems (Aeisyah, et al., 2014).

2.3 Wastewater Treatment Method

Chemicals are used during wastewater treatment in an assortment of processes to expedite disinfection. These chemical processes, which induce chemical reactions, are called chemical unit processes, and are used alongside biological and physical cleaning processes to achieve various water standards. There are several distinct chemical unit processes, including chemical coagulation, chemical precipitation, chemical oxidation and advanced oxidation, ion exchange, and chemical neutralization and stabilization, which can be applied to wastewater during cleaning. Physical methods of wastewater treatment accomplish removal of substances by use of naturally occurring forces, such as gravity, electrical attraction, and van der Waal forces, as well as by use of physical barriers.

In general, the mechanisms involved in physical treatment do not result in changes in chemical structure of the target substances. In some cases, physical state is changed, as in vaporization, and often dispersed substances are caused to agglomerate, as happens during filtration. Biological treatment methods use microorganisms, mostly bacteria, in the biochemical decomposition of wastewaters to stable end products. More microorganisms, or sludge, are formed and a portion of waste is converted to carbon dioxide, water and other end products. Generally, biological treatment methods can be divided into aerobic and anaerobic methods, based on availability of dissolved oxygen.

2.4 Bioremediation using *Saccharomyces cerevisiae* (Yeast)

In biological treatment such as halophilic bacteria, yeasts could be a reasonable approach for treatment of high salinity wastewater. *Saccharomyces cerevisiae* or yeasts are eucaryotic, heterotrophic, unicellular microorganisms with a variety of shapes ranging from spherical to egg-shaped (common shape) and ellipsoidal, and from cylindrical to considerably elongated and even filamentous (mycelium).Yeasts have a complex internal structure. The vegetative budding yeast cell, in the log growth phase, contains a very large vacuole and has rigid walls. Yeast produce in vary of size which typically measure between 3 – 8 µM in diameter as show in **Figure 2-1**. Mostly,

saccharomyces cerevisiae used in scientific research, baking, and fermentation (Held, 2010)

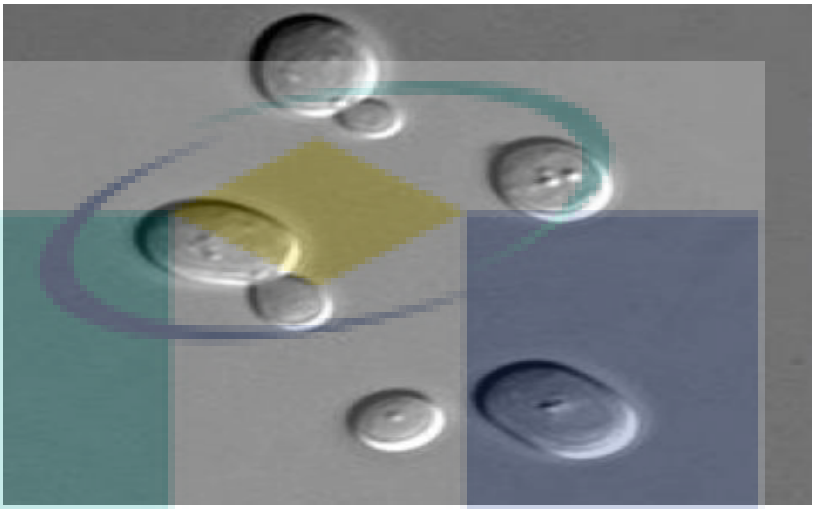


Figure 2-1: *Saccharomyces cerevisiae* (Held, 2010)

Saccharomyces cerevisiae or baker's yeast can be grown in either liquid medium or on the surface of solid agar plate. Presence of yeast extract and bactopeptone in the reagents contributed the yeast cells grow much more rapidly (Bergman, 2001). Besides that, yeast also producing the carbon dioxide (CO_2) and responsible for aerated structure which is not limited to produce gas (Rezaei, et al., 2014). Yeast consumes glucose as it carbon source and emits ethanol as waste. **Equation 1** below is showed the fermentation of glucose to ethanol and **Equation 2** is showed the reaction occurs when high of glucose concentration reacts with oxygen (Boyd, 1985).

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + Energy \qquad \textbf{Eq 1}$$

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 2CO_2 + Energy \qquad \textbf{Eq 2}$$

The application of yeast in the wastewater treatment has potential in the treatment and reuse of wastes containing solids and high concentrations of salt, fat and antibiotics. Yeasts were utilized for treatment of wastewater, and the recovered excess sludge could be reused (Dan, et al., 2002). Glycerol is the main important for maintaining osmotic equilibrium in yeast cells (Moreno-Garcia, et al., 2015) and yeast also used in treating high organic and salinity of wastewater (Dan, et al., 2002). The

optimum pH and concentration of Sodium chloride (NaCl) for yeast growth better between concentrations of 0-30 g/L but high concentration of NaCl greater than 60 g/L prevented growth of yeast at pH 5.6 (Marshall, & Odame-Darkwah, 1995). It also same with the medium chain fatty acid (C_6, C_8 and C_{10}) where prevent the growth of yeast and some bacteria (Moreno-Garcia, et al., 2015).

Yeasts can grow in temperatures ranging from 0 ^{o}C to 47 ^{o}C. The optimum temperature for most yeast is 20 ^{o}C to 30 ^{o}C. It is noted here that osmophilic yeasts are cable of growing in high osmotic pressure habitats such as high concentrations of salt or sugar which restrict the availability of moisture. On the other hand, yeasts can grow in a wide pH range (from 2.2 to 8.0). In general, yeasts grow well on media with acid reactions (3.8-4.0), whereas optimum pH values for bacteria growth range from 7.5 to 8.5. Katayama-Hirayama et al. (1994) cultured the yeast to treat the wastewater. The cultures were propagated in shaking flasks and incubated at 30 ^{o}C and completely degraded after 3 days. **Figure 2-2** showed yeast was grown and harvesting at different points (label A to G). Critical phase for sugar in the medium gradually declined at the first three harvest point (A, B, C) where it was the exponential growth phase for yeast. Yeast cell reacts with the available sugar into CO_2 and ethanol during the exponential growth phase. At point D is diauxic shift phase and at point E is in the early post-diauxic growth phase. Lastly, harvest points F and G were early and late stationary phase (Rezaei, et al., 2014).

