BIOLEACHING OF COPPER FROM WASTE PRINTED CIRCUIT BOARD BY POTENTIAL BACTERIA ISOLATED FROM A LANDFILL

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DEDICATION

This thesis is dedicated to my family, friends, and colleagues

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In preparing this thesis, I experienced many things, be it good or bad. It forces me to think outside my comfort zone to finish the laboratory procedures required, especially during the outburst of the pandemic Covid 19 recently. Limited time in the campus and laboratory is an obstacle that cannot be avoided. With that being said, I would like to thank both my supervisor, Dr Fazrena Nadia, and Assoc. Prof. Dr Hara, for their support throughout my research duration. Without them, I will not be able to progress at all in this study. My heartfelt appreciation also to the lecturers in MemoBio i-kohza for their assistance and help towards my research as well to my other postgraduates' colleagues, which helped whenever I need one. Last but not least to all my family and friends, thank you all for the support.

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ABSTRACT

Electronic waste has been identified as one of the fastest growing wastes generated globally that is predicted to surpass 111 million tons per year by 2050. E-waste is becoming a worrying issue not just for its high volume generated, but also due to high composition of heavy metals it contains. This may contribute to potential environmental pollution if e-waste is not managed properly. High volume of e-waste leads to a new prospect of material acquisition through the concept of urban mining by extracting the valuable metals from the e-waste. This metal is usually extracted from components such as waste printed circuit board which houses valuable metals such as copper. Conventional extracting processes, however, pose harmful environmental effects as well as incur high cost. The purpose of the study is to introduce an alternative greener method of extraction which uses microbial activity to extract metals namely bioleaching. This study focuses on the isolation of bacteria sourced from the sanitary landfill to be used as the bacterial strain for bioleaching accompanied by minimal pre-processing to reduce the amount of secondary pollution from the pre-processing. The strains were grown in low pH medium, utilising their tolerance towards low pH condition for the copper bioleaching process. Four bacterial strains were isolated. Using 16S rRNA gene sequencing, the isolates were identified as *Bacillus* sp. strain SE, *Bacillus* sp. strain SC, *Lysinibacillus* sp. strain SE2 and *Oryzobacter* sp. strain S1A. All the isolates showed some bioleaching activity with strain SC being able to extract the highest amount of copper from the bioleaching with value of up to 23.36 ppm through the two-step bioleaching method. Strain SC was used to further evaluate the copper extraction by using pure copper strip to evaluate the mobilisation of copper by the strain microbial activity and it managed to mobilise 818.1 ppm of copper. These results suggest that the standalone bioleaching activity by strain SC is proven to perform effectively.

ABSTRAK

Sisa elektronik telah dikenalpasti sebagai salah satu sisa yang paling cepat berkembang yang dihasilkan di seluruh dunia, diramalkan melepasi 111 juta tan setahun pada 2050. E-sisa menjadi isu yang membimbangkan bukan sahaja kerana jumlah yang tinggi yang dihasilkan, tetapi juga kerana komposisi logam berat yang tinggi yang dibawanya, yang boleh menyebabkan pencemaran alam sekitar yang berpotensi jika ia tidak diuruskan dengan betul. Jumlah e-sisa yang tinggi membawa kepada prospek baru untuk pemerolehan bahan melalui konsep perlombongan bandar, dengan mengekstrak logam berharga dari e-sisa. Logam ini biasanya diekstrak dari komponen seperti sisa papan litar bercetak yang merupakan sumber logam berharga seperti tembaga. Walau bagaimanapun, proses pengekstrakan konvensional mempunyai kesan alam sekitar yang berbahaya serta kos yang tinggi yang diperlukan untuk proses itu dilakukan. Kaedah pengekstrakan alternatif yang lebih hijau diperkenalkan yang menggunakan aktiviti mikrob untuk mengekstrak logam iaitu *bioleaching*. Kajian ini memberi tumpuan kepada pengasingan bakteria yang diperoleh dari tapak pelupusan sanitari untuk digunakan sebagai strain bakteria untuk *bioleaching* disertai dengan pra-pemprosesan yang minimum untuk mengurangkan jumlah pencemaran sekunder dari pra-pemprosesan. Strain ini ditanam dalam medium pH yang rendah, menggunakan toleran mereka terhadap keadaan pH yang rendah untuk *bioleaching* tembaga. Empat jenis bakteria telah diasingkan. Dengan menggunakan penjujukan gen rRNA 16S, pengasingan dikenal pasti sebagai *Bacillus* sp. strain SE, *Bacillus* sp. strain SC, *Lysinibacillus* sp. strain SE2 dan *Oryzobacter* sp. strain S1A. Semua pengasingan menunjukkan beberapa aktiviti *bioleaching* dengan strain SC dapat mengekstrak jumlah tembaga tertinggi dari *bioleaching* dengan nilai sehingga 23.36 ppm melalui kaedah *bioleaching* dua langkah. Strain SC digunakan untuk menilai lebih lanjut pengekstrakan tembaga dengan menggunakan jalur tembaga tulen untuk menilai mobilisasi tembaga oleh aktiviti mikrob terikan dan berjaya menggerakkan 818.1 ppm tembaga. Keputusan ini mencadangkan aktiviti *bioleaching* kendiri oleh strain SC boleh dijalankan.

