BIOLEACHING OF METALS FROM ELECTRONIC WASTE BY HETEROTROPHIC BACTERIA IN FLUIDIZED-BED BIOREACTOR

Thesis

Submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

MINIMOL M.

Registration No. 177088CH003

DEPARTMENT OF CHEMICAL ENGINEERING

NATIONAL INSTITUTE OF TECHNOLOGY KARNATAKA,

SURATHKAL, MANGALURU

September 2023

DECLARATION

I hereby declare that the research thesis entitled "**BIOLEACHING OF METALS FROM ELECTRONIC WASTE BY HETEROTROPHIC BACTERIA IN FLUIDIZED-BED BIOREACTOR**" which is being submitted to the **National Institute of Technology Karnataka, Surathkal** in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy** in Chemical Engineering is a *bonafide report of the research work carried out by me*. The material contained in this research thesis has not been submitted to any University or Institution for the award of any degree.

Minimol M.
177088CH003
Department of Chemical Engineering

Place: Surathkal
Date: 27.9.2023

CERTIFICATE

This is to certify that the research thesis entitled "**BIOLEACHING OF METALS FROM ELECTRONIC WASTE BY HETEROTROPHIC BACTERIA IN FLUIDIZED-BED BIOREACTOR**" submitted by **MINIMOL M (177088CH003)** as the record of the research work carried out by her *is accepted as the research thesis submission* in partial fulfilment of the requirements for the award of degree of **Doctor of Philosophy**.

M.B. Saidutta

Research Guide
Prof. M.B. Saidutta
Professor
Department of Chemical engineering
NITK, Surathkal

Research Guide
Prof. Vidya Shetty K.
Professor
Department of Chemical engineering
NITK, Surathkal

Chairman - DRPC
Department of Chemical Engineering
रासायनिक अभियांत्रिकी विभाग
National Institute of Technology Karnataka - Surathkal
राष्ट्रीय प्रौद्योगिकी संस्थान कर्नाटक, सुरत्कल
PO Srinivasnagar, Mangalore - 575025 Karnataka
पी.ओ. श्रीनिवासनगर, मंगलौर – ५७५०२५, कर्नाटक

ACKNOWLEDGEMENTS

I take this opportunity to acknowledge each and every individual who has contributed in different ways for my research work carried out in the Department of Chemical Engineering at NITK, Surathkal.

I wish to express a special gratitude to both my research guides, Prof. M. B. Saidutta and Prof. Vidya Shetty K., Department of Chemical Engineering, NITK, Surathkal for their valuable guidance with attention to detail, unfailing support, constant encouragement, and providing me the cognitive liberty throughout my PhD.

I earnestly thank Dr. D. V R. Murthy, Retired Professor, Department of Chemical Engineering, NITK, Surathkal for his valuable support and guidance in my Research work.

I sincerely thank the RPAC committee members, Prof. Jagannath Nayak, Department of Metallurgical & Materials Engineering and Dr. Keyur Raval, Department of Chemical Engineering. Their valuable suggestions, guidance and encouragement have helped me to improve my research work and gain confidence.

I express my sincere gratitude to the Director, NITK. I wish to extend my gratitude to all the HODs (Prof. Raj Mohan, B., Dr. Hari Mahalingam, Dr. Prasanna Belur, D., and Dr. P. E. Jagadeeshbabu) for providing support throughout the research work. I also wish to thank all Faculty members and Research Scholars in the Department of Chemical Engineering for their support and encouragement. I thank MoE, GOI for providing stipend throughout my research work.

I would like to extend my thanks to Mr. Sadashiva, Mrs. Trithila, Mrs. Vijetha, Mrs. Bhavya Shree, Mrs. Sandhya, Mr. Gnaneshwar, and all other non-teaching staffs of Department of Chemical Engineering for their timely help and support in the laboratory for the research work. I exclusively thank Mr. Suresh for his constant availability to rectify the issues in operating the bioreactor and ICP-OES. I also thank the Central Research Facility (CRF), for providing the instrumentation facilities.

I particularly appreciate my friends, Dr. Farisa Banu S, Analyst, SGS, Canada Inc., Soumya K. M, Research Scholar, Department of Chemical Engineering, NITK, Surathkal and Sreelakshmy K, Scientist, ISRO, Thiruvananthapuram for their continuous support and motivation right through.

I would also like to thank all my cousins and relatives for their persistent encouragement. Especially, Manjusha M, Aswin S, Athira M and Manu S for their help in their fields of expertise to complete my Research work.

Finally, I am grateful and my heartfelt thanks to my husband, Mr. Ashwin K for his persuasion to get things done, my parents, parents-in-law and brother-in-law, for their patience, support and care throughout the research work.

MINIMOL M

ABSTRACT

The technological novelty has led to a decline in the lifespan of electronic gadgets, consequently discarding these as electronic wastes (e-wastes). Printed Circuit Boards (PCBs) which form the major proportion of the e-wastes, can serve as secondary sources of metals. The escalating global consumption and pressing demand for base and precious metals due to exhaustion from their natural resources has necessitated recycling the wastes for metal recovery. Bioleaching is the microbe-mediated mobilization of metals, and it can treat e-wastes to recycle it into the circular economy. The present study aims to recover metals from PCBs using heterotrophic bacteria in a Fluidized-bed bioreactor (FBR). *Alcaligenes aquatilis* and *Chromobacterium violaceum* were chosen for Cu, Ag, and Au bioleaching, respectively, through screening studies. The performance of FBR for Cu bioleaching revealed 0.175 mm particle size, 5% inoculum (v/v), and 2% e-waste load (w/v) as the optimum conditions. The mechanism of Cu bioleaching by *A. aquatilis* through contact and non-contact modes with redoxolysis and complexolysis mechanisms was proposed. Both bacteria performed better in bioleaching of metals as individual cultures than as co-cultures. Comparison of the three bioleaching methods in the FBR proved that the two-step method using *C. violaceum* is efficient for precious metal bioleaching with 9% Au and 23.7% Ag bioleaching. In contrast, the spent medium of *A. aquatilis* could bioleach 52.2% Cu. The recovery could be improved through three sequential batch runs of one-step bioleaching in FBR with *A. aquatilis*, yielding cumulative recovery of 80% Cu and 28.6% Ag. The recovery was also improved through a two-stage sequential batch operation involving a first stage of three sequential batches using the spent medium of *A. aquatilis*, followed by a second stage involving three sequential batches of a two-step method with *C. violaceum*, accomplishing a cumulative recovery of 99% Cu, 36.8% Ag, and 21.9% Au. The process can potentially serve as a greener and more economical method for bioleaching.

Keywords: bioleaching, electronic waste, Fluidized-bed bioreactor, heterotrophic bacteria, sequential batch bioleaching

CONTENTS

LIST OF FIGURES

LIST OF TABLES

NOMENCLATURE

µ	-	micron
µg	-	microgram
µl	-	microlitre
AFM	-	Atomic Force Microscopy
ANOVA	-	Analysis of Variance
BSA	-	Bovine Serum Albumin
CFU	-	Colony Forming Unit
CSTR	-	Continuous Stirred Tank reactor
DNA	-	Deoxyribonucleic acid
EDS	-	Energy Dispersive X-ray Spectroscopy
E-waste	-	Electronic waste
FBR	-	Fluidized-bed Bioreactor
FESEM	-	Field Emission Scanning Electron Microscopy
g	-	gram
h	-	hour
HCl	-	Hydrochloric acid
HNO_3	-	Nitric acid
ICP-OES	-	Inductively Coupled Plasma Optical Emission Spectroscopy
LPM	-	Litres per minute
mg	-	Milligram
MIC	-	Minimum Inhibitory Concentration
min	-	minute
ml	-	millilitre
mm	-	millimetre
MTCC	-	Microbial Type Culture Collection Centre
mV	-	millivolt
nm	-	nanometre
PCBs	-	Printed Circuit Boards

ppm	-	Parts per million
rpm	-	Revolutions per minute
SDS-PAGE	-	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
sp.	-	species
UV	-	Ultra-violet
V	-	Volt
W_{AD}	-	Concentration of metal in the acid leachate
W_{BL}	-	Amount of metal bioleached
WHO	-	World Health Organization

CHAPTER 1

INTRODUCTION

1.1. ELECTRONIC WASTE GENERATION

The modernization of society and its adaptability to faster-growing digital communication technologies have accelerated the production of mobile phones with great versatility. The performance of mobile phones is equivalent to professional computers and cameras, making itself crucial for human living. Moreover, this paradigm can be extrapolated to other electronic devices too. The technological novelty has led to a revolutionary standard of living with a decline in the lifespan of electronic equipment consequently (Rautela et al. 2021). Launching of the new models of electronic devices makes obsolete products sorted into electronic waste (e-waste) (Chen et al. 2018; Mejame et al. 2018). The e-wastes include discarded computer parts, motherboards, CDs, mobile phones, televisions, refrigerators, and accessories (Liu et al. 2023). According to Liu et al. (2023), the global e-waste generation has increased by 21% from 2014 to 2019. In 2019, only 17.4% of e-waste was recycled, and the rest of the e-waste flow is uncertain. Asia generates the highest quantities of e-wastes, i.e., 46.4%, followed by America and Europe with 24.4% and 22.4%, respectively though the generation per inhabitant is the highest in Europe (16.2 kg/inhabitant) (Shahabuddin et al. 2023). India, being the third largest in e-waste generation as per 2019 reports, the electronics consumer market is expected to grow at a 16.1% compound annual growth rate between 2019 and 2025 (Singh et al. 2023).

1.2. RISKS ASSOCIATED WITH E-WASTES

Electronic goods contain metallic and non-metallic components. The non-metallic components include glass, ceramics, plastics, and rubber. The base metals like Cu, Zn, Al, Pb, Ni, Fe, etc., and precious metals like Au, Ag and Pd constitute the metallic fraction (Abdelbasir et al. 2018). The complex and heterogeneous nature of the wastes makes them hazardous due to the presence of noxious elements, flame retardants, and

other recalcitrants. The toxicity level depends on the device type, composition, and the prior treatment strategy adopted (Cesaro et al. 2018). It is noticed that only a minor proportion of e-waste is recycled. The legal obligations regarding the impact of treatment in developed countries and rudimentary treatment practices in developing countries contribute to this inadequacy. These limitations, however, have significance in environmental health. The informal discharge processes mobilize these substances into the environment (air, water, and soil) as various pollutants (Rautela et al. 2021). The hazardous constituents in the unprocessed or partially processed e-waste, mainly metals, leach out from the e-waste dumps and pollute water and soil. The health risks of e-waste are due to the contaminated air, water, food, and direct/indirect routes of exposure to the lethal chemicals in the water. The neighbourhood regions of the dump yard or e-waste processing sites are at health risk, which may extend further into the ecosystem. The chemicals in these wastes are powerful mutants, carcinogens, and neurointoxicants, making e-waste management vital (Heacock et al. 2015).

1.3. E-WASTE MANAGEMENT

The first step for managing electronic waste is to approximate the waste generated for well-planned execution. The export of e-wastes from developed to developing countries for recovery of its constituents is a primary concern in the management of e-waste. The neutralization of challenging issues like quantity, infrastructure, assessment, transportation, a mixture of other solid wastes, limited awareness, and incomplete implementation of regulations can achieve a proper e-waste management system. The system should have safe disposal, collection, treatment, and recycling technologies functioning. The wastes were generally treated by classical landfill and incineration systems. Recycling the metal constituents is needed to counter the exhaustion of resources, meet the growing population's demands, and reduce environmental risks associated with conventional mining (Ikhlayel 2018).

1.4. IMPLICATIONS OF ELECTRONIC WASTE MANAGEMENT

Recycling e-waste brings the critical economic incentive of recovering precious metals from scrap, like gold and silver. A ton of computer e-waste can substitute the gold

obtained from seventeen tons of gold ore and constitutes forty times more copper than its ore (Das et al. 2017). Accordingly, the escalating global consumption and pressing demand for precious metals due to exhaustion from their natural resources have necessitated recycling. The recovery of metals from the e-wastes is considered urban mining. The two major metallurgical strategies used for the recovery of metals are 1. Pyrometallurgy, a pyrolytic smelting process that requires high investments, produces toxic emissions, and 2. Hydrometallurgy, chemical-mediated extraction, discharges contaminated effluent. An alternative approach is Biohydrometallurgy using biological agents in the aqueous extraction of metals (Işıldar et al. 2018). Biohydrometallurgical studies are promoted as they are environmental friendly and help in the treatment to efficiently recover metals from electronic wastes containing metals at low concentrations. These advantages ensure the generation of detoxified or less toxic products, a clean environment, prevent the depletion of high-grade ores, and the wastes are considered secondary resources of metals (He and Kappler 2017).

1.5. EMPHASIS ON BIOLEACHING

The percentage recovery of metals from low-grade materials using conventional techniques is marginal. Sequences of environmental disasters in the mines have raised concerns regarding the use of chemical agents for metal recovery on a global scale. Biological methods can treat wastes, recover the valuables from these secondary ores and reduce the release of contaminated effluents. The prerequisite to develop the application of a cost-effective and sustainable approach for metal recovery is established by using microorganisms. The microorganisms act as leachants, flocculants, adsorbents, and oxidants, covering all the areas of biohydrometallurgy. An insight into the –omics (genome, transcriptome, proteome, secretome) of the organism will provide the relevance of the microbe in a particular process. Bioleaching or bio-oxidation as an initial step in the biohydrometallurgical process increases the recovery rate (He and Kappler 2017). Bioleaching is the solubilization of metals catalyzed by microbial cells or their metabolites (Martínez-Bussenius et al. 2017). Different nutritional classes of microorganisms, like lithotrophic bacteria, heterotrophic fungi/bacteria, and actinomycetes, can be used effectively as bioleaching agents. The chemolithotrophic

bacteria are widely studied and applied for the process but require stringent growth and bioleaching conditions (Marra et al. 2018). Heterotrophic bacteria can grow and carry out bioleaching in ambient physiological conditions. Only a very few of these genera, like *Chromobacterium* sp., *Pseudomonas* sp., and *Bacillus* sp. (Baniasadi et al. 2019), have been exploited due to its precious metal bioleaching efficiencies through cyanogenesis.

1.6. BIOREACTORS FOR BIOLEACHING

Microbial leaching with the subsequent release of soluble metals from its insoluble matrices is essential from an industrial perspective for waste management. Bioleaching requires a detailed study of the class of microbes employed, biotic and abiotic requirements for growth, adaptability, dissolution capability, and the physicochemical nature of the solids *in-vitro*. The mechanisms involved in bioleaching are anticipated from the habitat of the isolate and subjected to research. A profound understanding of the microbial mechanisms of leaching will channel the specific bioprocess requirements and its scale-up for industrial requirements.

The bioleaching studies in shake flasks allow precise and easier experimentation and observations on the various leaching parameters, the microbial flora, and their potential in bioleaching as well as in understanding the mechanism of bioleaching. However, large-scale implementation of the bioleaching operation necessitates using bioreactors like stirred tank reactors and column reactors. Bioreactors enable good mixing and mass transfer characteristics to obtain appreciable bioleaching efficiency even when processing large amounts of e-waste. The controlled conditions in the bioreactor allow free movement of the reactants and enhance the mass transfer in the multi-phase system. The practical implementation of any bioreactor on a large-scale needs prior tests and experiments on a lab scale and pilot scale to validate their performance. The bioreactors also serve as a model for appropriate in-situ processes like heap and dump leaching by indigenous microorganisms.

Further, these bioreactor simulations validate the possibility of leaching in refineries and other industries which benefit from the process. Fluidized-bed reactors have excellent solid-liquid, solid-gas, and gas-liquid mass transfer characteristics. This

has been widely used for multi-phase reactions (Tisa et al. 2014) as a multi-phase contactor for various applications. Thus, it is an ideal candidate to carry out aerobic bioleaching (Soleimani et al. 2009; Trivedi et al. 2022) involving solid, liquid, and gaseous phases. The present study focuses on screening a potential heterotrophic bacterial strain for bioleaching and optimizing the bioleaching process in a Fluidized-bed bioreactor for leaching Ag and Cu. A mechanistic study on the one-step Cu bioleaching from PCBs by the selected strain was proposed. The selected strains have been used as co-cultures to recover Au, Ag, and Cu simultaneously. A comparative study of the different bioleaching methods and the performance of the Fluidized-bed bioreactor operated in sequential batch mode for bioleaching using the selected heterotrophic bacterial strains are presented.

CHAPTER 2

REVIEW OF LITERATURE

2.1. ELECTRONIC WASTE

The increasing technological advances coupled with consumer demand for electrical and electronic items are the sole cause of electronic waste (e-waste) generation. The printed circuit board (PCB) of the e-waste is the core component contributing 3-6% of the total weight. The tiny life span of electronics contributes significantly to e-waste generation due to less efficient recycling and reuse (Priya and Hait 2017). The printed circuit board is heterogeneous and complex, constituting approximately 28% metallic fraction, and the non-metallic fraction represents plastics, rubber, glass, and ceramics. PCB contains metals like gold, silver, copper, zinc, nickel, lead, aluminium, and iron that are valuable and hazardous. The exact figure of the composition varies with the source of the obtained PCB (Awasthi et al. 2016).

2.2. ENVIRONMENTAL IMPACT OF PRINTED CIRCUIT BOARDS

In most developing and under-developed countries, the handling and disposal of PCBs are unregulated. Safety concerns arise due to its toxic constituents like metals and flame retardants released into the air, water, and soil, which are potential neurotoxicants and carcinogens. The 2013 WHO work group observed a need to raise awareness about electronic scrap as an environmental health threat internationally (Heacock et al. 2015). Previous research on health assessments of informal workers of e-waste recycling units in Ghana have shown the presence of elevated levels of heavy metals (Caravanos et al. 2013) and flame retardants (Wittsiepe et al. 2015) in their blood and urine samples. Their findings were not pathological but caused significant pulmonary issues. The breast milk samples of the women residing nearby had high concentrations of polychlorinated biphenyls. These chemicals pose a neonatal health risk more than adults since these chemicals act as developmental neurointoxicants, leading to cognitive deficiencies in infants. The pollutants also affect the metabolism of aquatic life, physiology, reproduction, development, and growth. The risks are associated with releasing these

chemicals into the atmosphere, water bodies, and biota of vulnerable areas with rudimentary recycling techniques and informal processes (Daum et al. 2017).

2.3. METAL RECOVERY FROM E-WASTE

Electronic materials can be treated directly through landfills and incineration. Metals could be recovered from the electronic scrap by Pyrometallurgy and Hydrometallurgy processes. These recycling techniques are generally employed for extracting the metals from their low-grade ores and other metal-containing matrices (Table 2.2). Electronic wastes are considered the secondary resource for precious and base metals with high purity.

2.3.1. Pyrometallurgy

Pyrometallurgy is the technique in which the non-metallic fraction of the PCBs is decomposed, and the metallic fraction is concentrated in an inert atmosphere or under a vacuum. A high temperature is required to remove the solders and consequently separate the metals and non-metals (Ning et al. 2017). Pyrolysis is a technique of choice due to its volume reduction capacity and pure yields. Most of the reported studies have used nitrogen gas to provide inert conditions, while a few have used vacuum pyrolysis (Zhou et al. 2010). The organic portion of the PCBs is decomposed to low molecular weight products like oils and finds its application as fuels/feedstock in manufacturing industries. The inorganic metals can be recycled and recovered (Zhou and Qiu 2010).

2.3.2. Hydrometallurgy

The modern hydrometallurgical methods mark the beginning after the discovery of aqua regia (a mixture of HNO_3 and HCl), the cyanidation process for the recovery of gold, and Bayer's process for alumina. Further, various chemical and biological leaching agents were discovered following the separation processes that are applied at present (Habashi 2005). Hydrometallurgy is the solubilization of metals in an acidic or alkaline lixiviant from its resource (leaching) and recovery from the pregnant solution. Some common leaching agents of precious metals include thiosulphate, cyanide, and halides; other metals are subjected to acid leaching. The extraction of metals from mobile phone PCBs requires more than one leaching agent. Silver was best solubilized using nitric acid,

copper, and gold in aqua regia. Leaching in aqua regia is the best method to achieve complete solubilization of most metals in PCBs (Petter et al. 2014).

2.3.3. Biohydrometallurgy for e-waste treatment

The metal accumulation and dissolution abilities of the microorganisms make it a suitable biological agent in hydrometallurgy, substituting chemical leaching agents for metal recovery. The microbe-mediated hydrometallurgical process is termed "Biohydrometallurgy." Both prokaryotic and eukaryotic microorganisms are reported for metal-microbe interactions in the natural biogeochemical cycles. Hence these organisms are exploited for bioremediation and hydrometallurgical applications from their ores (Gadd 1999; White et al. 1997). Prokaryotes include bacteria and actinomycetes, while yeasts, molds, and cyanobacteria are also studied for their metal speciation and further mobilization potential (Abdulla 2009; Gadd 1999; He and Kappler 2017; Pollmann et al. 2016). An essential prerequisite for applying these microorganisms for hydrometallurgy is their metal resistance mechanisms by efflux systems, enzymatic secretions, or biofilm formation (Chien et al. 2013).

2.4. MICROORGANISMS AS BIOLEACHING AGENTS

A brief overview of the bioleaching of metals from various sources, mechanisms, and microorganisms used is presented in Table 2.2.

2.4.1 Chemolithotrophic autotrophs

The bacteria classified under this group are well-studied for their bioleaching abilities. These bacterial communities can survive extreme environmental conditions, grow as oligotrophs, and oxidize metals into solution using oxygen from the air as the final electron acceptor. Chemolithoautotrophs derive energy from reduced inorganic chemicals (sulfides), use atmospheric carbon dioxide as a carbon source, and inorganic hydrogen serves to donate electrons. Additionally, the stringent acidic pH for growth at which metals remain soluble enhances the leaching. Most of the members of the lithotrophs are sulfur and iron oxidizers. The genera of bacteria effective for bioleaching metals from their surfaces include *Acidothiobacillus*, *Leptospirillum*, *Acidianus*, and *Sulfolobus* (Bosecker 1997; Cárdenas et al. 2016). Though lithotrophs are the preferred

organisms for bioleaching due to their low nutrient requirements, they cannot withstand high pH levels and can be applied only for sulfur-containing compounds (White et al. 1997).

2.4.2 Chemoorganotrophic heterotrophs

Most of the fungi (yeasts and molds) and bacteria constitute heterotrophs. The contribution to the mode of nutrition for heterotrophs is provided by organic compounds, which serve as the source of carbon, energy, and electrons. An investigation into the influence of metals on bacteria and fungi has revealed that the metals caused bacteriostatic and fungistatic activity but were not microbicidal (Monballiu et al. 2015). The glycolytic pathway and the tri-carboxylic acid cycle in the metabolism of organic compounds generate different types of organic acids that occupy a central position in the heterotrophic bioleaching (Faramarzi et al. 2004).

These organisms also can produce amino acids, proteins, and exopolysaccharides which facilitate metal solubilization. Heterotrophs contribute to the metal leaching of non-sulfur-containing solids and sustain higher pH. The problem associated with using heterotrophs for bioleaching is that the physiological pH range poses an issue of contamination for scale-up operations, and the requirement of an organic carbon source would not be cost-effective. Using fungi creates another drawback: it should be implemented as a spent medium bioleaching process since the metals would be entrapped in the mycelial network or adsorbed in the cells (Jain and Sharma 2004).

2.4.2.1 *Acinetobacter sp.* CR B2

Acinetobacter sp. CR B2 has shown its potential in the bioleaching of metals for wastes. These ubiquitous heterotrophic gram-negative coccobacilli appear as plump rods in chains or pairs when visualized under oil immersion. These non-sporing, non-pigmented cells are mucoid when encapsulated and budge by twitching motility due to the presence of fimbriae. The strict aerobes grow at temperature optima of 33-35^{o}C and pH 6.5. The nutrient requirements are not stringent, and they grow well in the presence of glutamate and aspartate in a mineral medium that supplies carbon and nitrogen sources. They have no limitations thriving on hydrophobic substrates as well. *Acinetobacter* sps.

are functional in bioremediation due to the presence of degradative plasmids. These plasmids facilitate the degradation of a wide variety of organic compounds. Moreover, the plasmids impart resistance to several antibiotics and heavy metals (Deshpande and Chopade 1994). *Acinetobacter* sp. (GenBank accession No. JF461086), isolated from the aerator water of the activated sludge process from the chemical industry, exhibited tolerance to an array of heavy metals, including Cr, Ni, Cu, Pb, Cd, Zn, Fe, and could reduce hexavalent chromium. Morphological changes on exposure to hexavalent chromium were also discerned (Narayani and Vidya Shetty 2012). The isolate has been an effective microorganism in the bioleaching of metals like Cu, Fe, Ag, Ni, and Zn (Jagannath et al. 2017) from e-waste.

2.4.2.2 *Ochrobactrum sp.* CR B4

These heterotrophic gram-negative rods exist as single, short oval forms with parallel sides and rounded ends. The cells are obligate aerobes and grow at temperature optima of 20-37°C. *Ochrobactrum* sps. are motile with peritrichous flagella. The cells form smooth, non-pigmented, translucent, low convex colonies on solid media. Chemoorganotrophy is established by utilizing amino acids, carbohydrates, and organic acids as carbon sources. They produce acids with most sugars, hydrolyze starch, and esculin. The cells produce enzymes like cytochrome oxidase, urease, and catalase. The genes for the biodegradation of pollutants are accommodated in their chromosomal DNA (Bergey's Manual of systematic bacteriology, 2nd Ed.). *Ochrobactrum* sp. (GenBank Accession No. JF824998) from the activated sludge aerator water has been reported to leach metals like Ag, Cu, and Pb to considerable quantities from fly ash (Taskeen Salim Shedde and Vidya Shetty K 2014). The isolate is also resistant to most metals and can reduce hexavalent chromium to Cr (III) (Narayani and Vidya Shetty 2014).

2.4.2.3 *Alcaligenes aquatilis*

Alcaligenes aquatilis, a heterotrophic bacterium previously isolated from the dust of a silversmith's workbench, is a gram-negative motile rod with peritrichous flagella. This culture is metal tolerant and has been reported for bioprecipitation of silver as nanoparticles (Kulal and Shetty Kodialbail 2021). The cells grow as small, convex,

smooth glistening colonies on solid media and form a slimy consistency with a pinkish tinge by the end of 24 hours in broth. The organism also has various enzyme functions like hydrolase and reductase activity for metal interactions.

2.4.2.4 *Chromobacterium violaceum*

Chromobacterium violaceum is a chemoorganotrophic, gram-negative coccobacilli with a polar flagellum. The cyanogenic bacterial cells are facultatively anaerobic and grow at optimum temperatures of 28-37°C. The cells thrive with the help of oxidases and reductases in aerobic and anoxic conditions. The cells on solid media develop as low convex, distinctive dark violet metallic sheen colonies. The genus is generally identified by its pigment (violacein) production, a natural antibiotic for treating cancers (Bergey's Manual of systematic bacteriology, 2nd Ed.). Most reports on bacterial heterotrophic bioleaching pertain to *Chromobacterium violaceum* for its ability to produce cyanide and leach the metals. Oxidative decarboxylation of glycine precursor exudes cyanide as a secondary metabolite. Iron and magnesium significantly stimulate cyanogenic pathways in gram-negative and gram-positive bacteria, respectively. At physiological pH, it exists as HCN and is volatile, whereas in the presence of salts, the volatility is reduced and hence complex with most of the transition metals (Faramarzi et al. 2004).

Well-studied reports on the use of chemolithotrophs (sulfur or iron oxidizers) and the mode of action of these organisms on the e-waste solids are available. The most commonly used lithotrophs for metal dissolution are *Acidothiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, *Thiobacillus thiooxidans*, *Sulfobacillus thermosulfidooxidans*, and *Sulfolobus* sp. This nutritional group of bacteria grows under extreme environmental conditions like very low pH and high temperatures and thrives in low nutritional habitats with inorganic energy sources (Bosecker 1997; Ilyas et al. 2007). Very few genera of heterotrophic fungi (both yeast and molds), bacteria, and actinomycetes have been functionally applied in the biometallurgy of e-wastes (Kumar et al. 2018). A few examples include the bacteria like *Pseudomonas* sp., *Chromobacterium* sp., *Acinetobacter* sp., and *Bacillus* sp. (Arshadi et al. 2016; Jagannath et al. 2017; Jujun

et al. 2015; Li et al. 2015; Liu et al. 2016). Fungi, like *Aspergillus* sp. and *Penicillium* sp., are also reported for metal dissolution from e-waste (Dave et al. 2018; Narayanasamy et al. 2017). Yeasts like *Rhodotorulla* sp. and *Candida* sp. are studied for their bioleaching abilities from other sources (Jain and Sharma 2004), and *Streptomyces* sp., of the actinomycete group, has the characteristics to biotransform the rock minerals (Abdulla 2009). The factors that influence the recovery of metals from e-waste are the type of microorganism, its nutritional requirements, the genetic makeup of the strain, growth, pH, temperature, oxygen requirement, e-waste particle size, pulp density, and mode of operation (Liu et al. 2016). This process can be done in heaps, tanks, and bioreactors for e-waste treatment and recovery of metals from the e-waste. A few genera of heterotrophs, like *Pseudomonas sp*. and *Bacillus sp*., are also reported for bioleaching of metals. The brief overview of the bioleaching of metals from e-waste conveys that bacterial heterotrophs could be of choice due to their ambient physiological requirements.

2.5. BIOLEACHING

Bioleaching is the microbe-mediated transformation or mobilization of metals from ores or metal wastes into the lixiviant (Priya and Hait 2017). The discovery of *Acidothiobacillus ferrooxidans* from the acid mine drainages has led to studies on its physiology and genetics for further application in biomining. This was noted due to the dissolution of copper from sulfidic ores (Ehrlich 2004). Metal bioleaching by lithotrophs from sulfidic ores is commercially implicated in the recovery of Au, Cu, Ni, U, and Co. Non-sulfidic ores do not contain the energy source for lithotrophs and can be leached by heterotrophs for metal recovery (Jain and Sharma 2004). Besides mining, bioleaching is a credible technique adapted from the natural biogeochemical cycles to detoxify valuable metals or redeem them from wastes like electronic scrap (Brandl 2002; Fu et al. 2016; Heydarian et al. 2018; Nie et al. 2015; Yang et al. 2014).

2.6. SIGNIFICANT MICROBIAL MECHANISMS AND MODES OF BIOLEACHING

A primary requirement for the microbe is to acclimatize itself to metal-containing waste. To establish this, the organism expresses the cryptic metal-resistant gene clusters in the plasmids or chromosomal DNA (Monballiu et al. 2015). Further, metal resistance

is also attained through an active efflux of metal ions or entrapment by metal chaperones (Martínez-Bussenius et al. 2017). Three main principles (Das et al. 2017) that govern the microbial leaching of metals through contact and non-contact modes are (represented in Fig. 2.1),

1. ***Redoxolysis*** accounts for metal solubilization through oxidation and reduction reactions. Under aerobic and acidic conditions, the microorganism oxidizes ferrous to ferric ions that act as oxidants for the insoluble metal forms. Additionally, ferric ion catalyzed sulphuric acid formation in the case of metal sulfides and dissimilatory reduction of ferric to ferrous ions result. This occurs in the following reaction cycle (Bosecker 1997; Vera et al. 2013).

2. ***Complexolysis*** is a consequence of the secretome of the organism complexing with the metals. The extracellular metabolites of the microbial cells either act as ligands, aid in chelation, or directly complex with the metals. Siderophore-mediated iron chelation, peptides binding the metals, and carboxylate anions from the carboxylic acids complexing with the metals churn out soluble forms of metal complexes (Gadd 2000).

3. ***Acidolysis*** is the generation of inorganic and organic acids and the dissolution of metals from the surface. The inorganic acid produced by the microorganism is usually sulphuric acid which plays a role in solubilizing metal sulfides. Another group of organisms excretes organic acids like citric, glutamic, and oxalic acids. The metals are displaced from their surfaces by the protons from these carboxylic acids (Bosecker 1997).

2.6.1. Contact bioleaching

The corrosion of the metal surface occurs by direct physical or close proximal interactions of the bacteria at the interface. This is a consequence of the loss of electrons by the metal to an electron acceptor that is in contact with the metal exterior. A series of redox reactions by the chemical species cause the release of metal ions into the solution. In aerobic conditions, oxygen is reduced, and anoxic leaching evolves hydrogen. Another critical factor contributing to contact leaching is the interaction in the biofilm matrix. The bacterial cells form a biofilm by producing extracellular polymeric substances (slime) on the material. The metals bind to the functional groups of the carbohydrates and proteins

that constitute the proteinaceous surface layers (Beech and Sunner 2004; Pollmann et al. 2016). Chemotaxis and attachment to preferred sites of imperfection on the metal surface mediate biofilm formation and dissolution (Bosecker 1997). The role of the bacteria is to create a micro-environment at the interphase for electron and mass transfer reactions of the bioleaching process (Bobadilla Fazzini et al. 2011). A recent development is using genetically modified organisms that express the metal binding peptides as receptors on the cell surfaces (Pollmann et al. 2016).

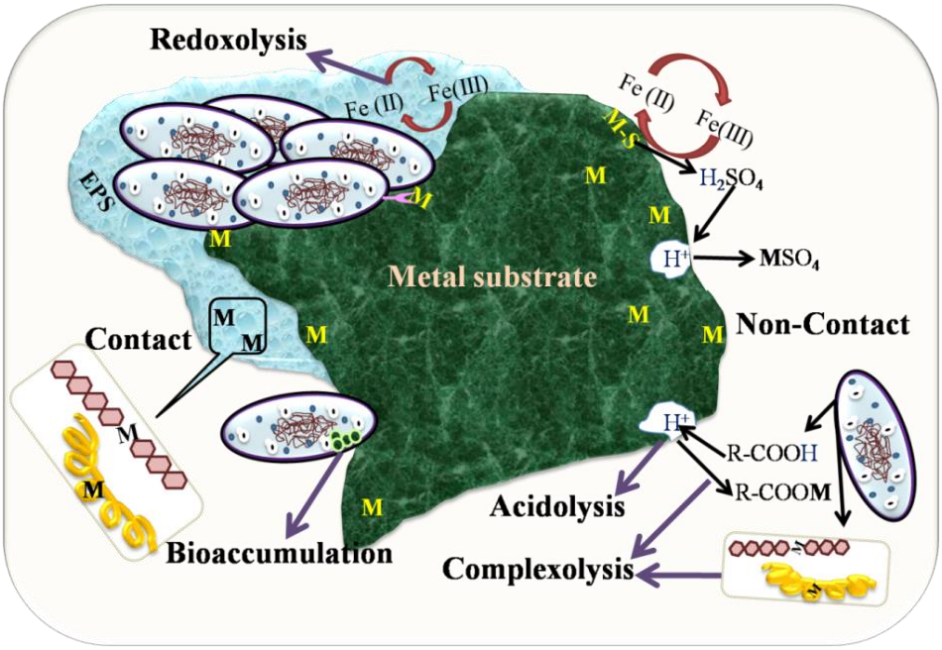


Fig. 2.1. Contact and Non-contact mechanisms of bioleaching (Minimol et al. 2021)

2.6.2. Non-contact bioleaching

The presence of metals in the environment of the planktonic state cells triggers the production of various secondary metabolites, including organic acids and enzymes (White et al. 1997). Even the release of CO_2 leads to carbonic acid attacks on mineral surfaces (Abdulla 2009). The increased Fe^{3+} concentration in the solution is expected to increase the redox potential (Yang et al. 2013). The suspended Fe^{2+} ions in the leaching medium lead to redoxolysis, as in the extracellular polymeric substance compartment around the cells in a biofilm. The acid exudes into the medium, resulting in a protonic attack on the critical bonds that hold the reduced metal to surfaces and solubilize them.

The organic acid anions function as a ligand for complex formation to a certain extent, along with the microbial secretion of chelating agents for complexolysis. Inorganic acids like sulphuric acid would be produced in the case of sulfur-oxidizing bacteria (Awasthi et al. 2016). Fermentation of organic compounds and catabolic pathways release ligands to form metal complexes. The low molecular weight chelating agents like siderophores solubilize $Fe^{3+,}$ facilitating redoxolysis. The extracellular polysaccharides produce complexes with the metal ions, although it has a signifying role in contact leaching (Jain and Sharma 2004). A novel lipoprotein characterized as licanantase from the secretome of the bacterium *Acidothiobacillus thiooxidans* has effectively enhanced the leaching rate of copper from chalcopyrite (Bobadilla Fazzini et al. 2011). The modes of action of the microbial cells on the metal-rich surface, both directly and indirectly, are illustrated in Fig. 2.1. Investigating the expressions of energy conservation genes and the role of planktonic and attached cells has better understood the combined mechanisms of contact and non-contact leaching from chalcopyrite ore (Zhu et al. 2012). Based on the mechanisms and modes of bioleaching, three methods could be applied to achieve metal dissolution from the surface of the solid metal concentrates (Rasoulnia and Mousavi 2016).

2.7. REACTORS IN BIOLEACHING

Bioleaching can be carried out in different types of reactors. In mechanically agitated stirred tank reactors, the internal moving components bring about contact between the phases (Foucher et al. 2003; Zhou et al. 2009). The rotating drum bioreactor rotates in the leaching liquor, wherein the perforations in the solid-containing drum allow solid-liquid contact. The drum rotations establish uniform mixing (Rodrigues et al. 2015). The packed columns allow only the lixiviant to pass through the solids, limiting the mass transfer in the system (Yang et al. 2013). In the pulsed-plate column, the solids are loaded on the perforated plates. The liquid and gas phases contact the solids through the perforations in the plates. The movement of particles within the plates increases bioleaching. Higher particle loading reserves free movement and reduces the yield (Jagannath et al. 2017). The advantages and disadvantages of these reactors are presented

in Table 2.1. An overview of the different bioreactors used for the bioleaching of metals is presented in Table 2.2.

Table 2.1. Advantages and disadvantages of various bioleaching reactors

S. No.	Bioleaching Reactor	Advantages	Disadvantages
1.	Stirred Tank Reactor	High volumetric productivity (Cancho et al. 2007). Mixing/Temperature control (Rouchalova et al. 2020; Zhou et al. 2009). Dominating microbial population (Chen et al. 2022).	Biofilm formation on the internal moving parts. High energy requirements. Negative influence on the bio-oxidation of Fe^{2+} (Rodrigues et al. 2015) Shearing force imposed by the agitator blade on the biomass (Xia et al. 2018a).
2.	Rotating Drum Reactor	Provision for high pulp densities and coarse particle size (Rodrigues et al. 2015)	Impaired bacterial growth at high solid loading. Energy requirements for drum rotation
3.	Pulsed-Plate Bioreactor	Pulsation improves renewal of interfacial area enhancing mass transfer in a fixed bed of solids (Jagannath et al. 2017).	Higher frequencies may cause cell damage. Lower frequencies limit mass transfer during bioleaching.

			Energy requirements for pulsation.
4.	Column bioreactor	Provides excellent mass transfer and used to simulate heap and dump bioleaching. Suitable for scale-up (Benzal et al. 2020a).	Clogging due to biomass. Channeling in the bed. Flow restrictions cause mass transfer limitation (Minimol et al. 2020).
5.	Air-Lift Reactor	No internal moving parts. Well defined fluid circulation and uniform mixing conditions. Clogging is eliminated and supports efficient mass transfer. High gas dispersion and mixing (Minimol et al. 2020)	Energy requirement on fluid transport. Shear due to collision of cells and particles. Limitation on the selection of media due to properties like viscosity or density affecting the bioleaching rate.

2.7.1. Fluidized-bed bioreactor for bioleaching

Fluidized beds are preferred over packed beds to provide efficient contact of the phases and overcome the mass transfer limitations. The technology is widespread for many industrial applications which involve multiple phase contacts. Owing to its efficient mixing and mass transfer characteristics, higher rates are achieved in fluidized-bed reactors resulting in smaller-size reactors. The fluidized bed columns do not have moving

parts like stirred tank systems. It prevents shear-induced damage to cells and may reduce energy consumption. Additionally, the surface renewability for contact is enhanced in the column. Operational glitches like biomass clogging and channeling, frequently observed in packed bed columns, are not encountered in fluidized-bed columns. Fluidized-bed columns have been reported to show excellent mass transfer in liquid-liquid, solid-liquid, and gas-liquid systems (Tisa et al. 2014). Thus, the absence of moving components, improved process efficiency, and comparable reduction in requisite bioreactor capacity persuade the option of a fluidized-bed bioreactor (Banerjee and Ghoshal 2016).

2.8. PROCESS ENGINEERING ASPECTS IN BIOLEACHING

Like other bioprocesses, bioleaching can be efficiently applied to recover metals from e-waste in various application scales. Bioleaching involves the use of microbes, and hence the biotic factors to be considered for its implication include the type of microorganism, the inoculum concentration, its growth rate, the cell genotype, the ability of the organism to resist the heterogeneity and toxicity of the e-waste (Minimol et al. 2020). Various abiotic factors like the physicochemical conditions, the e-waste loading, its size and composition, and nutrients required by the microorganism also affect bioleaching (Priya and Hait 2017). Based on the complete understanding of the principles and modes of bioleaching by a suitable microbe and bioreactor design, the process can be established by opting for either batch or continuous mode of operation. The choice of the bioleaching process operation depends on the type of microbe chosen, the inhibitory nature of the metals, and the metal-microbe interactions involved. The bioleaching efficiency can be maximized by adequate optimization of process conditions.