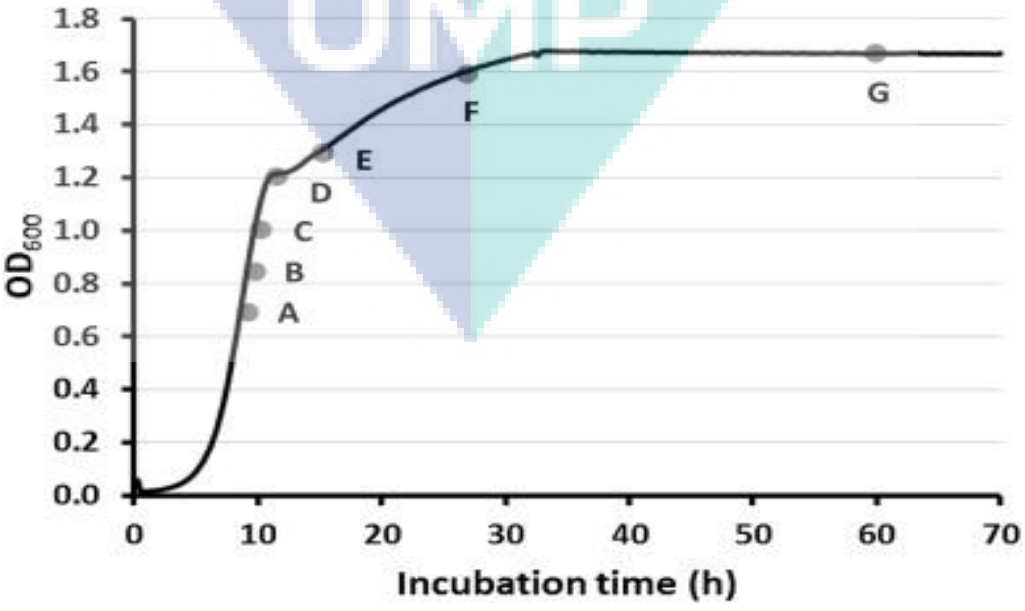


Figure 2-2: Growth curve of *Saccharomyces cerevisiae* and harvesting at different point for 72 hours (Rezaei, et al., 2014)

Figure 2-3 is showed growth of yeast cell has four main phases which are the lag phase, the exponential phase, the stationary phase and death phase. Firstly, the lag phase refers as the initial growth phase of yeast after the medium have been inoculated. The nutrient concentration, pH, temperature from the inoculating medium to the fresh medium and size of inoculating loop influences the length of the lag phase. For example, richer concentration of nutrient gives a larger concentration of metabolizing enzymes. Exponential growth will start when yeast cell used to their new environment at the end of the lag phase. Besides that, the number growth of yeast cell become multiply rapidly and the cell mass doubles regularly with time. Then, stationary phase is occurring when level of nutrient rapidly decrease and can no longer support the growth of yeast which it is remain constant. Lastly, due to nutrient depletion and toxic products in wastewater, the growth of yeast cannot survive the population and the death phase will begin (Boyd, 1985).

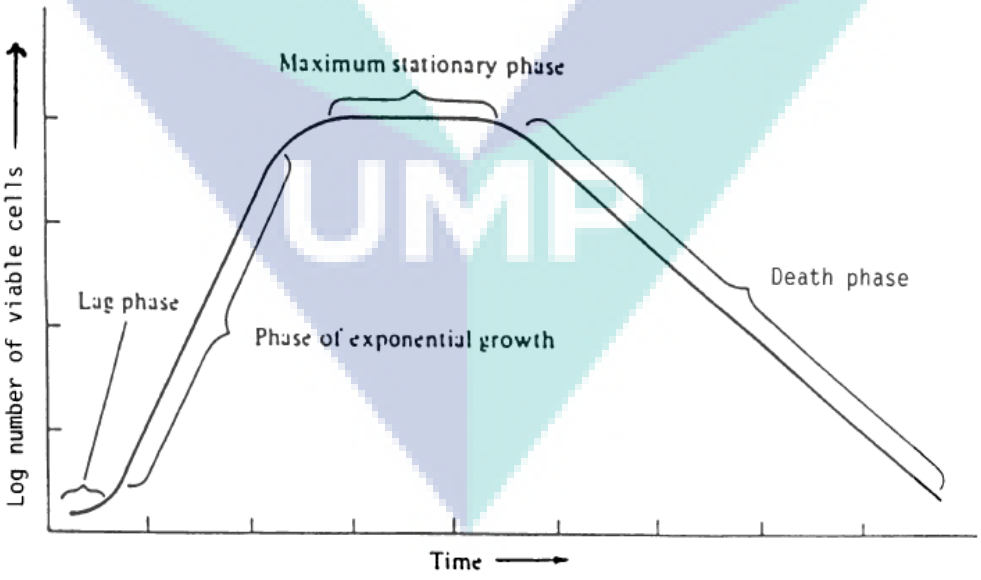


Figure 2-3: Typical Growth Curve of *Saccharomyces cerevisiae* (Boyd, 1985)

2.5 Electrocoagulation

Electrocoagulation is a novel method in wastewater treatment and this emerging technology combines the functions and advantages of conventional methods such as coagulation, flotation and electrochemistry in water and wastewater treatment. It applied the same concept as coagulation in which the coagulant is generated from its own electrode without any needs of chemical coagulant addition. Electrocoagulation process is reacted based on electrolysis principle that consist of metal electrodes and electric current. The oxidation and reduction reaction occurs at the anode and cathode, respectively.

The electrodes can generate coagulated species and metal hydroxides destabilized and aggregate the suspended solids. Electrocoagulation is considered as an economical and environment friendly because it is portable and only need an optimum space to locate the system. This process only used a simple and compact reactor. In fact, this method is a persuasive method in treating wastewater from review from previous study. It also stated as suitable for different type of industries. Other than that, it is said that the flocks produces in this process is larger. Plus, less bound water contains and no extensive chemical required.

2.6 Summary

Rare earth industrial wastewater can bring harm to living aquatic and environment. It is very vital to ensure the wastewater is treated properly. Chloride increases the electrical conductivity of water and thus increases its corrosivity. In metal pipes, chloride reacts with metal ions to form soluble salts, thus increasing levels of metals in drinking-water. In lead pipes, a protective oxide layer is built up, but chloride enhances galvanic corrosion. It can also increase the rate of pitting corrosion of metal pipes (WHO. SDE. WSH, 2003). There are three types of wastewater treatment which is physical treatment, chemical treatment and biological treatment. Therefore by using bioremediation hybrid with electrocoagulation system can help remove or reduce concentration of chloride in the wastewater.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter will describe about the materials and methods used in the study. This experiment will be conducted in two stages after preliminary testing of wastewater. The first stage is bioremediation hybrid and the second stage is electrocoagulation process. The overall methodology for this research is shown in **Figure 3-1**.