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LIST OF ABBREVIATIONS

AAS	-	Atomic Absorption Spectroscopy
Ag	-	Silver
Al	-	Aluminium
Au	-	Gold
Fe	-	Iron
PCR	-	Polymerase Chain Reaction
WPCB	-	Waste Printed Circuit Board

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CHAPTER 1

INTRODUCTION

1.1 Research Background

In one-decade, various elements of life have gone through an evolution. This scenario includes our daily technical equipment, which has gone through rapid evolution with newer technology improving our daily lives. However, the rapid improvement has caused a jump in the generation of e-waste over the years. A newer model of smartphone, for example, is being released more frequently with a massive gap in the performance from the last model, causing consumers to replace the older model quicker than a decade ago to experience the features set of a newer device. Not to mention the strategy of brands to shorten the lifespan of their products by limiting the upgrade option and reducing technical and software supports, forcing the consumer to replace their devices faster. This scenario has caused a jump in generating electrical and electronic waste (e-waste) worldwide. Statistically, in 2015 alone, e-waste was amounted to have been generated approximately 43.8 million tonnes globally, with the prediction that the numbers would exceed 50 million tonnes by 2020 (Tansel, 2017).

According to the Global E-waste Statistic Partner, in 2020, approximately 364-kilo tonnes of e-waste was generated in Malaysia alone, amounting to almost 11.1 kilograms of e-waste per capita generated. This number is almost double the approximated projection by the Department of Environment of Malaysia (DOE). The higher generation of e-waste concerns developed countries and developing countries. The statistic also indicates that another 547-kilo tonne of e-waste is exported. The DOE had initiated some effort; however, the problem is far from reaching its solution, for example, the existence of collection centers throughout the country; however, the low rate of recycling is still a significant concern added by the low numbers of the formal and legal processing facility

One of the concerns arising amid the increasing amount of e-waste is the illegal export of e-waste globally, especially from China and the United States of America. This precedent possesses a higher possibility of an increasing number of illegal processing facilities in which the process itself is harmful to the surrounding area and the facility's operator. This illegal processing facility usually uses a combustion process without regulation or safe operating procedure. First, the plastic part of the e-waste was burnt to retrieve the metal component, usually involving cable. Then, the insulation part was burnt to extract the copper inside the cable. Concerning the low recycling rate in Malaysia, the most common factor will be the misconception and habitual behaviour of just disposing e-waste to the landfill, signifying low exposure of the importance of e-waste to be recycled.

Most e-waste will have specific components called printed circuit boards (PCBs), which houses all the electronic parts that power up the entire system of the devices. As e-waste is being discarded, the PCB will be removed from the e-waste to salvage its valuable metals. Most of the time, Illegal recyclers will burn and salvage the metals from the waste printed circuit boards (WPCB). The boards are usually made of fibres and copper, which act as the conductor for the electrical circuit. The burning of this board will eventually release secondary pollutants in the form of toxic fumes, endangering the recycler and the surrounding community.

1.2 Problem Statement

Standard processing facility for e-waste usually operates by implementing a few procedures and methods, primarily to extract the metal component from the discarded e-waste. Metal is usually extracted as one of the efforts to reduce carbon emissions by mining a new metal compound. This procedure is also defined as urban mining with a more sustainable metal mining method (Arora *et al.*, 2017).

Extraction of metal from e-waste is usually categorized based on the approach of each method used. The standard method includes pyrometallurgical, hydrometallurgical, mechanical, and physical metal extraction. This process can also include a combination of any of the metallurgical processes. The pyrometallurgical method involves using high-temperature combustion to melt the plastic component from e-waste. High energy is required for this method as combustion and a high-temperature working environment (Wang *et al.*, 2017). As the most direct approach in processing e-waste to extract its metal component, this method can also induce a secondary contaminant in the form of toxic fume due to the combustion of the e-waste (Hannah and Ryn, 2019). Nevertheless, most informal and illegal e-waste processing facilities are prone to use this method as it was the fastest to obtain the metal component. Insulated copper wire and printed circuit board are burned to retrieve their copper component.

The metallurgical e-waste method was also usually done through mechanical and physical treatment. The mechanical process usually involves shredding the e-waste in small size and using the principle of electromagnetism to remove the metal from the shredded bits of the e-waste (Patel *et al.*, 2017; Debnath *et al.*, 2018). Mechanical shredding produces tiny particles of dust resulting from the shredding process. The overall cost of obtaining the equipment and operating the procedure is the highest compared to the other metallurgical process, which might not be economically viable for some processing facilities. The mechanical shredding process was also used as a pre-processing to the other metallurgical process.