2.8.1. Inoculum size and growth

The inoculum size is vital in the bioleaching of metals from e-waste by influencing the substrate utilization rate and metabolites released. The increase in the size of the inoculum was found to increase the production of organic acids to leach metals (Rasoulnia and Mousavi 2016), increase the bioleaching of heavy metals (Chen and Cheng 2019), and enhance copper recovery (Jagannath et al. 2017) from e-waste. Inoculum size beyond a specific limit results in turbidity and clogging in the bioreactor,

limiting the contact between the solids and cells within a short period leaving the bioleaching of metals incomplete (Jagannath et al. 2017). The advantage of optimizing inoculum size is to shorten the lag phase and obtain a higher growth rate while preventing clogging. The growth rate indirectly triggers the process, analogous to natural biogeochemical pathways at a faster rate and hence bioleaching (Rehm 2001). Pham and Ting (2009) have found that in the presence of e-waste, *Pseudomonas fluoresecens* has higher cyanide production due to a higher growth rate to achieve better bioleaching efficiency (Pham and Ting 2009).

2.8.2. E-waste Load

E-waste loading up to the optimum positively affects the bioleaching efficiency, whereas higher loading will limit the contact between the phases. Further, the available cell concentration will not be sufficient for the bioleaching of the entire loading (Jagannath et al. 2017). Increased loading of e-waste for bioleaching would also increase pH, toxic metals, and hazardous compounds (above MIC), causing a decrease in the bioleaching rate (Işıldar et al. 2016; Yang et al. 2014). To overcome the inhibitory effect on the specific growth rate of the organism and the production of organic acids, the bioleaching process can be accomplished by a two-step or spent medium bioleaching process (Narayanasamy et al. 2017). E-waste loading also affects specific metal dissolution; increased loading increases copper leaching by non-cyanide leaching agents, whereas gold removal is minimal, attributed to the inhibition of cyanide production (Pham and Ting 2009). Higher e-waste load will affect attrition within the particles and the cells where the mixing will be affected. The e-waste loading and particle size to be chosen also depend on the type of bioleaching system operated.

2.8.3. Particle size

The particle size of the solid is another crucial factor in the hydrodynamics of bioleaching, phase contact for efficient mass transfer, contact surface area, and the collision between the solids and the cells. Coarse particles have lesser surface area for contact, and the fine particles cause a collision of particles and attrition on the microbial cells, which are detrimental, thereby decreasing the bioleaching efficiency. The metal

leaching rate increases with the decrease in the size of e-waste (Arshadi and Mousavi 2014; Willner and Fornalczyk 2013; Zhu et al. 2011). A study by Li et al. (2015) reveals that the set range of particle size was the least significant for bioleaching and was not considered for the pre-treatment process. The order of significance of the biotic and abiotic factors in their study reports to be pH > e-waste loading > inoculum size > temperature > particle size. However, the significance of parameters may vary with different bioreactor systems and the microorganisms used.

2.9. CO-CULTURE OF MICROORGANISMS FOR BIOLEACHING OF METALS

The syntrophic behavior of naturally occurring microorganisms in the indigenous environment, like landfills with e-wastes and mines, has resulted in efficient bioleaching. Hence, the synergy in the co-culture of two or more organisms (consortium) can be exploited by understanding its single-cell genomics and their roles in bioleaching for industrial applications (Cárdenas et al. 2016; Islam et al. 2021). This would reveal the co-metabolic functions helpful in predicting the behavior of the microbial consortium and its constituents in designing and optimizing novel strategies for metal recovery (Martinez et al. 2015). A minimal system of this approach is the co-inoculation of two microbes for enhancing the metal bioleaching rate due to co-metabolic pathways and simultaneous recovery of two or more metals due to their individual functions. This is a consequence of coupled molecular processes in the system (Bosse et al. 2015). The co-inoculation can be a combination with two more lithotrophic bacteria (Cancho et al. 2007; Foucher et al. 2003; Zhou et al. 2009), two or more heterotrophs (Pradhan and Kumar 2012), or lithotrophs and heterotrophs (Baniasadi et al. 2019). In all these reports, the metal bioleaching has been enhanced, and different metals have been solubilized for recovery. The co-inoculation may be done by initially adding the individual inocula in specific concentrations in one-step bioleaching. In two-step bioleaching, the organisms grow together without metal substrates and then add the solids or grow them individually and mix them while adding the metal solids. This way, the bioleaching rate can be effectively increased and scaled up for metal recovery.

2.10. BIOLEACHING METHODS

The different methods of bioleaching are (i) One-step bioleaching, (ii) Two-step bioleaching, and (iii) Spent medium bioleaching. Although the methods are for metal mobilization (leaching or oxidation) from the e-waste solids, the action of microbial cells or their secretome may lead to other processes after solubilization. Metal substrate type and the microorganism used determines the bioleaching method for efficient metal recovery (Rasoulnia and Mousavi 2016). A few recent reports that employed different bioleaching methods are presented in Table 2.2.

2.10.1. One-step bioleaching

One-step bioleaching is a method in which the microorganism and the metal concentrates are simultaneously added and incubated for solubilization (Baniasadi et al. 2019). This method would lead to a more prolonged lag phase as the cells require time for acclimatization to the metal-containing media (Yang et al. 2008). The studies by Yang et al. (2008) on the bioleaching of metals from fly ash has revealed that the one-step method is feasible regarding metal yield and organic acid production only at lower fly ash concentration. The reason behind this is speculated that the higher concentration of metals and alkali could be toxic to the cells and hinder the metabolism, thus reducing organic acid production. Morphological changes may occur as the cells are exposed to a high concentration of toxic metals, and there might be deposition of e-waste particles on the dividing mycelia inhibiting the leaching process (Narayanasamy et al. 2017). All the cells inoculated may not be viable for growth or bioleaching in this method. The chances of biosorption of metals onto the live or dead biomass may decrease the metal yield. Jagannath et al. (2017) have reported the bioleaching of e-waste by *Acinetobacter* sp. in a pulsed-plate bioreactor. With an increase in the concentration of Cu^{2+} ions in the leaching medium as the bioleaching proceeds, the biosorption of Cu^{2+} ions occurs, which is revealed by the presence of Cu^{2+}on bacterial cell surfaces (Jagannath et al. 2017), which decreases the metal recovery. In the case of precious metal bioleaching, the organisms produce metabolites like hydrogen cyanide, and oxygen is required for the solubilization process. Since the cells are actively growing, the cells might utilize the oxygen available,

which may reduce the bioleaching efficiency where cyanide leaching is the target. Bioaccumulation of leached metals in the cells may lead to cell death when their concentration exceeds toxic levels (Baniasadi et al. 2019).

2.10.2. Two-step bioleaching

Two-step bioleaching is the addition of metal-containing solids after the pre-cultured microorganism has attained the exponential growth phase (Baniasadi et al. 2019). According to Yang et al. (2008), two-step bioleaching increases the organic acid production and metal yield by *Aspergillus niger*, and the lag phase is not applicable for bioleaching. In the studies by Narayanasamy et al. (2017) on the leaching of precious metals from PCBs by the two-step bioleaching method, since the fungi were pre-cultured in the first step, the production of organic acid and the mycelial growth rate increased as the cells were not exposed to metals in the first step. This has resulted in a faster rate of bioleaching in the second step. Several reports proved that two-step bioleaching of e-waste (Kumar et al. 2018; Natarajan and Ting 2013; Pradhan and Kumar 2012; Sodha et al. 2020; Tay et al. 2013) and other materials (Qayyum et al. 2019) could be efficiently employed with organisms that are susceptible and become metabolically inactive in the presence of heterogeneous composition of the metal source. The two-step bioleaching method is well established in organisms where redoxolysis is the mechanism of bioleaching. The growth and generation of ferrous ions occur in the first step, followed by the e-waste leaching in the second step (Nie et al. 2015). The other processes that might occur in two-step bioleaching are similar to one-step bioleaching, which includes bio-sorption, bio-accumulation, and precipitation of the metals in the leachate. However, the metal yield in two-step bioleaching may reduce due to biosorption or bioaccumulation of metals by the cell mass in the second step and due to precipitation of the metals by forming complexes with the metabolites.

2.10.3. Spent medium bioleaching

Spent medium bioleaching is the dissolution of metals from the solids in cell-free supernatant obtained after attaining the maximum cell density. The spent medium contains extracellular proteins and secondary metabolites. This strategy would overcome

the limitation of one-step bioleaching, in which the rate of production of metabolites is inhibited by the presence of metals, especially when the pulp densities are high (Baniasadi et al. 2019). Studies on spent medium bioleaching explain the role of extracellular metabolites to bioleach the metals from e-waste. Efforts to improve the production of secretory compounds that bioleach the metals without affecting the growth and metabolism of the organism have been carried out. This is accomplished by allowing the culture to grow and separate the cells, using the cell-free medium as a bioleaching lixiviant (Natarajan and Ting 2013). The microbial cells can be optimized or genetically engineered to produce a maximum amount of the metabolite, and the cell-free spent medium can be utilized for leaching. This method also offers the advantage that there will not be any biomass contamination of the recovered metals. As the cells are not in direct contact with the e-waste, a continuous or fed-batch system with cell separation can be employed, and bioleaching can be enhanced continuously (Natarajan and Ting 2015). Before selecting this bioleaching method, a detailed study of the leaching mechanism should be understood. Metal recovery may be higher in this process due to the absence of bio-sorption or bioaccumulation. However, there are high possibilities of loss of recoverable metals due to the precipitation of metals by enzymes or metabolites.

2.11. SEQUENTIAL BATCH BIOLEACHING

In batch bioleaching processes, depletion of nutrients in the media may occur, causing the microbial growth to reach a stationary phase, which limits the action of the organism on e-waste. Additionally, the culture medium gets saturated with the leached-out metals preventing further solubilization of the targeted metal from e-waste. These challenges can be circumvented by employing multistage or sequential bioleaching of the e-waste to enhance the metal solubilization by bioleaching. After each batch of bioleaching operation, when the metal concentration in the media remains constant or the metal solubilization rate becomes slower, the leachate from the contactor may be drained. Fresh media with inoculum may be added to the residual e-waste, and bioleaching may proceed. This process enhances the overall recovery, considering the cumulative recoveries from each sequential step. According to Jagannath et al. (2017), bioleaching of copper from e-waste in a pulsed plate bioreactor as a batch process yielded 23% recovery

of copper. In contrast, after five sequential bioleaching of the e-waste residue from each previous batch run, the cumulative recovery was 63.5% copper. This method can also be applied where different groups of microorganisms are employed for selective recovery of metals in each batch of the sequential batch operation. In a two-step bioleaching process by Işıldar et al. (2016), the e-waste was treated with chemolithotrophic acidophilic bacteria like *Acidithiobacillus ferrivorans* and *Acidithiobacillus thiooxidans* to solubilize copper in the first batch run. This was followed by the residue treatment by cyanogenic heterotrophic bacteria, *Pseudomonas fluorescens,* and *Pseudomonas putida,* to leach out the gold from the residue of the first step. Thus, selective bioleaching with maximization of target metal recovery may be attained in a single contactor.

Table 2.2. Review on the previous bioleaching studies for metal recovery from various sources

S. No	Metal substrate	Microorganism	Bioleaching method	Bioreactor	Bioleaching time	Maximum % Metal Recovery	Reference
1.	Chalcopyrite concentrate	Thermophiles	One-step	Continuous stirred tank reactor	14 days	66% Cu	(Cancho et al. 2007)
2.	Fly ash	*Aspergillus niger*	One-step	Shake flask	Not mentioned	98.7% Mn	(Yang et al. 2008)
3.	Sphalerite ore	*Sulfobacillus* sp. *Acidithiobacillus ferrooxidans*	One-step	Draft tube Fluidized bed Bioreactor	9 days	91.4% Zn	(Soleimani et al. 2009)
4.	Chalcopyrite concentrate	*Leptospirillum ferriphilum Acidithiobacillus caldus*	(Cell-mediated) Method not mentioned	Stirred tank reactor	44 days	75% Cu	(Zhou et al. 2009)
5.	PCBs	*Sulfobacillus thermosulfidooxidans Thermoplasma acidophilum*	One-step	Column	280 days	80% Zn 64% Al 86% Cu 74% Ni	(Ilyas et al. 2010)

6.	PCBs	*Pseudomonas fluorescens* *Chromobacterium violaceum* *Pseudomonas aeruginosa*	Two-step	Shake flask	7 days	83% Cu 73% Au 49% Zn 13% Fe 8% Ag	(Pradhan and Kumar 2012)
7.	Electronic scrap	*Chromobacterium violaceum*	Two-step Spent medium	Shake flask	8 days	30% Au	(Natarajan and Ting 2013)
8.	Copper ore	*Acidithiobacillus ferrooxidans* GF *Acidiphilium* sp. DX1-1	One-step	Column	102 days	20.11% Cu	(Yang et al. 2013)
9.	Platreef ore	*Metallosphaera hakonensis*	One-step	Column	304 days	93% Cu 75% Ni 53% Co	(Mwase et al. 2014)
10.	Electronic scrap	*Sulfobacillus thermosulfidooxidans*	One-step	Stirred tank reactor	15 days	91% Al 95% Cu 96% Zn 94% Ni	(Ilyas and Lee 2014)
11.	Mixed Cu and Au particles	*Pseudomonas chlororaphis*	Two-step	Novel designed	Until there was no	88.1% Cu 76.6% Au	(Jujun et al. 2015)

	separated from PCBs			bioreactor	further leaching		
12.	PCBs	*Sulfobacillus thermosulfidooxidans*	One-step	Rotating-drum reactor	8 days	85% Cu	(Rodrigues et al. 2015)
13.	PCBs	*Acidithiobacillus ferrooxidans*	One-step	Column reactor	28 days	94.8% Cu	(Chen et al. 2015)
14.	Electronic scrap	*Pseudomonas fluorescens* *Chromobacterium violaceum* *Pseudomonas aeruginosa*	Two-step Spent medium	Shake flask	8 days	30% Au	(Natarajan and Ting 2015)
15.	PCBs	*Bacillus megaterium*	Two-step	Shake flask	Not mentioned	3.6% Au 71.45% Cu	(Arshadi et al. 2016)
16.	PCBs	*Acidithiobacillus ferrivorans* *Acidithiobacillus thiooxidans* *Pseudomonas putida* *Pseudomonas fluorescens*	One-step Two-step (in sequence)	Shake flask	7+2 days	98.4% Cu 44% Au (in sequence)	(Işıldar et al. 2016)
17.	PCBs	*Acidithiobacillus ferrooxidans* SWUST-01	One-step	Stirred tank reactor	30 days	82.13% Al	(Fu et al. 2016)

18.	Power plant residual ash	*Aspergillus niger*	Spent medium	Bubble column bioreactor	7 days	83% V 30% Ni	(Rasoulnia and Mousavi 2016)
19.	Electronic scrap	*Chromobacterium violaceum*	Two-step Spent medium	Shake flask	8 days	30% Au	(Das et al. 2017)
20.	PCBs	*Acinetobacter* sp. Cr B2	One-step	Pulsed-plate bioreactor	1+1+1+1+1 days	63% Cu (in sequence)	(Jagannath et al. 2017)
21.	PCBs	*Leptospirillum ferriphilum* *Acidithiobacillus caldus*	(Cell-mediated) Method not mentioned	Stirred tank reactor	7 days	85.23% Zn 76.59% Cu 70.16% Al	(Xia et al. 2017)
22.	PCBs and Pyrite	Consortium of lithotrophs	Spent medium	Shake flask	9 days	93.4% Cu	(Wu et al. 2018)
23.	WEEE shredding dust	*Acidithiobacillus thiooxidans* *Pseudomonas putida*	Two-step (in sequence)	Shake flask	8 days+30 hours	>99% Ce, Eu, Nd 80% La, Y 48% Au	(Marra et al. 2018)
24.	Spent Lithium-ion batteries	*Acidithiobacillus thiooxidans* and *Acidithiobacillus*	Two-step	Shake flask	16 days	99.2% Li 50.4% Co 89.4% Ni	(Heydarian et al. 2018)

		ferrooxidans					
25.	PCBs	*Pseudomonas balearica* SAE1	Two-step	Shake flask	7 days	68.5% Au 33.8% Ag	(Kumar et al. 2018)
26.	PCBs	*Purpureocillium lilacinum* *Aspergillus niger* (dominant species)	Two-step	Stirred tank reactor	27 days	56.1% Cu 15.7% Al 20.5% Pb 49.5% Zn 8.1% Sn	(Xia et al. 2018a)
27.	PCBs	*Penicillium chrysogenum*	One-step Two-step Spent medium	Shake flask	21 days	47% Cu	(Xia et al. 2018b)
28.	Contaminated soil	*Aspergillus flavus*	One-step Two-step	Shake flask	15 days	18.16% Pb 49.66% Cd 65.73% Zn	(Qayyum et al. 2019)
29.	Sewage sludge	Consortium of Sulfur-oxidizers	One-step	Pilot-scale bioreactor	24 days	73-100% Mn 51-60% Zn 38-52% Ni 17-43% Cu 1-38% Cr	(Chen and Cheng 2019)

30.	PCBs	*Acidithiobacillus* (isolate)	One-step	Bioelectrical reactor	6 days	97% Cu	(Wei et al. 2019)
31.	Sulfidic gold ore concentrate and PCBs	*Roseovarius tolerans* and *Roseovarius mucosus*	One-step Two-step Spent medium	Shake flask	10 days	~100% Au (ore) 1.6% Au (PCBs)	(Kudpeng et al. 2020)
32.	PCBs	*Frankia* sp. DDNSF-0 and *Frankia casuarinae* DDNSF-02	One-step Two-step	Shake flask	30 days	75% Au 94% Cu	(Marappa et al. 2020)
33.	PCBs	*Leptospirillum ferriphilum*	Two-step	Indigenously designed reactor	Until complete recovery	99% Cu	(Sodha et al. 2020)
34.	Copper-gold ore	*Bacillus megaterium* *Pseudomonas aeruginosa*	Two-step Spent medium	Shake flask	4.5 days	>80% Au	(Gorji et al. 2020)
35.	PCBs	*Pseudomonas fluorescens*	Two-step	Shake flask	Not mentioned	54% Au	(Li et al. 2020)
36.	Sphalerite concentrate	*Leptospirillum ferriphilum*	One-step	Shake flask	20 days	96.96% Zn	(Sundramurthy et al. 2020)
37.	PCBs	*Bacillus megaterium*	Two-step	Shake flask	1.4 days	87.46% Au	(Zhou et al.

		Pseudomonas putida					2020)
38.	Sludge mining sediment	*Acidithiobacillus ferrooxidans*	Cell-mediated (Method not mentioned)	Stirred tank bioreactor	35	98.73% Zn 85.42% Fe 96.44% Cu	(Rouchalova et al. 2020)
39.	PCBs	*Acidithiobacillus ferrooxidans*	Spent medium	Stirred tank bioreactor (growth) Column (bioleaching)	6 hours	80% Cu	(Benzal et al. 2020a)
40.	PCBs	*Acidithiobacillus ferrooxidans*	Spent medium	Shake flask	2 days	95-100% Cu	(Benzal et al. 2020b)
41.	PCBs	*Bacillus megaterium* SAG1, *Lysinibacillus sphaericus* SAG2, *Bacillus* sp. SAG3 *Chromobacterium violaceum*	Two-step	Shake flask	7 days	87.5% Cu 73.6% Au	(Kumar et al. 2021)
42.	Mine tailings	*Acidithiobacillus ferrooxidans* *Sulfobacillus*	One-step	Shake flask	16 days	94% Zn	(Liao et al. 2021)

		thermosulfidooxidans					
43.	PCBs	*Pseudomonas aeruginosa*	Two-step	Shake flask	7 days	90% Ag 20% Au	(Merli et al. 2022)
44.	Zn plant purification residue	*Aspergillus niger*	Spent medium	Shake flask	7 days	98% Zn 100% Co 99% Mn	(Faraji et al. 2022)
45.	Contaminated sediment	Sulfur-oxidizing microorganisms	Two-step	Continuous stirred tank bioreactor	30 days	78% Zn 90% Ni 88% Cu 68% Cr	(Chen et al. 2022)

2.12. SCOPE AND OBJECTIVES

Scope of the Research Work

An increase in the use of contemporary electronic devices generates a significant quantity of electronic waste, which mandates treatment due to its hazardous nature. Of these wastes, the PCBs of mobile phones are rich in their metallic composition. The metals can be recovered efficiently from PCBs by employing the bioleaching process for their recycling. Based on the literature survey, the following research gaps were noted.

- The heterotrophic bacterial strains have not been applied widely for bioleaching applications.
- The bioleaching mechanism by heterotrophic bacterial strains and its mode of action on the PCB surfaces have not been clearly understood.
- Column bioreactors, especially Fluidized-bed columns, have excellent mass transfer and mixing characteristics which can be employed for processes like bioleaching.
- The application of heterotrophic bacterial bioleaching for metal recovery in a Fluidized-bed bioreactor has not been reported.

Speculation that heterotrophic bacterial bioleaching would be beneficial over lithotrophic bioleaching led to screening four previously reported metal-resistant heterotrophic bacterial cultures. Choosing an appropriate reacting system/bioreactor was crucial for the bioleaching process. This led to the hypothesis that heterotrophic bacterial bioleaching can efficiently recover metals from PCBs in a Fluidized-bed bioreactor due to its excellent mass transfer characteristics. In order to maximize the bioleaching efficiency in any bioreactor, it is necessary to study the effect of the process operating parameters such as particle size, inoculum size, and e-waste load and to optimize them. Before conducting any column bioreactor study, preliminary shake flask studies are necessary to test the media, microbe, and leaching activity with metal-microbe interactions. An investigation of the mechanism and mode of bioleaching would confer an in-depth understanding of the implication of the process. The simultaneous recovery of different metals may require different organisms to be used as co-cultures. Further, to

improve the metal recovery from e-waste, it may be necessary to develop a process adopting different bioleaching methods with different modes of operation, such as batch and sequential batch, to achieve maximum metal recovery. The following objectives were framed based on the identified research gaps and scope of the research work mentioned above.

Objectives

The current study aims to study the performance of a Fluidized-bed bioreactor in bioleaching for enhanced metal recovery and understand the bacterial heterotrophic mechanisms involved in the bioleaching of metals from printed circuit boards.

The precise objectives of the study are,

- Screening for a potent heterotrophic bacteria from the reported bioleaching strains
- To study the performance of fluidized bed bioreactor for bioleaching of metals from PCBs – effect of particle size, inoculum size, and solid loading
- To investigate the chemoorganoheterotrophic bacterial mechanisms and modes of action in the bioleaching of metals
- To study the effect of co-inoculation on bioleaching in fluidized bed bioreactor
- To comparatively assess one-step, two-step, and spent medium bioleaching methods in fluidized bed bioreactor
- To improve the recovery of metals by bioleaching with sequential batch mode

CHAPTER 3

MATERIALS & METHODS

3.1. CHEMICALS

Nutrient broth, Tryptone, Yeast extract, Beef extract, Peptone, Spirit blue agar, Agar agar, Bovine serum albumin (HiMedia Laboratories), NaCl (99.5%, Spectrum Reagents and Chemicals), Glycine (>99%, Sigma Aldrich), conc. HCL (35%), conc. HNO_3 (70%), L-Methionine (99%), Coomassie brilliant blue-G-250 (Loba Chemie Pvt. Ltd.), KH_2PO_4 (99%), $MgSO_4.7H_2O$ (99%), Methyl Red Indicator (Nice Chemicals Pvt. Ltd.), Methanol (99%, Rankem).

3.2. PCB PROCESSING

Mobile phone PCBs were collected from the service retailers. The make and model of the mobile phones from which the PCBs were procured were heterogeneous. These boards were pulverized in Wiley's mill (Jagannath et al. 2017) after the manual separation of the non-metallic components. A fine powder was obtained by further size reduction in a mixer. The crushed PCBs were sieved, and particles of different sizes, i.e., 1 mm, 0.75 mm, 0.6 mm, and 0.35 mm, were stored for the experiments. The particles of the above size fractions were further subjected to size reduction in a mixer to obtain the material with the average particle size of 0.175 mm.

3.3. QUANTIFICATION OF METALS

The PCB powder obtained in the pan after sieving was used for screening the bioleaching bacteria and the larger particle sizes for optimization. PCBs of different particle sizes were subjected to acid digestion in Aqua regia (1:3 conc. HNO_3:conc. HCl) (Kumar et al. 2018; Marra et al. 2018; Petter et al. 2014). This was done by taking 2 g of PCBs in a beaker to which, was added 20 ml of concentrated HCl and 5 ml of concentrated HNO_3 in a fume hood. An equal volume of distilled water (25 ml) was added and heated to boiling until clear leachate was obtained. The leachate with the

residue was left undisturbed and allowed to settle. The leachate was cooled and then filtered through a Whatman No. 1 filter paper. The filtrate was then analyzed in Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES, Agilent Technologies 5100) at 242.79 nm, 328.06 nm, 327.39 nm, 238.20 nm, 231.60 nm, 220.35 nm, and 213.85 nm for the quantification of Au, Ag, Cu, Fe, Ni, Pb, and Zn in the leachate respectively. The amount of metal leached per gram of e-waste (W_{AD}, mg/g) was calculated using Eq. (3.1),

$$\mathbf{W_{AD}\ (mg/g)} = \boldsymbol{c \times v/w} \qquad (3.1)$$

where *c* is the concentration of metal in the acid digest/leachate (mg/L), *v* is the total volume of the acid digest (L), and *w* is the weight of e-waste digested (g).

3.4. ACCLIMATIZATION OF MICROORGANISMS AND CULTURE CONDITIONS

Metal-resistant heterotrophic bacterial strains, *Acinetobacter* sp. CR B2 (Narayani and Vidya Shetty 2012), *Ochrobatrum* sp. CR B4 (Narayani and Vidya Shetty 2014), *Alcaligenes aquatilis* (GenBank accession number KP772325) previously isolated from the silver smith's workbench dust were obtained from the Department of Chemical Engineering, National Institute of Technology Karnataka. *Chromobacterium violaceum* (MTCC 2656), a cyanogenic bacterium reported for bioleaching of precious and base metals, was procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. The bacterial cultures were acclimatized by a serial sub-culture every 24 hours by increasing the PCB concentrations (1%, 2%, 4%, and 5% w/v) (Heydarian et al. 2018). The adapted cultures were maintained in a Nutrient agar medium at 4°C. As per the requirement, these cultures were freshly grown in Nutrient broth at 28°C at 80 rpm for 12-18 hours for sub-culture and used as inocula for all the bioleaching experiments.

3.5. SCREENING OF HETEROTROPHIC BACTERIA FOR THE BIOLEACHING OF METALS AND SELECTION OF BIOLEACHING MEDIUM

Duplicate experiments were conducted in 250 ml Erlenmeyer flasks with the four strains of heterotrophic bacteria mentioned under section 3.3 in four different bioleaching media. The compositions of the various media used for the study are presented in Table 3.1. Sterilized PCB powder was subjected to one-step bioleaching in 100 ml media in a flask with 2% (w/v) e-waste loading and 5% (v/v) inoculum.

Table 3.1. Media composition for screening

Medium	**Composition**
Medium 1 (Nutrient Broth)	0.5% peptone 0.5% NaCl 0.15% yeast extract 0.15% beef extract
Medium 2 (Pradhan and Kumar 2012)	1% tryptone 0.5% yeast extract 1% NaCl 0.5% glycine
Medium 3 (Jujun et al. 2015; Ruan et al. 2014)	0.5% beef extract 1% peptone 0.5% NaCl 0.44% glycine 0.2% methionine
Medium 4 (Castro et al. 2000)	2% glucose 2% peptone 0.2% yeast extract 0.075% KH_2PO_4 0.03% $MgSO_4.7H_2O$

The flasks were incubated at 30°C in a shaking incubator at 100 rpm for four days. 15 ml of samples were drawn every 24 hours up to 96 hours, and the cell-free supernatant was collected by centrifugation at 8000 rpm for 10 min. The sample was then filtered through a syringe filter with pore size 0.2 µ and analyzed for Au, Ag, Cu, Fe, Ni, Pb, and Zn in ICP-OES at 242.794 nm, 328.068 nm, 327.395 nm, 238.204 nm, 231.604 nm, 220.353 nm, and 213.857 nm respectively. The same experiments were carried out without the bacterial strain and served as the negative control. The amount of metal bioleached per gram of e-waste in mg/g (W_{BL}) was calculated using Eq. (3.1) similar to that used to calculate W_{AD}.

3.6. OPTIMIZATION OF Cu AND Ag BIOLEACHING FROM PCBs

Table 3.2. Experimental design for optimization of Ag and Cu bioleaching

Parameters	Particle size	Inoculum size	% E-waste Load
Effect of Particle size	**1 mm**	5%	2%
	0.75 mm	5%	2%
	0.6 mm	5%	2%
	0.35 mm	5%	2%
	0.175 mm	5%	2%
Effect of Inoculum size	Optimum size	**2%**	2%
	Optimum size	**5%**	2%
	Optimum size	**10%**	2%
Effect of E-waste load	Optimum size	Optimum inoculum	**2%**
	Optimum size	Optimum inoculum	**3.5%**
	Optimum size	Optimum inoculum	**5%**

The bioleaching of Cu and Ag by *A. aquatilis* was optimized by the One Factor at a Time (OFAT) method in shake flasks and Fluidized-bed Bioreactor (FBR). The experiments were conducted using the selected bioleaching media, which constitutes 0.5% Beef extract, 1% Peptone, 0.5% NaCl, 0.44% Glycine, and 0.2% Methionine. The

batch bioleaching experiments were conducted by varying the process parameters like particle size, inoculum size, and e-waste load, as shown in Table 3.1, and the metal recovery was determined.

3.6.1. Bioleaching in Shake Flasks

All the bioleaching experiments mentioned in Table 3.2 were conducted by adding the desired quantity of sterile PCBs to 100 ml of the bioleaching media in 250 ml Erlenmeyer flasks. The flasks were inoculated with the required volume (to obtain the desired inoculum size) of *A. aquatilis* culture at its exponential growth phase and incubated for 96 hours at 28±3°C in a shaker at 100 rpm. At every 12 hours, 10 ml of samples were drawn aseptically, and the flask was supplemented with 10 ml of sterile bioleaching media to substantiate the decrease in volume. The samples were centrifuged at 8000 rpm for 10 min to obtain the leachate free of the biomass and particles. The bioleachate was analyzed in ICP-OES, and the amount of metal bioleached per gram of PCB powder was calculated using Eq. (3.1) for the respective metals.

3.6.2. Fluidized-bed bioreactor

The Fluidized-bed bioreactor (FBR) used for the bioleaching of Cu and Ag from PCBs is shown in Fig. 3.1 with its dimensions. The column bioreactor was set up using a glass column of 600 mm in height and 40 mm in diameter. An air inlet is provided at the bottom of the column with an open stop cock. The airflow rate was set at 3 LPM using an airflow regulator and measured using a rotameter. A sintered glass disc of porosity grade 1 (100-160 microns) and a diameter of 40 mm was infused with the column at the bottom to serve the gas dispersion and support the bed of PCB powder in the column. The aseptic withdrawal of the samples from the column during the bioleaching process was facilitated by suction of the samples from the column using a 20 ml capacity syringe with a 0.2-micron filter, using a silicon sampling tube running from the bioleaching media to a Falcon tube.

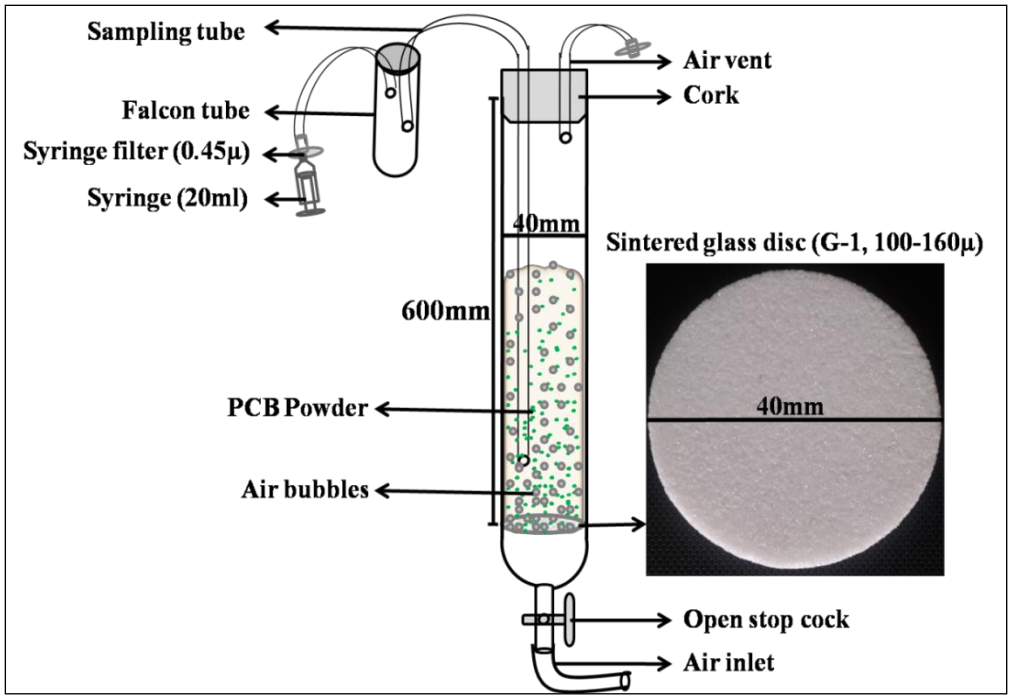


Fig. 3.1 Schematic illustration of Fluidized-bed bioreactor

3.6.3. Bartsch Test

The Bartsch test estimated the foam destruction property of different antifoaming agents (Darby et al. 2012). The test was performed by adding 0.01% of the antifoaming agents like PEG 600 and natural oils (rice bran oil, coconut oil, and sesame oil) to 5 ml of the bioleaching medium in a graduated screw-capped tube. No antifoaming agents were added to the control tube with media. The tubes were closed and shaken vigorously to induce foam. The total volume of the system (medium and foam) and the volume of the medium alone were noted. The antifoaming activity was calculated by subtracting the volume of the medium from the total volume of the system.

3.6.4. Lipolytic activity of the selected bacterial strains

Since the selected antifoaming agent was a natural oil, the lipolytic activity (ability to utilize lipids as a carbon source) of *A. aquatilis* and *C. violaceum* was tested by two different methods in duplicates. First, the organism was streaked in spirit blue agar medium plates containing the anti-foaming agent. The plates were incubated at 28°C for

24-48 hours and observed for the clearance zone around the streak. The second method was well-diffusion in agar medium with 2% substrate and 0.01% methyl red indicator (Samad et al. 1989). 100 µl of cell-free supernatant of 24 hours culture was added to the wells and observed for a zone of clearance up to 18 hours, followed by color intensification later for its lipolytic activity. The well-diffusion assay was also conducted with the sterile broth as a control.

3.6.5. Bioleaching in Fluidized-bed Bioreactor

The FBR was set up, and the optimization for metal bioleaching was carried out by varying the process parameters, as shown in Table 3.2. The required quantity of sterile PCBs was added to 250 ml of bioleaching media, followed by a specified volume of freshly growing (12-18 h) culture of *A. aquatilis* to achieve the desired inoculum size. The airflow rate was fixed at 3 LPM after estimating the minimum fluidization velocity with the highest e-waste load and particle size used in this study. 0.2% of an antifoaming agent (coconut oil) was added to reduce the initial frothing caused by gas dispersion in the bioleaching media. The bioleaching was conducted for 96 hours at an ambient temperature of 28±3°C, and 10 ml of the sample was withdrawn from the column (FBR) every 12 hours. After sampling, this volume loss was compensated by adding 10 ml of sterile bioleaching media to the column. The samples were centrifuged at 8000 rpm for 10 mins, and the leachate (supernatant) was quantified for Cu and Ag in ICP-OES, and the amount of metals per gram of PCB powder was calculated using Eq. (3.1).

3.7. STUDIES ON THE MECHANISMS OF ONE-STEP BIOLEACHING BY *A. aquatilis*

3.7.1. Influence of PCBs on pH, redox potential, and Fe Concentration

Three mechanisms that govern the bioleaching process are acidolysis, redoxolysis, and complexolysis via contact or non-contact modes. To investigate the mode of action of *A. aquatilis* during one-step bioleaching, the experiments were conducted in triplicates with and without PCBs in shake flask conditions, as described in section 3.5.1. The samples were withdrawn every 12 hours until 96 hours and analyzed

for the pH and the oxidation-reduction potential (ORP) of the bioleachate in the pH/mV meter-LMPH9 from LABMAN to confirm if the cells produce or mediate any acid production. Further, Fe plays a vital role in Cu mobilization through redox reactions. Hence, the concentration of Fe in the leachate was analyzed, along with the metal concentrations in an ICP-OES.

3.7.2. Effect of PCBs on extracellular protein concentration and cell viability

The cell-free samples withdrawn at different time intervals were quantified for the concentration of proteins by Bradford's method. The calibration was done using different concentrations of the standard protein, bovine serum albumin (BSA- HiMedia), ranging from 100-1000 µg/ml in deionized water. 250 ml of Bradford reagent was prepared by dissolving 25 mg Coomassie brilliant blue G-250 in 12.5 ml methanol. 25 ml of 85% ortho-phosphoric acid was added to this solution and diluted to 250 ml with deionized water. This reagent was filtered through Whatman No. 1 filter paper and stored in an amber reagent bottle at 4^0C for the assay. 3 ml of Bradford reagent was added to 100 µl of the standards and samples, mixed, and incubated for 5-10 min to obtain a blue colour. The intensity of the colour is directly proportional to the concentration of protein. The absorbance of the standards and samples was measured at 595 nm in a UV-Visible spectrophotometer (Genesys 180 from Thermo Fisher Scientific). The protein concentrations in the samples were obtained from the absorbance values of samples using the calibration plot of BSA.

To assess the cytotoxic effects of PCBs and metals bioleached, the viability of the bacterial cells in the presence and absence of PCBs was determined. This was achieved by the drop-plate method (Herigstad et al. 2001), an alternative to the spread plate technique. 1 ml of samples were aseptically collected from the flasks every 12 hours and were serially diluted. 10 µl of the dilutions were placed as drops on the surface of the sterile nutrient agar medium. The drops were allowed to dry and placed for incubation at 30°C for 24 hours. The petriplates were then observed for colony count, and the colony forming units (CFU/ml) were calculated using Eq. (3.2).

$$\text{CFU/ml} = \frac{\text{No. of colonies} \times \text{dilution factor}}{\text{volume plated}} \quad (3.2)$$

3.8. ESTIMATION OF DIFFERENTIAL PROTEIN EXPRESSION BY SDS-PAGE

10 ml of the cell-free samples withdrawn from the shake flasks after conducting the experiments in the presence and absence of PCBs at 48, 60, and 72 hours were lyophilized (FD5 series Freeze Dryer from Gold SIM International). The freeze-dried samples were reconstituted to 1 ml with molecular-grade water (HiMedia) to obtain the crude protein samples. To prepare the sample for analysis, 150 µl of the crude protein sample was heated at 95°C for 10 minutes with 50 µl loading dye. 20 µl of the prepared samples were loaded in the wells of 6% stacking and 18% separating gel along with the 5 µl ladder (~11-245 kDa from HiMedia) in one of the wells. The protein electrophoresis was carried out in the SDS-PAGE setup (BIO-RAD) at 100 V (power supply unit from BIO BEE) in 1X Tris-Glycine gel running buffer (HiMedia). The gel was then stained overnight in Coomassie blue and destained 2-3 times to visualize the protein bands for any differential expression in the presence of PCBs.

3.9. MICROMORPHOLOGICAL CHARACTERIZATION OF PCBs AND *A. aquatilis* CELLS

After the one-step bioleaching experimental run in the shake flask, the PCB residue was obtained by filtration of the reactor content through Whatman No. 1 filter paper to obtain the residue and the filtrate. The residue was used to visualize any cell attachments on PCB particles. The filtrate was centrifuged at 10,000 rpm for 10 mins at 4°C to pellet out the free cells in the bioleaching medium. The PCB residue and the cell pellet samples were freeze-dried through lyophilization and stored at -80°C until they were characterized using Field Emission Scanning Electron Microscope (FESEM–7610FPLUS, Jeol, Japan). A small portion of the residue was washed in sterile distilled water 3-4 times by vortexing to remove the attached cells. These particles were dried at 80°C for 6-8 hours in hot air oven and stored in a desiccator until they were characterized using FESEM and Energy Dispersive Spectroscopy (EDS) for their micromorphological

characteristics. The crushed PCB powder before bioleaching was also analyzed to discern its morphology and elemental composition.