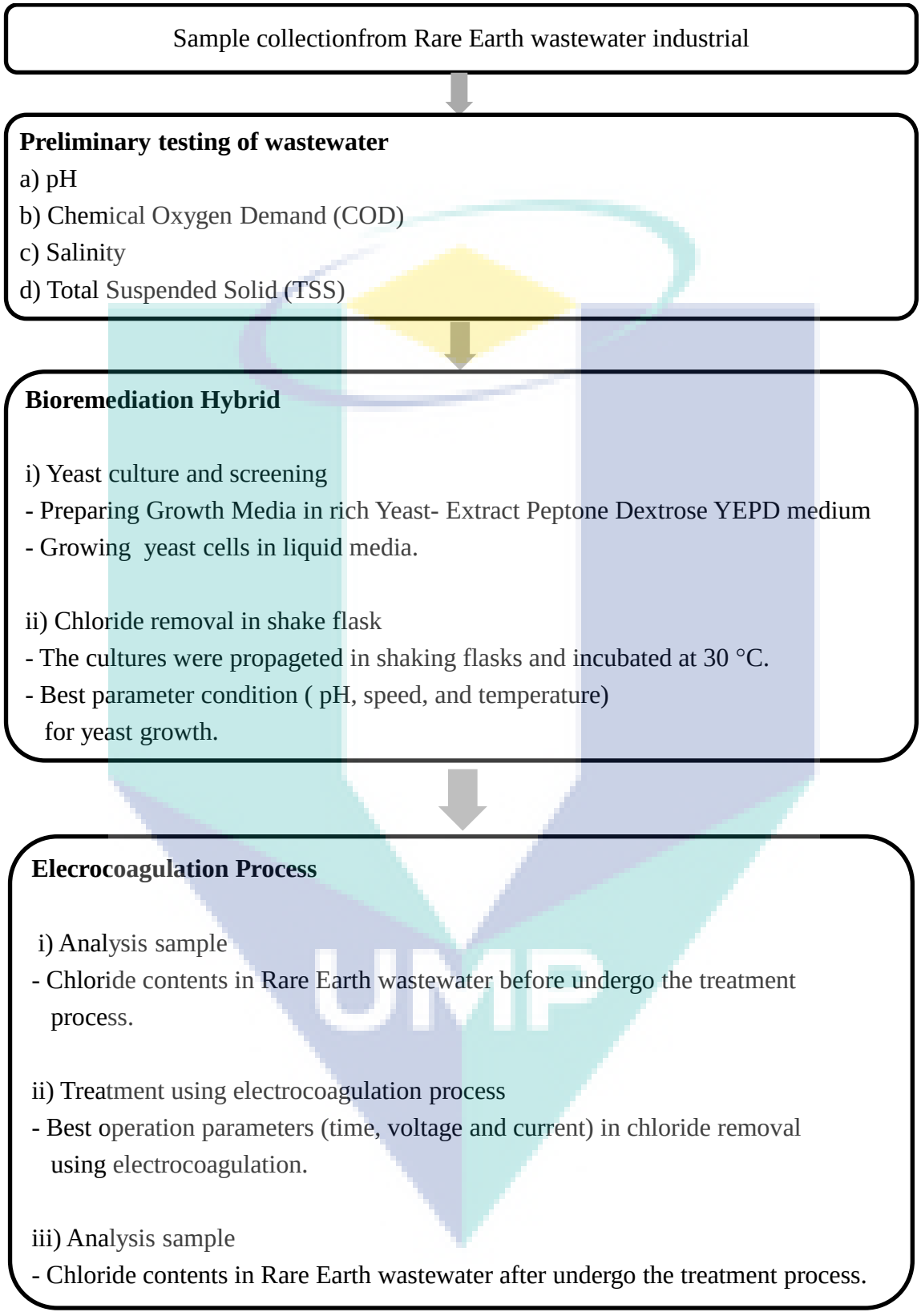


Figure 3-1: The overall methodology

3.2 Materials

The materials that were used in this research are Mauri-Pan Instant Yeast (*Saccharomyces cerevisiae*) that obtained from AB Mauri Malaysia Sdn. Bhd. All chemical and reagent used in this study were YEPD medium (Yeast Extract, peptone, glucose), and 70% of Ethanol. Actual rare earth wastewater sample are collect from Lynas (M) Sdn Bhd located at Jalan Gebeng 3, kawasan perindustrian gebeng, 26080 kuantan, pahang.

3.3 Equipment and Instruments

Equipment and instruments used in experiment are Hirayama HVE-50 Autoclave, Stackable Incubator Shaker, Memert Microbiological incubator (BE600), pH Meter, UV/Vis spectrophotometer 21 D-Mitonoy, Laminar flow cabinet (ISOCIDE) and analytical balance. **Table 3-1** showed the function of equipments and instruments used for the experiments.

Table 3-1: The equipment/ instrument and the function used for the experiments

Equipment/ Instrument	Function
Hirayama HVE-50 Autoclave	Sterilizing equipments and YEPD medium or agar at 120^{o}C for 15 minutes
Stackable Incubator Shaker (Infors, CH-4102)	To mix and growth yeast in the shake flask at specific temperature and speed
Memert microbiological incubator (BE600)	To keep the culture of yeast at 30^{o}C for 24 hours
pH meter model Mettler Toledo Delta 320	To check the pH meter of sample
UV/Vis spectrophotometer (Hitachi, U-1800)	To determine the growth of yeast at 600nm (wavelength)
Laminar flow cabinet (ISOCIDE)	Use for bioremediation treatment
Analytical balance	To weight the nutrient for yeast

3.4 Analysis of Chloride Concentration

Chloride concentration was tested before and after the treatment. The samples were filtered with filter paper before analysis. The chloride concentration of the wastewater sample will be measured by standard method using a Hach spectrophotometer (DR2400). Firstly, 10 ml of sample was filled in a sample and another sample was filled with 10 ml of deionized water as a blank. Then, 0.8 ml of mercuric thiocyanate solution were added into each sample and mixed the solution. After that, 0.4 ml of ferric ion solution were added into each sample cell and mixed the solution. If the chloride consists in sample, solution was changed to orange color. The chloride reading was measured using Hach spectrophotometer (DR2400). The flowchart of HACH method to measure chloride concentration as shown in **Figure 3-2**.

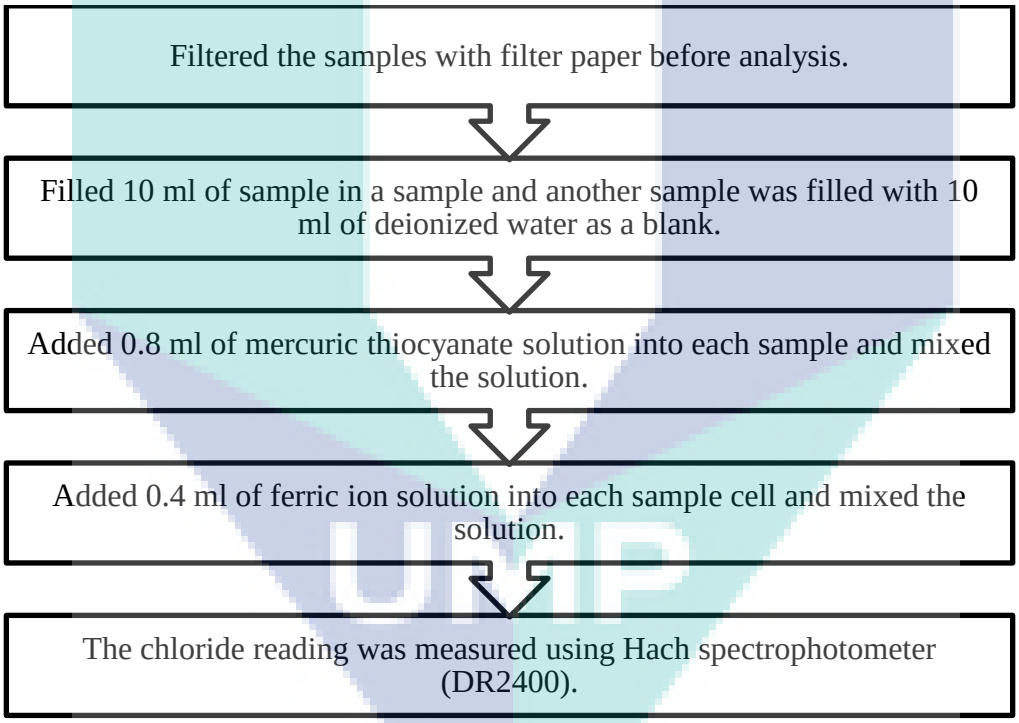


Figure 3-2: HACH method to measure chloride concentration

3.5 Bioremediation Treatment using Yeast

The experiments were conducted in laminar flow cabinet to avoid contamination of culture and wiped with 70% of ethanol. Biological treatment using Yeast has two stages which are preparation and growth of the yeast cell in YEPD (Yeast, Peptone, & Dextrose) medium and Yeast in shake flask.