The hydrometallurgical process is commonly used for extracting metal according to their type by using a specific leaching agent and has the highest efficiency compared to the other method. The hydrometallurgical process, commonly known as acid leaching, uses strong acid and other chemical reagents to act as leaching agents for a specific type of metal (Santos *et al.*, 2011; Priya and Hait, 2018). Although the efficiency of this method, the downside of this procedure is that the leachate produced from this process is a powerful acid that is classified as a secondary contaminant

(Pramila and Bhawana, 2012). This leachate can be harmful and risk to the environment and the people handling the process.

In order to overcome the weaknesses of the hydrometallurgical method, another method was introduced in which microbial activity was used to replace strong reagents in the acid leaching method. This method was named bioleaching. Bioleaching was usually carried out by acidophile and thermophile strain, for example, *Acidithiobacillus ferrooxidans* (Biosci *et al.*, 2012) or fungi such as *Aspergillus niger* (Bosecker, 1997). However, due to lower efficiency than the other method, bioleaching was usually carried out as a hybrid method, usually involving mechanical shredding as a pre-process step (Priya and Hait, 2017). That being said, a much more sustainable and environmentally friendly approach by limiting the pre-processing through mechanical shredding should be prioritized. Therefore, another bioleaching application was often used as a complementary method in leaching out metals from the leachate produced from the hydrometallurgical method.

Malaysia, an equatorial climate country, possesses high possibilities and adaptability for microbial activity to perform optimally. Most of the studies done for bioleaching involved bacterial strain acquired from a temperate climate and none from tropical climate countries like Malaysia. This open possibility to acquire and investigate new strains of bacterial for bioleaching from this country.

1.3 Research Objectives

The objectives of the research are :

(a) To isolate and characterize microbes from sanitary landfill for copper bioleaching of waste printed circuit board (WPCB)

(b) To perform bioleaching of waste printed circuit board (WPCB) using the isolated strain from the landfill with direct bioleaching and spent medium bioleaching.

(c) To evaluate the bioleaching ability using the strain using pure copper as the target bioleaching.

1.4 Research Scope

This research utilized the microbes grown from Jeram Sanitary Landfill as the primary microbial agents for bioleaching. This condition limits the outcomes of this research based on the microbial activity from the microbes attained from the Jeram Sanitary Landfill, which acclimate to the Malaysian equatorial climate and conditions and the condition of the landfill mentioned. Furthermore, the condition of bioleaching was also acclimated to mimic the Malaysian climate.

This research also used different conditions of WPCB compared to common bioleaching. The usage of heavy machinery to ground the WPCB was limited, and the WPCB was only cut into the size of 2 cm x 2cm compared to the shredded WPCB with a size smaller than 5 mm used in common bioleaching. This research is also limited to the extraction of copper from WPCB by bioleaching without noting any other metals bioleaching by the acquired strains.

1.5 Significance of Study

The Bioleaching process has been introduced and used widely in copper extraction, usually by using bacterial species like *Acidithiobacillus ferrooxidans* as microbial activity sources. This research, however, utilized the abundant bacterial species in Malaysia in which readily adapted to this country's environment and are readily available to be used. The current bioleaching process also includes heavy mechanical processes that often cause fine dust pollution and defeat the purpose of going towards a greener alternative to metal recycling; hence, this research minimized the usage of heavy machinery and reduced the mechanical ground process to reduce and limit the production of fine dust pollution. As mentioned, this research derives the current metallurgical method favoring a much more environmentally viable method through bioleaching. It alters the current procedure further to reduce secondary and tertiary pollution production.

1.6 Research Goal

Isolated species from Malaysia sanitary landfills are hypothesized to display bioleaching activity on chosen e-waste and compatibility with the chosen leaching media. In addition, the isolated bacteria are expected to be ground up to work to future bioleaching work or research in Malaysia especially, to be one of the alternatives for mitigating the ever-increasing e-waste issue.

This research is also expected to contribute to biotechnology, especially in waste management, opening to more research and discussion in the future. Furthermore, this research will also contribute to more development towards recycling and reuse of materials, especially regarding e-waste, in a suit of the Sustainable Development Goals structured by The United Nations, under the Goal 12: Responsible Consumption and Production.

Despite its performance almost matching up to the conventional metal extracting method, bioleaching is often treated as a complementary process, extracting leftover metals from the leachate of the conventional method or often being pre-processed with other metallurgical processes. This research aims to discard the previous bioleaching process and have this process as a standalone process, with minimal pre-processing of the e-waste. Incoherent, to reduce the amount of secondary and tertiary pollution often caused by conventional metallurgical processes aiming to pursue a greener and more environmentally friendly method for e-waste metal recycling following The United Nation Sustainable Development Goals, Goal 12: Responsible Consumption and Production objectives.

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