3.10. COMPATIBILITY TEST FOR GROWTH

The two selected bacterial strains, *A. aquatilis* and *C. violaceum*, were subjected to compatibility tests in duplicates for growth by cross steak method (James and Mathew 2017; Santiago et al. 2017). The bacterial cultures were inoculated by streaking them perpendicular to each other in Nutrient agar plates and incubated for 24 hours at 28°C. The uninoculated plates served as control. The plates were observed for any antagonistic activity at the intersecting points of the streaks.

3.11. CO-INOCULATION OF *A. aquatilis* AND *C. violaceum* FOR BIOLEACHING

The freshly grown bacterial cultures, *A. aquatilis,* and *C. violaceum*, individually and co-inoculated in different ratios, were used for the bioleaching of Au, Ag, and Cu from PCBs. The bioleaching experiments were conducted in FBR and shake flasks under the previously optimized conditions. The 5% inoculum was added in three ratios, 1:1, 1:3 3:1, of *C. violaceum*: *A. aquatilis* for co-inoculation. The one-step bioleaching was carried out for 96 hours. The FBR and shake flask operated under the same conditions, but uninoculated served as the negative control. 10 ml of samples were withdrawn every 12 hours, and an equal volume of sterile bioleaching media was added to substantiate the volume loss. The samples were centrifuged for 10 min at 8000 rpm, and the cell-free leachate was analyzed in an ICP-OES for Au, Ag, and Cu concentrations. Further, the amount of metals leached (W_{BL}) per gram of PCBs (mg/g) was calculated using Eq. (3.1). The percentage bioleaching of these metals was calculated using Eq. (3.3).

$$\textbf{\% Bioleaching} = (W_{BL}/W_{AD})*100 \qquad (3.3)$$

where W_{BL} is the amount of metals leached per gram of PCBs (mg/g), and W_{AD} is the amount of metal per gram of PCB sample (mg/g) initially.

3.12. COMPARISON OF BIOLEACHING METHODS IN FLUIDIZED-BED BIOREACTOR AND SHAKE FLASKS

A comparative study was performed on the different bioleaching methods, i.e., One-step bioleaching, Two-step bioleaching, and Spent-medium bioleaching. The bioleaching experiments were carried out using *A. aquatilis* and *C. violaceum* in a shake flask and FBR under the previously optimized conditions of 0.175 mm particle size, 5% inoculum, and 2% e-waste load for Au, Ag, and Cu recovery.

3.12.1. One-step bioleaching method

A one-step bioleaching process is performed by inoculating the bacterial strain and adding metal substrates (Qayyum et al. 2019) to the medium. Consequently, metal solubilization can be achieved during the microbial growth phases. To conduct the bioleaching experiments in FBR, the air was supplied at a rate of 3 LPM from the bottom of the column. Then, 250 ml of sterile bioleaching medium and 0.2% coconut oil for foam destruction were added to the column. Further, 2% (w/v) of crushed PCB powder was added as the initial e-waste load, and the column was inoculated with 5% (v/v) of the respective bacterial strains. The same procedure was followed for conducting the bioleaching experiments in a shake flask using a 100 ml bioleaching medium without airflow and in the absence of the anti-foaming agent.

The bioleaching was performed for up to 96 hours in both FBR and shake flask. The FBR and the shake flask experiments were conducted at a room temperature of about 28±3°C. The shake flasks were incubated in an orbital shaker at 100 rpm shaking conditions. 10 ml of samples were aseptically withdrawn at the sampling intervals of 12 hours, and the same volume of sterile medium was added to substantiate the volume loss. The samples withdrawn were centrifuged at 8000 rpm for 10 mins, and the bioleachate was obtained as supernatant. The leachates were analyzed in ICP-OES for Au, Ag, and Cu concentrations. The amount of metal bioleached (W_{BL}) per gram of PCB particles (mg/g) was calculated using Eq. (3.1). Further, the percentage of bioleaching was calculated using Eq. (3.3).

3.12.2. Two-step bioleaching method

The two-step bioleaching was carried out separately using both the bacteria, *A. aquatilis* and *C. violaceum*. In the two-step bioleaching method (Natarajan and Ting 2015; Qayyum et al. 2019), the first step was established by culturing the bacteria in 250 ml bioleaching medium with 5% (v/v) inoculum in 500 ml Erlenmeyer flask at 28±3°C with 100 rpm shaking for 24 hours in the absence of PCBs. The bioleaching was carried out in the second step, where 250 ml of this 24 hour grown culture and 2% (w/v) of PCB powder as e-waste load were added to the FBR with an airflow rate of 3 LPM. Similarly, the cultures were grown in 250 ml Erlenmeyer flasks containing 100 ml bioleaching medium with 5% (v/v) inoculum as step 1. The bioleaching in the shake flask was initiated by adding 2% (w/v) PCB powder to the 24 hour grown culture, followed by incubation in an orbital shaker in step 2. The bioleaching process proceeded for 96 hours as described for one-step bioleaching in section 3.12.1, and the metal recovery was calculated.

3.12.3. Spent medium bioleaching method

The cell-free supernatant of the fully grown microbial culture containing the extracellular metabolites and proteins is used for bioleaching in this method (Natarajan and Ting 2015). The cultures were grown in 500 ml Erlenmeyer flasks with 250 ml bioleaching medium for FBR experiments and in 250 ml flasks with 100 ml medium for shake flask studies with 5% (v/v) inoculum for 24 hours at 28±3°C and 100 rpm shaking. The cell-free supernatants were obtained by centrifugation at 8000 rpm for 10 min. 250 ml of the obtained culture supernatant and 2% (w/v) of PCBs were added to the FBR for bioleaching with an airflow of 3 LPM. Similarly, 2% (w/v) of PCB powder was added to the conical flask containing 100 ml of cell-free supernatant for shake flask experiments. The bioleaching processes in both systems were carried out for 96 hours, followed by the determination of the metal recovery and bioleaching percentage, as explained for one-step bioleaching in section 3.12.1.

3.13. SEQUENTIAL BATCH BIOLEACHING IN FBR FOR ENHANCED METAL RECOVERY

3.13.1. Sequential batch bioleaching by one-step method

One-step bioleaching of Ag and Cu was performed under the optimized conditions of FBR in three sequential batches (96 hours each for 12 days). All three batches of the experiment were conducted with *A. aquatilis* following the operating conditions explained in section 3.12.1, starting with the initial e-waste load of 2% (w/v) in batch 1. Further, the subsequent sequential batch experiments were conducted using the e-waste residue of the preceding batch. After removing the bioleachate from FBR, Ag and Cu bioleaching in batch 2 was proceeded by adding fresh bioleaching medium and inoculum. The e-waste residue of the first batch served to be the e-waste load for the second batch. Similarly, the third batch of Ag and Cu bioleaching was continued with the e-waste residue of the second batch. The bioleachates obtained every 12 hours in all the sequential batch experiments were analyzed in an ICP-OES for their metal concentrations. The percentage bioleaching of the metals in each batch with reference to the initial e-waste load fed (to Batch 1) was calculated. Further, the cumulative bioleaching percentage after every batch was also determined. The cumulative metal bioleaching percentage after any "N" number of sequential batches was calculated using Eq. (3.4).

$$\textbf{Cumulative metal bioleaching percentage} = \sum_{n=1}^{N} \frac{W_{BL_n}}{W_{AD}} \times 100 \quad (3.4)$$

where W_{BLn} is the amount of metal bioleached at any stage (n) per gram of PCB taken initially, n = 1, 2, 3…, and W_{AD} is the metal content in the PCBs

3.13.2. Two-stage sequential metal recovery by spent-medium and two-step method

Sequential bioleaching of Au, Ag, and Cu was attempted by employing two stages: stage 1 was operated with the spent medium of *A. aquatilis,* and stage 2 was operated with two-step bioleaching of *C. violaceum*. Each stage was comprised of three sequential batches. Each batch was operated for 96 hours and the entire process with two

stages and six batches ran for 24 days. In the first stage, the first three batches of bioleaching were carried out using the spent medium of *A. aquatilis* for Cu and Ag bioleaching in FBR following the procedure described in section 3.12.3. The bioleaching of Au and Ag was continued in the subsequent three batches in the second stage following the two-step method with *C. violaceum*, as described in section 3.12.2. A 2% (w/v) initial e-waste load was subjected to bioleaching in batch 1. The subsequent sequential batches were accomplished with the e-waste residue from the preceding batch up to six batches, as explained in Section 3.13.1 for sequential batch bioleaching using the one-step method.

The bioleachates obtained after every 12 hours in all the sequential batch experiments were analyzed in an ICP-OES for their metal concentrations. The cumulative metal bioleaching percentage after "N" No. of sequential batches was calculated using Eq. (3.4). After the six batches of sequential bioleaching, the leftover Au, Ag, and Cu were quantified as described in section 3.2 after acid digestion.

3.14. STATISTICAL ANALYSIS

All the bioleaching experiments were conducted in triplicates unless otherwise mentioned. The results are represented as their mean and standard deviation as error bars. All the calculated metal recovery data from different bioleaching methods were subjected to One-Way Analysis of Variance (ANOVA) using Tukey's test to estimate the mean differences between the methods at each time interval. The alphabets in the results indicate this difference at a 0.05 level of significance ($p \leq 0.05$). Origin Pro 9 was the software tool for statistical tests and graphical representations.

CHAPTER 4

RESULTS & DISCUSSION

In this research work, the bioleaching of metals from PCBs was studied. The study involved screening heterotrophic bacteria and suitable media for bioleaching of Au, Ag, and Cu, followed by optimization of process parameters to achieve maximum metal recovery in shake flask and FBR with a one-step bioleaching method. The mechanism of bioleaching is also proposed. The studies on co-cultures, one-step, two-step, and spent medium bioleaching methods are presented and compared in terms of metal recovery and bioleaching efficiency. In order to improve the metal recovery, a two-stage process, which combined the spent media bioleaching and two-step bioleaching, was carried out, and the results are presented in this chapter. This chapter presents the findings of the current study and the interpretation of the results, along with a detailed discussion.

4.1. PCB PROCESSING AND METAL QUANTIFICATION

The PCBs were collected, processed and quantified for their metal composition.

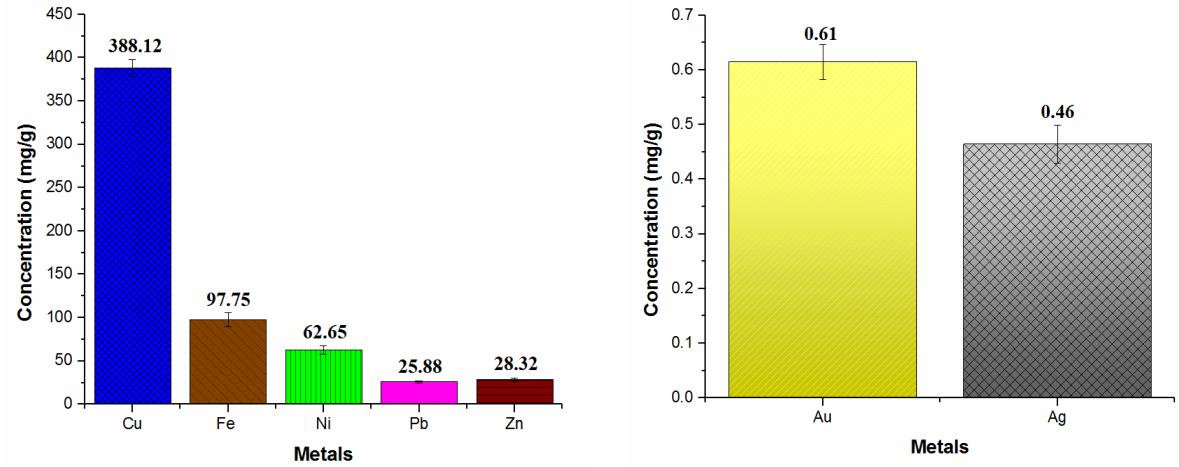
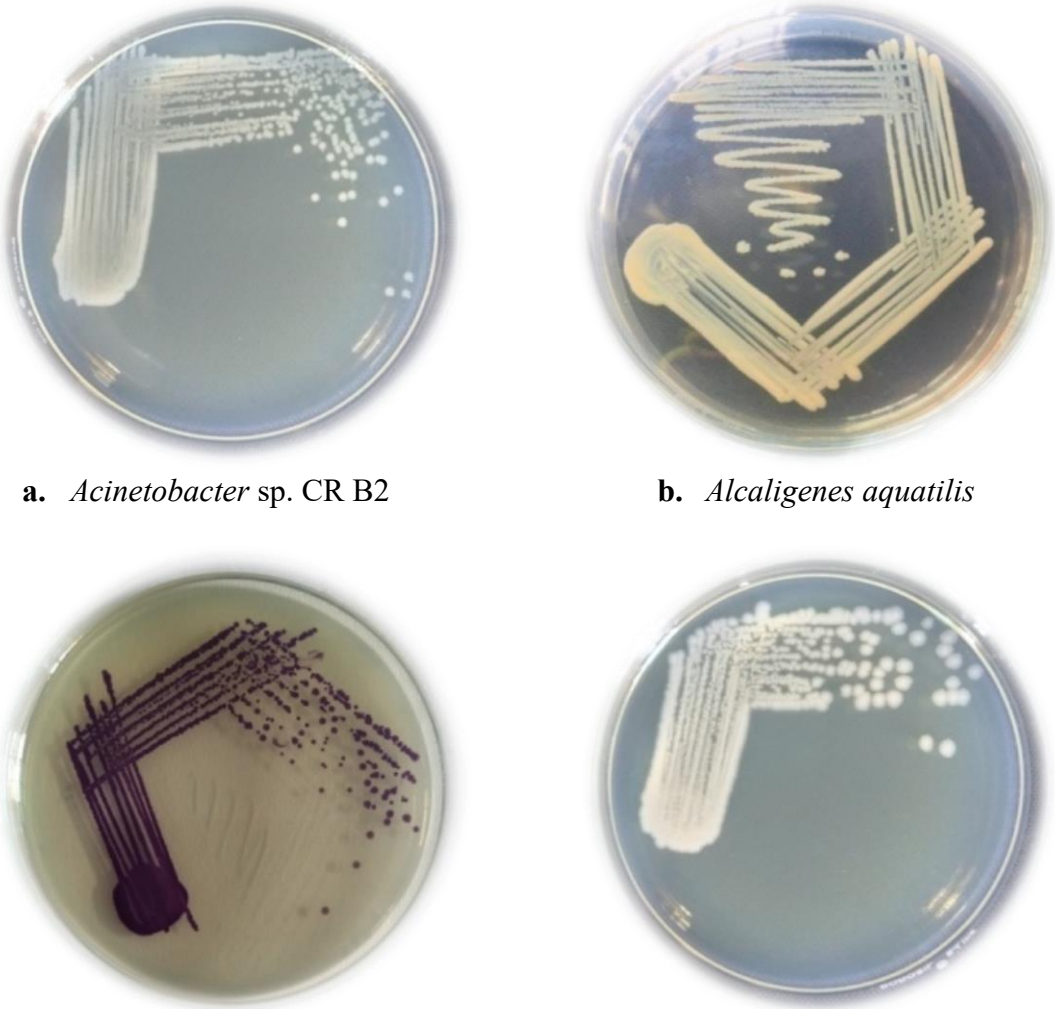


Fig. 4.1. Concentration of metals (W_{AD} mg/g) in PCB powder (<0.175mm) on acid digestion with Aqua regia

On acid digestion with aqua regia, the initial metallic composition of PCB powder as milligrams of metal per gram of PCB powder (W_{AD} mg/g) was determined. The amount of Au, Ag, Cu, Ni, Fe, Pb, and Zn present in one gram of the size-reduced PCB powder (<0.175 mm) to be used for the screening of bioleaching bacteria is graphically presented in Fig. 4.1. This shows that PCBs comprise of high concentration of Cu, 388.12 (mg/g) followed by Fe, 97.75 (mg/g), Ni, 62.65 (mg/g), Zn, 28.32 (mg/g), and Pb 25.88 (mg/g). Precious metals like Au, 0.61 (mg/g), and Ag, 0.46 (mg/g) were also present in the PCBs. Hence, it is confirmed that the PCBs of mobile phones are a rich resource of metals and can be used as a secondary ore for the recovery of metals for reuse, thereby reducing their metal toxicity.

4.2. SCREENING OF HETEROTROPHIC BACTERIA FOR BIOLEACHING OF METALS

This section presents the results of screening a suitable heterotrophic bacterial strain for its bioleaching potential. Four metal-resistant cultures subjected to screening studies are (i) *Acinetobacter* sp. CR B2 due to its bioreduction (Narayani and Vidya Shetty 2012) and bioleaching (Jagannath et al. 2017) abilities; (ii) *Alcaligenes aquatilis* due to its metal resistance and the ability to thrive in the presence of Ag (Kulal and Shetty Kodialbail 2021); (iii) *Chromobacterium violaceum* (MTCC 2656) since it was previously reported for precious metal bioleaching; and (iv) *Ochrobactrum* sp. CR B4 due to its heavy metal resistance and reduction property (Narayani and Vidya Shetty 2014). These bacterial cultures are shown in Fig. 4.2. The bacterial strains were also screened in four different media to select a suitable bioleaching medium for the recovery of metals from PCBs, and the results of this study are discussed in this section.



a. *Acinetobacter* sp. CR B2 **b.** *Alcaligenes aquatilis*

c. *Chromobacterium violaceum* **d.** *Ochrobactrum* sp. CR B4

Fig. 4.2. Heterotrophic bacterial strains used for the bioleaching studies, **a.** *Acinetobacter* sp. CR B2, **b.** *Alcaligenes aquatilis*, **c.** *Chromobacterium violaceum,* and **d.** *Ochrobactrum* sp. CR B4.

4.2.1. Bioleaching of metals in Medium 1

The bioleaching of metals in Medium 1 (Nutrient broth, a general-purpose medium) was carried out using the four test organisms for 96 hours. The results are illustrated in Fig. 4.3. *Chromobacterium violaceum* proved to be the best for bioleaching of precious metals in this medium with 0.03 mg/g Au bioleached on day 3 and 0.06 mg/g Ag bioleached on day 4. Similar precious metal bioleaching efficiencies have been reported by *C. violaceum* due to its ability to produce biogenic cyanides by expressing

the HCN synthase operon (Liu et al. 2016). *A. aquatilis* could leach out a maximum of 0.05 mg/g of Ag from the PCBs on day 4. *Acinetobacter* sp. and *Ochrobactrum* sp. leached out a relatively lesser amount of Ag. Au bioleaching by the other bacterial strains except *C. violaceum* was negligible, with a maximum of 2 µg/g by *Acinetobacter* sp., Cu bioleaching by *A. aquatilis* was the maximum at day 3 with 57.75 mg/g, and Cu was solubilized by *A. aquatilis* in high concentrations in Medium 1. This could be attributed to prominent bacterial action and shows that the organism has maximum Cu bioleaching efficiency.

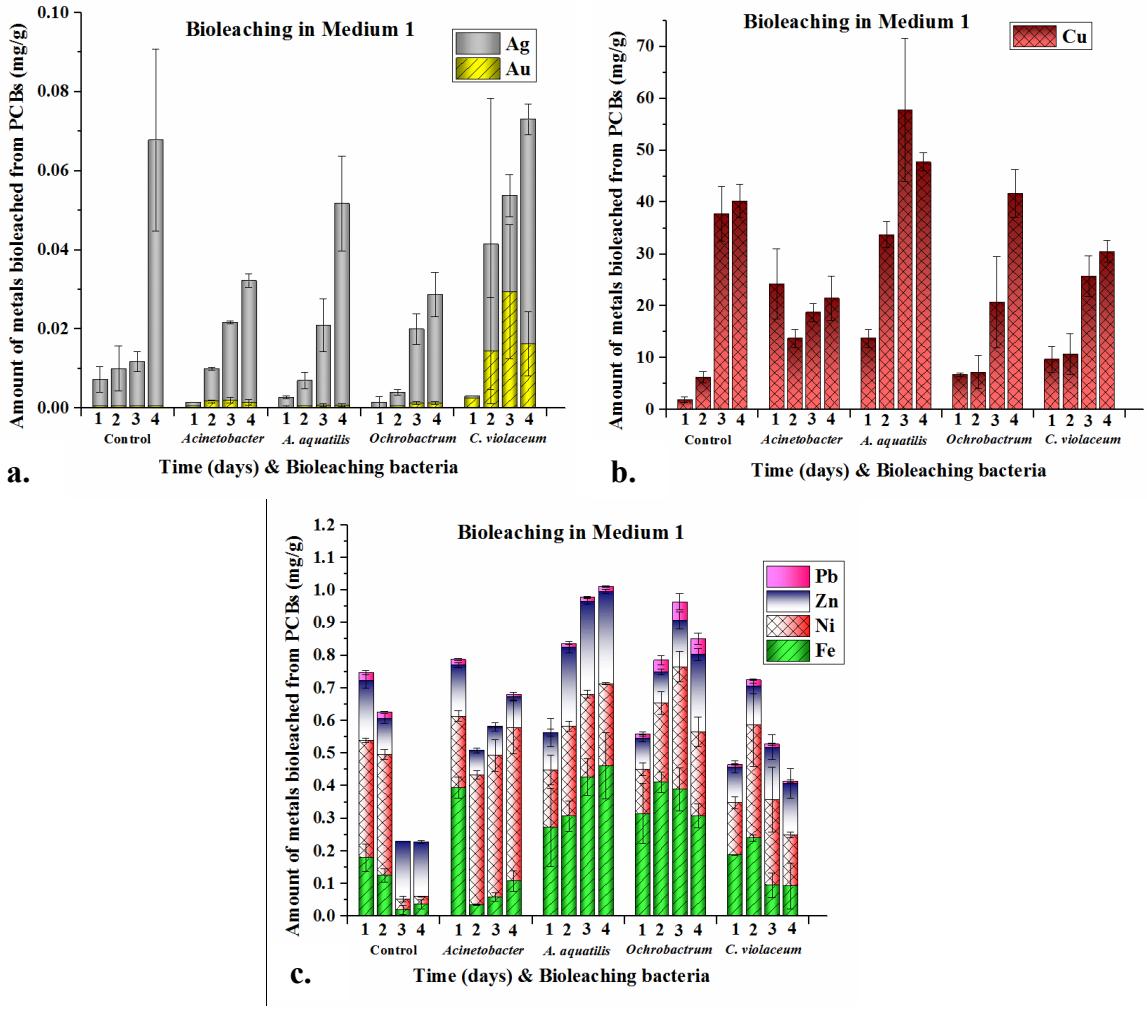


Fig. 4.3. Bioleaching of metals in Medium 1 from Day 1-4, **a.** Bioleaching of Au & Ag, **b.** Bioleaching of Cu and **c.** Bioleaching of Fe, Ni, Pb & Zn

The other bacterial strains leached out Cu in concentrations lesser/similar to that of the control, proving that the organisms could not leach Cu in sufficient quantities in Medium 1. The maximum recovery of other metals through bioleaching in the leaching liquor was, 0.46 mg/g (day 4) Fe by *A. aquatilis*, 0.47 mg/g (day 4) Ni by *Acinetobacter* sp., 0.05 mg/g (day 4) Pb by *Ochrobactrum* sp., and 0.28 mg/g Zn by *A. aquatilis* at day 3. The bacteria utilize the components in the medium for its growth and metabolism and in the process leach out the metals into the medium. The presence of metals in control may be attributed to the ability of the medium components to complex with the metals present in the PCBs.

4.2.2. Bioleaching of metals in Medium 2

Bioleaching studies were carried out in Medium 2, Luria Bertani broth supplemented with 0.5% Glycine. Fig. 4.4 indicates that a maximum of 177.75 mg/g Cu is leached out by *Acinetobacter* sp. followed by 163.5 mg/g Cu by *A. aquatilis* in Medium 2 on the fourth day. Ag bioleaching by *A. aquatilis* was the highest of about 0.03 mg/g on day 4. 0.06 mg/g of Au was solubilized by *C. violaceum* on day 3 of the bioleaching process. The results show that medium 2 was better in bioleaching Ag, Cu, and Au than Medium 1. The oxidative decarboxylation of glycine precursor in the medium enhances the bacteria's cyanogenic pathway, consequently increasing the bioleaching of precious metals from PCBs. A very high concentration of Cu in the PCBs might consume the chelating agents responsible for the bioleaching of precious metals. The unused glycine in the media complexes with cupric or cuprous ions releases free cyanide, and removes the Cu layers in the process. Hence the precious metals are exposed to the free cyanide/cells and get bioleached (Gorji et al. 2020). A similar influence of glycine additive on Au and Ag bioleaching has been reported by Kumar et al. (2018) in their studies on bioleaching by *Pseudomonas balearica* SAE1 strain.

The results of the screening in the present study reveal that *A. aquatilis* had the highest Ag bioleaching efficiency, *Acinetobacter* sp. and *A. aquatilis* showed high Cu bioleaching efficiencies, and *C. violaceum* exhibited maximum Au bioleaching efficiency in Medium 2. The strains which showed maximum solubilization of other metals in

medium 2 are *Ochrobatrum* sp. for Fe (0.74 mg/g), *Acinetobacter* sp. for Pb (0.04 mg/g) and Ni (0.38 mg/g), and *Ochrobactrum* sp. for Zn (0.3 mg/g) which occurred on day 1. The medium components in the control could leach out modest quantities of the base metals. However, the higher concentration of the metals in lixiviant with bacterial cells than that with control has proved the bioleaching potential of the bacteria.

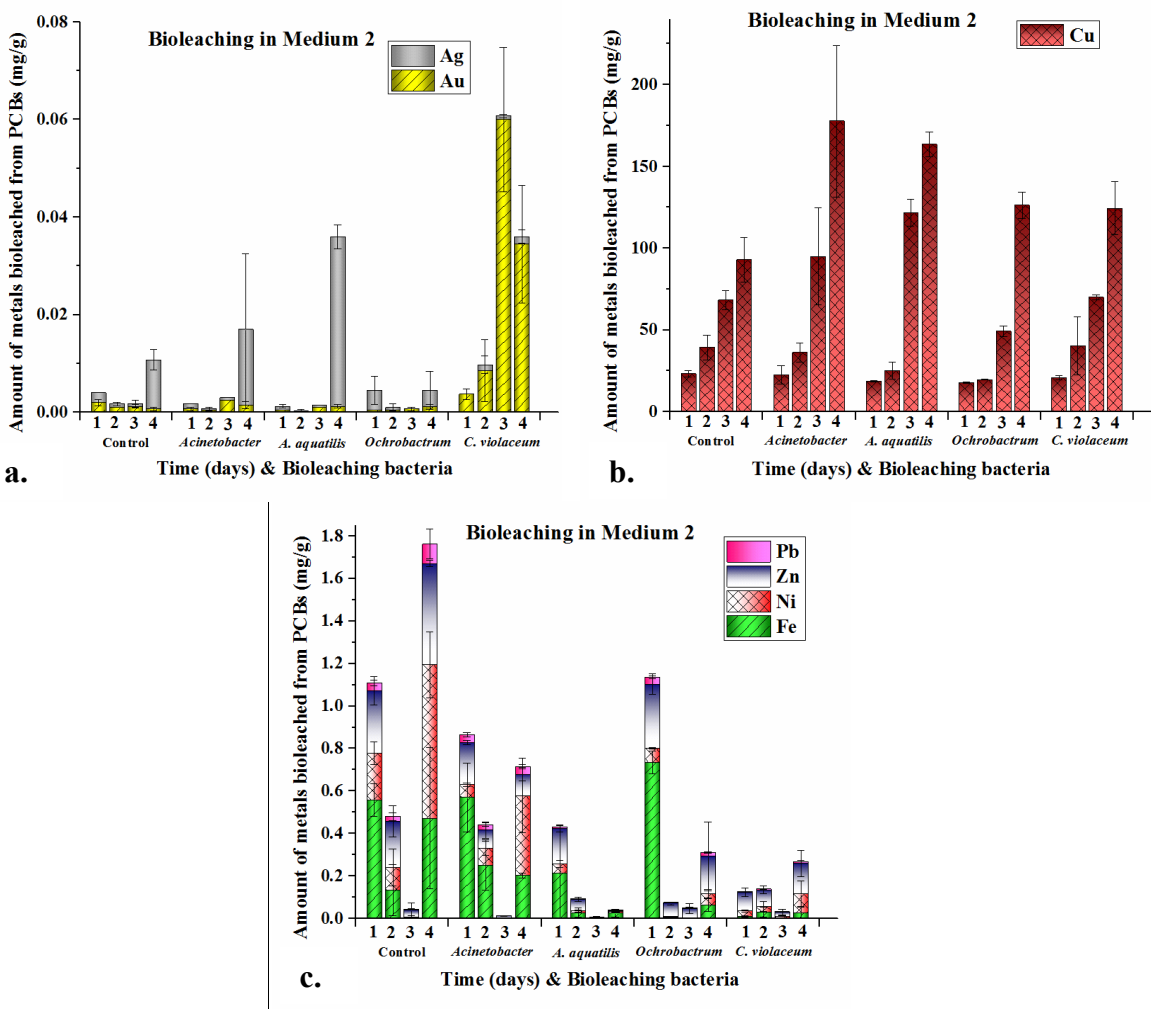


Fig. 4.4. Bioleaching of metals in Medium 2 from Day 1-4, **a.** Bioleaching of Au & Ag, **b.** Bioleaching of Cu and **c.** Bioleaching of Fe, Ni, Pb & Zn

4.2.3. Bioleaching of metals in Medium 3

Medium 3 contained the essential nutrients available in medium 1 and 2, along with amino acids like glycine and methionine as additives to improve the bioleaching of precious metals. Fig. 4.5 indicates that *C. violaceum* could mobilize a maximum amount

of Au (0.07 mg/g on day 3), and *A. aquatilis* leached out a maximum Ag (0.06 mg/g on day 4) among the test organisms in Medium 3. The maximum solubilization of Cu was also achieved with *A. aquatilis* amounting to 122.6 mg/g Cu leached on day 3. *Acinetobacter* sp. bioleached a maximum amount of Fe (0.53 mg/g) and Ni (1.73 mg/g), which occurred on day 4, *Ochrobactrum* sp. could bioleach maximum amount of Pb (0.04 mg/g) on day 2 and *A. aquatilis* bioleached maximum of Zn (1.74 mg/g) on day 4.

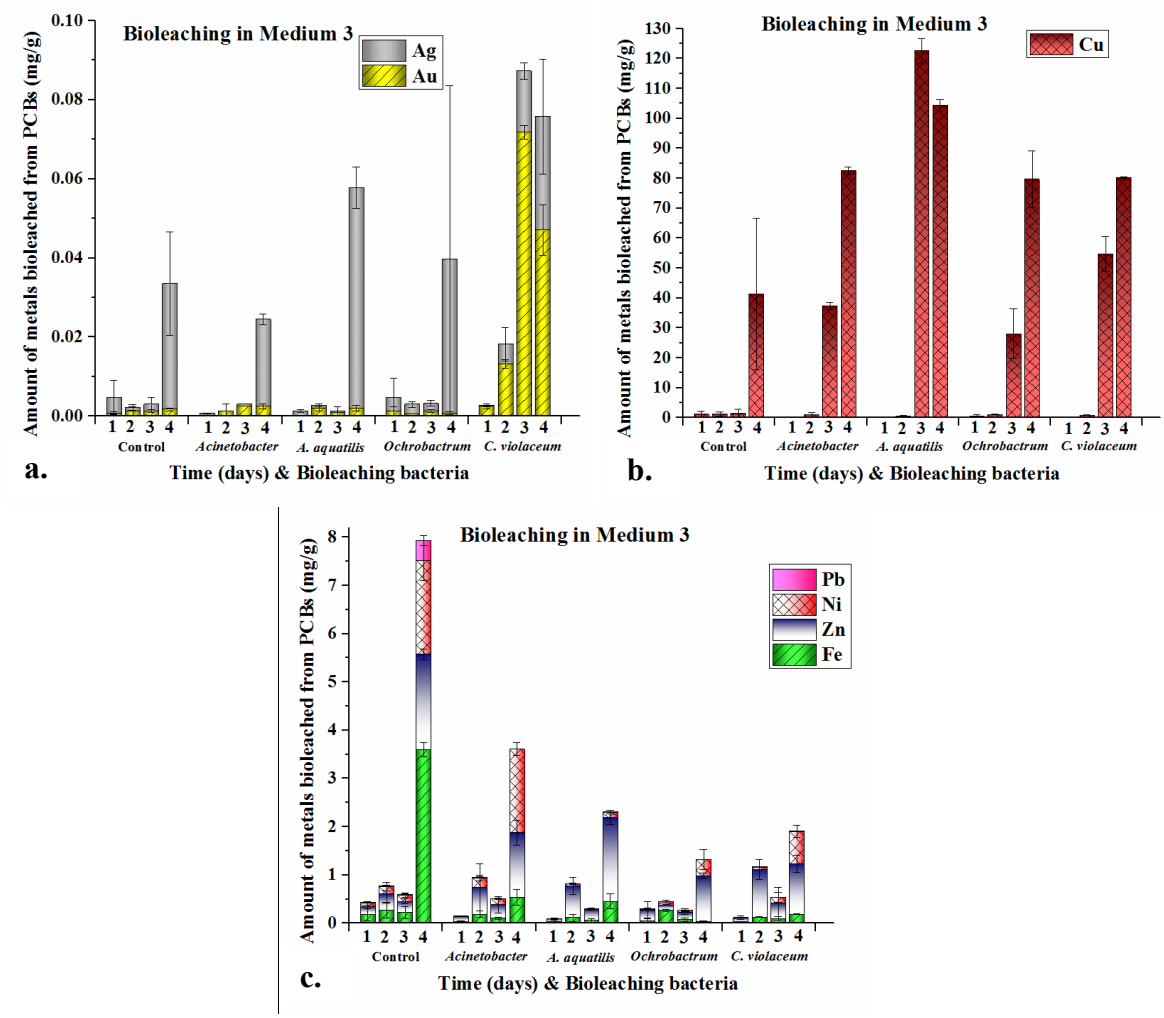


Fig. 4.5. Bioleaching of metals in Medium 3 from Day 1-4, **a.** Bioleaching of Au & Ag, **b.** Bioleaching of Cu and **c.** Bioleaching of Fe, Ni, Pb & Zn

A substantial increase in Au, Ag, Cu, and Zn bioleaching was observed on the third day. The bioleaching potential of the bacteria in this medium was confirmed by the leaching of a larger amount of metals in the presence of bacteria than that in control. The

comparison of Fig. 4.5 with Fig 4.3 and Fig.4.4 reveals that the bioleaching of Au and Ag by *C. violaceum* and *A. aquatilis,* respectively, was found to be higher in Medium 3 as compared to that in Medium 1 and 2. This may be the result of increased cyanide production augmented by the addition of methionine, as reported by Ruan et al. (2014), who studied the bioleaching of Au, Ag, and Cu by *Pseudomonas chlororaphis* in the same medium. Ferrous ions in the leaching lixiviant may also levitate the bioleaching of Cu by redox reactions (Rodrigues et al. 2015).

4.2.4. Bioleaching of Metals in Medium 4

Medium 4, which consisted of 2% glucose, 2% peptone, 0.2% yeast extract, 0.075% KH_2PO_4, and 0.03% $MgSO_4.7H_2O$, is a complete medium that provided carbon and nitrogen sources for the growth of the organism. The bioleaching results studied in Medium 4 are presented in Fig. 4.6. The bioleaching of Au was the maximum by C. violaceum, with 0.029 mg/g achieved on day 4, much less than that obtained with the other media studied. *A. aquatilis* proved to be the best for Ag bioleaching of 0.02 mg/g Ag in Medium 4, but the amount leached was the least in comparison to that in the other media. The maximum amount of Cu was solubilized (18.3 mg/g) by *A. aquatilis* in this medium. It is known that a particular group of heterotrophs like *Pseudomonas* sp. and fungi like *Aspergillus niger* and *Penicillium* sp. are found to produce organic acids (gluconic acid) from glucose (Castro et al. 2000). Castro et al. (2000), in their studies assessed the involvement of acidolysis mechanism in bioleaching, using this medium to ensure whether the organisms are acid-producing. However, the experiments conducted in Medium 4 in this study showed that the medium was unsuitable for the bioleaching of metals from PCBs by the test organisms. A maximum amount of Fe (1.55 mg/g) was leached out by *Ochrobactrum* sp. on day 1, Ni (0.58 mg/g) by *Acinetobacter* sp. on day 4, Pb (0.12 mg/g) by *Ochrobactrum* sp., on day 2 and 0.73 mg/g of Zn by *A. aquatilis* on day 3.

During the time course of the bioleaching process, the amount of metals bioleached was found to increase with time and then decrease in different media, as observed in Fig. 4.3, 4.4, 4.5, and 4.6. During the period of contact of e-waste particles

with the cells, along with the solubilization of metals from e-waste, other processes, such as metal uptake and biosorption by the cells, may co-occur (Jagannath et al. 2017). When the bioleaching rate is higher than the uptake or biosorption rate, a net increase in concentration can occur, leading to an increase in the percentage of bioleaching. However, when the metal concentration in the medium is very high or above some threshold value, the rate of uptake and biosorption may dominate over the rate of bioleaching, thus resulting in a decrease in the concentration of metal in the medium.

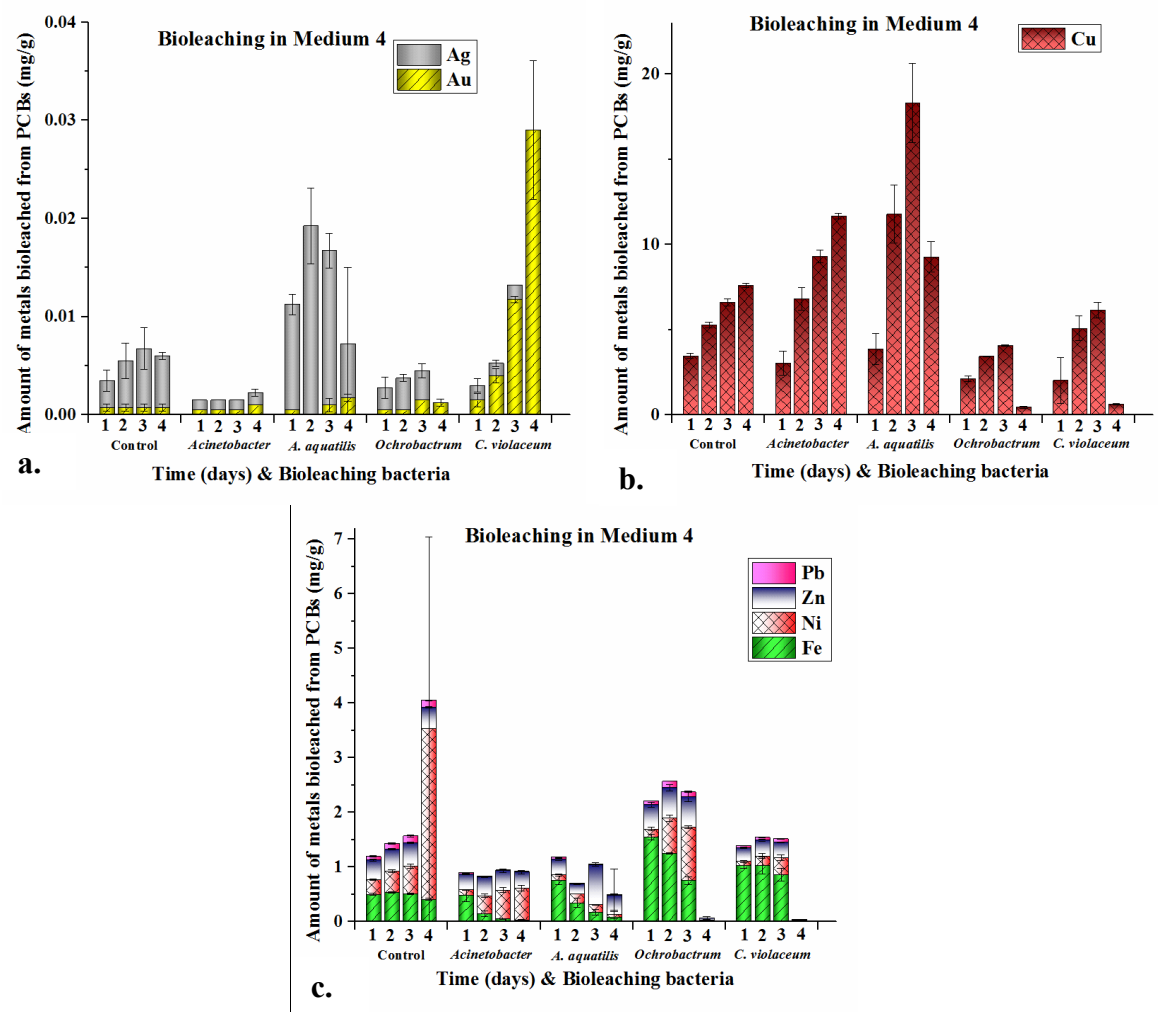


Fig. 4.6. Bioleaching of metals in Medium 4 from Day 1-4, **a.** Bioleaching of Au & Ag, **b.** Bioleaching of Cu and **c.** Bioleaching of Fe, Ni, Pb & Zn

When the concentration of metal ions in the medium is very high, the mass transfer rate from the bulk liquid to the cell surface may enhance. Further, an enhanced

rate of adsorption or uptake of the leached metals by the cells leads to a decrease in metal concentration. The low efficiency in bioleaching of Au and Ag may be attributed to their presence at low concentrations and their embedment deeper in the matrix of other constituents of PCBs. Thus, they are less accessible to the secretory compounds in the media. Further, Cu, which may be present in high concentrations due to its faster solubilization, can selectively complex with the precious metal-binding ligands making it unavailable for leaching Au and Ag. From Fig. 4.3-4.6 it was observed that Fe concentration was found to decrease whenever there is an increase in the bioleaching of Cu by *A. aquatilis*. This may be due to the recyclable role of Fe in the redox reactions for the bioleaching of Cu and other metals from electronic scrap materials as represented in the following reactions (Zhu et al. 2011).

$$4Fe^{2+} + O_2 + 4H^+ \xrightarrow{\text{Cells}} 4Fe^{3+} + 2H_2O$$

$$2Fe^{3+} + Cu^0 \rightarrow 2Fe^{2+} + Cu^{2+}$$

The results indicate that *A. aquatilis* leached out minimal concentrations of hazardous metals like Ni and Pb along with the other metals, thus reducing the toxicity of the lixiviant after the bioleaching process. The reason explained by Marra et al. (2018) for the absence/lesser bioleaching of Pb is that the sulfate salts of Pb may be insoluble and result in precipitation. The results of the control experiments show that the leaching of Pb is higher in the absence of bacteria in Medium 3 that contains sulfur.