3.5.1 Preparation and Growth of the Yeast Cells

The flowchart of preparation and growth of the yeast cell method are described as shown in **Figure 3-3**.

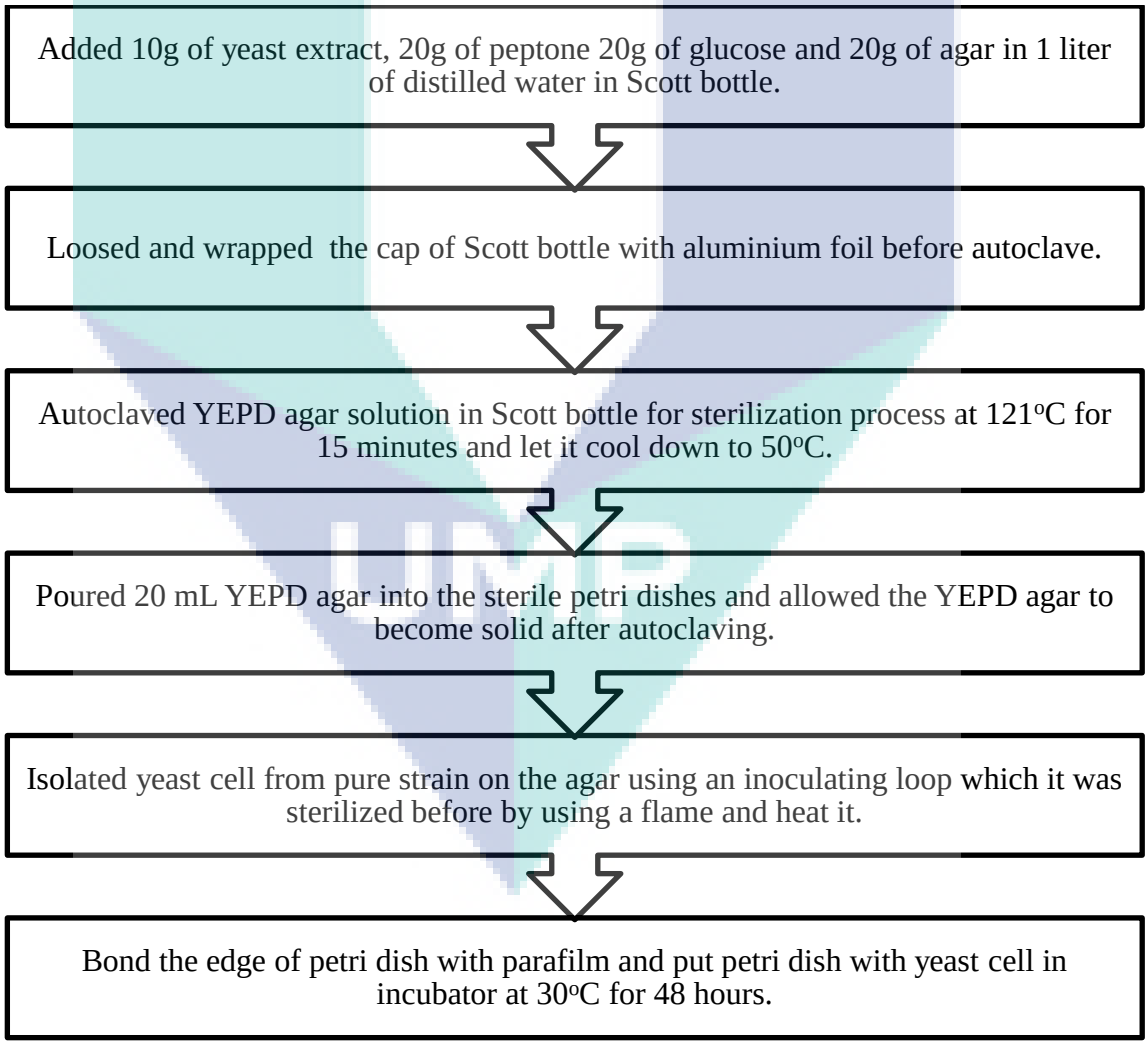


Figure 3-3: Preparation and growth of the yeast cell method

Yeast growth were grown on YEPD agar which were containing the following composition: 1.0% w/v yeast extract, 2.0% w/v peptone, 2.0% glucose and 2.0% w/v agar (Rezaei, et al., 2014) where 10g of yeast extract, 20g of peptone 20g of glucose and 20g of agar is suspended in 1 liter of distilled water in Scott bottle. The cap of Scott bottle was loosed and wrapped with aluminium foil before autoclave. After the preparation, YEPD agar solution in Scott bottle was autoclaved for sterilization process at 121 ^{o}C for 15 minutes and let it cool down to 50 ^{o}C. After autoclaving, approximately of 20ml YEPD agar was poured into the sterile petri dishes and allowed the YEPD agar to become solid (Nam, & Powel, 2014). Then, streaking method where yeast cell were isolate from pure strain on the agar using an inoculating loop which it was sterilized before by using a flame and heated until it has red glow to remove the contamination or particles from inoculating loop (Sherman, 2002). After streaking of the yeast, edge of petri dish was bind with parafilm to avoid contamination. Petri dish with yeast cell was put in incubator at 30 ^{o}C for 48 hours. **Figure 3-4** shows yeast cell on the agar.

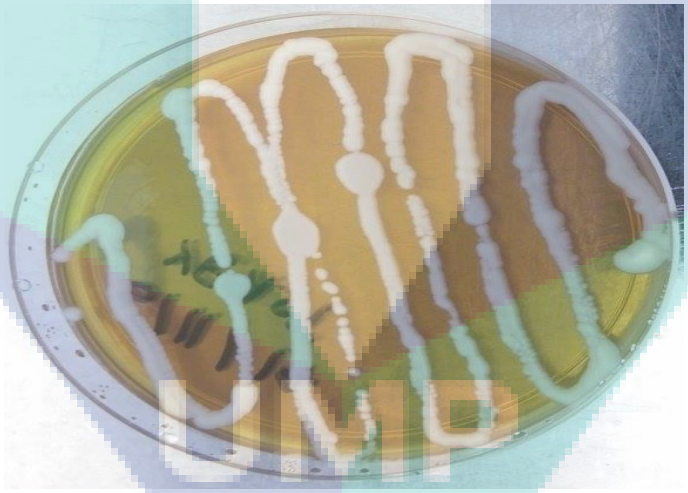


Figure 3-4: Yeast cell on the agar

YEPD medium broth was prepared with 10g of yeast extract, 20g of peptone and 20g of glucose in 1 liters of distilled water and then, the solution was autoclaved with 20ml of universal bottle at121^{o}C for 15 minutes and wrapped with aluminum foil (Sherman, 2002). After 48 hours, the culture of yeast cells on the agar was inoculated by using an inoculating loop where it was sterilized by using flame (Sherman, 2002). After cooling the inoculating loop, a loopful of yeast from the petri dish with yeast culture was placed into 20 ml of YEPD

medium broth it was repeated for three loops of yeast cell into universal bottle. Then, the cap of universal bottle with yeast cell was bind with parafilm and placed into microbial incubator for 24 hours at 30^{o}C (Nam, & Powel, 2014). **Figure 3-5** shows flowchart of the YEPD medium broth procedure.

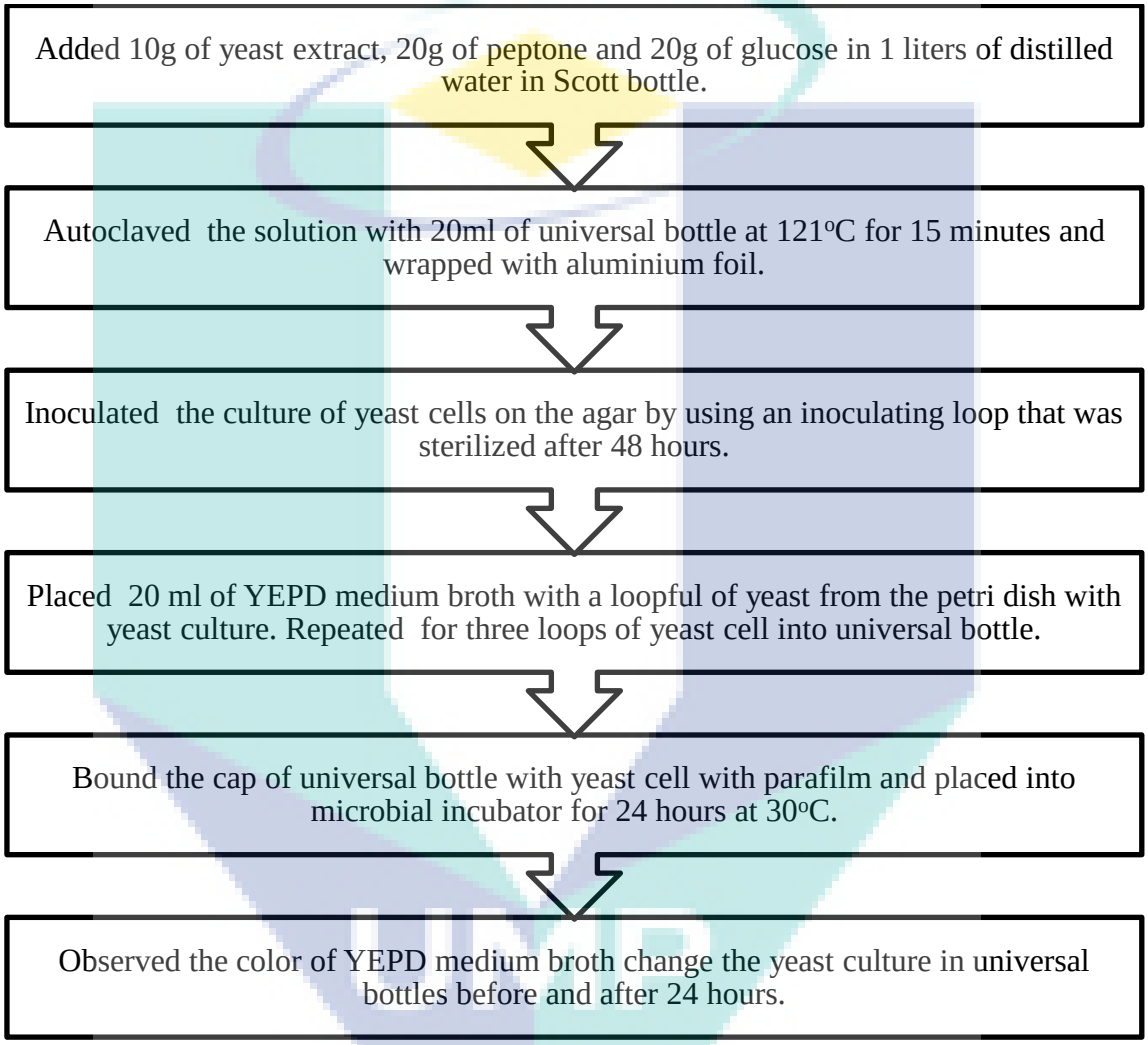
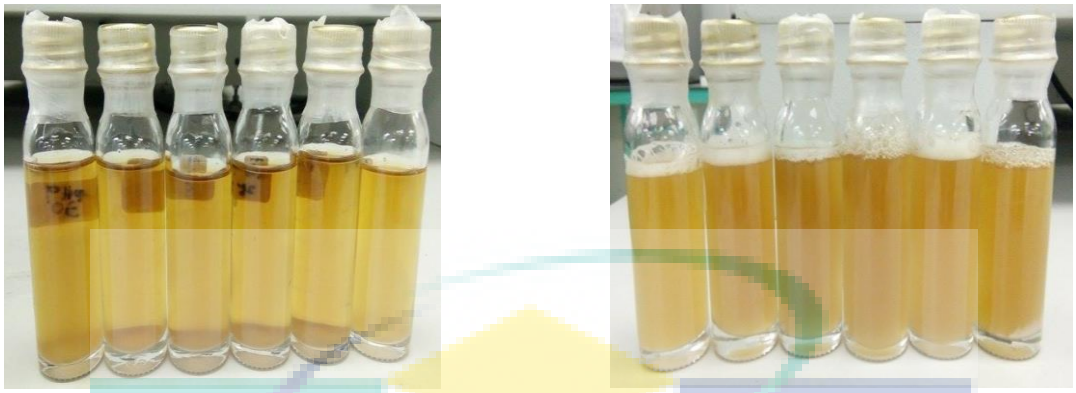


Figure 3-5: YEPD medium broth procedure

After that, yeast culture in universal bottles before and after 24 hours where color of YEPD medium broth change to cloudy. **Figure 3-6** shows a) Before yeast culture in universal bottle and b) After yeast culture in universal bottle.



a) b)

Figure 3-6: Yeast culture in universal bottles (a) before and (b) after 24 hours

3.5.2 Yeast in Shake Flask

After 24 hours, 20 ml of YEPD broth in universal bottle was transferred into a 250ml sterilized Erlenmeyer flask containing 200ml of wastewater at the different pH where the optimal pH range of yeast groth from pH 4 to 6 and plug with cotton wool and cover with aluminium foil. These cultures were put in an orbital shaking incubator at 30 ^{o}C for 24 hours at 120 rpm (Ali, & Khan, 2014). The range of temperatures from 20 ^{o}C to 40 ^{o}C (Nam, & Powel, 2014) and speed of orbital shaking incubator between 100 rpm to 180 rpm where the yeast strains were able to grown. The growth of *S. cerivisiae* cells was determined using UV/Vis spectrophotometer 21 D-Mitonoy with optical density (OD) measurement at 600nm wavelength at interval of 1 hour (Nam & Powel, 2014) where the cultures of yeast were continuous shaking for 70 h (Rezaei et al., 2014). **Figure 3-7** shows flowchart of chloride removal in shake flask procedure.