The dissolution of Cu was maximum in Medium 2 (42%), it was found that Medium 3 facilitated substantial quantity of Cu bioleaching (32%) and a higher amount of Ag bioleaching (12%) with *A. aquatilis*. Hence, Medium 3 was selected for further studies on improving bioleaching efficiency. Au was also effectively leached out in this medium by *C. violaceum* (12%). From the results obtained after screening for a suitable media as represented in Fig. 4.7 and focusing on the simultaneous recovery of Au, Ag, and Cu, *Chromobacterium violaceum* and *Alcaligenes aquatilis* were selected for further bioleaching studies. To implement bioleaching on a larger scale, it is recommended to enhance the contact between the e-waste particles and cells. This can be achieved by good contactors proven effective in mixing and multiphase contacting. In view of this,

the performance of a Fluidized-bed bioreactor which has been proven to be an excellent multiphase contactor for adequate mixing and mass transfer was studied for the bioleaching of metals from PCBs by *A. aquatilis* in Medium 3.

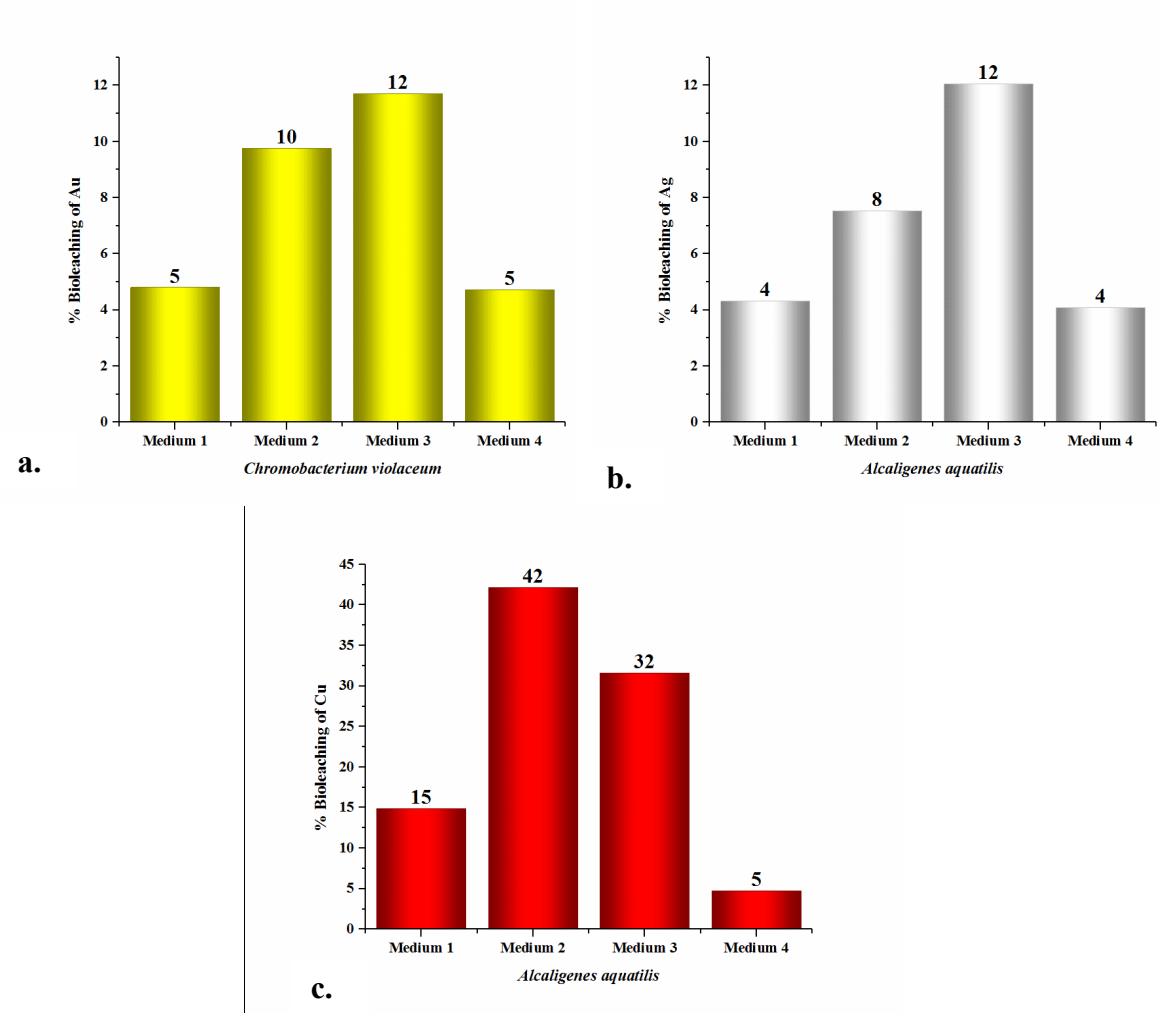


Fig. 4.7. Maximum bioleaching efficiency of metals in four different media **a.** Percentage bioleaching of Au, **b.** Percentage bioleaching of Ag and **c.** Percentage bioleaching of Cu

4.3 PERFORMANCE OF FBR FOR Ag AND Cu BIOLEACHING FROM PCBs

This section presents the results and discussions on the optimization of bioleaching studies with respect to the effect of particle size, inoculum size, and e-waste load in FBR and shake flask conditions to maximize Cu and Ag recovery.

4.3.1. BARTSCH TEST

Excessive foaming of the bioleaching medium in the FBR necessitated the Bartsch test to select suitable antifoam. In this study, the Bartsch test estimates the foam destruction properties of PEG 600, Coconut oil, Rice bran oil, and Sesame oil. The results are represented in Fig. 4.8.

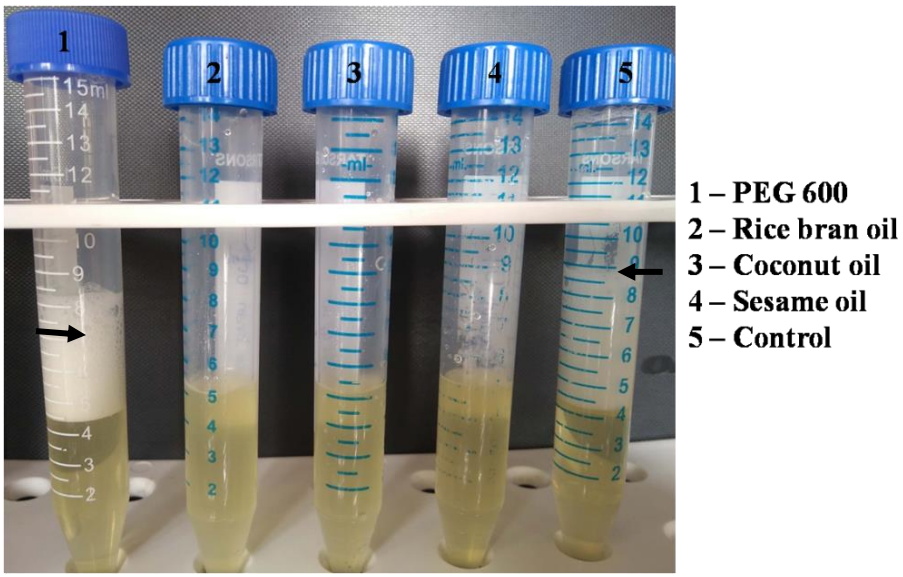


Fig. 4.8. Foam destruction property of different antifoaming agents

The foam destruction by natural oils proved more rapid than PEG 600 and control. The antifoaming activity of PEG 600 was 5 ml, and all three natural oils used as antifoams had almost the same antifoaming activity of 0.1 ml. Coconut oil was selected as the anti-foaming agent due to its availability. The defoaming property for oil-based antifoams was bridging-dewetting and bridging-stretching mechanisms, which control the foam for a longer time in a bioprocess. During the bridging-dewetting process, the foam film becomes thin and deform into a lens shape causing an oil bridge. The film moves to the next layer/opposite surface dewetting away from the bridge through capillary forces resulting in film rupture. During bridging-stretching mechanism, the oil forms a bridge between the foam films and stretches with time making the film unstable. The film disintegrates at the thinnest regions and destroys the foam structure. PEG is the most

investigated antifoam, but its ineffectiveness agrees with the antifoaming activity of PEG conducted by (Routledge 2012).

4.3.2. LIPOLYTIC ACTIVITY OF *A. aquatilis* AND *C. violaceum*

A qualitative estimation of the lipolytic activity of *A. aquatilis* and *C. violaceum* was performed by streaking the cultures on the surface of the spirit blue agar medium with the selected antifoaming agent (coconut oil).

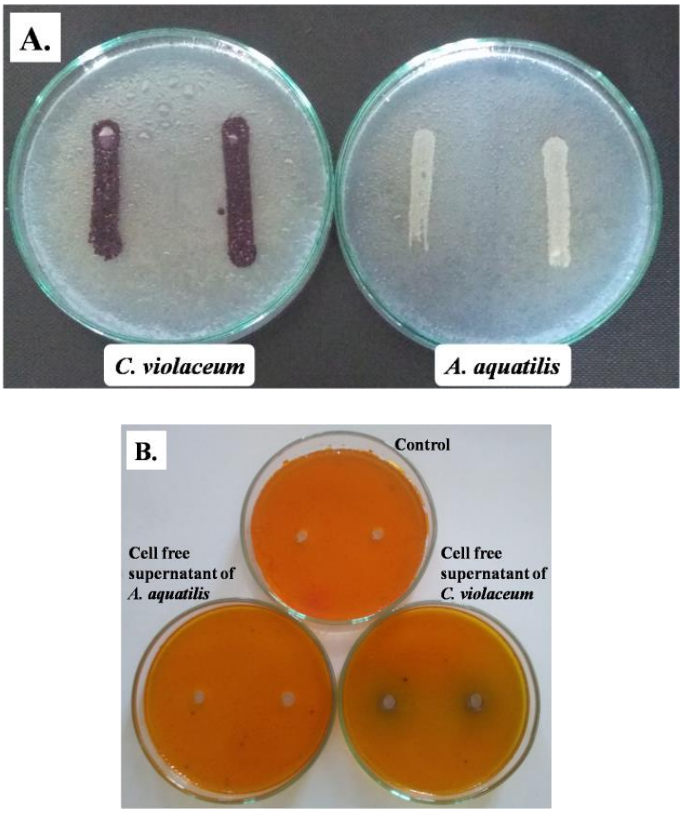


Fig. 4.9. Lipolytic activity of *A. aquatilis* and *C. violaceum* **(A)** Growth and coconut oil degradation in Spirit blue agar medium and **(B)** Well diffusion of cell-free supernatant of the cultures in plain agar with methyl red indicator.

Proper growth of the organism was observed, and there was no zone of clearance around the streak, as evident in Fig. 4.9 (A), which confirmed that the organism grew well without utilizing the oil as its nutrient source. As evident from Fig. 4.9 (B), the same was inferred after comparing the well-diffusion with cell-free supernatant and sterile

broth where there was no initial zone of clearance followed by color intensification in plain agar with coconut oil and methyl red indicator.

4.3.3. Cu bioleaching in FBR and shake flask conditions

(a) Effect of Particle size

The crushed PCBs were size separated as 1 mm, 0.75 mm, 0.6 mm, and 0.35 mm to study the particle size effect. These different-sized particles were further crushed to obtain a uniform particle size of 0.175 mm. The distribution of Au, Ag, and Cu in crushed PCBs was found to vary with the size fraction of the particles, as presented in Fig. 4.10.

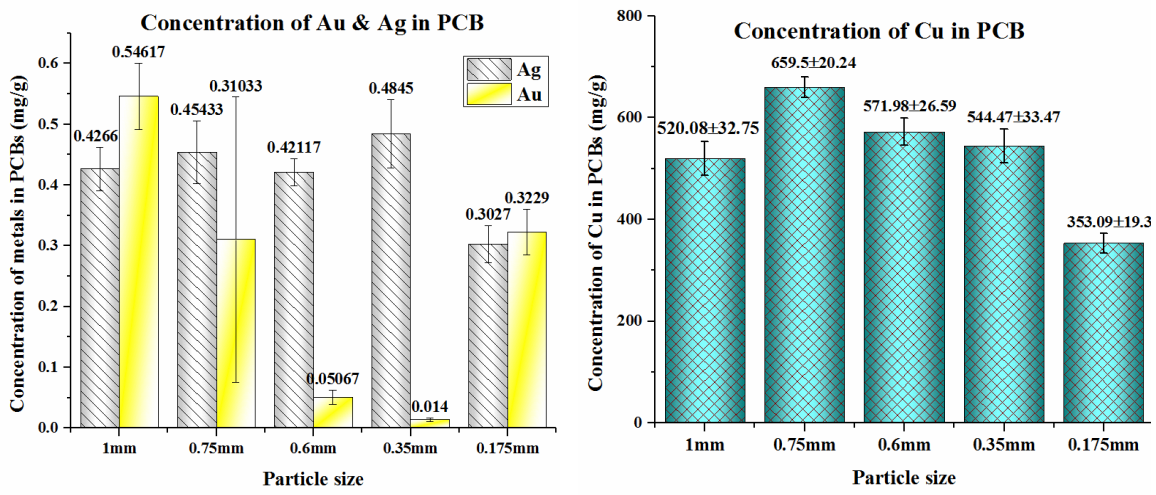


Fig. 4.10. Concentration of Au, Ag, and Cu in PCBs of different particle sizes

The concentration of Cu per gram of crushed PCB was the highest in the particles of 0.75 mm size. The higher amount of Cu was retained in larger particle sizes of 0.75 mm, 0.6 mm, and 0.35mm. In contrast, the highest and lowest particle sizes of 1 mm and 0.175 mm were found to have comparatively lesser Cu concentrations. Ag was almost evenly distributed in all the size fractions and found to be relatively high in 0.35 mm particles. Au was retained in the larger size fraction. This heterogeneous distribution of metal concentrations in various particle sizes mandated a further crushing of all the particle sizes to <0.35 mm, which yielded an average size of 0.175 mm and was also used as one of the sizes for particle size optimization.

The effect of particle size was studied with 5% (v/v) inoculum and 2% (w/v) e-waste load for the bioleaching of Cu from PCBs under shake flask and FBR conditions. Fig. 4.11 (a) and (b) show the effect of particle size on Cu bioleaching in shake flask and FBR, respectively. A maximum of 169.45 mg/g of Cu was leached at 84 hours with 0.175 mm particle size in a shake flask. In FBR, a maximum of 160.52 mg/g Cu was found to be bioleached in 96 hours with 0.6 mm particle size. As observed in Fig. 4.11 (a), the Cu bioleaching was not found to be initiated up to 36 hours in a shake flask with any of the particle sizes except that with the least size of 0.175 mm.

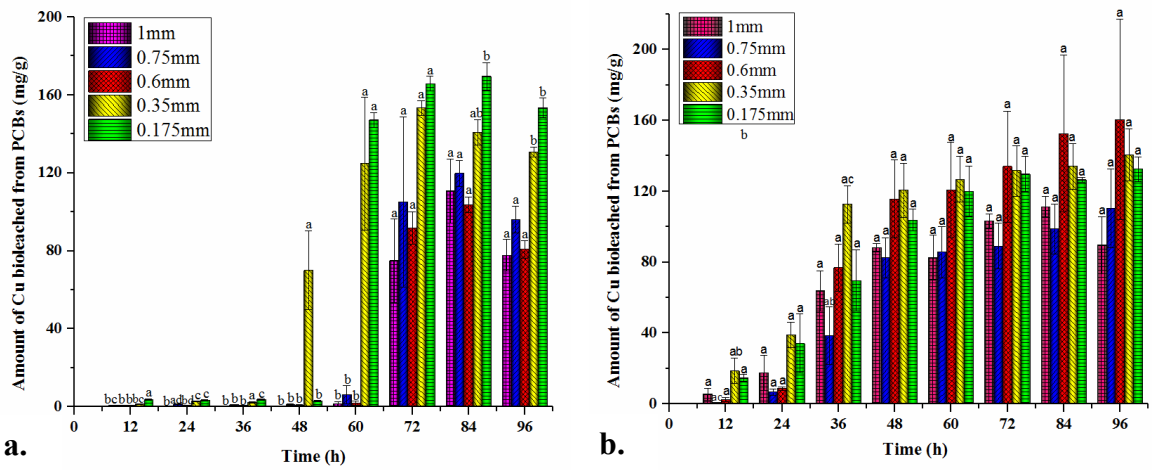


Fig. 4.11. Effect of particle size on Cu bioleaching from PCBs. **a.** Cu bioleaching in Shake flask **b.** Cu bioleaching in Fluidized-bed bioreactor (Lowercase alphabets above the error bars indicate the statistically significant difference/similarity at 0.05 significance level).

Though Cu was found to have been bioleached from the 12th hour onwards with a particle size of 0.175 mm, considerable bioleaching was observed only at the 60th hour and later. The bioleaching of Cu was initiated at 48 hours with 0.35 mm particles and from 72 hours for the particles of size larger than 0.35 mm. The maximum bioleaching of Cu was observed with the particles of 0.175 mm in size, even though the copper content was the least in this fraction, showing that the least size favors the bioleaching process owing to the large surface area available for the bioleaching reactions. The amount of leached Cu increased with time up to a certain level, then decreased with a further increase in time. The maximum bioleaching was achieved in the 84th hour with all the

particle sizes except that with 0.35 mm size, wherein the maximum amount of Cu was observed in 72 hours. As the cells grow, they facilitate bioleaching, and thus the concentration of copper increases. However, when the concentration of copper exceeds a certain level in the medium, the rate of adsorption of copper may exceed the rate of bioleaching, which may lead to a reduction in the copper level in the medium.

The results from One-way ANOVA showed that the particle size variation significantly affected Cu bioleaching in the shake flask. In contrast, a statistically insignificant effect of particle size on the bioleaching of Cu was observed in the FBR. Particle size establishes contact surfaces that consequently affect a system's collision and mass transfer between the metal substrate and cells (Arshadi and Mousavi 2014). The collisions result in non-uniform mass transfer characteristics and surface area of contact in the FBR due to size gradation along the column length, leading to variation in the contact surface area. In a shake flask, due to the rotational shaking movement, the mixing and mass transfer characteristics are uniform in the entire reactor volume. The insignificant effect of particle size in FBR was confirmed from the mean comparisons of the amount leached with various particle sizes at all the time intervals using Tukey's test in One-way ANOVA, which revealed statistical insignificance at a 0.05 significance level. Birloaga et al. (2013) have also reported that the particle size effect is insignificant in Au and Cu recovery from waste PCBs by hydrometallurgical methods. A maximum recovery of around 160 mg/g Cu was obtained with particles of 0.175 mm in size in a shake flask. The PCBs were crushed to obtain an average size of 0.175 mm and were used for optimizing the inoculum size and e-waste load parameters.

(b) Effect of Inoculum size

Cu bioleaching with inoculum sizes 2%, 5%, and 10% (v/v) was performed under a shake flask and FBR conditions using the optimum particle size of 0.175 mm and 2% (w/v) e-waste load. Fig. 4.12 (a and b) present the effect of inoculum size on the bioleaching of Cu in the shake flask and FBR, respectively. The inoculum size did not affect the Cu bioleaching significantly until 60 hours in the FBR. However, there was a statistically significant difference in Cu bioleaching with various inoculum sizes from 72

hours and further. As determined through ANOVA, Cu bioleaching was significantly affected by the inoculum size in shake flask studies. From Fig. 4.12 (a) and (b), the maximum recovery of Cu is achieved with an inoculum size of 5% (v/v), and hence, it is the optimum inoculum size in both the shake flask and FBR.

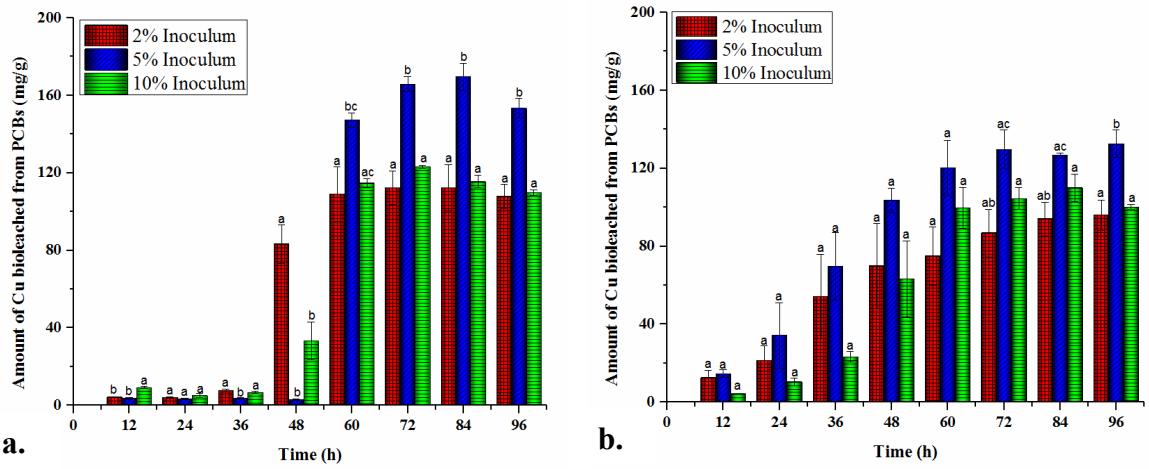


Fig. 4.12. Effect of Inoculum size on Cu bioleaching from PCBs. **a.** Cu bioleaching in Shake flask **b.** Cu bioleaching in Fluidized-bed bioreactor (Lowercase alphabets above the error bars indicate the statistically significant difference/similarity at 0.05 significance level).

The bioleaching of Cu was almost similar in the shake flask inoculated with 2% and 10% (v/v) inoculum. With 5% inoculum size, the Cu recovery from PCBs reached a maximum of 169.45 mg/g at 84 hours and decreased slightly after that in the shake flask. In FBR, a maximum of 132.55 mg/g Cu was found to have bioleached with 5% inoculum at the end of 96 hours. The Cu recovery using 5% and 10% (v/v) inoculum had no statistically significant mean difference until 84 hours, but the difference was evident at 96 hours. As observed in Fig. 4.12 (a), considerable bioleaching in the shake flask was observed at 48 hours with the lowest inoculum size of 2% (v/v), whereas a considerable amount was bioleached at the 60th hour with other inoculum sizes, indicating that the initial rate of bioleaching was the maximum with the lowest inoculum size. With the lowest inoculum size, cell toxicity may occur earlier by the bioleached copper, thus causing no further bioleaching as indicated by almost the same amount of bioleached Cu after 60 hours. However, considerable bioleaching was observed only at 60 hours with

the inoculum size of 5% (v/v) and 10% (v/v) showing delayed initiation of bioleaching. Cell overgrowth was observed at a high inoculum size of 10 % (v/v), which caused nutrient and oxygen deprivation in the earlier period. Oxygen plays a vital role as an electron acceptor in bacterial redox reactions, and this oxygen deprivation, along with nutrient deficiency, might have hindered the bioleaching rate with an initial inoculum of 10% relative to the lower inoculum sizes used (Li et al. 2015, 2020).

A gradual increase in the Cu concentration with time in FBR inoculated with all the tested inoculum sizes was noted. The media inoculated with 2% inoculum achieved turbidity only after 18 hours of inoculation, and this lower growth rate may have resulted in a lower rate of bioleaching. There was visible turbidity in the bioleaching media with 10% inoculum within 12 hours of inoculation. This may have limited the mixing conditions and mass transfer characteristics in the FBR that do not favor high bioleaching efficiency. The optimum inoculum size of 5% (v/v) provides sufficient growth rate and favorable mixing/mass transfer characteristics in the FBR leading to maximum bioleaching of Cu. Jagannath et al. (2017), in their studies on heterotrophic bioleaching of Cu in a Pulsed-plate bioreactor, have also reported that inoculum size above 9% resulted in the overgrowth of cells and decreased bioleaching efficiency. In the present study, the Cu bioleaching efficiency of 47.99% in the shake flask and 37.54% in FBR could be achieved under ambient conditions using heterotrophic bacteria as the bioleaching agent with an optimum inoculum size of 5% (v/v). Chen and Cheng (2019) have also studied the effect of inoculum size on thermophilic bioleaching of metals from sewage sludge in a pilot-scale stirred bioreactor using chemolithotrophic bacteria and have obtained bioleaching percentage of 17-39% as the inoculum size was varied in the range of 5-20% (v/v), which also shows that the bioleaching efficiency depends on the inoculum size.

(c) Effect of E-waste load

2%, 3.5%, and 5% (w/v) of E-waste load was used to determine the optimum load for Cu bioleaching under FBR and shake flask conditions using 0.175 mm particles and 5% (v/v) inoculum. Fig. 4.13 (a) and (b) present the effect of e-waste load on the bioleaching of Cu.

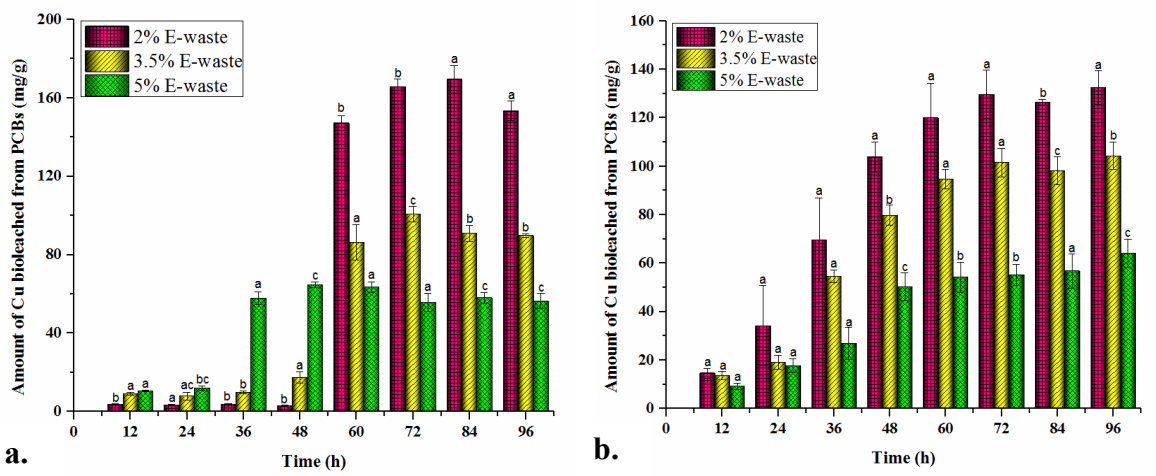


Fig. 4.13. Effect of E-waste load on Cu bioleaching from PCBs. **a.** Cu bioleaching in Shake flask **b.** Cu bioleaching in Fluidized-bed bioreactor (Lowercase alphabets above the error bars indicate the statistically significant difference/similarity at 0.05 significance level).

This showed that a 2% (w/v) e-waste load could significantly recover a maximum of 169.45 mg/g Cu at 84 hours and 132.55 mg/g of Cu at 96 hours in the shake flask and FBR, respectively. One-way ANOVA revealed that the e-waste load variations had a statistically significant effect on the Cu bioleaching efficiency. The results show that an increase in e-waste load has limited the bioleaching efficiency in both systems. The minimum e-waste load of 2% (w/v) showed maximum bioleaching in the shake flask and FBR. Xia et al. (2017) observed a similar trend in their studies on metal recycling from pre-treated PCBs using mixed thermophilic organisms in a stirred tank reactor, and the Cu recovery was reported to have decreased with increasing pulp densities. A gradual increase in the Cu concentration with time is observed in FBR with all three e-waste loads. In the shake flask, statistically significant bioleaching was observed from the 36th hour onwards with a maximum e-waste load of 5% (w/v), though the Cu concentration remained almost constant later. A considerable extent of bioleaching could be achieved only from 60 hours onwards with an e-waste load of 2% (w/v) and 3.5% (w/v), with the maximum being at 2% (w/v). The results show that a lower e-waste load favors the bioleaching process. At high e-waste loads, the cells may be exposed to a larger available surface area of PCBs which is heterogeneous in composition. This could result in

inhibitory effects on bacterial growth and metabolism during bioleaching. This effect was indirectly assessed by Rodrigues et al. (2015) in verifying the redox potential (Eh) using thermophilic bacteria in a rotating drum reactor for Cu extraction from PCBs. The other possible reasons for a decrease in bioleaching at higher e-waste load could be attributed to cell toxicity at higher concentrations of leached metals and inadequate mixing, instigating mass transfer limitations in the system, thereby minimizing the contact between the cells and substrate.

4.3.4. Ag bioleaching in FBR and shake flask conditions

(a) Effect of Particle size

Particle sizes 1 mm, 0.75 mm, 0.6 mm, 0.35 mm, and 0.175 mm were studied for the bioleaching of Ag in shake flasks and FBR with 5% (v/v) inoculum and 2% (w/v) e-waste load. Fig. 4.14 (a and b) indicates that 0.175 mm is the optimum size at which a maximum Ag recovery of 0.05 mg/g at 96 hours and 0.028 mg/g at 84 hours could be achieved in the shake flask and FBR, respectively. In FBR, the Ag solubilization occurred gradually with time, whereas a sudden increase in the Ag concentration was observed at 60 hours and continued steadily until 96 hours in shake flask conditions.

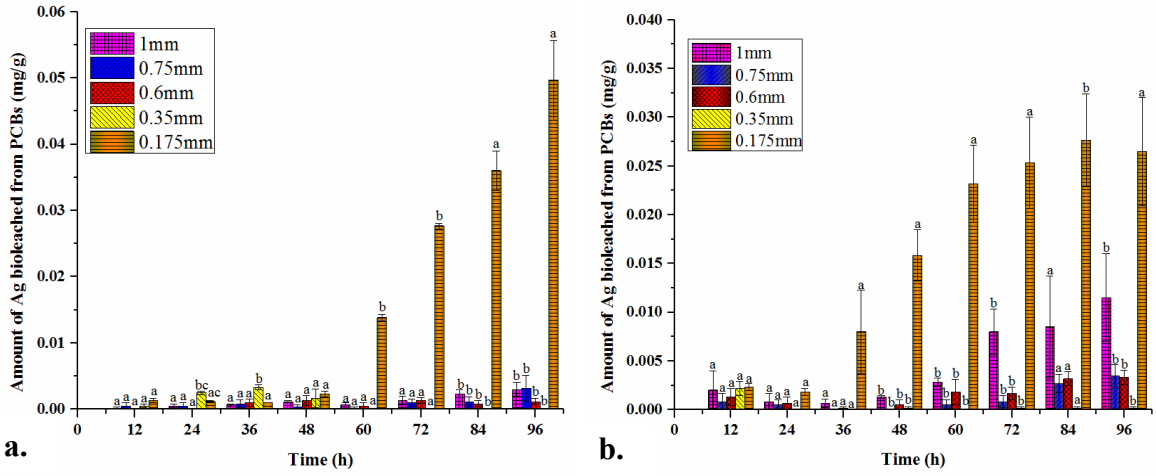


Fig. 4.14. Effect of particle size on Ag bioleaching from PCBs. **a.** Ag bioleaching in Shake flask **b.** Ag bioleaching in Fluidized-bed bioreactor (Lowercase alphabets above the error bars indicate the statistically significant difference/similarity at 0.05 significance level).

A further incubation time of up to 96 hours with *A. aquatilis* has proven to solubilize more Ag from the PCBs. Ruan et al. (2014) have also observed an increase in the Ag bioleaching efficiency of *P. chlororaphis* up to the culture time of 72 hours (Ruan et al. 2014). Ag bioleaching can be a result of redoxolysis (Jadhav and Hocheng 2013), complexolysis (Kumar et al. 2018), or acidolysis (Dave et al. 2018). The particle size of 0.175 mm was the optimum, and the particle sizes used were also found to exhibit a statistical mean difference through ANOVA. This confirmed its effect on Ag bioleaching from PCBs in FBR and shake flask conditions, unlike in Cu bioleaching (FBR).

(b) Effect of Inoculum size

The effect of inoculum size (2%, 5%, and 10% (v/v)) on Ag bioleaching in FBR and shake flask conditions were studied with 0.175 mm size particles and 5% (w/v) e-waste load. From Fig. 4.15 (a and b), it is evident that inoculum size did not significantly affect the Ag bioleaching of FBR in contradiction to shake flask bioleaching. A maximum of 0.064 mg/g Ag was solubilized by *A. aquatilis* when the inoculum size was 2 %. However, the bioleaching of Ag was found to decrease as the inoculum size was increased with 0.05 mg/g Ag with 5% and 0.03 mg/g with 10% inoculum at 96 hours in shake flask bioleaching. The bioleaching pattern gradually increased with time until maximum Ag dissolution was achieved. At higher inoculum size, the medium was turbid earlier, within 12 hours of the bioleaching period; hence, the Ag bioleaching was lesser. Similar results were obtained by Li et al. (2020) while studying the effect of initial additions of *Pseudomonas fluorescens* for Au bioleaching. Excessive initial bacterial load causes a quick nutrient deficiency as a consequence of cell multiplication and causes earlier cell death (Li et al. 2020). In FBR, the Ag bioleaching was highest at about 0.026 mg/g at 72 hours with 2% inoculum, 0.028 mg/g at 84 hours with 5% inoculum, and 0.029 mg/g at 96 hours with 10% inoculum. The ineffectiveness of inoculum size in the FBR confirmed through ANOVA may be attributed to the selectivity of the cell/secreta to leach out other metals in high concentrations efficiently rather than Ag.

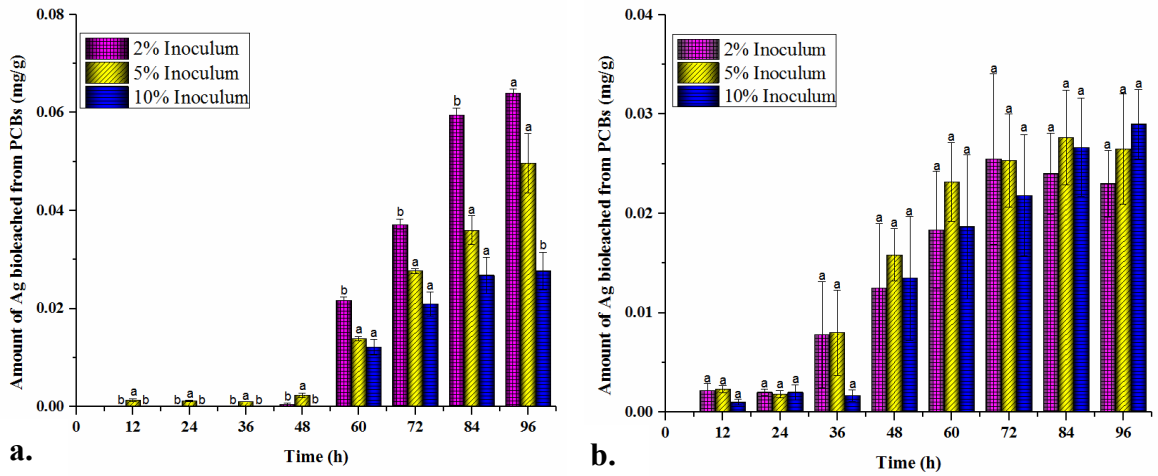


Fig. 4.15. Effect of Inoculum size on Ag bioleaching from PCBs. **a.** Ag bioleaching in Shake flask **b.** Ag bioleaching in Fluidized-bed bioreactor (Lowercase alphabets above the error bars indicate the statistically significant difference/similarity at 0.05 significance level).

(c) Effect of E-waste load

The effect of e-waste loading (2%, 3.5%, and 5% (w/v)) on Ag bioleaching was carried out in an FBR and shake flasks with 5% (v/v) inoculum and 2% (w/v) e-waste load. Fig. 4.16 (a and b) clearly explains that shake flask conditions could solubilize higher Ag concentration than in FBR conditions. A maximum of 0.05 mg/g Ag was leached out in the shake flask at 96 hours with a 2% e-waste load. This was followed by a 5% e-waste load from which 0.02 mg/g Ag was solubilized at 96 hours, and in the leaching medium with 3.5% e-waste load, a maximum of 0.003 mg/g Ag was bioleached at 60 hours. In the shake flask, the Ag bioleaching from 2% e-waste load was observed suddenly at 60 hours, gradually increasing. In FBR, 0.028 mg/g Ag was the maximum recovery at 84 hours with 2% e-waste load, followed by 3.5% e-waste load, from which 0.022 mg/g Ag was found to bioleach at 96 hours. 5% e-waste load had a minimum Ag in the bioleaching medium, with a recovery of 0.014 mg/g at 60 hours. Ag bioleaching was significantly affected by the e-waste load in both FBR and shake flask conditions, proved by Tukey's test in ANOVA. Previous studies on *Acidothiobacillus ferrooxidans* for metal bioleaching show that at lower loading of the metal substrate, the metals could be extracted more successfully since higher pulp density affects the multiplication of cells

and provides lesser oxygen to the cells, thereby decreasing the bacterial activity (Rouchalova et al. 2020).

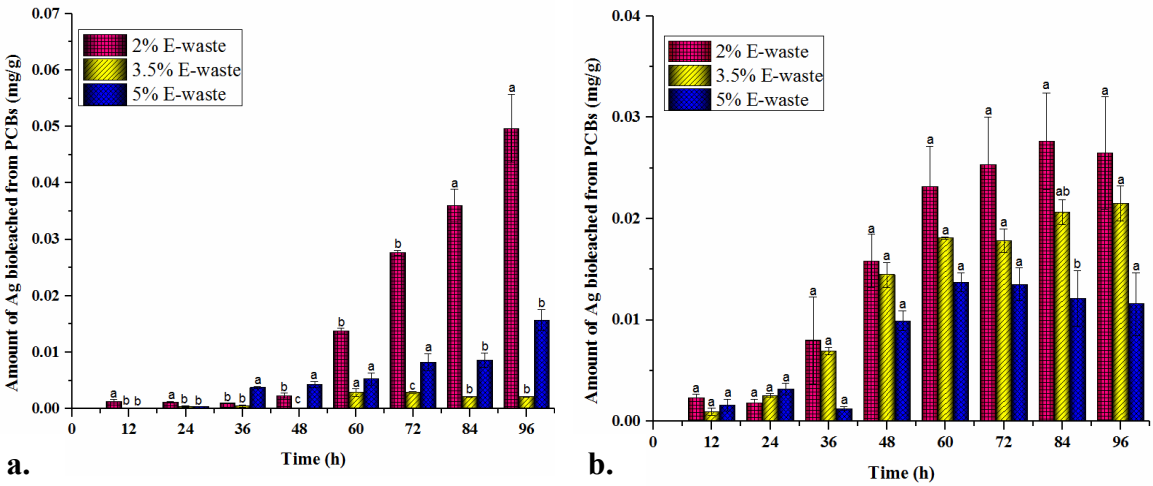


Fig. 4.16. Effect of E-waste load on Ag bioleaching from PCBs. **a.** Ag bioleaching in Shake flask **b.** Ag bioleaching in Fluidized-bed bioreactor (Lowercase alphabets above the error bars indicate the statistically significant difference/similarity at 0.05 significance level).

The higher e-waste loading conditions in the FBR may result in the attrition of cells, with the e-waste particles causing cell damage, resulting in lesser bioleaching. At lower loadings, the bioleaching had increased due to the good mixing conditions in the bioreactor, which resulted in higher bioleaching. However, the results show that beyond a specific limit of e-waste loading, the attrition effect may dominate the mixing effect. With a higher e-waste load, the heterogeneous total surface area of e-waste available in the reactor would be higher. This leads to greater exposure of cells to the e-waste both in terms of exposure surface and contact frequency. The toxic substances present on the surfaces may inhibit the growth/metabolism of the bacterial cells. Alternatively, the other constituents released in higher concentrations from higher e-waste loads may form stable complexes with the bioleaching metabolites of the cells competing with the metals from PCBs (Li et al. 2020). These results show that the higher e-waste load adversely affects bioleaching efficiency.