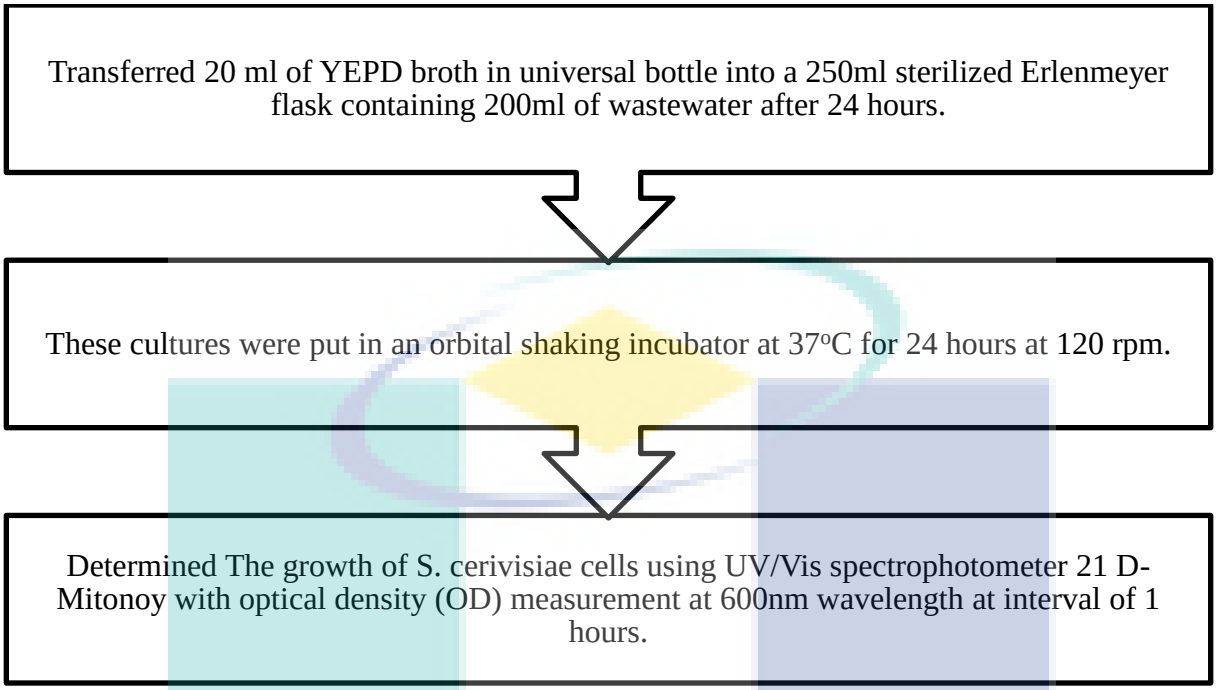


Figure 3-7: Chloride removal in shake flask procedure

After that, reductions of organic matters in wastewater were tested after 24 hours such as chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solid (TSS) and turbidity.

3.6 Electrocoagulation Process

The experimental setup is shown in **Figure 3-8**. EC experiments were conducted in a lab-scale EC cell having a total volume of 1 L. The total effective electrode area was 90 cm^2 and the spacing between the electrodes was 11 mm. A digital DC power supply (Smart Power, 1 kW SMART Programmable DC Power Supplies was used to give regulated electricity current to the EC cell is shown in **Figure 3-9**. All runs were performed at constant temperature (25^0C), mixing speed (200 rpm), current 2 Ampere, and with 1 L of wastewater solution. At the end of the run, the solution was filtered and then the filtrate was analyzed; the electrodes were washed thoroughly with water to remove any solid residues on the surfaces, dried, and reweighted (M. Kobya, 2013). Reductions of chloride in wastewater were tested after treatment.

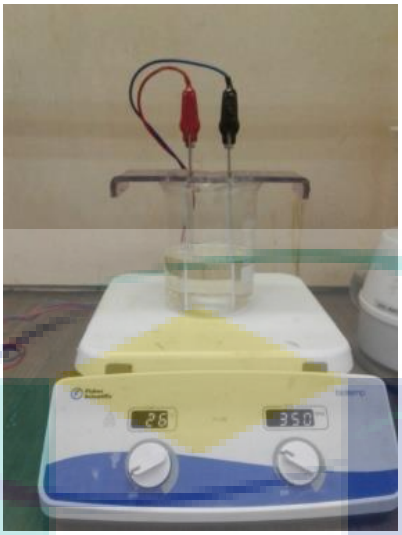


Figure 3-8: Experiments conducted in Lab scale

Figure 3-9: Electrocoagulation (EC) system

3.7 Summary

The growth curve of *S. cerivisiae* was studied using UV-spectrophotometer and also yeast in shake flask. After the optimum condition is obtained from bioremediation studies, the solution undergoes the process of electrocoagulation. For each time, voltage and current was

studies. After treatment, each concentration of solution was determined by HACH method to identify percentage of chloride removal.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter discuss about the result and discussion that obtain from the laboratory experiment. The subtopics that will be discussed on the characteristic rare earth wastewater, growth parameters for *Saccharomyces cerevisiae* and electrocoagulation for chloride removal.

4.2 Characteristic Rare Earth Wastewater

The wastewater sample from Rare Earth was analysed by UMP Consultancy & Training Sdn Bhd. The main characteristics of untreated rare earth wastewater specifically Chloride, Chemical Oxygen Demand (COD), and pH value. **Table 4-1** showed the characteristics of the wastewater sample.

Table 4-1: Characteristics of Wastewater Sample

No.	**Wastewater parameters**	**Result of analysis mg/L**	**"Environment Quality Act 1974:Environment Quality (Sewage &Industrial Effluents) Regulations 1979" (2012) Standard B**
1	Chloride	12,000 mg/L	2.0
2	COD	915 mg/L	100
3	pH	2.94	5.5 – 9.0

From **Table 4-1**, the result of analysis shows the untreated rare earth wastewater in this study has high chloride concentration which is 12,000 mg/L. Furthermore, this rare earth

wastewater also has high COD concentration and acidic pH value. Table 1 also show that the untreated rare earth wastewater has high especially chloride concentration value which has exceeded the limit of Regulations Sewage & Industrial Effluents (mg/L) under Malaysia's Department of Environment (DOE) regulations. Thus, treatment of rare earth wastewater is important in reducing the chloride concentration before being released into water bodies. Bioremediation hybrid with electrocoagulation system has potential in removing chloride concentration and other parameters as well.

4.3 Growth Parameters for Saccharomyces cerevisiae

4.3.1 Different Concentration of Samples

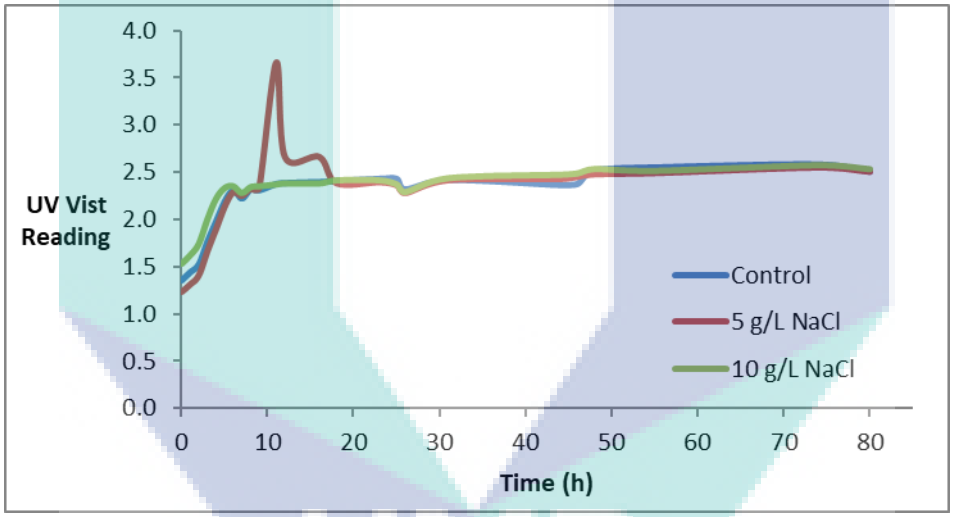


Figure 4-1: Saccharomyces cerevisiae growth for control, 5 g/L and 10 g/L simulated chloride wastewater (NaCl).

The growth of *Saccharomyces cerevisiae* was conducted by measuring the cell density or optical density (OD) to monitor the growth of *Saccharomyces cerevisiae*. The different concentration of samples has been studied to determine the *Saccharomyces cerevisiae* growth which are the control, 5 g/L and 10 g/L simulated chloride wastewater (NaCl) as shown in Figure 4-1. The figure 4-1 showed for control concentration, the growth of *Saccharomyces cerevisiae* population increased dramatically for the first 7 hours with OD increased from 1.3 to 2.2. For 10 g/L NaCl concentration, the growth of *Saccharomyces cerevisiae* population also increased dramatically for the first 8 hours with OD increased from 1.5 to 2.3. On the other hand, there is a significant fluctuates in the growth of *Saccharomyces cerevisiae* with the increasing time. It

showed that *Saccharomyces cerevisiae* grew and adapted well under condition 10 g/L NaCl in suitable nutrient medium. As a result, microbial mass or population of *Saccharomyces cerevisiae* increased with suitable time and also the growth parameters will show the different microorganisms growing under the different culture conditions (L. Shuler, M., & Kargi, F., 2002).

4.3.2 Different pH Conditions

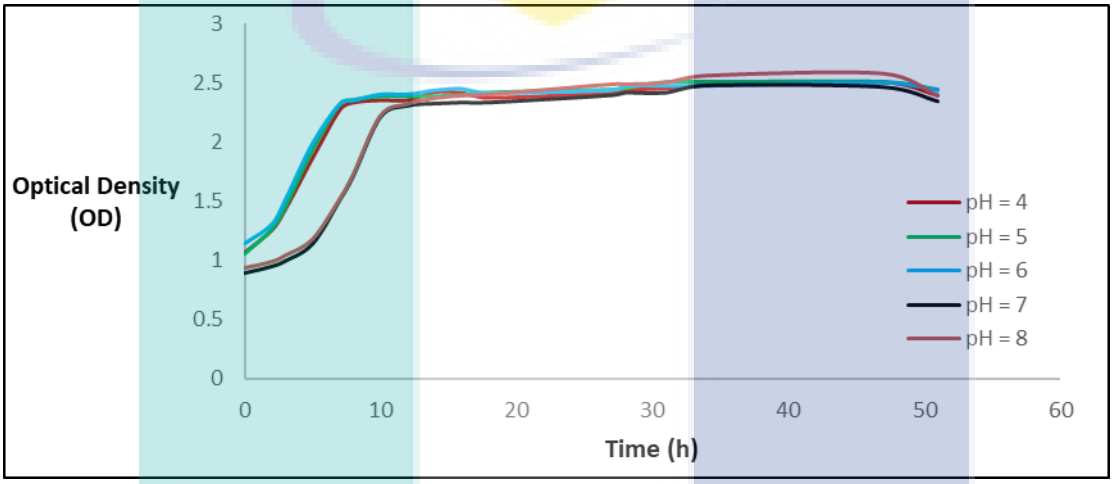


Figure 4-2: Yeast growth for standard at different pH

From previous study, it was reported that most organisms grew actively at pH range of 2 to 3 and natural pH of the environment between 5 and 9 (Brock et al., 2006). However, most of biological treatment of wastewater occurs generally at neutral pH and meet the demand of industries (Bitton, 2005). Hence, further experiment was carried out to determine the suitable pH value when using *Saccharomyces cerevisiae* for chloride removal. From figure 4-2, there have pH varies to analysis yeast growth which are for pH 4, pH 5, pH 6, pH 7 and pH 8. For pH 4, the growth of *Saccharomyces cerevisiae* increased for the first 7 hours with OD increased from 1.1 to 2.3 which same as pH 5, the growth of *Saccharomyces cerevisiae* increased for the first 7 hours with OD increased from 1.1 to 2.3. For pH 6, *Saccharomyces cerevisiae* able to increase growth for first 8 hours with OD increased from 1.1 to 2.3. For pH 7, the growth of *Saccharomyces cerevisiae* increased for the first 10 hours with OD increased from 0.8 to 2.2. Lastly, for pH 8, the growth of *Saccharomyces cerevisiae* increased for the first 10 hours also with OD increased from 0.9 to 2.2. This showed that the *Saccharomyces cerevisiae* was suitable for growth in the pH 7 condition of the chloride condition.

4.3.3 Different Condition of The Environment

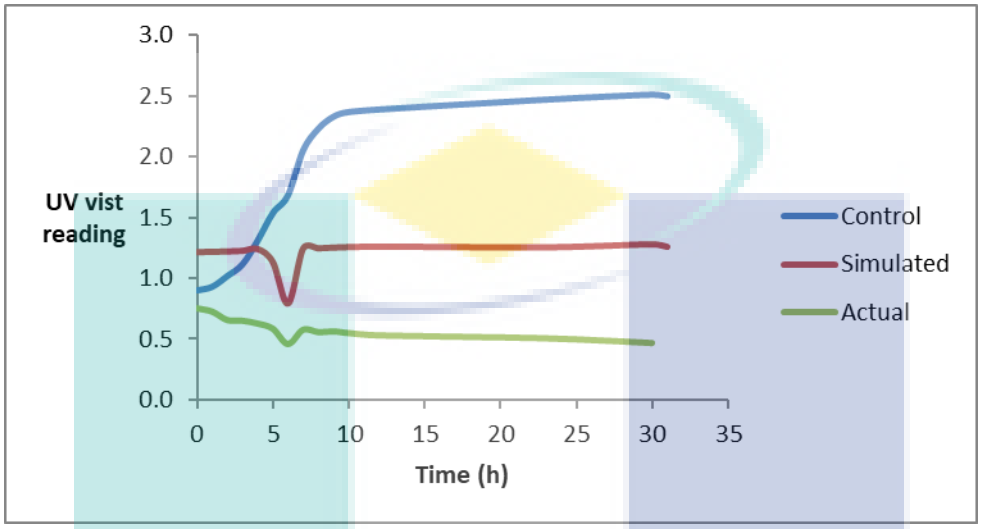


Figure 4-3: Yeast growth for standard, simulated chloride (NaCl) and actual wastewater.

Figure 4-3 shows a typical profile of yeast at different sample. Three parameters have been analysed the yeast growth which are control, simulated chloride wastewater and actual wastewater. The results showed that for control the growth of yeast population increased dramatically for the first 10 hours with OD increased from 0.8 to 2.4. For sample simulated chloride wastewater and actual wastewater, the growth of yeast population both is significant fluctuates from 0 to 6 hours. But then, the growth of yeast increased dramatically from 6 to 7 hours with OD increased from 0.8 to 1.2 and 0.4 to 0.6 for simulated NaCl and actual wastewater respectively.