Similar bioleaching experiments conducted by Ruan et al. (2014) using *Pseudomonas chlororaphis* in shake flasks yielded 0.39 mg/L amounting to 0.024 mg/g Ag and 2.6 g/L, i.e., 162.5 mg/g Cu, which was lesser than the Ag dissolution with comparable Cu solubilization achieved in FBR and shake flask in the present study. An e-waste load of 2% (w/v) was the optimum, similar to Cu bioleaching. The possible reason for an initial low bioleaching rate in the shake flask could be attributed to the growth period of the bacterial cells in a metal-containing environment of up to 24 hours and the initial interfacial steps of up to 48-60 hours. The initial interfacial steps of attachment and pre-colonization of the cells to the e-waste surface (contact bioleaching) or the cell/metabolite mediated generation of Fe^{3+} for the Cu mobilization (indirect bioleaching) exhibit a low microbial activity (bioleaching) (Safar et al. 2020). This delay in the initiation of Ag and Cu bioleaching was circumvented in the FBR, as the continuous air supply favors the rapid growth of the bacteria and leads to the early initiation of Cu bioleaching.

Arshadi and Mousavi (2014), who reported the statistical optimization conducted for simultaneous Cu and Ni recovery, showed that the most influential factors for the bioleaching of each metal are different. Similarly, the current study shows that the most influential factors for Cu bioleaching are inoculum size and e-waste load but not particle size, whereas e-waste load and particle size and not inoculum size for Ag bioleaching in FBR. In shake flask bioleaching, all three parameters were statistically significant for Ag and Cu dissolution into the bioleaching medium. The optimal conditions of Cu bioleaching, i.e., 0.175 mm particle size, 5% (v/v) inoculum, and 2% (w/v) e-waste load, were used for further studies.

4.4. MECHANISM OF ONE-STEP BIOLEACHING OF Cu FROM PCBs BY *A. aquatilis*

Three mechanisms, namely, acidolysis, redoxolysis, and complexolysis, can achieve the solubilization of metals. Hence, the pH, ORP, Fe concentration, and protein concentration in the bioleachates, along with the cell viability, were estimated in the presence and absence of PCBs to uncover these mechanisms under shake flask

conditions. Further, FESEM-EDS analysis of PCBs and cells was performed to understand the mode of action of *A. aquatilis* on PCBs.

4.4.1. Variation of pH and ORP during the one-step bioleaching process

The pH and ORP changes during the bioleaching process are evidenced in Fig. 4.17. There is a noticeable increase in the pH from approximately 6.2 to 9 (in the presence and absence of PCBs). A decrease in the ORP from +46 mV to -113 mV with PCBs and from +48.3 mV to -115 mV without PCBs of the bioleachates was noted with time. The pH or ORP changes in the presence and absence of PCBs were similar in trend, which reveals that the increase in pH and decrease in ORP was due to the normal bacterial metabolism and not an effect of PCBs. This makes clear that the bioleaching proceeded under alkaline conditions, and a redox potential was not generated due to pH changes. The pH changes of cyanogenic isolates during the Cu bioleaching were alike and proceeded under alkaline conditions (Arab et al. 2020).

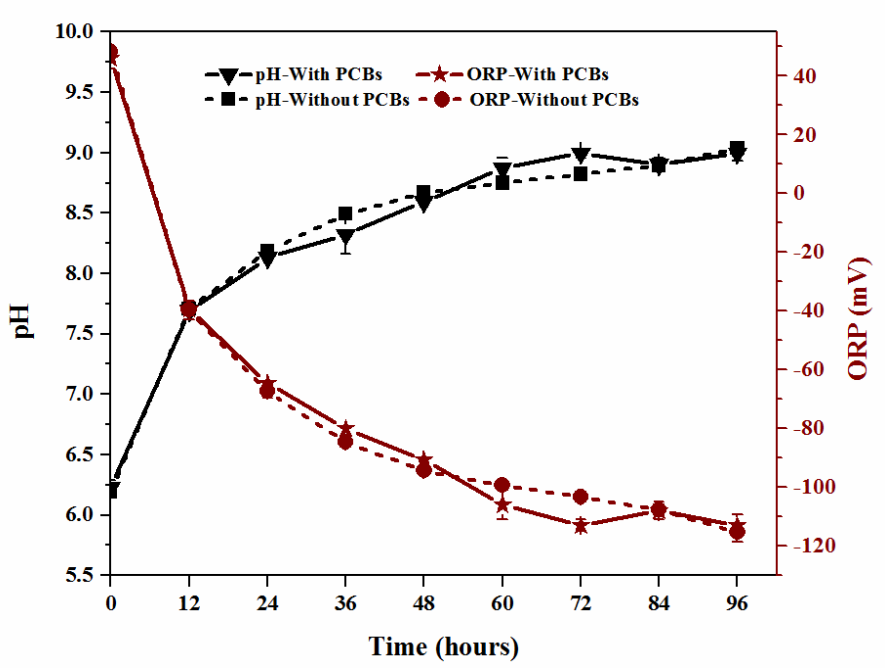


Fig. 4.17. Variation of pH and ORP during one-step bioleaching of Cu

The pattern of pH changes with or without PCBs was similar to that observed with the pH patterns with or without PCBs by a consortium of *Aspergillus niger* strains

reported by Argumedo-Delira et al. (2019). These results contradict the observations Aston et al. (2022) reported, where the heterotrophic bacteria *Gluconobacter oxydans* produce organic acids, thereby decreasing the pH of the bioleaching lixiviant. The patterns of pH and ORP changes in the present study do not agree with that of the pH and ORP changes witnessed in the studies (Fu et al. 2016; Rodrigues et al. 2015; Trivedi et al. 2022; Wu et al. 2019; Xia et al. 2018b) that used acidophilic lithotrophs or organic acid producing fungal strains. An acidolysis mechanism would display a decrease in pH and an increase in the ORP, as evidenced in these reports where metal oxidation resulted due to electron transfer from the metal to protons generated from the acids. Hence, it may be concluded that Cu oxidation by *A. aquatilis* was not caused by neutralization reactions of the acids through a protonic attack.

4.4.2. Significance of Fe in one-step bioleaching of Cu

The Fe concentration in the bioleaching liquor has an influence on Cu solubilization. As observed in Fig. 4.18, the Cu bioleaching rate was maximum during 48 to 60 hours. During the same period, there was a rapid decrease in the Fe concentration from 19.29 mg/L to 3.69 mg/L, possibly due to Fe^{2+} to Fe^{3+} conversion by *A. aquatilis*. From 60 to 96 hours, there was a further decrease in the Fe concentration, which increased the Cu bioleaching. *A. aquatilis*, a heterotrophic bacterium that has proved to be a potent bioleaching agent for Cu recovery under ambient conditions, could leach out iron simultaneously. The Fe thus leached out is used for Cu mobilization from the PCBs. These results are similar to those obtained by Rodrigues et al. (2015), in which the variation in Fe concentration was observed where Cu extraction was achieved by the bioleaching of PCBs using iron-oxidizing, thermophilic bacteria in a rotating drum reactor. Their results prove that the initial iron concentration had a vital role in the chemolithotrophic bioleaching process. *A. aquatilis* could have used redoxolysis for Cu bioleaching as one of the mechanisms during this process through electron transfer (Shi et al. 2016).

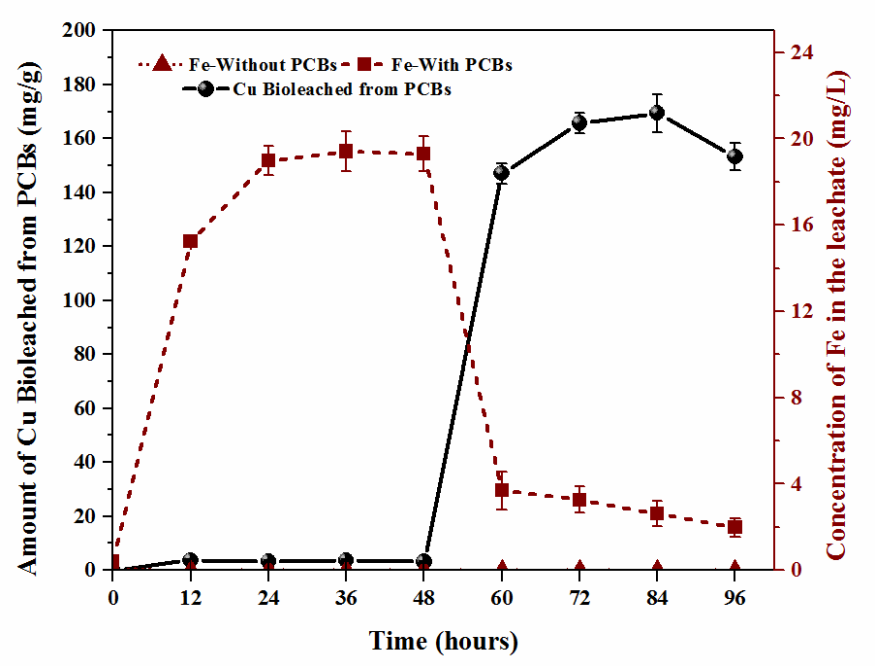


Fig. 4.18. Influence of Fe in Cu recovery from PCBs

4.4.3. Extracellular protein concentration and cell viability of *A. aquatilis*

This section discusses the variation in extracellular protein concentration and cell viability during the bioleaching process using *A. aquatilis*. Fig. 4.19a distinguishes the effect of PCBs on cell viability (also shown in Appendix I) during the bioleaching period. *A. aquatilis* cells inoculated with approximately 10^7 cells (5% v/v) in the absence of PCBs grow for 24 hours and remain viable with 10^8 -10^{10} cells with marginal variations until 96 hours. A similar trend was observed in the presence of PCBs, but a cytotoxic effect was noticed from 60 hours with a sharp decline in the number of cells and resulted in complete cell death by 96 hours. This shows a decrease in cell viability as the Cu concentration increases and with the exhaustion of nutrients. These results align with the toxicity assay findings of Benzal et al. (2020), where the cells of *Acidothiobacillus ferrooxidans* were unaffected until 48 hours of Cu bioleaching. The cytotoxic effects may result only after this time interval with the upsurge in Cu and decline of nutrient supply in the bioleaching lixiviant.

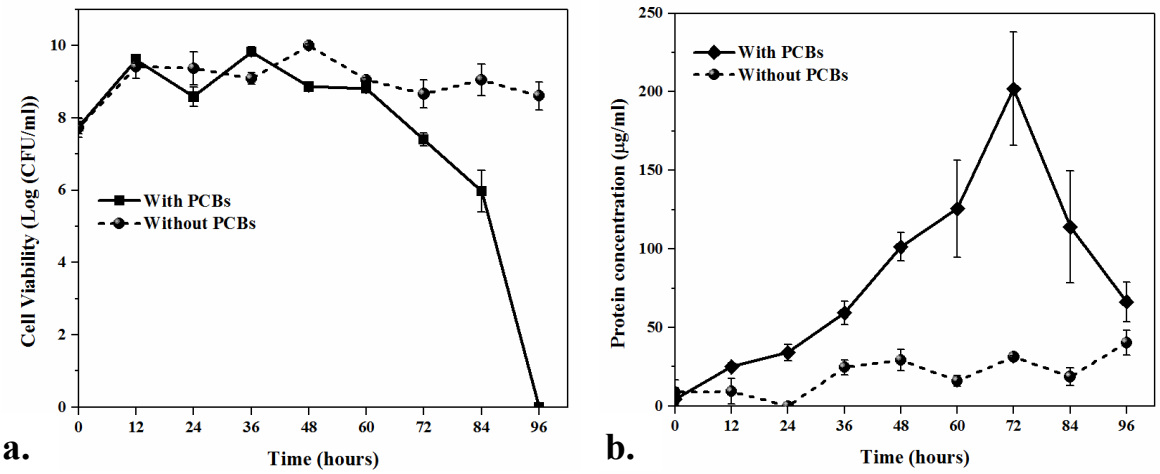


Fig. 4.19. Variations of viability and protein concentration in the presence and absence of PCBs, **a.** Cell viability and **b.** Extracellular protein concentration

The extracellular protein concentrations in the bioleachates were estimated in the presence and absence of PCBs and represented in Fig. 4.19b and the calibration curve of the standard protein in Appendix II. The protein concentration in the bioleachate with PCBs is higher than that without PCBs. An increase in the concentration with a maximum of 202 µg/ml of secreted protein was estimated at 72 hours of bioleaching. The proteins secreted in the absence of PCBs were relatively much lower and were approximately stable with trivial fluctuations. This clarifies that a differential protein expression could have ensued with PCBs.

A comparable study by Argumedo-Delira et al. (2019) showed a slight difference in the protein concentration of *Aspergillus niger* with and without PCBs. The difference in the concentration of proteins in our study was noteworthy and thus played a vital role in the bioleaching of PCBs. The results obtained earlier from the comparative study of bioleaching methods showed that the Cu bioleaching efficiency was the maximum with the spent medium of *A. aquatilis,* followed by the two-step method. This showed that the secretome or the culture in its stationary phase of the bacterial strain with secondary metabolites and extracellular proteins had a prominent role in Cu bioleaching. A steep decline in the protein concentration from 72 hours coincides with the lower metabolic activity caused by the decline in cell viability after 60 hours, necessitating a fresh inoculum and medium for complete mineralization. This decrease can also be attributed

to the complexation of Cu (or other metals) to the secreted proteins in the extracellular space (Shi et al. 2016).

4.4.4. Differential protein expression by *A. aquatilis* during one-step bioleaching

The results obtained from the difference in protein concentrations with and without PCBs directed its confirmation through an electrophoretic separation of proteins in the bioleachates. The bioleachates from the time intervals of 48, 60, and 72 hours were considered discerning the time intervals at which a steady increase in Cu bioleaching and highest protein concentration was observed. The polyacrylamide gel picture with the separated and stained protein bands is shown in Fig. 4.20. Three protein bands approximately of sizes 63 kDa, 75 kDa, and slightly above 25 kDa were prominent in all the lanes (indicated by dotted arrows). This depicts that these proteins are indigenous to the *A. aquatilis* strain in the presence or absence of PCBs. The thickness of the bands immediately above 25 kDa in lanes 3 and 5 are visibly higher than in lanes 2 and 4, indicating their over-expression in the presence of PCBs at 48 and 60 hours. Three new faint protein bands were observed in lane 3 at 48 hours with PCBs, i.e., at the initiation of Fe^{2+} to Fe^{3+} conversion. These differentially expressed proteins were between 25 to 48 kDa in size and were not spotted after 48 hours; therefore, these three proteins may perhaps aid in the Fe conversions. Three new bands were evident in lanes 3 and 5, which were proteins expressed with PCBs at 48 and 60 hours, respectively. One of these proteins was between the size range of 25 and 35 kDa, another approximately 11 to 17 kDa, and the third was around ≤11 kDa in size. The thickness of the protein bands at 11 kDa and between 11 and 17 kDa was much higher at 60 hours than at 48 hours, indicating that these proteins and the over-expressed protein <25 kDa had a vital role in Cu bioleaching. Another observation was that no protein band separated in lane 7 with PCBs at 72 hours. This might be due to the interference caused by high metal concentrations in the protein sample though the highest protein concentration was noted at this time interval.

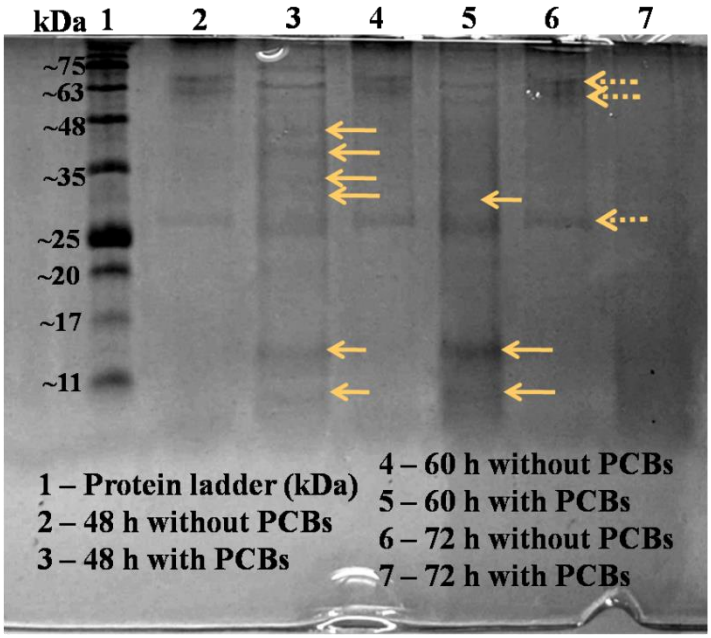


Fig. 4.20. Differential extracellular protein expression of *A. aquatilis* in the presence and absence of PCBs during one-step bioleaching

2-dimensional partial proteome analysis revealed several differentially expressed proteins during the growth of *Acidithiobacillus ferrooxidans*. Identifying these proteins that function to up/downregulate the energy metabolism during the bacterial growth on iron, sulfur, and metal substrates disclosed the two energy pathways (Ramírez et al. 2004). Bobadilla Fazzini et al. (2011) identified a lipoprotein Licanantase that was differentially expressed and enhanced chalcopyrite bioleaching (for Cu recovery) by a lithotroph, *Acidithiobacillus thiooxidans*. An advanced 2-dimensional SDS-PAGE was used to separate the secretome proteins and identify the differential spots through Liquid Chromatography – Mass Spectrometry (LC-MS/MS) analysis. The present study focused on confirming differential protein expression in the presence of PCBs and hence used a 1-dimensional SDS-PAGE. A similar pattern of bands was obtained in a study conducted by Vimalnath and Subramanian (2018) with and without Pb to understand the mechanism of its biosorption by *Pseudomonas aeruginosa* in a 1-dimensional SDS-PAGE. Therefore, the separation of extracellular proteins with and without PCBs in SDS-PAGE revealed (i) overexpression of a protein (>25kDa) at 48 and 60 hours in the presence of

PCBs, (ii) three new bands present at 48 hours were not there at 60 hours (25-48 kDa) indicating their role in Fe^{2+} to Fe^{3+} conversion, and (iii) three differentially expressed prominent bands (one between 25-35 kDa, one between 11-17 kDa and another ≤11 kDa) obtained in the presence of PCBs at 48 and 60 hours proving their significance in bioleaching.

4.5. CHARACTERIZATION OF PCBS AND *A. aquatilis* CELLS

The micromorphology of free and attached cells on PCB surfaces after bioleaching was studied through FESEM analysis. The characterization of PCBs was carried out in FESEM and EDS to understand the difference in the surfaces and elemental composition before and after bioleaching.

4.5.1. Variation in PCB surfaces and *A. aquatilis* cell morphology

The differences in the surface characteristics of PCBs before and after one-step bioleaching are presented in Fig. 4.21. Accordingly, rod-like and solid structures that contain metals on their surfaces or inner layers were observed. From Fig. 4.21 a, c, and e, it was clear that there were fissures or cracks in the PCB particles before bioleaching under 1000x, 5000x, and 10000x magnifications, respectively. This might have been caused during the austere crushing conditions and serves as the attachment sites of cells for bioleaching. Apart from this, the PCB surfaces were smooth before bioleaching. Fig. 4.21 b, d, and f exhibit the changes in PCB surfaces of the bioleached residue. From 1000x and 5000x magnifications, it was clear that the smooth surface of the rod-shaped PCB particles had been altered after bioleaching. There were pits formed on the surfaces of particles in the residue as a result of microbial activity on the surfaces for bioleaching. The modification of surface roughness and formation of pits indicates the leaching of metals from PCBs, as such an observation matched with those made by Calgaro et al. (2015) after the chemical leaching of PCBs with standard aqua regia solution. A 10000x magnification of the surface of PCB residue revealed a completely rough and eroded surface formed as a result of corrosion by the *A. aquatilis* cells.

PCBs before Bioleaching **PCBs after Bioleaching**

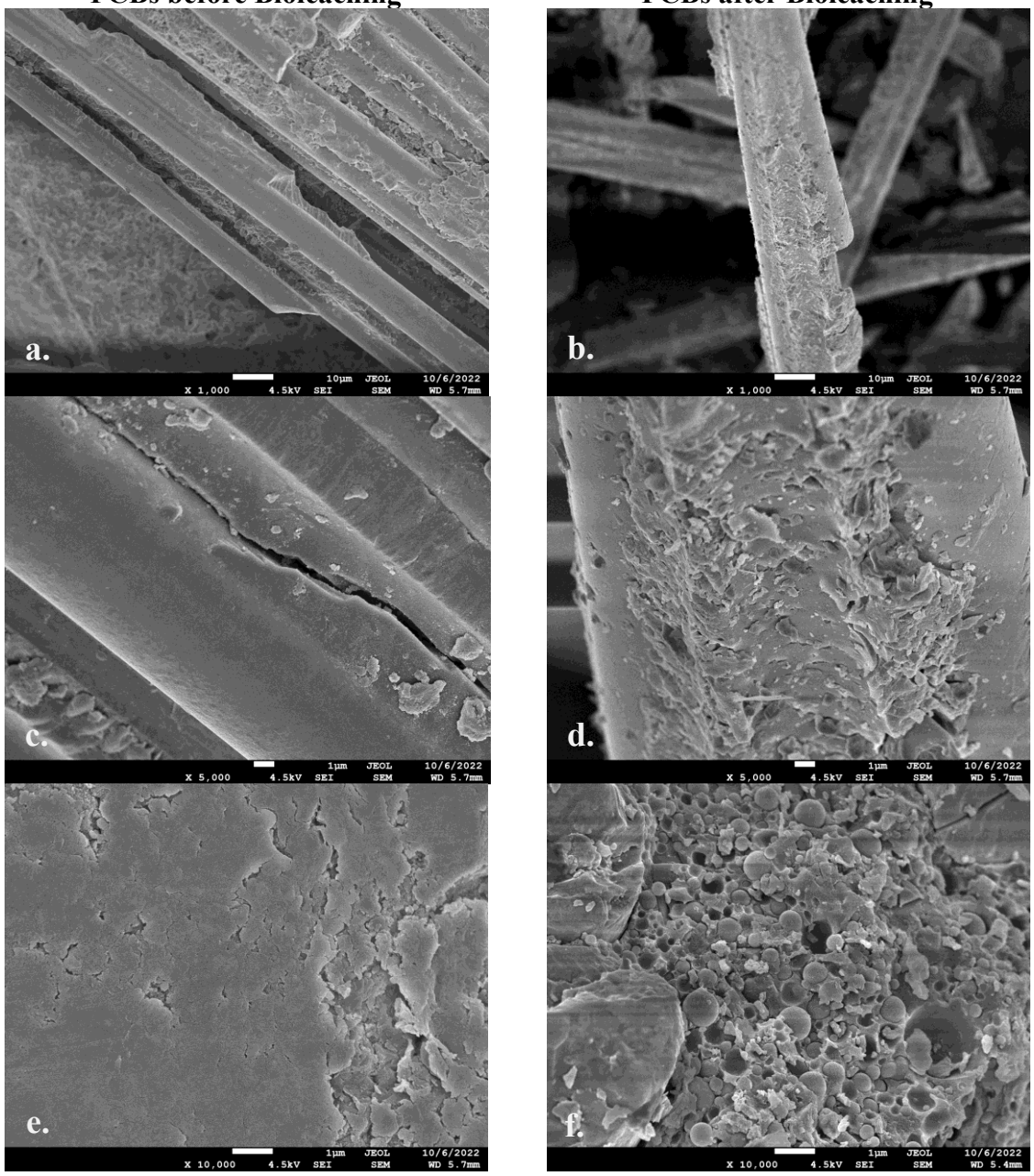


Fig. 4.21. Variation in the surface characteristics of PCBs. **a,c,e.** Surfaces of PCB before bioleaching at 1000x, 5000x, and 10000x, respectively. **b,d,f.** Surfaces of PCB residue after bioleaching at 1000x, 5000x, and 10000x, respectively.

Aminian-Dehkordi et al. (2020) noted comparable microfissure initiation and particle erosion in one-step bioleaching by *Bacillus megaterium* for Au bioleaching from PCBs. The corroded surfaces provide more sites for cell attachment and metabolic

activity when the residue is used for sequential bioleaching. These results also agreed with the observations of Shabani et al. (2013) after the bioleaching of Cu from copper oxide ore by *Pseudomonas aeruginosa* and Heydarian et al. (2018) after bioleaching of lithium-ion batteries by acidophiles. These studies reported the morphological changes during a two-step bioleaching that increased the porosity caused by bacterial activity. As noticed in Fig. 4.20f, several spherical structures and powdery clumps were formed in the residue, which may be attributed to bio-precipitation after the solubilization of metals from PCBs by *A. aquatilis*. In Fluidized-bed columns, the time scale of mixing may be much smaller than the time scale of the precipitation reaction (Bałdyga 2016). So, before the precipitation reaction can lead to the formation of new nuclei of precipitate, the turbulence may sweep away the reacting molecules, thus preventing precipitation. In shake flasks, precipitation overtakes after sufficient bioleaching occurs due to the higher time scale of mixing compared to FBR. Similarly, on characterizing the sphalerite ore before and after bioleaching of Zn by *Leptospirillum ferriphilum*, Sundramurthy et al. (2020) found a layer of elemental sulfur on the surface of the residue due to sulfur reduction.

The micromorphology of the free *A. aquatilis* cells from the bioleachate has been captured under 5000x, 10000x, and 15000x magnifications and displayed in Fig. 4.22. Most of the cells observed under 5000x (Fig. 4.22a) magnification have deformations in cell structure or cell-wall disintegration after bioleaching. A magnified image of the same field at 15000x (Fig. 4.22b) showed the deterioration with profound clarity and exposed the precipitates on the cells as powdery clumps. A bio-precipitation was also noticed by Hubau et al. (2018) during the bioleaching of PCBs by acidophiles. A cluster of free cells visualized under 10000x (Fig. 4.22c) from another field endorsed all of these observations (deformed cells, precipitates, and cell rupture) with precision. The aggregation of cells might be a defensive mechanism to evade the metal concentrations, as Vimalnath and Subramanian (2018) discussed in the presence of Pb during their biosorption studies with *Pseudomonas aeruginosa*. To the best of our knowledge, there are no reports on the characterization of bacterial-free cells in the lixiviant after bioleaching. These results show that cell damage occurs with increased metal

concentrations in the lixiviant. These were also the reasons for a decline in the cell viability and protein concentration in the presence of PCBs after 72 hours.

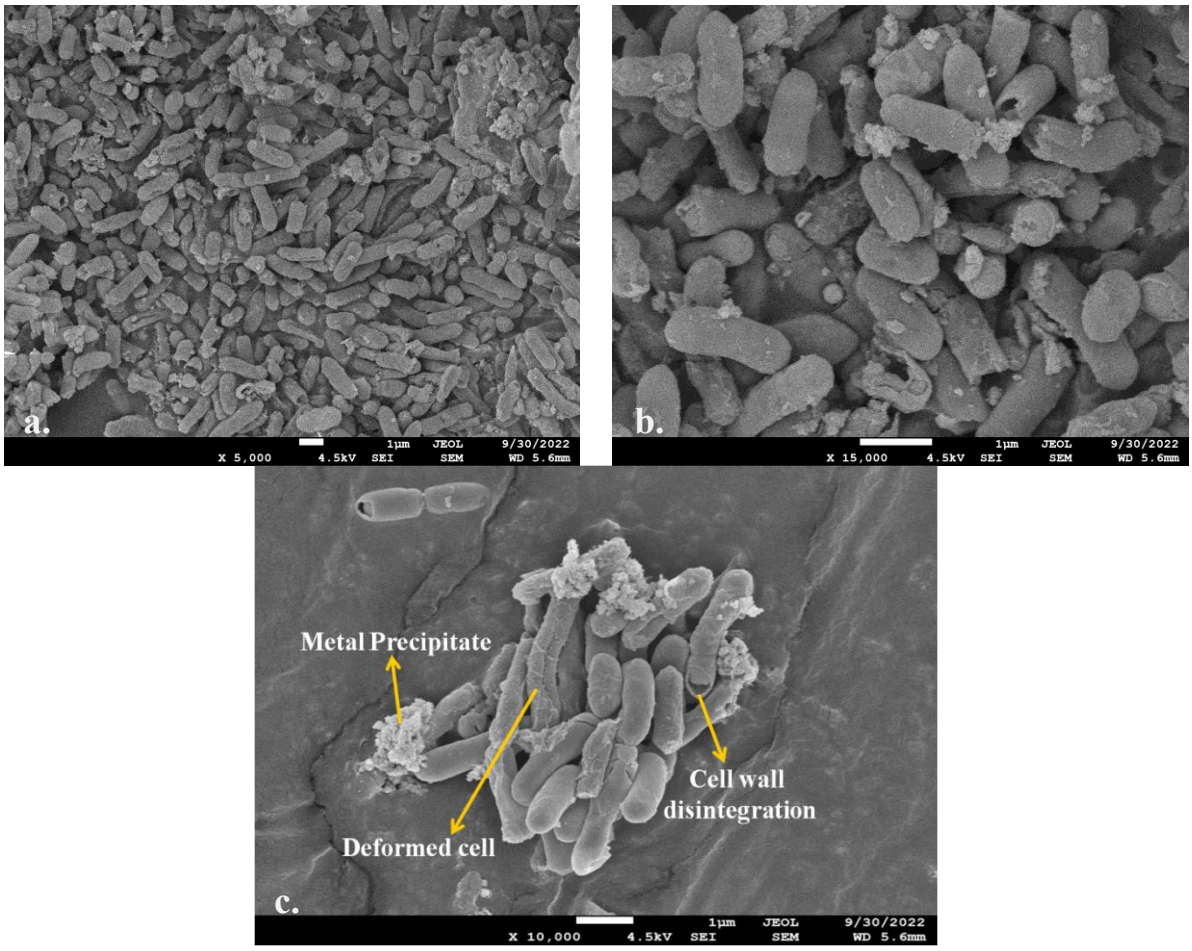


Fig. 4.22. *A. aquatilis* cell morphology after bioleaching. **a.** Morphological changes at 5000x, **b.** Morphological changes at 15000x and **c.** Morphological changes at 10000x

4.5.2. Elemental composition of PCBs before and after one-step bioleaching

Fig. 4.23 and Table 4.1 discloses the elemental composition on the surface of PCB particles before and after one-step bioleaching. Fig. 4.22a had peaks detected for Cu and Au with weight percentages of 1.4 and 52.8, respectively. These results do not agree with the results of metal quantification (Section 4.1) through acid digestion followed by ICP-OES analysis, where the amount of Cu was found to be higher than that obtained by EDS analysis which analyzes the metals on the surface. This proves that Cu is present in low concentrations on the surface of PCB particles, and a major amount is in the particle

interiors. These observations can be supported by the report of Rodrigues et al. (2015) through SEM micrographs where Cu was present as internal layers in PCBs and was exposed only after removing the outer coating.

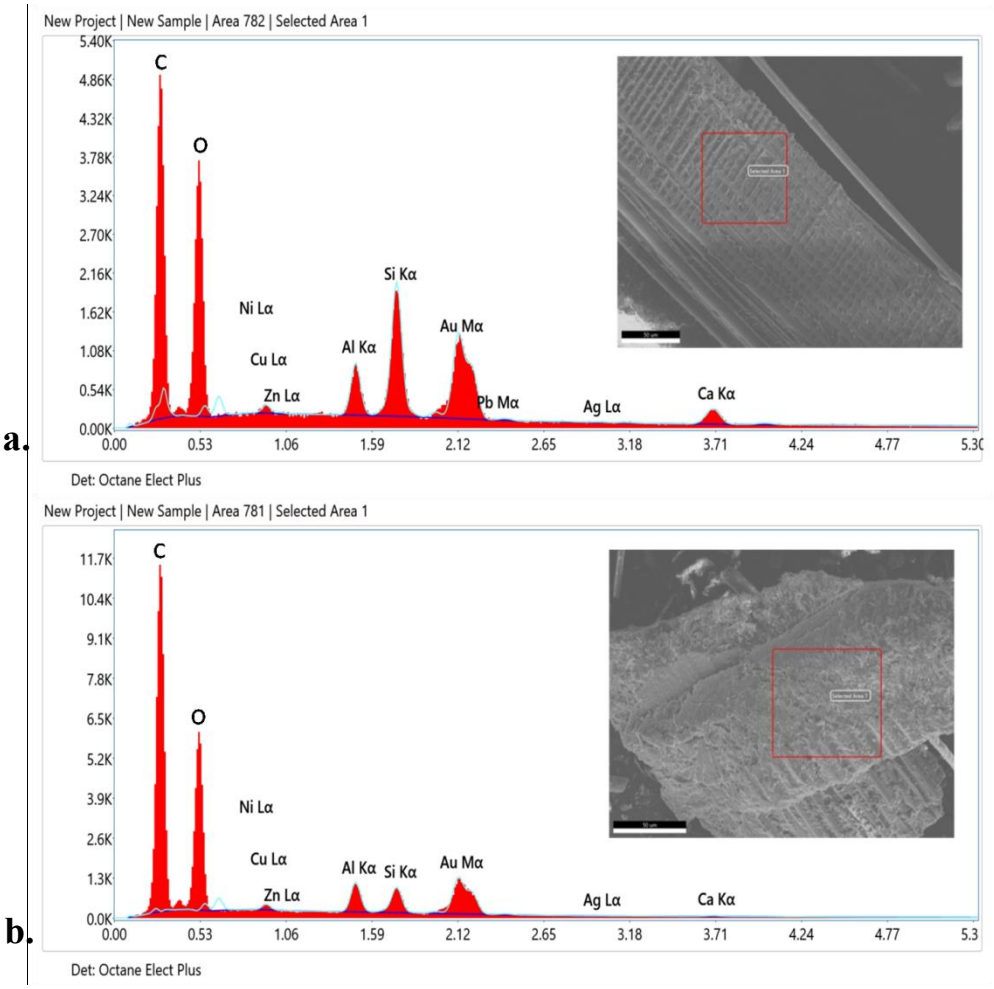


Fig. 4.23. Elemental composition of PCBs. **a.** EDS spectrum of PCBs before bioleaching and **b.** EDS spectrum of PCBs after one-step bioleaching

The above discussions are supported by the results of EDS analysis presented in Fig. 4.23b, where an increase in weight percentage of Cu and Au to 2.8 and 66.8 after one step bioleaching was observed, confirming the presence of a substantial fraction of Cu and Au in the particle interiors. The weight percentage of Ag showed a slight decrease after bioleaching from 0.7 to 0.5, which shows that the solubilization of Ag from the particle surface has occurred, and the weight percentage was approximately similar to that obtained with the earlier quantification using ICP-OES (section 4.1) implying

uniform distribution of Ag in the PCB particles. Similar observations were reported by Marappa et al. (2020) and Narayanasamy et al. (2017), with a decrease in the concentration of metals in the residue after one-step bioleaching. The higher weight percentage of Au compared to the other metals in EDS spectra, unlike ICP-OES quantification results, might be due to interference caused by the gold sputtering on the surface of the samples.

Table 4.1. Elemental composition of PCBs before and after one-step bioleaching

Element	Weight % Before bioleaching	Weight % After bioleaching
Al K	5.9	10.1
Si K	17.6	10.0
Ca K	13.1	1.8
Mn K	8.2	8.0
Fe L	0.0	0.0
Ni L	0.0	0.0
Cu L	1.4	2.8
Zn L	0.2	0.0
Ag L	0.7	0.5
Au M	52.8	66.8
Pb M	0.1	0.0

4.5.3. Cell attachment on PCB surfaces in one-step bioleaching

Cell attachment is an important step during contact bioleaching and was visualized in FESEM. Fig. 4.24 reveals the *A. aquatilis* cell attachment on PCBs during a one-step bioleaching process. It is evident from Fig. 4.24a, focused at 3000x, that the cells are embedded in the exopolysaccharides on the entire surface of the PCB particle. Similar results have been discerned through Atomic force microscopic images captured by Vera et al. (2013) during the mechanistic study on bio-oxidation of metal sulfides.

Another field was focused on the rod-shaped structures (Fig. 4.24b), which revealed that most cells were attached in the depressions or crevices and few on the plain surface. A similar attachment of cells on porous solid support was observed by Hubau et al. (2018) through Cryo-SEM during the bioleaching of PCBs and Sand et al. (2001) in an Atomic force microscopic image.

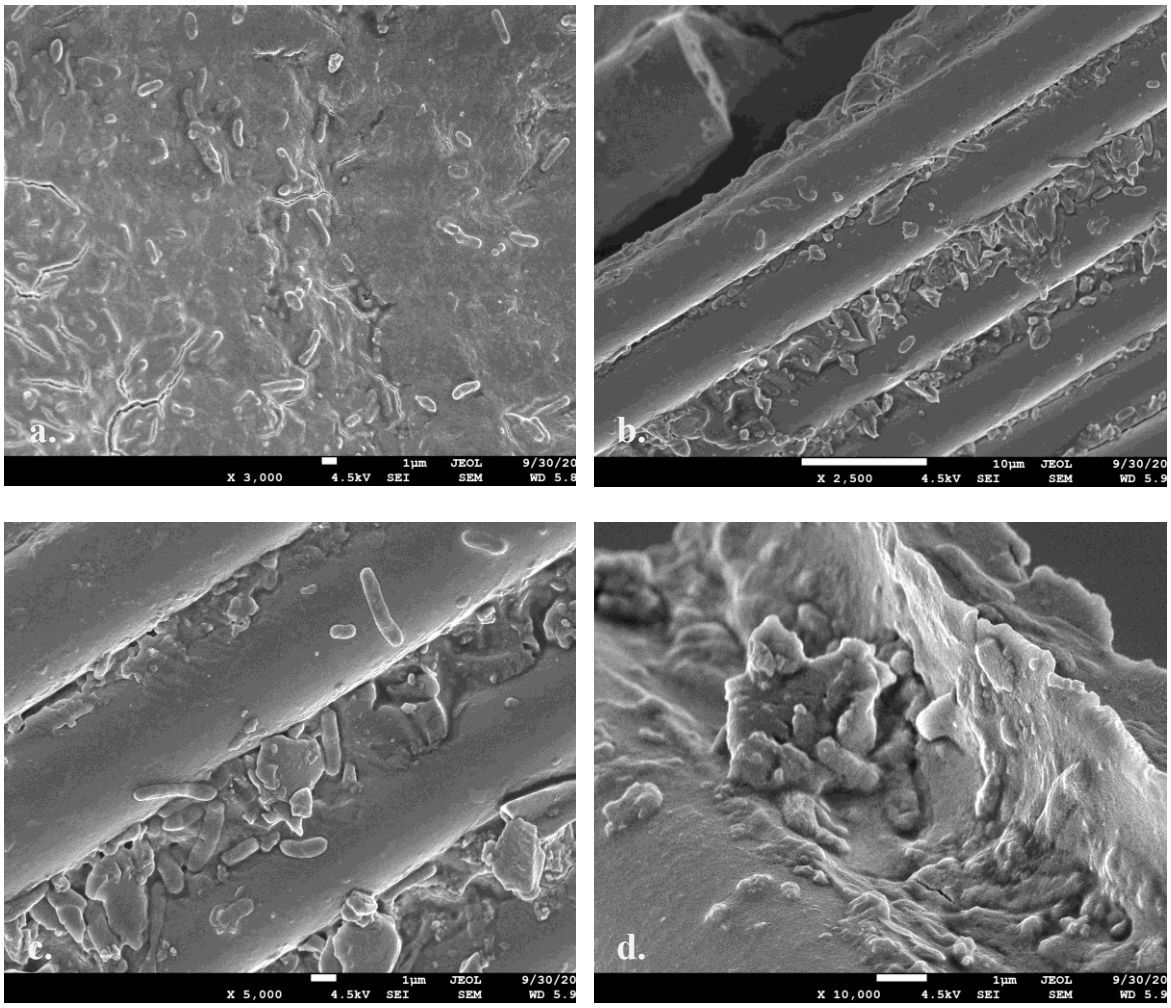


Fig. 4.24 Cell attachment on PCB surfaces in one-step bioleaching. **a.** Cell attachment at 3000x, **b.** Cell attachment at 2500x, **c.** Cell attachment at 5000x and **d.** Exopolysaccharide over the surface of cell-attached PCB particle

Magnification at 5000x (Fig. 4.24c) further confirmed the cell attachment to PCB surfaces with better resolution. An exopolysaccharide layer over the cells and PCB surface was observed at 10000x (Fig. 4.24d). It was also inferred that most of the free

cells in the bioleachate showed cell damage, as in Fig. 4.22. In contrast, most of the adhered cells have no cell disruptions where the exopolysaccharide layer protects the attached cells, as reported by Vimalnath and Subramanian (2018) during the biosorption of Pb by *Pseudomonas aeruginosa* as a defense mechanism. This confirmed a contact mode of bioleaching and the action of secreted secondary metabolites/extracellular proteins during the one-step method for Cu mobilization.

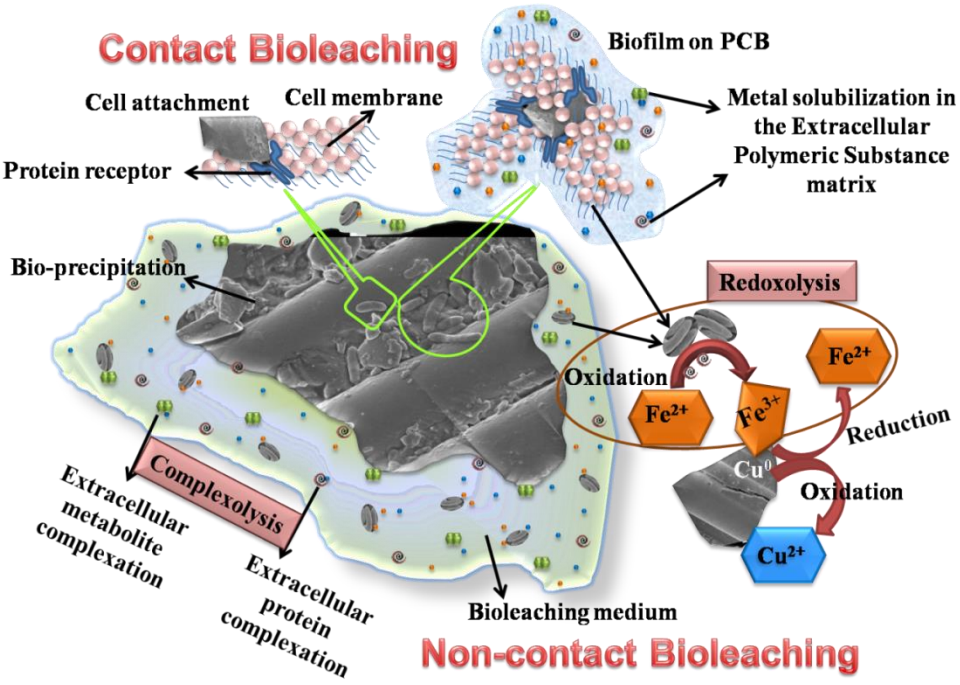


Fig. 4.25. Possible mode and mechanisms of one-step bioleaching by *A. aquatilis*

Based on the observations from the role of Fe in Cu solubilization, difference in protein concentration, protein separation through SDS-PAGE, and FESEM micrographs, the possible mechanism for initial Cu dissolution is redoxolysis. Redoxolysis is a sequence of oxidation and reduction of Fe through which Cu metal is solubilized from the PCBs. The Fe^{2+} in the medium is oxidized by *A. aquatilis* through enzymatic Fe^{2+} to Fe^{3+} conversions that generate Fe^{3+}, which comes into contact with metallic Cu in the PCBs. Cu^{0} in the PCBs gets oxidized to Cu^{2+} by transferring its electrons to Fe^{3+}, reducing itself to Fe^{2+} again, and the cycle continues. From the cell attachment studies in section 4.5.3, it was found that a contact mode of bioleaching could also occur along with the action of Fe

in the bioleaching medium. Hence the redoxolysis mechanism of Cu bioleaching proceeds in contact and non contact modes. Further bioleaching of Cu continues through redox reactions coupled with complexolysis mechanism i.e., complexation with the overexpressed or differentially expressed proteins in the bioleaching medium. This complexation and solubilization occur in the bioleaching medium through a non-contact mode. The same processes of complexolysis ensue at PCB-cell interfaces in a contact mode. These processes have been depicted in Fig. 4.25 (Chen et al. 2015; Rodrigues et al. 2015; Sodha et al. 2020; Wu et al. 2018).

4.6. COMPATIBILITY TEST FOR THE GROWTH OF *A. aquatilis* and *C. violaceum*

The results presented in section 4.2 have confirmed that *A. aquatilis* was efficient in bioleaching of Cu and Ag, whereas *C. violaceum* for Au bioleaching.

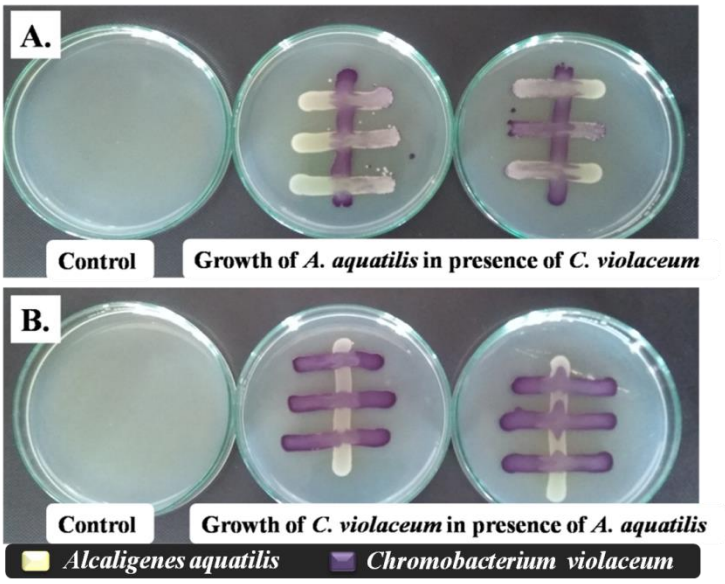


Fig. 4.26. Compatibility test for the growth of *A. aquatilis* and *C. violaceum* (A) Growth of *A. aquatilis* in the presence of *C. violaceum* (B) Growth of *C. violaceum* in the presence of *A. aquatilis*.

So, it was hypothesized that the use of these two bacteria as co-cultures in one step bioleaching process may facilitate the simultaneous leaching of Cu, Ag, and Au from

PCBs and improve their bioleaching efficiencies. An improvement in bioleaching efficiencies by mixing two or more microbial cultures has been reported by several researchers (Baniasadi et al. 2019). However, the application of bacteria as co-cultures in bioleaching necessitates their mutual compatibility for growth in the same system. The cross-streak method estimated the compatibility of *A. aquatilis* and *C. violaceum*. From Fig. 4.26 (A and B), it is clear that the bacterial cultures can grow in harmony, and no antagonistic activities exist. If there were any growth inhibition at the intersecting points of the streaks of the two cultures, then the cultures would exhibit amensalism (Santiago et al. 2017).

4.7. CO-INOCULATION OF *A. aquatilis* AND *C. violaceum* FOR BIOLEACHING

The effect of co-inoculation in different ratios of *C. violaceum* to *A. aquatilis* (1:1, 1:3, and 3:1) for simultaneous bioleaching of Au, Ag, and Cu was carried out. The bioleaching was performed with the optimized conditions of 0.175 mm particle size, 5% inoculum, and 2% e-waste load under a shake flask and FBR conditions. The 5% (v/v) inoculum was provided in three different ratios and compared with the results of bioleaching by individual cultures.

4.7.1. Co-Inoculation in Shake Flasks

The results of bioleaching of Au, Ag, and Cu obtained with co-inoculation studies in a shake flask are illustrated in Fig. 4.27, and the statistical mean difference of the results obtained from the ANOVA table is presented in Appendix III. The results indicate that *A. aquatilis* and *C. violaceum* play a significant role as sole strains in Cu and Au bioleaching, respectively. The Ag bioleaching pattern demonstrates that the co-inoculated cultures have better Ag dissolution abilities than their constituent cultures. A significant mean difference in Au bioleaching was noticed with *C. violaceum* as a sole strain rather than *A. aquatilis* or co-cultures. Higher bioleaching of Au could be obtained with a mixing ratio of 3:1 compared to that with *A. aquatilis* or other mixing ratios in co-cultures at all time intervals, with a maximum of 7.2% at 48 hours. The bioleaching of Au with *A. aquatilis* as a sole strain and in the mixing ratio of 1:1 was almost equal to or lesser than the Au leaching in control, whereas the inoculum in 1:3 ratio could leach out

slightly higher Au than these at 36 hours. The strains as co-cultures significantly enhanced the Ag bioleaching in comparison to individual cultures, with 70.3%, 37.7%, and 33.4% being achieved at 96 hours in a system inoculated with 3:1, 1:1, and 1:3 ratios, respectively. The individual cultures of *C. violaceum* and *A. aquatilis* had a bioleaching efficiency of 25.7% and 16.4% at 96 hours which was approximately equal and lesser, respectively, than the control. The Cu bioleaching with co-cultures was found to be lesser than that with the individual strain of *A. aquatilis,* with the mean difference being statistically significant at all the time intervals.

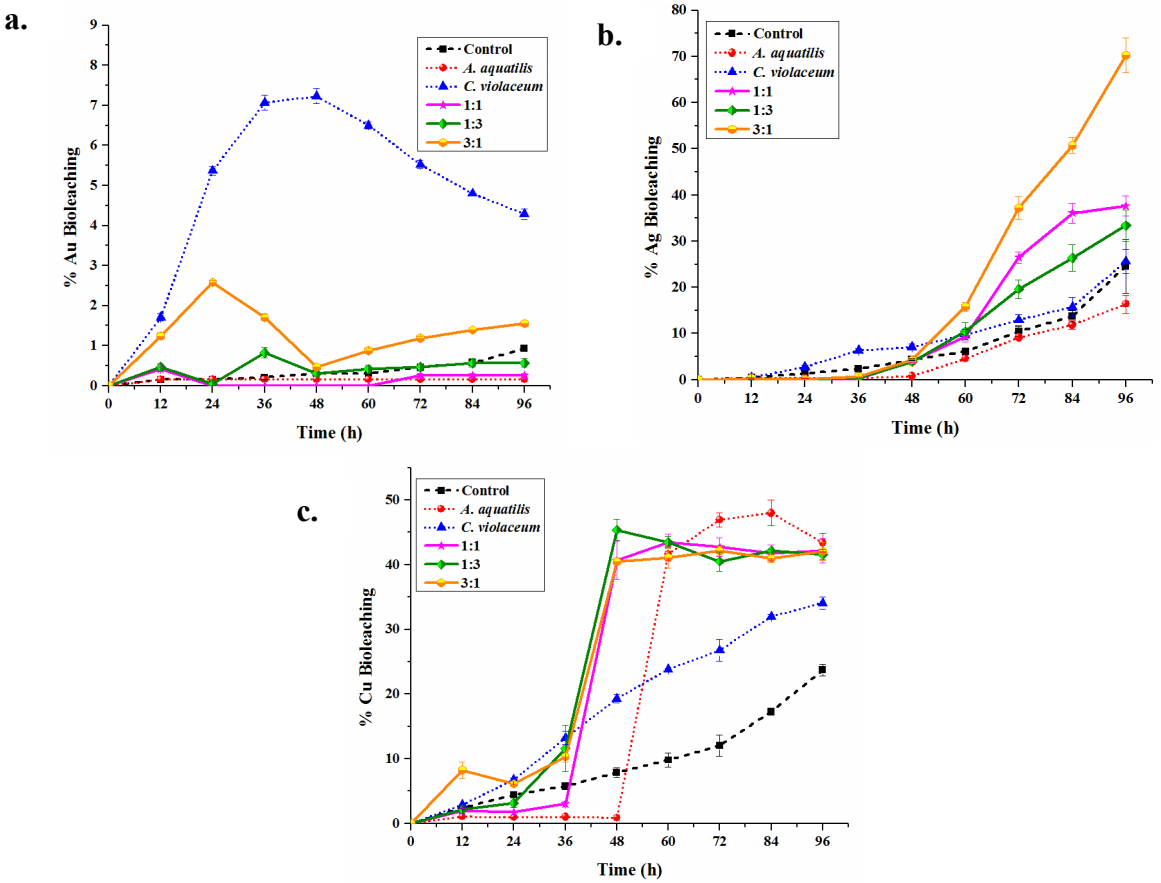


Fig. 4.27. Bioleaching of metals by individual and co-inoculated cultures of *A. aquatilis* and *C. violaceum* in shake flask **a.** Bioleaching of Au, **b.** Bioleaching of Ag and **c.** Bioleaching of Cu.

Furthermore, the effect of co-cultures at different inoculum ratios was compared, and the variation in Cu bioleaching with inoculum ratio was noticed to be statistically

insignificant after 48 hours. This indicated that the co-inoculation did not affect the Cu bioleaching in the shake flask. *A. aquatilis* leached out a maximum of 47.9% of Cu from PCBs in 84 hours. The co-culture inocula mixed in different ratios could yield 40-42% of Cu between 72-96 hours. *C. violaceum* individually solubilized 34.1% Cu which was higher than the control with 23.7% Cu at 96 hours. These results show that the bioleaching efficiency is the maximum with the sole strain of *A. aquatilis,* followed by that with co-cultures and then with a sole strain of *C. violaceum*. The efficacy of *A. aquatilis* as a sole strain in Cu bioleaching is evidenced in these studies.

4.7.2. Co-Inoculation in FBR

The bioleaching with the co-cultures of the strains was performed in the FBR and the results are compared with bioleaching by the individual cultures. The bioleaching percentages of Au, Ag, and Cu in FBR are shown in Fig. 4.28, and their statistical mean differences through ANOVA are presented in Appendix III.

(a) Au Bioleaching by *A. aquatilis* and *C. violaceum*

The Au bioleaching trend of the selected bacterial strains as individual cultures, co-cultures with different inoculum ratios, and uninoculated control in the FBR is discussed in this section. The results are presented in Fig. 4.28a. The bioleaching of Au from PCBs by the single strains or co-cultures is evidenced against the uninoculated media. *C. violaceum* individually demonstrated a higher Au bioleaching with 9.2% (w/w) at 48 hours than those with sole *A. aquatilis* or as co-culture. *A. aquatilis* showed no noticeable bioleaching ability as a sole strain. The co-cultures exhibited a statistically significant difference in Au bioleaching only for up to 24 hours. A maximum Au bioleaching of 3.3%, 2%, and 3.4% (w/w) was achieved with 1:1, 1:3, and 3:1 inoculum ratios at 24 hours and decreased subsequently. The Au bioleaching trends of the co-cultures follow the bioleaching pattern of *C. violaceum* for up to 24 hours and then that of *A. aquatilis*. This denotes that *C. violaceum* had a significant role in the bioleaching of Au rather than *A. aquatilis* during the process. The bioleaching medium contains amino acid additives like glycine and methionine to accomplish cyanidation, which has been proven to facilitate the accomplishment of enhanced Au bioleaching efficiency as

reported by Li et al. (2020), wherein the bioleaching efficiency was found to be 54% at pH 9 using *Pseudomonas fluorescens*, which was higher than the Au bioleaching in the current study where the process was carried out at an initial pH of 6 – 6.5. According to Kumar et al. (2018), the cyanide available for precious metal bioleaching was competitively consumed for Cu bioleaching, reducing precious metal bioleaching. Since the Cu concentration in their e-waste was minimal, Au bioleaching of 68.5% was reported. This contradicts the results of the present study, wherein the concentration of Cu in the PCBs is the highest. Moreover, Ag mobilization may also occur utilizing the biogenic cyanide produced by *C. violaceum* resulting in a low Au bioleaching efficiency.

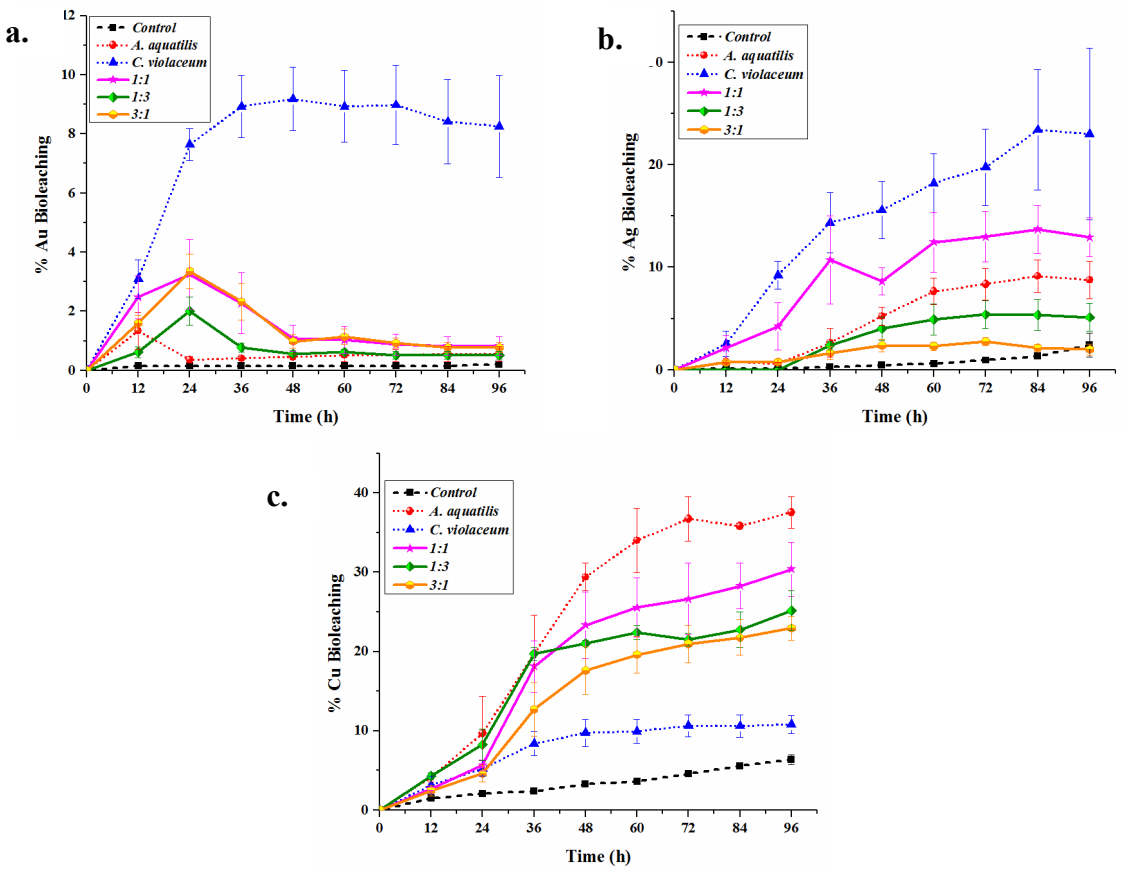


Fig. 4.28. Bioleaching of metals by individual and co-inoculated cultures of *A. aquatilis* and *C. violaceum* in FBR **a.** Au Bioleaching, **b.** Ag Bioleaching, and **c.** Cu Bioleaching.

(b) Ag Bioleaching by *A. aquatilis* and *C. violaceum*

Similar to Au bioleaching, *C. violaceum* individually exhibits a higher Ag bioleaching of about 23.4% (w/w) at 84 hours, as evident in Fig. 4.28b. The 1:1 ratio of the mixed inoculum attains a bioleaching percentage of 13.7% (w/w) followed by 9.1% (w/w) Ag using *A. aquatilis* alone at 84 hours. The other mixing ratios had lesser Ag bioleaching percentages but more than the control. A noticeable Ag bioleaching trend of a 1:1 ratio of the inocula falls between the individual strains' bioleaching patterns. The Ag bioleaching trend increased with 1:1 ratio inoculation that follows the *C. violaceum* up to 36 hours, slightly decreased at the 48th hour, and further increased analogous to that of the Ag bioleaching pattern of *A. aquatilis*.

The results also indicate a significant increase in the Ag bioleaching with time that had the individual strains and 1:1 ratio as inoculants. It is apparent that both bacterial strains play a role in the bioleaching of Ag, from which *C. violaceum* had the prime function in the FBR though *A. aquatilis* was found to have higher bioleaching efficiency in shake flask conditions during the screening experiments. Moreover, *A. aquatilis*, isolated from Silversmith's workbench dust, possessed a tolerance mechanism to the metal and was reported to facilitate silver nanoparticle synthesis (Kulal and Shetty Kodialbail 2021). A maximum of 7.08% Ag was solubilized by the *C. violaceum* strain when used individually, as reported by Pradhan and Kumar (2012), which increased marginally to 8.42% on mixing *C. violaceum* with *Pseudomonas aeruginosa* in two-step bioleaching process for 7 days. A higher Ag bioleaching of 23.4% was achieved with the same bacterial strain of *C. violaceum* within 3.5 days which was a relatively lesser bioleaching time accomplished with a one-step bioleaching process in the present study. Similarly, *Pseudomonas chlororaphis*, a cyanogenic isolate, could leach out 12.1% Ag (Ruan et al. 2014), which is comparatively lesser than the *C. violaceum* used individually and as a co-inoculant with *A. aquatilis* in a 1:1 ratio.

(c) Cu Bioleaching by *A. aquatilis* and *C. violaceum*

As evidenced in Fig. 4.28c, Cu bioleaching of about 37.5% (w/w) could take place at 96 hours with *A. aquatilis* inoculated experiment, which was higher than that

achieved in either a co-inoculated system, the system inoculated with only *C. violaceum,* or in control experiments. The Cu solubilization of 30.4%, 25.2%, and 22.9% (w/w) was yielded by 1:1, 1:3, and 3:1 ratios, respectively, at 96 hours and was found to be lower than that with *A. aquatilis* as individual culture. This shows that *A. aquatilis* had a greater significance in Cu bioleaching. The redox mechanism could instigate the Cu bioleaching by *A. aquatilis* as there is a simultaneous Fe bioleaching in the system. These Fe^{2+} ions in the leachate are oxidized to Fe^{3+} by the bacterium. Further, Cu^{0} would supply electrons to the Fe^{3+} ions and oxidize as Cu^{2+}, reducing Fe^{3+} to Fe^{2+} for the next cycle of redoxolysis (Pourhossein and Mousavi 2019; Rodrigues et al. 2015). *C. violaceum* independently had a 10.8% Cu bioleaching efficiency at 96 hours which was higher than the control. The pattern of Cu bioleaching in the co-cultured trials fall in between the bioleaching pattern of the strains inoculated distinctly. The inoculation with an equal and higher fraction of *A. aquatilis* demonstrates better Cu bioleaching efficiency than the process with a higher fraction of *C. violaceum*. This endorses that *A. aquatilis* contributed largely to Cu bioleaching with co-culture. Further, the Cu bioleaching achieved in the present study is slightly higher than that obtained using a lithotrophic, iron-oxidizing strain of *Acidothiobacillus ferrooxidans* that solubilized 32.44% Cu from Computer circuit boards (Annamalai and Gurumurthy 2019).

The Au and Cu bioleaching percentages attained in the current study is much higher than the results reported by Chi et al. (2011) using *C. violaceum*, with 11.31% Au and 24.6% Cu in a longer bioleaching time of 8 hours obtained after supplementing O_2 with H_2O_2. The same report has suggested reducing the Cu concentration from the PCBs using an appropriate technique for enhancing the Au bioleaching efficiency. Pradhan and Kumar (2012) conducted a similar bioleaching study, employing cyanogenic strains of *P. aeruginosa*, *P. fluorescens,* and *C. violaceum* (MTCC 2656) by a two-step method in shake flasks. Their results showed that a co-culture of *P. aeruginosa* and *C. violaceum* could leach out a maximum of 83% Cu, 46% Au, and 8% Ag, whereas *C. violaceum,* as a single strain, could leach out almost 79% Cu, 69% Au and 7% Ag after 7 days. The present study reports a higher Ag bioleaching percentage in a lesser time of 4 days than that reported by Pradhan and Kumar (2012). However, the lower bioleaching of Au and

Cu reported in the present study, in comparison to that reported by Pradhan and Kumar (2012), could be due to the lesser bioleaching period (4 days) involved the difference in the bioleaching medium and bioleaching method (one step) employed in the present study. Under similar physiological conditions of pH 7 and 25°C in the same bioleaching medium of the present study, Ruan et al. (2014) could achieve 8.2% Au, 12.1% Ag, and 52.3% Cu bioleaching using *Pseudomonas chlororaphis* after optimizing to improve the CN- production. Relatively higher bioleaching of Au and Ag, but lesser Cu bioleaching efficiency is achieved in the present study. The co-culture of *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans* (lithotrophic bacteria) increased the bioleaching rate of Cu from chalcopyrite ore as reported by Bosse et al. (2015), which exhibited the evidence of the occurrence of synergistic effect through co-metabolism using coupled molecular processes that combine the polysulfide, iron oxidation, and thiosulfate pathways than the constituent bacterial cultures separately (Bosse et al. 2015). Several reports on mixing thermophiles and mesophiles, lithotrophs and heterotrophs, using two or more lithotrophs or heterotrophs have efficiently increased the metal extraction efficiencies (Arshadi and Mousavi 2014; Baniasadi et al. 2019; Pradhan and Kumar 2012; Xin et al. 2009). However, the results of the present study show that *C. violaceum* and *A. aquatilis* can co-exist in FBR and perform their individual functions but cannot outperform the Au, Ag, and Cu bioleaching efficiency through coupled intermolecular pathways as a mixture. So, further studies on bioleaching were conducted with single strains of bacteria.

4.8. COMPARATIVE ASSESSMENT OF BIOLEACHING METHODS FOR METAL RECOVERY

In order to improve the metal recovery by bioleaching, the bioleaching of Au, Ag, and Cu was carried out by three different methods, *viz*., (i) one-step method where PCBs were added with the inoculum, (ii) two-step method in which PCB powder was added to a grown bacterial culture, and (iii) spent-medium method where the PCB powder was added to the cell-free supernatant of the bacterial culture for bioleaching. *A. aquatilis* and *C. violaceum* as sole strains were used in shake flask and FBR to assess the different

bioleaching methods. The results of the study are presented in the following sections, and the statistical mean difference of the results between the three methods and the control is shown in Appendix IV, obtained from the mean comparison table of one-way ANOVA.

4.8.1. Bioleaching Methods for Au Recovery

Figure 4.29 evidences the Au bioleaching trend of *A. aquatilis* and *C. violaceum* with different bioleaching methods in shake flask and FBR experiments. Both systems show that *C. violaceum* has the Au solubilizing capacity with all three bioleaching methods. Moreover, a maximum Au bioleaching of about 11.25% in FBR at 72 hours and 9.08% at 24 hours in a shake flask were obtained by a two-step method employing *C. violaceum*. As reported in two-step bioleaching by Kumar et al. (2021), the same strain of *C. violaceum* could bioleach 73.6% Au which is higher than the results presented in the current study. This difference can be attributed to their optimized conditions of 1% e-waste load containing only 0.08 mg/g Au, higher initial pH 9, and a more extended bioleaching period of 7 days.

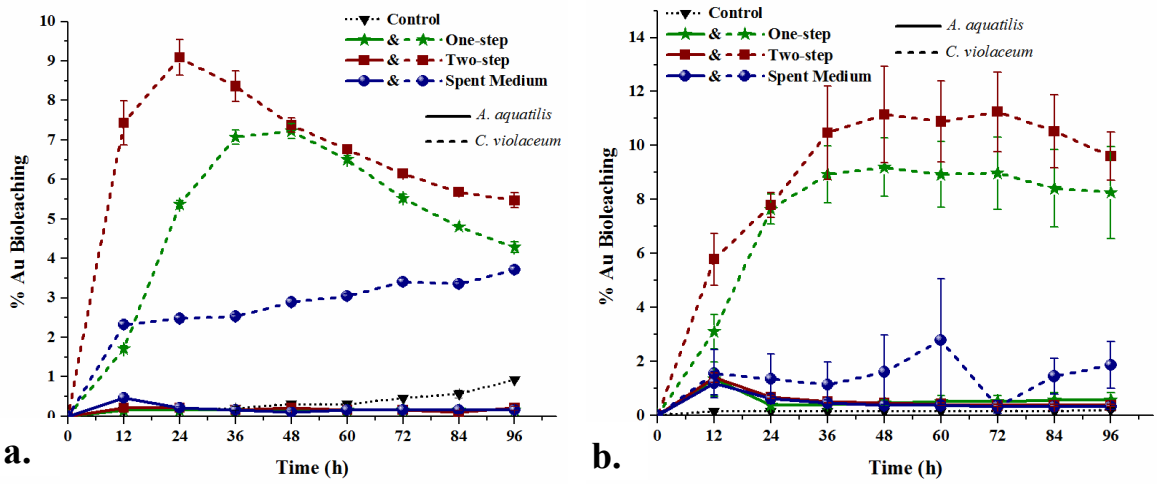


Fig. 4.29. Au Recovery with different bioleaching methods using *A. aquatilis* and *C. violaceum* in **a.** Shake Flask and **b.** Fluidized-bed Bioreactor

On the contrary, the present study uses a higher e-waste load of 2% containing a much higher Au concentration of about 0.32 mg/g. The process was also carried out for a shorter bioleaching period of 4 days at an initial natural pH of 7 that increases gradually with time. Following this was a one-step method with 9.19% and 7.23% Au bioleaching

in a shake flask and FBR at 48 hours, respectively. The cell-free spent medium of *C. violaceum* could bioleach Au of 2.79% in FBR and 3.72% in shake flask conditions at 60 and 96 hours, respectively. These results show that Au bioleaching occurs with the grown/actively growing cells of *C. violaceum* that produce cyanide as a secondary metabolite. The steady increase in the Au solubilization in one-step bioleaching could result from cyanide production by the actively growing cells with time. The absence of PCBs in the log phase of growth accumulates the metabolite and is utilized for Au cyanidation as the PCBs are introduced in the two-step method. Additionally, the PCBs used in the current study contain high amounts of Cu that can be preferentially utilized by the cyanide produced, thus reducing the Au recoveries (Kumar et al. 2021; Merli et al. 2022). According to Merli et al. (2022), a pre-treatment to remove Cu present in high concentration in the PCBs was necessary to recover 44% Au by cyanogenic *P. aeruginosa* employing a two-step method. In both these methods, the Au bioleaching proceeds until the cyanides are exhausted. In the two-step method, the cells are precluded from the high concentrations of metals and other toxic chemicals in the PCBs. This would otherwise lead to the death of a small fraction of cells from the inoculum as in the one-step method and might hinder the normal growth processes (Kumar et al. 2018). This could be one of the reasons that the two-step bioleaching of Au with *C. violaceum* was much more effective than the one-step method.

Under the same bioleaching conditions, *A. aquatilis* could bioleach Au, where the recovery was approximately 0.6% with any of the methods in the FBR. This was much lesser than the Au bioleaching by *C. violaceum* but higher than the uninoculated negative control, resulting in Au bioleaching as less as 0.2% in the FBR. Under shake flask conditions, *A. aquatilis* could leach out only negligible concentrations of Au, i.e., a maximum of 0.4%, much less than the negative control (0.9%) with a spent-medium method. This shows that the *A. aquatilis* cells are not cyanogenic or have no cellular/metabolic activity for the solubilization of Au present in the PCBs, whereas *C. violaceum* could serve as Au bioleaching agent with the maximum potential employing a two-step method in either shake flask or FBR conditions.

4.8.2. Bioleaching Methods for Ag Recovery

The Ag bioleaching efficiencies of *A. aquatilis* and *C. violaceum* with different methods in shake flask and FBR are shown in Fig. 4.30. The results obviate that the operating conditions of the shake flask are favorable for the bioleaching of Ag by *C. violaceum*. Moreover, the spent-medium bioleaching method enhanced Ag mobilization in the shake flasks to a maximum of 91.84%. This infers that extracellular metabolites and proteins play a vital role in Ag mobilization. Additionally, the mixing conditions and oxygen mass transfer characteristics in the shake flask imparted through mechanical rotational motion in the system with the spent medium may be better than that in one-step or two-step methods. The media fluid properties such as viscosity and density increase in the presence of cells leading to reduced mixing and mass transfer efficiencies. Ag solubilization of about 52.47% and 25.66% were achieved with two-step and one-step methods, respectively, by *C. violaceum*. From the shake flask studies, it is apparent that the fully grown cells in the stationary phase and their secondary metabolites enhance the Ag bioleaching in the two-step method rather than the cells growing in the presence of PCBs in the one-step method. The presented results of shake flask experiments are much higher than those obtained by (Kumar et al. 2018), where *P. balearica* SAE1, a cyanogen, could bioleach only 33.8% Ag after 7 days of employing a two-step method. A similar investigation on Ag bioleaching using *P. aeruginosa* optimized for cyanide production using a pre-treated lower e-waste load, higher initial pH, and more extended bioleaching period of 7 days resulted in 90% Ag recovery with a two-step method (Merli et al. 2022). The results of the present study reveal a slightly higher Ag recovery with spent-medium bioleaching. However, the two-step bioleaching yields were comparatively lesser due to the chosen experimental conditions that were previously optimized for Cu bioleaching in FBR. The Ag leaching in the uninoculated control in the shake flask was 24.56% which was in proximity to the one-step method of bioleaching. The Ag mobilization by *A. aquatilis* was relatively lesser than the uninoculated flasks indicating that the shake flask conditions did not favor the Ag solubilization by *A. aquatilis*.

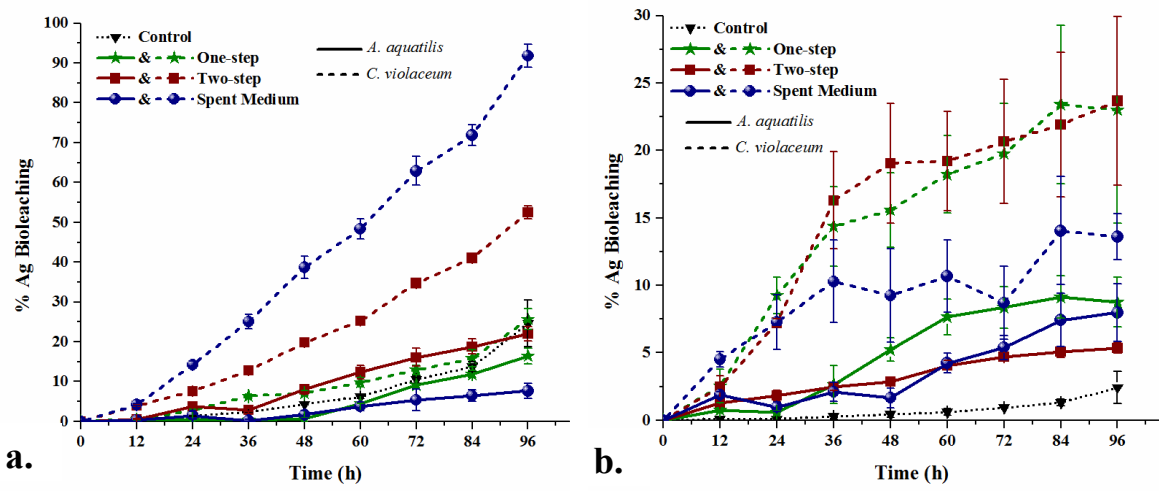


Fig. 4.30. Ag Recovery with different bioleaching methods using *A. aquatilis* and *C. violaceum* in **a.** Shake Flask and **b.** Fluidized-bed Bioreactor

Ag bioleaching in FBR bodes well with the cell-mediated one-step and two-step methods of bioleaching by *C. violaceum,* in contrast to the results of shake flask studies. The Ag recovery was 23.68% and 23.4% at 96 and 84 hours, respectively, with two-step and one-step bioleaching methods, whereas the spent medium could bioleach 13.6% at 96 hours in FBR. It can be deciphered that the *C. violaceum* cells grown in the presence or absence of PCBs in the one-step or two-step methods were active bioleaching agents rather than the extracellular compounds in the FBR conditions. The maximum Ag bioleaching by *A. aquatilis* was around 9.14% with the one-step method, 7.98% with a spent medium method, and 5.34% with the two-step method, which were all higher than the control. The *A. aquatilis* cells bioleached Ag during the growth phases and mobilized maximum concentrations during the stationary phase. The uninoculated trials did not exhibit significant Ag leaching, which was only 2.42% in the FBR conditions. Both the bacterial strains could solubilize Ag in the FBR, showing maximum efficiencies with the two-step method by *C. violaceum* and the one-step method by *A. aquatilis.*

In contrast, only *C. violaceum* revealed a prominent Ag bioleaching in shake flask conditions. The results indicated that the bioleaching of Ag is the maximum in the spent medium of *C. violaceum* in the shake flask and the one-step or two-step methods by *C. violaceum* in the FBR. Oxygen transfer plays a significant role in determining the activity of cells, and the continuous supply of oxygen in FBR eliminates any mass transfer

limitations. This ensures the proper transfer of oxygen required in the one-step and two-step methods, which take place in the presence of cells leading to the simultaneous occurrence of both contact and non-contact modes of bioleaching. However, in the shake flask, oxygen limitations may exist in the media leading to lower growth and activity of cells in the one-step and two-step methods, thus making the spent medium method more effective in Ag bioleaching involving only the non-contact mode.

4.8.3. Bioleaching Methods for Cu Recovery

Fig. 4.31 displays the Cu recovery by *A. aquatilis* and *C. violaceum* with different bioleaching methods conducted in shake flasks and FBR. The spent medium of *A. aquatilis* showed a prominent Cu bioleaching trend with the highest Cu recovery of 53.59% at 36 hours and 52.17% at 96 hours in the shake flask and FBR, respectively. A bioleaching efficiency of 93.4% was achieved with the bacteria-free cultural supernatant of iron-sulfur-oxidizing bacteria (a consortium of lithotrophs with *Leptospirillum ferriphilum* and *Sulfobacillus thermosulfidooxidans* being the predominant species) in 9 days (Wu et al. 2018). Followed by this, a two-step method could recover 43.07% at 36 hours and 31.46% at 96 hours in shake flask and FBR, respectively. From both these methods, it can be discerned that higher Cu bioleaching by *A. aquatilis* ensued only after the stationary phase of growth sets, which was evident in the shake flask results. The obtained data are in parity with the results of Natarajan and Ting (2015) using 2% e-waste load with approximately 50% Cu recovery by the spent-medium method followed by the two-step method. With the one-step method, *A. aquatilis* could bioleach 47.99% Cu at 84 hours and 37.54% at 96 hours in a shake flask and FBR, respectively. In any of the bioleaching methods, Cu bioleaching gradually increases with time. It reaches maximum recovery only at 96 hours in the FBR, whereas, in shake flask conditions, the maximum is achieved at earlier time intervals. In a one-step method, the cells in the inoculum may undergo stress due to the presence of PCBs and hence a delay in the initiation of noticeable Cu bioleaching until 48 hours. This initiation was achieved much earlier with spent-medium and two-step methods since the cells were grown separately without PCBs before bioleaching. In these cases, the cells have already reached their stationary phases, and the extracellular secretory compounds are instantly available for

Cu bioleaching as the PCBs are added. This phenomenon was evidenced in heterotrophic bacteria (Aston et al. 2022) or fungi (Faraji et al. 2022; Trivedi et al. 2022; Xia et al. 2018b) that release organic acids to accomplish metal recovery through a spent medium bioleaching approach. Similarly, the spent medium of iron-sulfur oxidizers (lithotroph) was employed to generate a biogenic lixiviant containing Fe^{3+} through bio-oxidation of the supplied Fe^{2+} (Wu et al. 2018) for 100% Cu recovery. Additionally, the protein elements of the secretome can also be associated with enhancing the bioleaching rate (Bobadilla Fazzini et al. 2011).

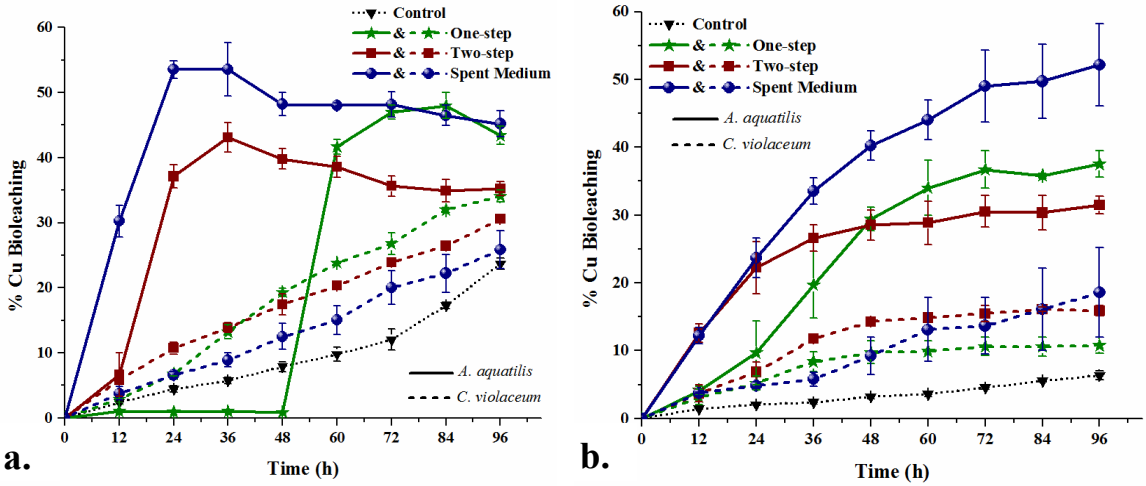


Fig. 4.31. Cu Recovery with different bioleaching methods using *A. aquatilis* and *C. violaceum* in **a.** Shake Flask and **b.** Fluidized-bed Bioreactor

Under the same bioleaching conditions, *C. violaceum* also contributed to trivial Cu solubilization relative to *A. aquatilis* from the PCBs to a maximum of 34.09%, 30.57%, and 25.85% at 96 hours in shake flasks with one-step, two-step and spent medium methods respectively. In FBR conditions, *C. violaceum* could mobilize only 18.58% at 96 hours with spent medium, followed by 16.09% at 84 hours with the two-step method and 10.8% at 96 hours with the one-step method. This signifies that *C. violaceum* more efficiently bioleaches Cu only in the presence of cells rather than the cell-free supernatant as in Au bioleaching. The Cu recovery by *C. violaceum* in the current study does not concur with the findings of Kumar et al. (2021), which reported 87.5% Cu recovery with the same strain. This might be due to the relatively low Cu concentrations in the PCBs used for their study, i.e., 23.39 mg/g Cu whereas the present

study used PCBs containing 353 mg/g Cu. Further, their optimal experimental conditions of e-waste load, higher initial pH, and more extended bioleaching periods contributed to their high Cu recoveries. The uninoculated control had only 6.37% and 23.72% Cu bioleaching in FBR and shake flask at 96 hours, which was lower than the results obtained with the three bioleaching methods. These findings disclose that maximum Cu bioleaching efficiencies were achieved with the spent medium of *A. aquatilis* followed by a one-step method contrary to Au and Ag bioleaching by *C. violaceum*.