4.4 Electrocoagulation (EC) for Chloride Removal

The experiment was proceeded with the electrocoagulation system. The comparison between stainless steel (SS) and aluminium (Al) plate were done using the simulated chloride wastewater and actual wastewater from rare earth. The current was fixed at 2 A, and retention time was recorded.

Table 4-2: Percentage removal of chloride by using bioremediation hybrid with EC in chloride simulated wastewater

Plates	Initial	Final Cl^-,	Retention time,	Current, A	Percentage

	Cl^-, ppm	ppm	min		removal, %
Stainless steel (SS)	12,000	1,000	10	2	92
Aluminum (Al)	12,000	2280	30	2	81

Table 4-2 showed the comparison between the stainless steel and aluminum plate. The samples directly run into the EC after broth was added. Current was fixed at 2 Ampere. The percentage removal (%) of chloride was highest recorded when using SS plate which is 92% and time taken was 10 min compared to Al which the percentage removal (%) of chloride is 81 % and time taken is 30 min. This shows that the SS is more suitable to use as plate in chloride removal using bioremediation hybrid with EC.

Table 4-3: Percentage of chloride by using bioremediation hybrid with EC in actual wastewater

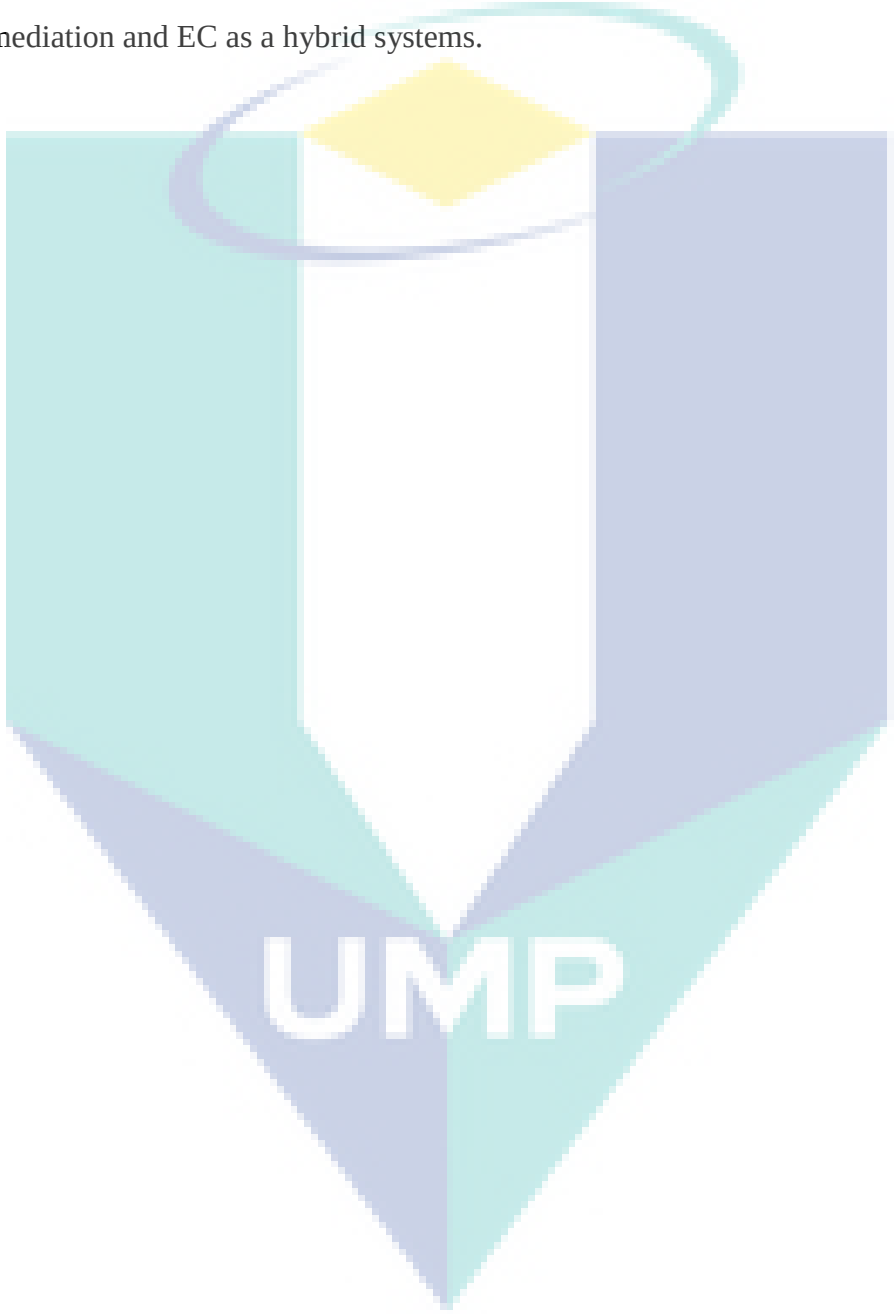
Plates	Initial Cl^-, ppm	Final Cl^-, ppm	Retention time, min	Current, A	Percentage removal, %
Stainless steel (SS)	12,000	3200	15	2	73.3
Aluminum (Al)	12,000	5400	30	2	55

Table 4-3 showed the percentage of chloride by using bioremediation hybrid with EC in actual wastewater. The percentage removal (%) of chloride was highest recorded when using SS plate which was 73.3 %, and time taken was 15 min. as for Al, the percentage removal (%) of chloride was 55% and time taken was 30 min. The percentage removal (%) of chloride was lowered compared when analyse using the simulated chloride wastewater. This may occur because of the present of other chemical in the actual wastewater that may interfered with the result (Brock et al., 2006)

.

4.5 Summary

The results obtained show that the *Saccharomyces cerevisiae* can growth in chloride condition and in actual wastewater. Then the wastewater was further analyse with electrocoagulation (EC) as to determine the chloride removal by using bioremediation and EC as a hybrid systems.



CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Bioremediation by using *Saccharomyces cerevisiae* is used as a pre-treatment to reduce the chloride concentration, followed by electrocoagulation process in reducing chloride from rare earth wastewater. From this research, it is found out that Saccharomyces cerevisiae cells grew and adapted well under condition 10 g/L NaCl in suitable nutrient medium. Saccharomyces cerevisiae was able to growth in control (10 hr), simulated chloride wastewater (6 hr) and actual wastewater (6 hr) with OD increased from 0.8 to 2.4, 0.8 to 1.2 and 0.4 to 0.6 respectively. Besides that, the optimum Saccharomyces cerevisiae able to growth in standard pH 7 at first 10 hours with OD increased from 1.1 to 2.1. Thus, the samples were treated directly using electrocoagulation system. The result shows Stainless steel (SS) plate able to remove chloride concentration which 92 % removal at 10 minutes and 2 Ampere when using the simulated chloride wastewater. As for actual wastewater, SS plate able to remove chloride concentration which 73.3 % removal at 15 minutes and 2 Ampere. These results may be useful as a guideline for the rare earth industry to treat especially reducing chloride concentration in the rare earth wastewater. Bioremediation hybrid with electrocoagulation system offers an attractive method to treat wastewater.

5.2 Recommendation for future works

The bioremediation hybrid with electrocoagulation system has an ability to remove the chloride from rare earth wastewater but does not meet requirement from DOE. To improve the effectiveness more pre-treatment should be carrying out before through process bioremediation and electrocoagulation.

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