To recapitulate the findings,

1. Au bioleaching was maximum with two-step followed by one-step methods using *C. violaceum*, whereas *A. aquatilis* did not contribute to Au mobilization.
2. Ag bioleaching was better with two-step and one-step methods by *C. violaceum* analogous to Au recovery. However, significant Ag solubilization was accomplished with the spent medium of *C. violaceum*. Though in trivial quantities, *A. aquatilis* did contribute to Ag bioleaching through cell-mediated methods.
3. Contradicting the precious metal bioleaching, maximum Cu recovery was achieved with the spent medium of *A. aquatilis,* followed by its one-step bioleaching method. Further, *C. violaceum* solubilized considerable quantities of Cu with all three methods.

A previous report by Natarajan and Ting (2015) on a pH-adjusted spent medium of *C. violaceum* could recover 30% Au and 95.7% Cu, and the two-step method yielded 11.3% Au and 86.2% Cu. The current study deviates from this report revealing that the two-step and one-step methods contributed to higher Au bioleaching by *C. violaceum* and agrees that the Cu bioleaching was efficient with the spent medium of *A. aquatilis*. A comparison between one-step and two-step methods by Marappa et al. (2020) using *Frankia* sp. exposed that a two-step over one-step bioleaching was effective for Au and Cu recovery. Similar conclusions were made by Narayanasamy et al. (2017) for Ag and Cu bioleaching by *A. niger* DDNS1. The findings of the present study support these results for Au and Ag recovery in shake flask, displaying a deviation with similar Ag

bioleaching in FBR by *C. violaceum*. However, the present study, where the one-step was more effective than the two-step method with *A. aquatilis* for Cu recovery, contradicts the results reported by Narayanasamy et al. (2017). A comparative study of the three bioleaching methods was carried out with two strains of iodide oxidizing bacteria as an alternative to cyanogenic bacteria by Kudpeng et al. (2020) for gold recovery from e-waste with meager Au recovery of <2%. These bacterial strains could bioleach 100% and 34% Au from the ore concentrate by a two-step method followed by spent medium and one-step methods.

Table 4.2. Comparison of the bioleaching efficiencies of the present study with previous reports

S. No.	Microorganism used	Bioleaching method	Recovery	Reference
1.	*Penicillium chrysogenum*	Spent medium One-step Two-step	Cu-76% Cu-25% Cu-47%	(Xia et al. 2018b)
2.	*Roseovarius tolerans* *Roseovarius mucosus*	Two-step Spent-medium One-step	Au-34% Au-28% Au-1.6%	(Kudpeng et al. 2020)
3.	*Chromobacterium violaceum*	Two-step One-step Spent medium	Au-11.3% Au-9.2% Au-3.7%	Present study
4.	*Chromobacterium violaceum*	One-step Two-step Spent medium	Ag-23.7% Ag-23.4% Ag-13.6%	Present study
5.	*Alcaligenes aquatilis*	Spent medium Two-step One-step	Cu-52.1% Cu-31.5% Cu-37.5%	Present study

The outcome of Au bioleaching in the present study partly agrees with this, showing that the two-step method had maximum Au solubilizing capability. Hence, two-step methods by *C. violaceum* for Au and Ag bioleaching and spent-medium of *A. aquatilis* for Cu recovery were chosen for further experiments to enhance the recovery through sequential batches. The metal bioleaching efficiency of the present study has been compared with the above studies in Table 4.2. The choice of a suitable bioleaching method depends on the microbial strain, its growth and metabolic activity, the metal to be leached, specific metal-microbe interactions, mixing, and mass transfer in the reacting systems leading to the bioleaching process.

4.9. SEQUENTIAL BATCH BIOLEACHING IN FBR FOR ENHANCED METAL RECOVERY

The bioleaching by either of the methods could not completely solubilize the metals in a single batch and mandated further bioleaching of the residue. The reasons could be attributed to (i) the exhaustion of nutrients that diminish the microbial activity and hinder the bioleaching process, (ii) the achievement of maximum solubility that interrupts further metal solubilization, (iii) the high concentration of the leached metal inhibits further microbial activity, (iv) the high concentration of specific metals like Cu, (v) the same bacterial strain may not bioleach all the metals of interest, and (vi) the co-inoculation strategy was not effective for simultaneous metal recovery. So, a sequential metal recovery was carried out in the FBR with 2% (w/v) initial e-waste load by removing the leachate after the first batch and contacting the residue with fresh medium and inoculum in the subsequent batches. The bioleaching efficiency in the FBR was improved in two ways, (i) Ag and Cu were recovered in three sequential batches by a one-step method, and (ii) Au, Ag, and Cu bioleaching was performed in two stages using the spent medium of *A. aquatilis* for three sequential batches in the first stage for Cu mobilization, followed by Au and Ag bioleaching with a two-step method by *C. violaceum* in the second stage. The results of these studies are presented and discussed in the following sections.

4.9.1. Sequential metal recovery by one-step method

Ag and Cu bioleaching efficiency by *A. aquatilis* with a one-step method was improved by increasing the number of sequential batches, as presented in Fig. 4.32. A maximum Ag bioleaching of 10.57%, 11.89%, and 6.11% at 96 hours was obtained in the first, second, and third batches, respectively. This consequently increased the cumulative Ag recovery to 22.46% after two batches and 28.58% (0.09 mg/g) after three sequential batches. Similarly, the cumulative Cu bioleaching reached 80% at 84 hours (282.55 mg/g), with 35.3% in the first, 29.31% in the second, and 15.41% in the third batch. Moreover, further addition of sequential batches of bioleaching would have further increased the recovery. These findings build on the evidence of bioleaching by Jagannath et al. (2017) with an enhancement of recovery from 23% to 63% as the number of batches increased from 1 to 5 batches (each batch for 48 hours) in a Pulsed-plate bioreactor using a heterotrophic one-step bioleaching method.

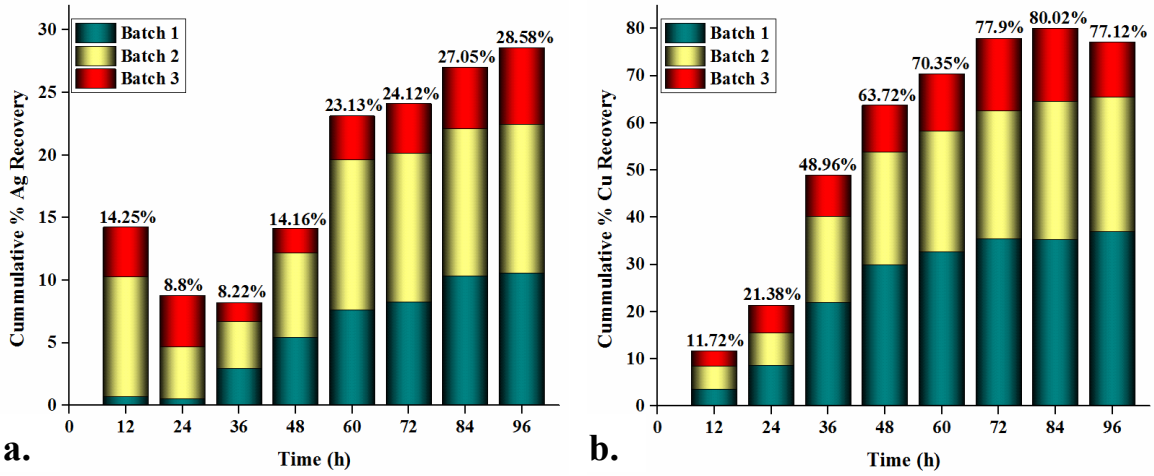


Fig. 4.32. Sequential batch bioleaching by the one-step method in FBR **a.** Cumulative Ag recovery, and **b.** Cumulative Cu recovery

A one-step bioleaching study conducted by Arab et al. (2020) showed that five strains of cyanogenic isolates from the e-waste landfill could recover 31-96% Cu from Cu powder with a bioleaching period of 10 days in shake flask conditions. The highest recovery obtained from their investigation with 0.1% pulp density can be comparable to the cumulative Cu recovery from a higher e-waste load (2% w/v) and 12 days. It is clear

that Cu bioleaching is highest in the first batch and decreases in the following batches due to the decrease in the Cu concentration after each batch of bioleaching. Therefore, the metals are less accessible to the organism or metabolites in the second or subsequent batches, thus reducing the sensing metal concentration.

Concurrently, Ag has the highest bioleaching efficiency in the second batch after considerable removal of Cu occurred but decreased in the third batch. This obviates that a high concentration of Cu interfered with the initial bioleaching rate of Ag due to lesser accessibility. The available reports on bioleaching show that the one-step method was rarely used due to growth limitations in the presence of e-waste (Monballiu et al. 2015) that would result in lesser metal recovery, which can be circumvented through sequential batch bioleaching (Srichandan et al. 2014). The advantage of using this method is that a pre-growth step or obtaining a cell-free supernatant after pre-growth was not required, and a sequential batch process can substantiate the limitations.

4.9.2 Two-stage sequential metal recovery by spent-medium and two-step method

The comparative study on the bioleaching methods (Section 4.8) revealed that the spent medium of *A. aquatilis* was efficient in Cu bioleaching and the two-step method using *C. violaceum* for Au and Ag bioleaching. Hence these two methods were combined to recover the metals in two stages by sequential batch bioleaching with 2% (w/v) initial e-waste load and the residue from the previous batch as e-waste load in the subsequent batches. The two-stage sequential batch bioleaching was established by Cu recovery using the spent medium of *A. aquatilis* for three batches (stage 1), followed by Au and Ag recovery with a two-step method of *C. violaceum* for the subsequent three batches (stage 2). The results of the cumulative metal recovery are presented in Fig. 4.33. The maximum Au, Ag, and Cu recovery was estimated in all six batches and tabulated in Table 4.3. This contributed to a cumulative Au bioleaching of 21.9%, 36.75% Ag, and 99.43% Cu at 12 hours and 96 hours, respectively, after two stages of sequential batch experiments. The leftover residue on acid digestion had 64% Ag and 0.15% Cu which further authenticated the cumulative bioleaching percentage obtained for these metals.

The Cu bioleaching in both stages proceeded until 96 hours, as observed for the one-step method in sequential batches. Stage 1 with the spent medium of *A. aquatilis* resulted in Cu solubilization of 92.96% after the three batches. Further bioleaching of the residue for Au and Ag mobilization with the two-step method of *C. violaceum* additionally contributed to achieving 99% Cu recovery along with precious metals in the present study.

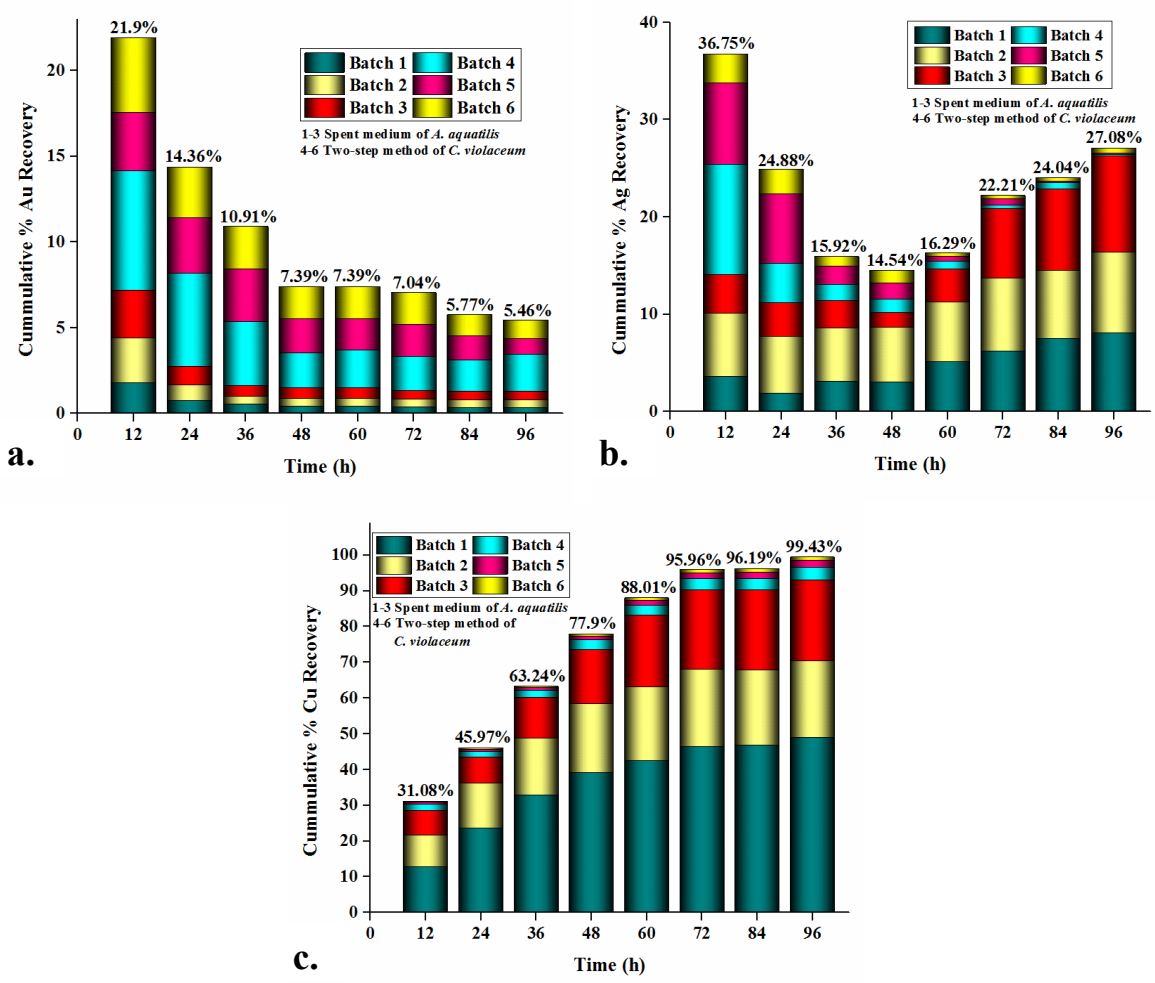


Fig. 4.33. Two-stage sequential batch bioleaching (Stage 1 with spent medium of *A. aquatilis* and Stage 2 of two-step method by *C. violaceum)* in FBR. **a.** Cumulative Au recovery **b.** Cumulative Ag recovery, and **c.** Cumulative Cu recovery

A 100% Cu extraction from a single PCB plate was reported by Sodha et al. (2020), wherein they used bio-generated ferric iron lixiviant with a 9% $FeSO_4$ supplement

to maintain the oxidation-reduction potential high with stringent acidophilic conditions in five cycles with each cycle for 4 hours using *Leptospirillum ferriphilum* dominated consortium of lithotrophic iron-oxidizing bacteria. The biogenic Fe(III) of *Acidithiobacillus ferrooxidans* could recover 80% Cu after 3 cycles of column bioleaching in 6 hours (Benzal et al. 2020a).

Table 4.3. Cumulative metal recovery after each batch during the sequential bioleaching experiments in two stages

Bioleaching Methods	**Batch**	**% Au Recovery**		**% Ag Recovery**		**% Cu Recovery**	
Spent medium method – *A. aquatilis*	**1**	1.78	12 hours	3.58	12 hours	48.89	96 hours
	2	4.41		10.08		70.42	
	3	7.20		14.08		92.96	
Two-step method – *C. violaceum*	**4**	14.16		25.42		96.47	
	5	17.57		33.75		98.47	
	6	21.90		36.75		99.43	

The present study is a relatively slow process that uses the natural conditions of the cell-free supernatant to achieve maximum efficiencies without any stringent bioleaching conditions, which is an advantage over lithotrophs that mandates rigorous bioleaching conditions. A study by Pakostova et al. (2018) reported a cumulative 87% (32 days) and 50% (49 days) Cu extracted from the two fractions of Kupferschiefer ore, respectively, with acid and bioleaching (bio-generated ferric iron from a consortium of iron-oxidizing acidophiles). Ag bioleaching from the fourth and fifth batches in the current work was maximum at 12 hours, contributed majorly by *C. violaceum* and moderately by the spent medium of *A. aquatilis*. As the bioleaching time extended, the role of *C. violaceum* was trivial, but the spent medium of *A. aquatilis* significantly contributed to Ag mobilization. From this, it is apparent that both organisms promoted Ag recovery. It was noticed that the highest Au and Ag mobilization was obtained from the fourth batch after Cu removal. A marginal role of the spent medium of *A. aquatilis* in Au bioleaching was observed at 12 hours, which decreased considerably during the bioleaching period. A study conducted by Işıldar et al. (2016) employed a step-wise

recovery using a two-step method by a mixture of chemolithotrophs (*A. ferrivorans* and *A. thiooxidans*) for Cu bioleaching with 98.4% yield in 7 days followed by heterotrophic cyanogens (*P. putida* and *P. fluorescens*) for Au mobilization of about 44% in two days from the residue. This was analogous to the current work that used heterotrophs in both stages with three batches each for Cu & Au solubilization, and a spent-medium method was employed for Cu recovery.

Mwase et al. (2014) investigated and observed that the *Metallosphaera hakonensis* mediated bioleaching stage could remove 93% Cu in 304 days, followed by 90.3% Au in the cyanide leaching (chemical-mediated) stage in 60 days from Platreef ore in column studies. Compared to these findings, the present study reveals a higher bioleaching rate within a short period. Since the Cu concentration was high in the PCBs, the first three batches focused on the solubilization of Cu from the initial 2% e-waste load using the spent medium of *A. aquatilis*. The process was continued for Au and Ag bioleaching from the residue employing a two-step method with *C. violaceum* for another three batches. The findings of the study endorse the hypothesis that sequential batch bioleaching with the same or different methods can achieve higher/maximum metal recovery. The high concentration of Cu in a single batch saturates the medium and inhibits further leaching (Işıldar et al. 2016). Benzal et al. (2020), in their toxicity assays on Cu bioleaching using *Acidothiobacillus ferrooxidans,* found that the microbial metabolic activity is not affected up to 48 hours due to Cu bioleaching. The attainment of inhibitory or toxic concentration levels may occur later. The high concentrations of bioleached metal in the media and depletion of nutrients may also show an inhibitory effect on cell growth and microbial activity for Cu mobilization.

Further, the metals readily available on the outer surfaces are extracted by the metabolites or ligands produced by the microbial cells. Owing to diffusional mass transfer limitation and exhaustion of the secretory bioleaching compounds, the inner layers and interior of PCBs containing Cu remain unaffected, thus resulting in limited bioleaching of Cu in a single batch. This necessitates further solubilization of the leftover Cu from the residue, which can be achieved by subjecting it to bioleaching in the subsequent batches with fresh bioleaching media and inoculum. Large-scale applications

to treat high quantities of e-wastes require several batches for complete metal recovery. Comparing the Cu recovery of the one-step method and spent medium sequential bioleaching for three batches yielded 80% and 92.96%, respectively. One or more additional batches of one-step bioleaching would substantiate the complete Cu recovery rather than setting up an additional bioreactor for the growth and cell separation units to obtain a cell-free biogenic lixiviant required to establish the spent medium bioleaching process. Hence, the one-step method can also be chosen over the spent-medium method for large-scale applications if the cell toxicity/viability issue caused by the PCBs is trivial. Metal-specific bacterial strain selection and employing it in different stages can accomplish a complete metal recovery from the PCBs.

5. SUMMARY & CONCLUSIONS

5.1. Summary

In the present study, the bioleaching of metals from waste PCBs was carried out using metal-resistant chemoorganoheterotrophic bacterial strains. The bioleaching of Ag, Au, Cu, Fe, Ni, Pb, and Zn from the PCB powder of mobile phones using the metal-resistant heterotrophic bacterial strains, *Acinetobacter* sp., CR B2, *Ochrobactrum* sp., CR B4, *Chromobacterium violaceum* and *Alcaligenes aquatilis* was investigated through shake flask experiments. Medium 3 was selected for further bioleaching experiments. *A. aquatilis* was selected as the bioleaching agent due to its high Ag and Cu bioleaching efficiencies. *C. violaceum* was also chosen for its Au and Ag mobilization ability. The effect of particle size, inoculum size, and e-waste load on Ag and Cu bioleaching was studied in a shake flask and fluidized bed bioreactor. Based on the statistical significance, the influential factors were found to be inoculum size and e-waste load for Cu bioleaching, whereas particle size and e-waste load for Ag bioleaching. The selected optima were 0.175mm particle size, 5% inoculum, and 2% e-waste load. With a view to maximize Cu recovery, the optimum process conditions of Cu bioleaching were used for further studies, along with attempts to maximize Ag and Au bioleaching under these optimum conditions by using various strategies.

The mechanism of one-step bioleaching of Cu was studied through analysis of various parameters like pH, ORP, Fe concentration, extracellular protein concentration, cell viability, and differential protein expression assessed during and after the process. The experiments in the presence and absence of PCBs confirmed the cell-mediated redoxolysis mechanism, and an extracellular complexolysis mechanism contributed to Cu bioleaching. After bioleaching, the FESEM micrographs of *A. aquatilis* cells evidenced the morphological changes, cell disruptions, and metal precipitation. The micrographs of the residue with cells disclosed the cell attachment on the PCB surface. This validates a contact mode of bioleaching and the non-contact mechanisms in the extracellular space. The FESEM-EDS analysis of PCBs before and after one-step bioleaching displayed the changes in surface characteristics.

Co-inoculation of *C. violaceum* and *A. aquatilis* in 1:1, 1:3, and 3:1 ratios was assessed for simultaneous recovery and improvement of Au, Ag, and Cu bioleaching. This was compared to the bioleaching efficiencies of the individual cultures. In FBR and shake flask, the Au and Cu bioleaching was maximum by the individual cultures of *C. violaceum* and *A. aquatilis,* respectively. Hence, the individual strains proved effective in FBR for Au, Ag, and Cu bioleaching. A comparative assessment of the one-step, two-step, and spent medium bioleaching methods for Au, Ag, and Cu recovery was conducted in a shake flask and FBR with *A. aquatilis* and *C. violaceum* cultures. From the experimental results, the spent medium of *A. aquatilis* for Cu recovery and a two-step method with *C. violaceum* for Au and Ag recovery were chosen for sequential batch experiments. Sequential batch experiments were carried out under the optimized conditions with a one-step method (three batches) using the residue of the preceding batch as an e-waste load. The same was done as a two-stage process with bioleaching in a spent medium of *A. aquatilis* as the first stage with three sequential batches, followed by the second stage of the two-step method of *C. violaceum* with three sequential batches for maximum metal recovery. Based on these studies, the following conclusions were drawn.

5.2. Conclusions

- *A. aquatilis* was selected for Cu and Ag bioleaching and *C. violaceum* for Au and Ag bioleaching, based on screening.
- Medium 3 supplemented with glycine and methionine, was selected as the bioleaching medium as it favored the bioleaching of Au, Ag, and Cu.
- The optimum conditions for Cu bioleaching in FBR were 0.175 mm particle size, 5% inoculum, and 2% e-waste load, which resulted in 37.5% recovery of Cu.
- The action of Fe in Cu mobilization through a redoxolysis mechanism was proved as a major mechanism involved in the bioleaching of Cu by *A. aquatilis*, whereas the complexolysis mechanism played a dominant role after the activity of cells diminished.

- The occurrence of both contact and non-contact mode of bioleaching by *A. aquatilis* cells is proposed based on the visualization of the surface morphology of PCBs through FESEM before and after bioleaching.
- *A. aquatilis* was more competent for Cu (37.5%) bioleaching and *C. violaceum* for Au (9.1%) and Ag (23.4%) bioleaching as individual strains in the FBR.
- Co-inoculation studies in the FBR revealed that *A. aquatilis* and *C. violaceum* as co-cultures were less effective in bioleaching Cu, Au, and Ag than the individual strains.
- On comparison of one-step, two-step, and spent medium methods of bioleaching in the FBR, the two-step method using *C. violaceum* was found to be the best method for precious metal bioleaching with 9% Au and 23.7% Ag bioleaching, whereas the spent medium of *A. aquatilis* with which 52.2% Cu could be bioleached was found to be the best for Cu bioleaching.
- The metal recovery through bioleaching could be improved by three sequential batch runs of one-step bioleaching in FBR with *A. aquatilis*, yielding a cumulative recovery of 80% Cu and 28.6% Ag.
- A further improvement in the recovery was achieved through a two-stage sequential batch operation involving a first stage of three sequential batches using the spent medium of *A. aquatilis*, followed by a second stage involving three sequential batches of two-step method with *C. violaceum*, accomplishing a cumulative recovery of 99% Cu, 36.8% Ag, and 21.9% Au.

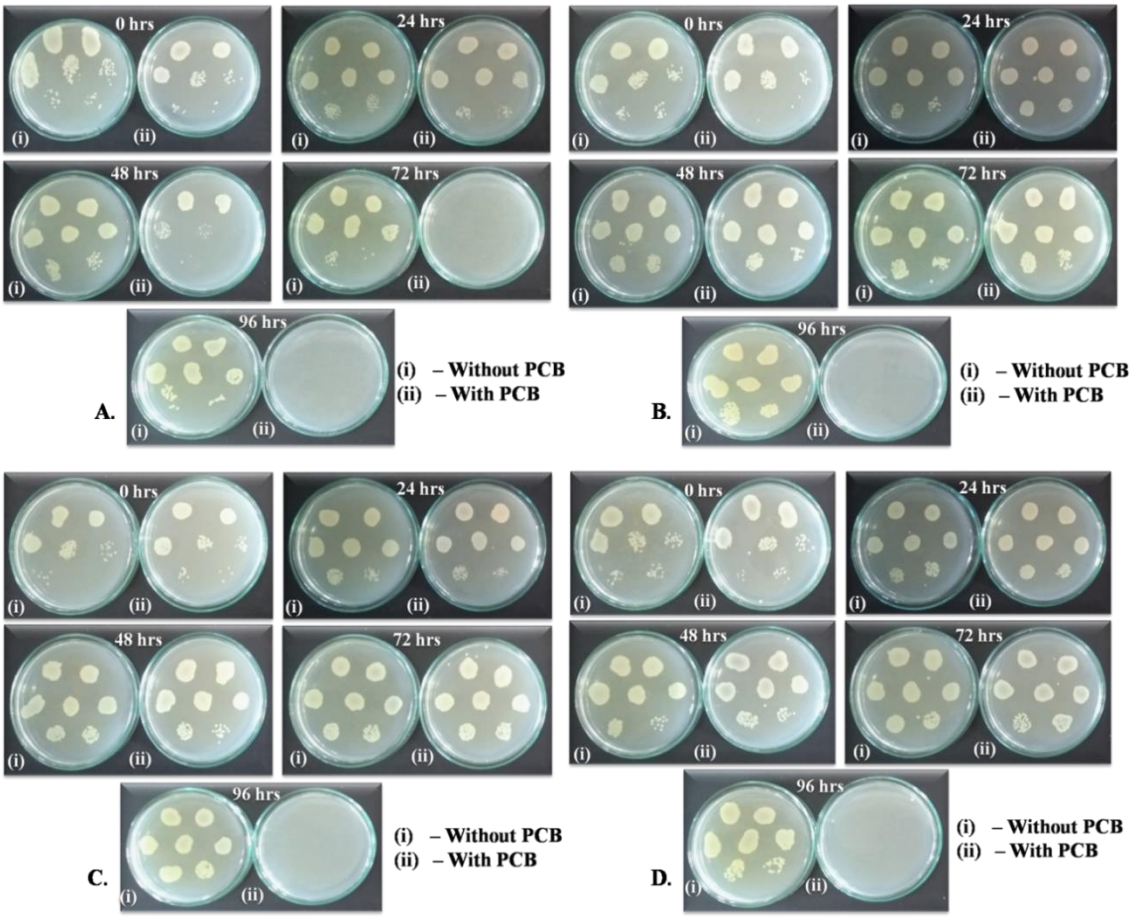
It may be concluded that FBR serves as a suitable bioreactor in efficiently bioleaching precious metals like Au and Ag and base metals like Cu from PCBs using the cells of *A. aquatilis* and *C. violaceum.* The process efficiency can be enhanced by improving the metal recovery through sequential batch runs in multiple stages. The bioleaching process developed in the present study can be conducted under ambient conditions and may easily be adopted in large-scale industries to treat e-waste for metal recovery. The process can potentially serve as a greener and more economical method for metal recovery.

5.3. Future Perspectives

- Study the effect of mixing the cultures (at stationary phase) or their spent medium for simultaneous metal solubilization.
- Identification of the overexpressed and differentially expressed proteins of *A. aquatilis* in the presence of PCBs.
- Scrutinize the mechanisms and mode of Ag mobilization in one-step bioleaching from PCBs by *A. aquatilis*.
- Selective recovery of the metals from the bioleachate in metallic form for reuse.
- Decipher the mechanisms during two-step and spent medium bioleaching methods for different metals.
- Investigation on the simultaneous recovery of different metals from PCBs using more specific bacterial isolates as a consortium.

APPENDIX I

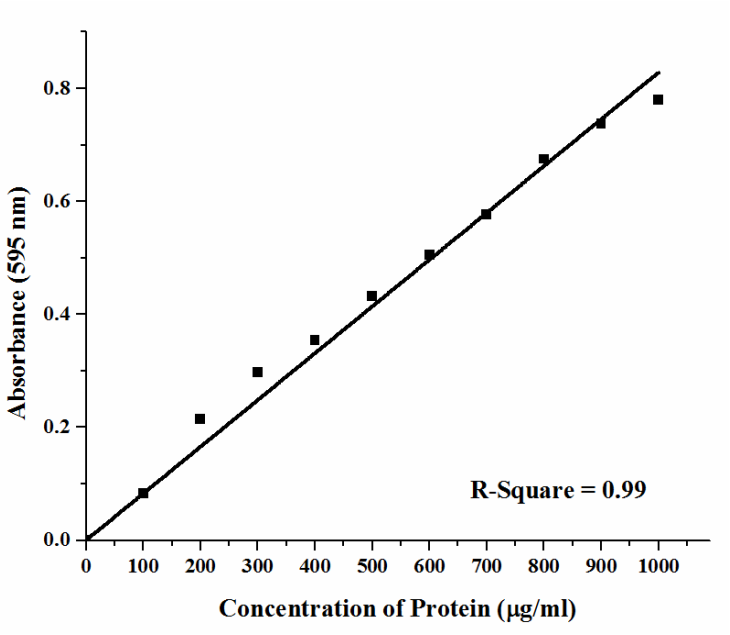
This appendix displays the loss of cell viability of *A. aquatilis* by the Drop Plate Method in the presence of PCBs during the one-step bioleaching period.



Viability Testing of *A. aquatilis* by Drop Plate Method. **(A.)** Cell viability in Medium 1 with (i) and without (ii) PCBs in each dilution (each drop represents each dilution from 10^{-1} to 10^{-7}) every 24 hours. **(B.)** Cell viability in Medium 2 with (i) and without (ii) PCBs in each dilution every 24 hours. **(C.)** Cell viability in Medium 3 with (i) and without (ii) PCBs in each dilution every 24 hours. **(D.)** Cell viability in Medium 4 with (i) and without (ii) PCBs in each dilution every 24 hours.

APPENDIX II

This appendix shows the calibration curve of the standard protein, Bovine Serum Albumin, used for estimating protein concentration in the bioleachate by Bradford's method.



Calibration curve of the standard protein (Bovine serum albumin)

APPENDIX III

Appendix III exhibits the statistical significance of co-inoculation studies through a One-way Analysis of Variance for Au, Ag, and Cu bioleaching in shake flask and FBR.

Table 1a. Bioleaching percentage of Au in FBR and Shake flasks after co-inoculation of *C. violaceum* and *A. aquatilis* (Lowercase alphabets indicate the statistically significant difference/similarity as superscripts at 0.05 significance level)

T (h)	% Bioleaching of Au (Mean±SD at α=0.05)											
	FBR						Shake flasks					
	Control	*A. aquatilis*	*C. violaceum*	1:1	1:3	3:1	Control	*A. aquatilis*	*C. violaceum*	1:1	1:3	3:1
12	0.15 ± 0^{a}	1.34 ± 0.6^{ab}	3.09 ± 0.6^{ac}	2.48 ± 0.7^{a}	0.62 ± 0.2^{ab}	1.6 ± 0.3^{a}	0.15 ± 0^{b}	0.15 ± 0^{b}	1.7 ± 0.09^{a}	0.41 ± 0.05^{b}	0.47 ± 0.1^{b}	1.24 ± 0.1^{c}
24	0.15 ± 0^{bc}	0.36 ± 0.1^{ac}	7.64 ± 0.5^{d}	3.25 ± 1.2^{ae}	2.01 ± 0.5^{ce}	3.36 ± 0.6^{e}	0.15 ± 0^{b}	0.15 ± 0^{b}	5.37 ± 0.1^{a}	0 ± 0^{b}	0.05 ± 0.05^{b}	2.58 ± 0.1^{c}
36	0.15 ± 0^{b}	0.41 ± 0.1^{b}	8.93 ± 1^{a}	2.27 ± 1^{b}	0.77 ± 0.2^{b}	2.32 ± 0.6^{b}	0.21 ± 0.05^{b}	0.15 ± 0^{b}	7.07 ± 0.2^{a}	0 ± 0^{b}	0.83 ± 0.1^{c}	1.7 ± 0.1^{d}
48	0.15 ± 0^{b}	0.46 ± 0.1^{b}	9.18 ± 1.1^{a}	1.08 ± 0.4^{b}	0.57 ± 0.1^{b}	0.98 ± 0.2^{b}	0.31 ± 0^{b}	0.15 ± 0^{b}	7.23 ± 0.2^{a}	0 ± 0^{bc}	0.31 ± 0^{b}	$0.46\pm0b^{d}$
60	0.15 ± 0^{b}	0.52 ± 0.2^{b}	8.93 ± 1.2^{a}	1.03 ± 0.4^{b}	0.62 ± 0.1^{b}	1.13 ± 0.3^{b}	0.31 ± 0^{a}	0.15 ± 0^{ad}	6.5 ± 0.1^{b}	0 ± 0^{cd}	0.41 ± 0.1^{a}	0.88 ± 0.1^{e}
72	0.15 ± 0^{b}	0.52 ± 0.2^{b}	8.98 ± 1.3^{a}	0.88 ± 0.4^{b}	0.52 ± 0.1^{b}	0.93 ± 0.2^{b}	0.46 ± 0^{b}	0.15 ± 0^{c}	5.52 ± 0.1^{a}	0.26 ± 0.05^{bc}	0.46 ± 0.1^{b}	1.19 ± 0.1^{d}
84	0.15 ± 0^{b}	0.57 ± 0.3^{b}	8.41 ± 1.4^{a}	0.83 ± 0.3^{b}	0.52 ± 0.1^{b}	0.77 ± 0.2^{b}	0.57 ± 0.1^{a}	0.15 ± 0^{b}	4.8 ± 0^{c}	0.26 ± 0.05^{b}	0.57 ± 0.1^{a}	1.39 ± 0^{d}
96	0.21 ± 0.05^{b}	0.57 ± 0.3^{b}	8.26 ± 1.7^{a}	0.83 ± 0.3^{b}	0.52 ± 0.1^{b}	0.77 ± 0.2^{b}	0.93 ± 0^{b}	0.15 ± 0^{cd}	4.28 ± 0.1^{a}	0.26 ± 0.05^{c}	0.57 ± 0.1^{ce}	1.55 ± 0^{f}

Table 1b. Bioleaching percentage of Ag in FBR and Shake flasks after co-inoculation of *C. violaceum* and *A. aquatilis* (Lowercase alphabets indicate the statistically significant difference/similarity as superscripts at 0.05 significance level)

T (h)	% Bioleaching of Ag (Mean±SDat α=0.05)											
	FBR						Shake flasks					
	Control	*A. aquatilis*	*C. violaceum*	1:1	1:3	3:1	Control	*A. aquatilis*	*C. violaceum*	1:1	1:3	3:1
12	0.11 ± 0.1^{a}	0.77 ± 0.1^{a}	2.53 ± 1.2^{a}	2.15 ± 1.2^{a}	0 ± 0^{a}	0.72 ± 0.3^{a}	0.44 ± 0.1^{ad}	0.44 ± 0.1^{ad}	0.5 ± 0^{abd}	0.11 ± 0.06^{acd}	0.06 ± 0.05^{cd}	0.33 ± 0^{d}
24	0.11 ± 0.1^{ab}	0.61 ± 0.1^{ab}	9.25 ± 1.4^{ac}	4.24 ± 2.3^{a}	0 ± 0^{ab}	0.77 ± 0.3^{ab}	1.38 ± 0.1^{b}	0.39 ± 0.1^{c}	2.81 ± 0.3^{a}	0.17 ± 0^{c}	0.17 ± 0^{c}	0.11 ± 0.05^{c}
36	0.28 ± 0.2^{ab}	2.64 ± 1.4^{ab}	14.37 ± 2.9^{ac}	10.74 ± 4.3^{a}	2.37 ± 0.4^{ab}	1.65 ± 0.6^{ab}	2.37 ± 0.1^{b}	0.33 ± 0^{c}	6.33 ± 0.5^{a}	0.22 ± 0.06^{c}	0.39 ± 0.05^{c}	0.72 ± 0.05^{c}
48	0.44 ± 0.2^{bc}	5.23 ± 0.9^{ac}	15.58 ± 2.8^{d}	8.64 ± 1.3^{a}	4.02 ± 1.1^{ac}	2.37 ± 0.6^{ac}	4.35 ± 0.2^{a}	0.77 ± 0.1^{b}	7.1 ± 0.6^{c}	4.02 ± 0.24^{a}	3.91 ± 0.43^{a}	4.24 ± 0.2^{a}
60	0.61 ± 0.2^{b}	7.65 ± 1.3^{ab}	18.22 ± 2.9^{ac}	12.44 ± 2.9^{a}	4.9 ± 1.5^{ab}	2.31 ± 0.4^{b}	6.17 ± 0.4^{ac}	4.57 ± 0.1^{bc}	9.75 ± 1.2^{ab}	9.31 ± 1.14^{ab}	10.46 ± 1.87^{a}	15.8 ± 0.9^{d}
72	0.94 ± 0.1^{b}	8.37 ± 1.5^{bc}	19.77 ± 3.7^{a}	12.99 ± 2.5^{ac}	5.4 ± 1.4^{bc}	2.75 ± 0.4^{b}	10.52 ± 1^{b}	9.14 ± 0.1^{b}	12.94 ± 1.2^{bd}	26.48 ± 1.22^{cd}	19.66 ± 2^{cd}	37.22 ± 2.4^{a}
84	1.32 ± 0.3^{b}	9.14 ± 1.6^{b}	23.4 ± 5.9^{a}	13.71 ± 2.3^{ab}	5.34 ± 1.5^{b}	2.15 ± 0.3^{b}	13.77 ± 1.2^{b}	11.89 ± 1^{b}	15.8 ± 2.1^{b}	36.12 ± 2.15^{c}	26.43 ± 2.9^{a}	50.77 ± 1.8^{d}
96	2.42 ± 1.2^{bc}	8.75 ± 1.8^{ac}	23.02 ± 8.4^{a}	12.94 ± 1.9^{abc}	5.12 ± 1.3^{bc}	1.98 ± 0.5^{bc}	24.56 ± 5.8^{a}	16.41 ± 2^{ab}	25.66 ± 2.7^{a}	37.66 ± 2.15^{ac}	33.42 ± 3.37^{ac}	70.26 ± 3.7^{d}

Table 1c. Bioleaching percentage of Cu in FBR and Shake flasks after co-inoculation of *C. violaceum* and *A. aquatilis* (Lowercase alphabets indicate the statistically significant difference/similarity as superscripts at 0.05 significance level)

T (h)	% Bioleaching of Cu (Mean±SDat α=0.05)											
	FBR						Shake flasks					
	Control	*A. aquatilis*	*C. violaceum*	1:1	1:3	3:1	Control	*A. aquatilis*	*C. violaceum*	1:1	1:3	3:1
12	1.46±0.1^{a}	4.14±0.6^{a}	3.15±1.1^{a}	2.7±0.2^{a}	4.29±0.4^{a}	2.44±0.6^{a}	2.42±0.2^{b}	1.05±0.1^{b}	2.88±0.02^{b}	1.96±0.4^{b}	2.18±0.6^{b}	8.25±1.3^{a}
24	2.07±0.2^{a}	9.68±4.7^{a}	5.23±1^{a}	5.64±0.7^{a}	8.23±2^{a}	4.6±1^{a}	4.44±0.5^{a}	0.95±0.1^{b}	6.77±0.3^{c}	1.73±0.02^{bd}	3.16±0.7^{ad}	6.14±0.4^{ac}
36	2.39±0.3^{b}	19.72±4.9^{a}	8.38±1.5^{ab}	18.12±3.2^{a}	19.72±0.8^{a}	12.7±3.4^{ab}	5.77±0.5^{ac}	1.03±0.1^{bc}	13.22±1.1^{ad}	3.02±0.5^{ce}	11.62±3.5^{a}	10.32±0.8^{ae}
48	3.25±0.3^{b}	29.39±1.7^{ac}	9.74±1.7^{be}	23.33±4.2^{a}	21±0.4^{a}	17.61±3ad^{e}	7.88±0.7^{b}	0.87±0.1^{c}	19.31±0.6^{d}	40.65±3^{a}	45.39±1.6^{a}	40.49±0.5^{a}
60	3.62±0.4^{b}	34.01±4^{a}	9.93±1.5^{bc}	25.55±3.7^{ac}	22.39±0.9^{ac}	19.58±2.3^{c}	9.8±1.1^{b}	41.66±1.1^{a}	23.84±0.2^{c}	43.44±1.3^{a}	43.44±0.9^{a}	41.1±1.6^{a}
72	4.56±0.3^{b}	36.73±2.8^{ac}	10.63±1.4^{be}	26.64±4.5^{a}	21.52±0.8^{ade}	20.96±2.4^{ade}	12.03±1.6^{b}	46.94±1.1^{c}	26.78±1.7^{a}	42.71±1.4^{c}	40.5±1.5^{c}	42.15±0.8^{c}
84	5.56±0.2^{b}	35.83±0.3^{ac}	10.61±1.4^{b}	28.27±2.9^{a}	22.75±2.3^{ad}	21.76±2.2^{ad}	17.27±0.5^{b}	47.99±2^{a}	31.98±0.4^{c}	41.8±1.2^{d}	42.14±0.2^{d}	40.97±0.6^{d}
96	6.37±0.6^{b}	37.54±2^{ac}	10.8±1.2^{b}	30.38±3.4^{a}	25.17±2.5^{ad}	22.94±1.5^{ad}	23.72±0.9^{b}	43.41±1.4^{a}	34.09±0.9^{c}	42.12±1.9^{a}	41.56±0.8^{a}	42.07±1.4^{a}

APPENDIX IV

Appendix IV exhibits the statistical mean difference between the bioleaching methods through a One-way Analysis of Variance for Au, Ag, and Cu bioleaching.

Table 2a. Au Recovery in FBR and Shake flasks employing different bioleaching methods using *A. aquatilis* and *C. violaceum* (Lowercase alphabets indicate the statistically significant difference/similarity as superscripts at 0.05 significance level).

T (h)	% Bioleaching of Au (Mean±SD at α=0.05) using *A. aquatilis*							
	FBR				Shake Flask			
	Control	One-step method	Two-step method	Spent-medium method	Control	One-step method	Two-step method	Spent-medium method
12	0.15 ± 0^{a}	1.34 ± 0.6^{a}	1.39 ± 0.1^{a}	1.19 ± 0.4^{a}	0.15 ± 0^{a}	0.15 ± 0^{a}	0.21 ± 0.05^{a}	0.46 ± 0^{b}
24	0.15 ± 0^{ac}	0.36 ± 0.1^{a}	0.67 ± 0.05^{ab}	0.62 ± 0.1^{ab}	0.15 ± 0^{a}	0.15 ± 0^{a}	0.21 ± 0.05^{a}	0.21 ± 0.05^{a}
36	0.15 ± 0^{ac}	0.41 ± 0.05^{a}	0.52 ± 0.05^{ab}	0.46 ± 0.09^{ac}	0.21 ± 0.05^{a}	0.15 ± 0^{a}	0.15 ± 0^{a}	0.15 ± 0^{a}
48	0.15 ± 0^{ac}	0.46 ± 0.09^{ab}	0.46 ± 0^{ab}	0.36 ± 0.05^{a}	0.31 ± 0^{ac}	0.15 ± 0^{a}	0.21 ± 0.05^{a}	0.1 ± 0.05^{ab}
60	0.15 ± 0^{a}	0.52 ± 0.2^{a}	0.41 ± 0.05^{a}	0.36 ± 0.05^{a}	0.31 ± 0^{a}	0.15 ± 0^{a}	0.15 ± 0^{a}	0.15 ± 0^{a}
72	0.15 ± 0^{a}	0.52 ± 0.2^{a}	0.36 ± 0.05^{a}	0.31 ± 0^{a}	0.46 ± 0^{a}	0.15 ± 0^{a}	0.15 ± 0^{a}	0.15 ± 0^{a}
84	0.15 ± 0^{a}	0.57 ± 0.2^{a}	0.36 ± 0.05^{a}	0.31 ± 0^{a}	0.57 ± 0.1^{a}	0.15 ± 0^{b}	0.1 ± 0.05^{b}	0.15 ± 0^{b}
96	0.21 ± 0.05^{a}	0.57 ± 0.2^{a}	0.36 ± 0.05^{a}	0.31 ± 0^{a}	0.93 ± 0^{b}	0.15 ± 0^{a}	0.21 ± 0.05^{a}	0.15 ± 0^{a}
% Bioleaching of Au (Mean±SD at α=0.05) using *C. violaceum*								
12	0.15 ± 0^{ac}	3.10 ± 0.6^{a}	5.78 ± 0.9^{ab}	1.55 ± 0.8^{ac}	0.15±0c	1.7 ± 0.09^{b}	7.43 ± 0.5^{a}	2.32 ± 0.09^{b}
24	0.15 ± 0^{a}	7.64 ± 0.5^{b}	7.79 ± 0.4^{b}	1.34 ± 0.9^{a}	0.15 ± 0^{d}	5.37 ± 0.1^{b}	9.08 ± 0.4^{a}	2.48 ± 0.09^{c}
36	0.15 ± 0^{b}	8.93 ± 1^{a}	10.48 ± 1.7^{a}	1.14 ± 0.8^{b}	0.21 ± 0.05^{d}	7.07 ± 0.1^{b}	8.36 ± 0.3^{a}	2.53 ± 0.05^{c}
48	0.15 ± 0^{b}	9.19 ± 1^{a}	11.15 ± 1.7^{a}	1.6 ± 1.3^{b}	0.31 ± 0^{c}	7.23 ± 0.1^{a}	7.38 ± 0.1^{a}	2.89 ± 0.1^{b}
60	0.15 ± 0^{ad}	8.93 ± 1.2^{ac}	10.89 ± 1.5^{bc}	2.79 ± 2.2^{a}	0.31 ± 0^{c}	6.5 ± 0.09^{a}	6.76 ± 0.1^{a}	3.05 ± 0.05^{b}
72	0.15 ± 0^{b}	8.98 ± 1.3^{a}	11.25 ± 1.4^{a}	0.31 ± 0.2^{b}	0.46 ± 0^{d}	5.52 ± 0.1^{b}	6.14 ± 0.1^{a}	3.41 ± 0.09^{c}
84	0.15 ± 0^{b}	8.41 ± 1.4^{a}	10.53 ± 1.3^{a}	1.45 ± 0.6^{b}	0.57 ± 0.1^{a}	4.8 ± 0^{b}	5.68 ± 0.05^{c}	3.36 ± 0.05^{d}
96	0.21 ± 0.05^{b}	8.26 ± 1.7^{a}	9.60 ± 0.8^{a}	1.86 ± 0.8^{b}	0.93 ± 0^{d}	4.28 ± 0.1^{b}	5.47 ± 0.1^{a}	3.72 ± 0.09^{c}

Table 2b. Ag Recovery in FBR and Shake flasks employing different bioleaching methods using *A. aquatilis* and *C. violaceum* (Lowercase alphabets indicate the statistically significant difference/similarity as superscripts at 0.05 significance level).

T (h)	% Bioleaching of Ag (Mean±SD at α=0.05) using *A. aquatilis*							
	FBR				Shake Flask			
	Control	One-step method	Two-step method	Spent-medium method	Control	One-step method	Two-step method	Spent-medium method
12	0.11 ± 0.1^{b}	0.77 ± 0.1^{bc}	1.27 ± 0.1^{ac}	1.87 ± 0.2^{a}	0.44 ± 0.1^{a}	0.44 ± 0.1^{a}	0.55 ± 0.06^{a}	0.33 ± 0^{a}
24	0.11 ± 0.1^{ac}	0.61 ± 0.1^{a}	1.82 ± 0.4^{ab}	0.99 ± 0.3^{a}	1.38 ± 0.06^{a}	0.39 ± 0.06^{a}	3.8 ± 0.1^{b}	1.49 ± 0.9^{a}
36	0.28 ± 0.2^{a}	2.64 ± 1.4^{a}	2.48 ± 0.2^{a}	2.09 ± 0.6^{a}	2.37 ± 0.06^{a}	0.33 ± 0^{b}	2.81 ± 0.3^{a}	0.22 ± 0.2^{b}
48	0.44 ± 0.2^{c}	5.23 ± 0.8^{a}	2.86 ± 0.1^{ac}	1.65 ± 0.7^{bc}	4.35 ± 0.2^{c}	0.77 ± 0.1^{b}	8.04 ± 1.1^{a}	1.71 ± 0.9^{bc}
60	0.61 ± 0.2^{b}	7.65 ± 1.3^{a}	4.07 ± 0.3^{bc}	4.24 ± 0.7^{ac}	6.17 ± 0.4^{b}	4.57 ± 0.1^{b}	12.33 ± 1.8^{a}	3.8 ± 1.1^{b}
72	0.94 ± 0.06^{bc}	8.37 ± 1.5^{a}	4.68 ± 0.2^{ac}	5.4 ± 0.8^{a}	10.52 ± 1^{ab}	9.14 ± 0.1^{ab}	16.08 ± 2.2^{b}	5.34 ± 2.7^{a}
84	1.32 ± 0.2^{bc}	9.14 ± 1.5^{a}	5.07 ± 0.4^{ac}	7.43 ± 2^{a}	13.77 ± 1.2^{abd}	11.89 ± 0.9^{b}	18.78 ± 1.9^{a}	6.5 ± 1.4^{bc}
96	2.42 ± 1.1^{a}	8.75 ± 1.8^{a}	5.34 ± 0.3^{a}	7.98 ± 2.1^{a}	24.56 ± 5.8^{a}	16.41 ± 1.9^{ac}	22.02 ± 1.8^{ac}	7.65 ± 1.9^{bc}
% Bioleaching of Ag (Mean±SD at α=0.05) using *C. violaceum*								
12	0.11 ± 0.1^{ac}	2.53 ± 1.2^{a}	2.48 ± 0.8^{a}	4.51 ± 0.5^{ab}	0.44 ± 0.1^{b}	0.5 ± 0^{b}	3.96 ± 0.1^{a}	4.18 ± 0.2^{a}
24	0.11 ± 0.1^{b}	9.25 ± 1.3^{a}	7.21 ± 0.3^{a}	7.27 ± 2^{a}	1.38 ± 0.06^{b}	2.81 ± 0.2^{b}	7.6 ± 0.4^{c}	14.26 ± 0.7^{a}
36	0.28 ± 0.2^{bc}	14.37 ± 2.9^{a}	16.3 ± 3.6^{a}	10.3 ± 3^{ac}	2.37 ± 0.06^{b}	6.33 ± 0.4^{b}	12.77 ± 0.3^{c}	25.05 ± 1.7^{a}
48	0.44 ± 0.2^{bc}	15.58 ± 2.7^{a}	19.05 ± 4.4^{a}	9.25 ± 3.4^{ac}	4.35 ± 0.2^{b}	7.1 ± 0.6^{b}	19.77 ± 0.2^{c}	38.65 ± 2.8^{a}
60	0.61 ± 0.2^{bc}	18.22 ± 2.8^{a}	19.22 ± 3.6^{a}	10.68 ± 2.6^{ac}	6.17 ± 0.4^{b}	9.75 ± 1.1^{b}	25.22 ± 0.7^{c}	48.34 ± 2.5^{a}
72	0.94 ± 0.06^{bc}	19.77 ± 3.7^{a}	20.65 ± 4.6^{a}	8.7 ± 2.7^{ac}	10.52 ± 1^{b}	12.94 ± 1.1^{b}	34.69 ± 0.7^{c}	62.88 ± 3.6^{a}
84	1.32 ± 0.2^{bc}	23.4 ± 5.8^{a}	21.91 ± 5.3^{a}	14.04 ± 4^{ac}	13.77 ± 1.2^{b}	15.8 ± 2.1^{b}	41.02 ± 0.5^{c}	71.85 ± 2.5^{a}
96	2.42 ± 1.1^{a}	23.02 ± 8.3^{a}	23.68 ± 6.2^{a}	13.6 ± 1.7^{a}	24.56 ± 5.8^{a}	25.66 ± 2.6^{a}	52.47 ± 1.6^{b}	91.84 ± 2.9^{c}

Table 2c. Cu Recovery in FBR and Shake flasks employing different bioleaching methods using *A. aquatilis* and *C. violaceum* (Lowercase alphabets indicate the statistically significant difference/similarity as superscripts at 0.05 significance level).

T (h)	% Bioleaching of Cu (Mean±SD at α=0.05) using *A. aquatilis*							
	FBR				Shake Flask			
	Control	One-step method	Two-step method	Spent-medium method	Control	One-step method	Two-step method	Spent-medium method
12	1.46±0.1^{b}	4.14±0.5^{b}	12.59±1.3^{a}	12.2±1.2^{a}	2.42±0.1^{a}	1.05±0.07^{a}	6.69±3.3^{a}	30.23±2.4^{b}
24	2.07±0.1^{c}	9.68±4.7^{ac}	22.26±3.8^{a}	23.68±2.9^{a}	4.44±0.4^{b}	0.95±0.07^{b}	37.12±1.7^{a}	53.52±1.3^{c}
36	2.39±0.2^{d}	19.72±4.9^{ab}	26.66±1.9^{a}	33.57±1.9^{ac}	5.77±0.5^{b}	1.03±0.09^{b}	43.07±2.2^{a}	53.59±4^{a}
48	3.25±0.3^{c}	29.39±1.7^{a}	28.56±2.2^{a}	40.24±2.1^{b}	7.88±0.7^{d}	0.87±0.09^{b}	39.8±1.5^{c}	48.22±1.8^{a}
60	3.62±0.3^{d}	34.01±4^{a}	28.86±3.2^{ab}	44.04±2.9^{ac}	9.8±1^{c}	41.66±1^{a}	38.57±1.5^{a}	48.03±0.3^{b}
72	4.56±0.2^{d}	36.73±2.8^{ac}	30.55±2.3^{bc}	49.05±5.2^{a}	12.03±1.6^{c}	46.94±1^{a}	35.62±1.5^{b}	48.17±1.9^{a}
84	5.56±0.2^{c}	35.83±0.3^{b}	30.35±2.5^{b}	49.74±5.4^{a}	17.27±0.5^{c}	47.99±1.9^{a}	34.9±1.6^{b}	46.48±1.5^{a}
96	6.37±0.6^{c}	37.54±1.9^{ac}	31.46±1.3^{bc}	52.17±6^{a}	23.72±0.8^{c}	43.42±1.4^{a}	35.21±1^{b}	45.19±2^{a}
% Bioleaching of Cu (Mean±SD at α=0.05) using *C. violaceum*								
12	1.46±0.1^{a}	3.15±1.1^{a}	3.71±1.2^{a}	3.78±0.1^{a}	2.42±0.1^{ab}	2.88±0.02^{ab}	5.86±0.5^{ac}	3.78±1^{a}
24	2.07±0.1^{ac}	5.23±0.9^{a}	6.84±1.4^{ab}	4.86±0.4^{a}	4.44±0.4^{b}	6.77±0.2^{b}	10.72±0.9^{a}	6.62±0.5^{b}
36	2.39±0.2^{c}	8.38±1.5^{a}	11.79±0.3^{ab}	5.81±1^{ac}	5.77±0.5^{a}	13.22±1^{b}	13.89±0.7^{b}	8.88±1^{a}
48	3.25±0.3 ^{ac}	9.74±1.6^{a}	14.31±0.5^{ab}	9.23±2.7^{a}	7.88±0.7^{ad}	19.31±0.6^{bc}	17.44±1.6^{ac}	12.5±2^{a}
60	3.62±0.3 ^{ac}	9.93±1.5^{a}	14.87±0.1^{ab}	13.12±4.7^{a}	9.8±1^{ad}	23.84±0.2^{bc}	20.31±0.3^{ac}	15.04±2.2^{a}
72	4.56±0.2 ^{ac}	10.63±1.3^{a}	15.5±1.2^{ab}	13.68±4.1^{a}	12.03±1.6^{b}	26.78±1.6^{a}	23.91±0.5^{a}	20.04±2.5^{a}
84	5.56±0.2^{a}	10.61±1.4^{a}	16.09±0.3^{a}	16.13±6^{a}	17.27±0.5^{ad}	31.98±0.3^{bc}	26.44±0.4^{ac}	22.22±2.9^{a}
96	6.37±0.6^{a}	10.8±1.1^{a}	15.87±0.7^{a}	18.58±6.5^{a}	23.72±0.8^{a}	34.09±0.9^{bc}	30.57±0.2^{ac}	25.85±2.9^{a}

REFERENCES

Abdelbasir, S. M., Hassan, S. S. M., Kamel, A. H., and El-Nasr, R. S. (2018). "Status of electronic waste recycling techniques: a review." *Environ. Sci. Pollut. Res.*, 25(17), 16533–16547.

Abdulla, H. (2009). "Bioweathering and biotransformation of granitic rock minerals by actinomycetes." *Microb. Ecol.*, 58(4), 753–761.

Aminian-Dehkordi, J., Mousavi, S. M., Marashi, S. A., Jafari, A., and Mijakovic, I. (2020). "A Systems-Based Approach for Cyanide Overproduction by Bacillus megaterium for Gold Bioleaching Enhancement." *Front. Bioeng. Biotechnol.*, 8(June), 1–18.

Annamalai, M., and Gurumurthy, K. (2019). "Enhanced bioleaching of copper from circuit boards of computer waste by Acidithiobacillus ferrooxidans." *Environ. Chem. Lett.*, 17(4), 1873–1879.

Arab, B., Hassanpour, F., Arshadi, M., Yaghmaei, S., and Hamedi, J. (2020). "Optimized bioleaching of copper by indigenous cyanogenic bacteria isolated from the landfill of e-waste." *J. Environ. Manage.*, 261(December 2019), 110124.

Argumedo-Delira, R., Gómez-Martínez, M. J., and Soto, B. J. (2019). "Gold bioleaching from printed circuit boards of mobile phones by aspergillus niger in a culture without agitation and with glucose as a carbon source." *Metals (Basel).*, 9(5).

Arshadi, M., and Mousavi, S. M. (2014). "Simultaneous recovery of Ni and Cu from computer-printed circuit boards using bioleaching: Statistical evaluation and optimization." *Bioresour. Technol.*, 174, 233–242.

Arshadi, M., Mousavi, S. M., and Rasoulnia, P. (2016). "Enhancement of simultaneous gold and copper recovery from discarded mobile phone PCBs using Bacillus megaterium: RSM based optimization of effective factors and evaluation of their interactions." *Waste Manag.*, 57, 158–167.

Aston, J. E., Thompson, V. S., Fujita, Y., and Reed, D. W. (2022). “Metabolic flux modeling of Gluconobacter oxydans enables improved production of bioleaching organic acids.” *Process Biochem.*, 122(P2), 350–356.

Awasthi, A. K., Zeng, X., and Li, J. (2016). “Integrated bioleaching of copper metal from waste printed circuit board—a comprehensive review of approaches and challenges.” *Environ. Sci. Pollut. Res.*, 23(21), 21141–21156.

Bałdyga, J. (2016). “Mixing and fluid dynamics effects in particle precipitation processes.” *KONA Powder Part. J.*

Banerjee, A., and Ghoshal, A. K. (2016). “Biodegradation of real petroleum wastewater by immobilized hyper phenol-tolerant strains of Bacillus cereus in a fluidized bed bioreactor.” *3 Biotech*, 6(2), 1–4.

Baniasadi, M., Vakilchap, F., Bahaloo-Horeh, N., Mousavi, S. M., and Farnaud, S. (2019). “Advances in bioleaching as a sustainable method for metal recovery from e-waste: A review.” *J. Ind. Eng. Chem.*, 76, 75–90.

Beech, I. B., and Sunner, J. (2004). “Biocorrosion: Towards understanding interactions between biofilms and metals.” *Curr. Opin. Biotechnol.*, 15(3), 181–186.

Benzal, E., Cano, A., Solé, M., Lao-Luque, C., Gamisans, X., and Dorado, A. D. (2020a). “Copper Recovery from PCBs by Acidithiobacillus ferrooxidans: Toxicity of Bioleached Metals on Biological Activity.” *Waste and Biomass Valorization*, 11(10), 5483–5492.

Benzal, E., Solé, M., Lao, C., Gamisans, X., and Dorado, A. D. (2020b). “Elemental Copper Recovery from e-Wastes Mediated with a Two-Step Bioleaching Process.” *Waste and Biomass Valorization*, 11(10), 5457–5465.

Birloaga, I., Michelis, I. De, Ferella, F., Buzatu, M., and Vegliò, F. (2013). “Study on the influence of various factors in the hydrometallurgical processing of waste printed circuit boards for copper and gold recovery.” *Waste Manag.*, 33(4), 935–41.

Bobadilla Fazzini, R. A., Levican, G., and Parada, P. (2011). “Acidithiobacillus

thiooxidans secretome containing a newly described lipoprotein Licanantase enhances chalcopyrite bioleaching rate." *Appl. Microbiol. Biotechnol.*, 89(3), 771–780.

Bosecker, K. (1997). "Bioleaching: Metal solubilization by microorganisms." *FEMS Microbiol. Rev.*, 20(3–4), 591–604.

Bosse, M., Heuwieser, A., Heinzel, A., Nancucheo, I., Melo Barbosa Dall'Agnol, H., Lukas, A., Tzotzos, G., and Mayer, B. (2015). "Interaction networks for identifying coupled molecular processes in microbial communities." *BioData Min.*, 8(1), 1–17.

Brandl, H. (2002). "Metal-microbe-interactions and thier biotechnological applications for mineral waste treatment." *Recent Res. Dev. Mircobiology*, 6(January 2002), 571–584.

Calgaro, C. O., Schlemmer, D. F., Silva, M. D. C. R. Da, Maziero, E. V., Tanabe, E. H., and Bertuol, D. A. (2015). "Fast copper extraction from printed circuit boards using supercritical carbon dioxide." *Waste Manag.*, 45, 289–297.

Cancho, L., Blázquez, M. L., Ballester, A., González, F., and Muñoz, J. A. (2007). "Bioleaching of a chalcopyrite concentrate with moderate thermophilic microorganisms in a continuous reactor system." *Hydrometallurgy*, 87(3–4), 100–111.

Caravanos, J., Clarke, E. E., Osei, C. S., and Amoyaw-Osei, Y. (2013). "Exploratory Health Assessment of Chemical Exposures at E-Waste Recycling and Scrapyard Facility in Ghana." *J. Heal. Pollut.*, 3(4), 11–22.

Cárdenas, J. P., Quatrini, R., and Holmes, D. S. (2016). "Genomic and metagenomic challenges and opportunities for bioleaching: a mini-review." *Res. Microbiol.*, 167(7), 529–538.

Castro, I. M., Fietto, J. L. R., Vieira, R. X., Trópia, M. J. M., Campos, L. M. M., Paniago, E. B., and Brandão, R. L. (2000). "Bioleaching of zinc and nickel from silicates using Aspergillus niger cultures." *Hydrometallurgy*, 57(1), 39–49.

Cesaro, A., Belgiorno, V., Vaccari, M., Jandric, A., Chung, T. D., Dias, M. I., Hursthouse, A., and Salhofer, S. (2018). "A device-specific prioritization strategy based

on the potential for harm to human health in informal WEEE recycling." *Environ. Sci. Pollut. Res.*, 25(1), 683–692.

Chen, S. Y., and Cheng, Y. K. (2019). "Effects of sulfur dosage and inoculum size on pilot-scale thermophilic bioleaching of heavy metals from sewage sludge." *Chemosphere*, 234, 346–355.

Chen, S. Y., Wu, J. Q., and Sung, S. (2022). "Effects of sulfur dosage on continuous bioleaching of heavy metals from contaminated sediment." *J. Hazard. Mater.*, 424(PA), 127257.

Chen, S., Yang, Y., Liu, C., Dong, F., and Liu, B. (2015). "Column bioleaching copper and its kinetics of waste printed circuit boards (WPCBs) by Acidithiobacillus ferrooxidans." *Chemosphere*, 141, 162–168.

Chen, Y., Chen, M., Li, Y., Wang, B., Chen, S., and Xu, Z. (2018). "Impact of technological innovation and regulation development on e-waste toxicity: A case study of waste mobile phones." *Sci. Rep.*, 8(1), 1–9.

Chi, T. D., Lee, J. C., Pandey, B. D., Yoo, K., and Jeong, J. (2011). "Bioleaching of gold and copper from waste mobile phone PCBs by using a cyanogenic bacterium." *Miner. Eng.*, 24(11), 1219–1222.

Chien, C. C., Lin, B. C., and Wu, C. H. (2013). "Biofilm formation and heavy metal resistance by an environmental Pseudomonas sp." *Biochem. Eng. J.*, 78, 132–137.

Darby, R. A., Cartwright, S. P., Dilworth, M. V, and Bill, R. M. (2012). "Which yeast species shall I choose? *Saccharomyces cerevisiae* versus *Pichia pastoris*." *Methods Mol. Biol.*, 866, 11–23.

Das, S., Natarajan, G., and Ting, Y.-P. (2017). "Bio-extraction of precious metals from urban solid waste." 020004, 020004.

Daum, K., Stoler, J., and Grant, R. J. (2017). "Toward a more sustainable trajectory for e-waste policy: A review of a decade of e-waste research in Accra, Ghana." *Int. J. Environ.*

Res. Public Health.

Dave, S. R., Sodha, A. B., and Tipre, D. R. (2018). "Microbial technology for metal recovery from e-waste printed circuit boards." *J. Bacteriol. Mycol. Open Access*, 6(4), 241–247.

Deshpande, L. M., and Chopade, B. A. (1994). "Plasmid mediated silver resistance in Acinetobacter baumannii." *Biometals*, 7(1), 49–56.

Ehrlich, H. L. (2004). "Beginnings of rational bioleaching and highlights in the development of biohydrometallurgy : A brief history." 4(2), 102–112.

Faraji, F., Golmohammadzadeh, R., and Rashchi, H. S. F. (2022). "An investigation of bioleaching and valorization of hazardous zinc plant purification residue using Aspergillus niger." *Int. J. Environ. Sci. Technol.*, (0123456789).

Faramarzi, M. A., Stagars, M., Pensini, E., Krebs, W., and Brandl, H. (2004). "Metal solubilization from metal-containing solid materials by cyanogenic Chromobacterium violaceum." *J. Biotechnol.*, 113(1–3), 321–326.

Foucher, S., Battaglia-Brunet, F., D'Hugues, P., Clarens, M., Godon, J. J., and Morin, D. (2003). "Evolution of the bacterial population during the batch bioleaching of a cobaltiferous pyrite in a suspended-solids bubble column and comparison with a mechanically agitated reactor." *Hydrometallurgy*, 71(1–2), 5–12.

Fu, K., Wang, B., Chen, H., Chen, M., and Chen, S. (2016). "Bioleaching of Al from Coarse-Grained Waste Printed Circuit Boards in a Stirred Tank Reactor." *Procedia Environ. Sci.*, 31, 897–902.

Gadd, G. M. (1999). *Fungal Production of Citric and Oxalic Acid: Importance in Metal Speciation, Physiology and Biogeochemical Processes*.

Gadd, G. M. (2000). "Bioremedial potential of microbial mechanisms." 271–279.

Gorji, M., Hosseini, M. R., and Ahmadi, A. (2020). "Comparison and optimization of the bio-cyanidation potentials of B. megaterium and P. aeruginosa for extracting gold from

an oxidized copper-gold ore in the presence of residual glycine." *Hydrometallurgy*, 191(March 2019), 105218.

Habashi, F. (2005). "A short history of hydrometallurgy." *Hydrometallurgy*, 79(1–2), 15–22.

He, J., and Kappler, A. (2017). "Recovery of precious metals from waste streams." *Microb. Biotechnol.*, 10(5), 1194–1198.

Heacock, M., Kelly, C. B., and Asante, K. A. (2015). "E-waste and harm to vulnerable populations: a growing global problem." *Env. Heal. Perspect 205 doi 101289ehp1509699*, 550(5), 550–555.

Herigstad, B., Hamilton, M., and Heersink, J. (2001). "How to optimize the drop plate method for enumerating bacteria." *J. Microbiol. Methods*, 44(2), 121–129.

Heydarian, A., Mousavi, S. M., Vakilchap, F., and Baniasadi, M. (2018). "Application of a mixed culture of adapted acidophilic bacteria in two-step bioleaching of spent lithium-ion laptop batteries." *J. Power Sources*, 378(September 2017), 19–30.

Hubau, A., Minier, M., Chagnes, A., Joulian, C., Perez, C., and Guezennec, A. G. (2018). "Continuous production of a biogenic ferric iron lixiviant for the bioleaching of printed circuit boards (PCBs)." *Hydrometallurgy*, 180(July), 180–191.

Ikhlayel, M. (2018). "An integrated approach to establish e-waste management systems for developing countries." *J. Clean. Prod.*, 170, 119–130.

Ilyas, S., Anwar, M. A., Niazi, S. B., and Afzal Ghauri, M. (2007). "Bioleaching of metals from electronic scrap by moderately thermophilic acidophilic bacteria." *Hydrometallurgy*, 88(1–4), 180–188.

Ilyas, S., and Lee, J. C. (2014). "Bioleaching of metals from electronic scrap in a stirred tank reactor." *Hydrometallurgy*, 149, 50–62.

Ilyas, S., Ruan, C., Bhatti, H. N., Ghauri, M. A., and Anwar, M. A. (2010). "Column bioleaching of metals from electronic scrap." *Hydrometallurgy*, 101(3–4), 135–140.

Işıldar, A., Rene, E. R., Hullebusch, E. D. van, and Lens, P. N. L. (2018). “Electronic waste as a secondary source of critical metals: Management and recovery technologies.” *Resour. Conserv. Recycl.*, 135(July), 296–312.

Işıldar, A., Vossenberg, J. van de, Rene, E. R., Hullebusch, E. D. van, and Lens, P. N. L. (2016). “Two-step bioleaching of copper and gold from discarded printed circuit boards (PCB).” *Waste Manag.*, 57, 149–157.

Islam, A., Swaraz, A. M., Teo, S. H., Taufiq-Yap, Y. H., Vo, D. V. N., Ibrahim, M. L., Abdulkreem-Alsultan, G., Rashid, U., and Awual, M. R. (2021). “Advances in physiochemical and biotechnological approaches for sustainable metal recovery from e-waste: A critical review.” *J. Clean. Prod.*, 323(September), 129015.

Jadhav, U., and Hocheng, H. (2013). “Extraction of silver from spent silver oxide-zinc button cells by using Acidithiobacillus ferrooxidans culture supernatant.” *J. Clean. Prod.*, 44, 39–44.

Jagannath, A., Vidya Shetty, K., and Saidutta, M. B. (2017). “Bioleaching of copper from electronic waste using Acinetobacter sp. Cr B2 in a pulsed plate column operated in batch and sequential batch mode.” *J. Environ. Chem. Eng.*, 5(2), 1599–1607.

Jain, N., and Sharma, D. K. (2004). “Biohydrometallurgy for nonsulfidic minerals - A review.” *Geomicrobiol. J.*, 21(3), 135–144.

James, D., and Mathew, S. K. (2017). “Compatibility Studies on Different Endophytic Microbes of Tomato Antagonistic To Bacterial Wilt Pathogen.” *IJABR*, 7(1), 190–194.

Jujun, R., Jie, Z., Jian, H., and Zhang, J. (2015). “A Novel Designed Bioreactor for Recovering Precious Metals from Waste Printed Circuit Boards.” *Sci. Rep.*, 5, 1–10.

Kudpeng, K., Bohu, T., Morris, C., Thiravetyan, P., and Kaksonen, A. H. (2020). “Bioleaching of gold from sulfidic gold ore concentrate and electronic waste by roseovarius tolerans and roseovarius mucosus.” *Microorganisms*, 8(11), 1–16.

Kulal, D., and Shetty Kodialbail, V. (2021). “Visible light mediated photocatalytic dye

degradation using Ag2O/AgO-TiO2nanocomposite synthesized by extracellular bacterial mediated synthesis - An eco-friendly approach for pollution abatement." *J. Environ. Chem. Eng.*, 9(4), 105389.

Kumar, A., Saini, H. S., and Kumar, S. (2018). "Bioleaching of Gold and Silver from Waste Printed Circuit Boards by Pseudomonas balearica SAE1 Isolated from an e-Waste Recycling Facility." *Curr. Microbiol.*, 75(2), 194–201.

Kumar, A., Saini, H. S., Şengör, S., Sani, R. K., and Kumar, S. (2021). "Bioleaching of metals from waste printed circuit boards using bacterial isolates native to abandoned gold mine." *BioMetals*, 34(5), 1043–1058.

Li, J., Liang, C., and Ma, C. (2015). "Bioleaching of gold from waste printed circuit boards by Chromobacterium violaceum." *J. Mater. Cycles Waste Manag.*, 17(3), 529–539.

Li, J., Wen, J., Guo, Y., An, N., Liang, C., and Ge, Z. (2020). "Bioleaching of gold from waste printed circuit boards by alkali-tolerant Pseudomonas fluorescens." *Hydrometallurgy*, 194(July 2019).

Liao, X., Ye, M., Li, S., Liang, J., Zhou, S., Fang, X., Gan, Q., and Sun, S. (2021). "Simultaneous recovery of valuable metal ions and tailings toxicity reduction using a mixed culture bioleaching process." *J. Clean. Prod.*, 316(July), 128319.

Liu, K., Tan, Q., Yu, J., and Wang, M. (2023). "A global perspective on e-waste recycling." 2.

Liu, R., Li, J., and Ge, Z. (2016). "Review on Chromobacterium Violaceum for Gold Bioleaching from E-waste." *Procedia Environ. Sci.*, 31, 947–953.

Marappa, N., Ramachandran, L., Dharumadurai, D., and Nooruddin, T. (2020). "Recovery of Gold and Other Precious Metal Resources from Environmental Polluted E-waste Printed Circuit Board by Bioleaching Frankia." *Int. J. Environ. Res.*, 14(2), 165–176.

Marra, A., Cesaro, A., Rene, E. R., Belgiorno, V., and Lens, P. N. L. (2018). "Bioleaching of metals from WEEE shredding dust." *J. Environ. Manage.*, 210, 180–190.

Martínez-Bussenius, C., Navarro, C. A., and Jerez, C. A. (2017). "Microbial copper resistance: importance in biohydrometallurgy." *Microb. Biotechnol.*, 10(2), 279–295.

Martinez, P., Vera, M., and Bobadilla-Fazzini, R. A. (2015). "Omics on bioleaching: current and future impacts." *Appl. Microbiol. Biotechnol.*, 99(20), 8337–8350.

Mejame, P. P. M., Kim, Y. M., Lee, D. S., and Lim, S.-R. (2018). "Effect of technology development on potential environmental impacts from heavy metals in waste smartphones." *J. Mater. Cycles Waste Manag.*, 20(1), 100–109.

Merli, G., Becci, A., and Amato, A. (2022). "Recovery of precious metals from printed circuit boards by cyanogenic bacteria: Optimization of cyanide production by statistical analysis." *J. Environ. Chem. Eng.*, 10(3), 107495.

Minimol, M., Shetty K, V., and Saidutta, M. B. (2020). "Process engineering aspects in bioleaching of metals from electronic waste." *Handb. Environ. Chem.*, Springer Science and Business Media Deutschland GmbH, 27–44.

Minimol, M., Vidya Shetty, K., and Saidutta, M. B. (2021). "Biohydrometallurgical methods and the processes involved in the bioleaching of WEEE." *Environ. Manag. Waste Electr. Electron. Equip.*, 89–107.

Monballiu, A., Cardon, N., Tri Nguyen, M., Cornelly, C., Meesschaert, B., and Chiang, Y. W. (2015). "Tolerance of Chemoorganotrophic Bioleaching Microorganisms to Heavy Metal and Alkaline Stresses." *Bioinorg. Chem. Appl.*, 2015.

Mwase, J. M., Petersen, J., and Eksteen, J. J. (2014). "A novel sequential heap leach process for treating crushed Platreef ore." *Hydrometallurgy*, 141, 97–104.

Narayanasamy, M., Dhanasekaran, D., Vinothini, G., and Thajuddin, N. (2017). "Extraction and recovery of precious metals from electronic waste printed circuit boards by bioleaching acidophilic fungi." *Int. J. Environ. Sci. Technol.*, 15(1), 119–132.

Narayani, M., and Vidya Shetty, K. (2012). “Characteristics of a novel Acinetobacter sp. and its kinetics in hexavalent chromium bioreduction.” *J. Microbiol. Biotechnol.*, 22(5), 690–698.

Narayani, M., and Vidya Shetty, K. (2014). “Reduction of hexavalent chromium by a novel Ochrobactrum sp. - microbial characteristics and reduction kinetics.” *J. Basic Microbiol.*, 54(4), 296–305.

Natarajan, G., and Ting, Y. P. (2013). “Two-step bioleaching and spent medium leaching of gold from electronic scrap material using Chromobacterium violaceum.” *Adv. Mater. Res.*, 825, 270–273.

Natarajan, G., and Ting, Y. P. (2015). “Gold biorecovery from e-waste: An improved strategy through spent medium leaching with pH modification.” *Chemosphere*, 136, 232–238.

Nie, H., Zhu, N., Cao, Y., Xu, Z., and Wu, P. (2015). “Immobilization of Acidithiobacillus ferrooxidans on Cotton Gauze for the Bioleaching of Waste Printed Circuit Boards.” *Appl. Biochem. Biotechnol.*, 177(3), 675–688.

Ning, C., Lin, C. S. K., Hui, D. C. W., and McKay, G. (2017). “Waste Printed Circuit Board (PCB) Recycling Techniques.” *Top. Curr. Chem.*, 375(2), 1–36.

Pakostova, E., Grail, B. M., and Johnson, D. B. (2018). “Bio-processing of a saline, calcareous copper sulfide ore by sequential leaching.” *Hydrometallurgy*, 179(April), 36–43.

Petter, P. M. H., Veit, H. M., and Bernardes, A. M. (2014). “Evaluation of gold and silver leaching from printed circuit board of cellphones.” *Waste Manag.*, 34(2), 475–482.

Pham, V. A., and Ting, Y. P. (2009). “Gold Bioleaching of Electronic Waste by Cyanogenic Bacteria and its Enhancement with Bio-Oxidation.” *Adv. Mater. Res.*, 71–73, 661–664.

Pollmann, K., Kutschke, S., Matys, S., Kostudis, S., Hopfe, S., and Raff, J. (2016).

"Novel Biotechnological Approaches for the Recovery of Metals from Primary and Secondary Resources." *c*, 6(2), 54.

Pourhossein, F., and Mousavi, S. M. (2019). "A novel step-wise indirect bioleaching using biogenic ferric agent for enhancement recovery of valuable metals from waste light emitting diode (WLED)." *J. Hazard. Mater.*, 378(December 2018), 120648.

Pradhan, J. K., and Kumar, S. (2012). "Metals bioleaching from electronic waste by Chromobacterium violaceum and Pseudomonads sp." *Waste Manag. Res.*, 30(11), 1151–1159.

Priya, A., and Hait, S. (2017). "Comparative assessment of metallurgical recovery of metals from electronic waste with special emphasis on bioleaching." *Environ. Sci. Pollut. Res.*, 24(8), 6989–7008.

Qayyum, S., Meng, K., Pervez, S., Nawaz, F., and Peng, C. (2019). "Optimization of pH, temperature and carbon source for bioleaching of heavy metals by Aspergillus flavus isolated from contaminated soil." *Main Gr. Met. Chem.*, 42(1), 1–7.

Ramírez, P., Guiliani, N., Valenzuela, L., Beard, S., and Jerez, C. a. (2004). "Differential Protein Expression during Growth of Acidithiobacillus ferrooxidans on Ferrous Iron , Sulfur Compounds , or Metal Sulfides." *Society*, 70(8), 4491–4498.

Rasoulnia, P., and Mousavi, S. M. (2016). "Maximization of organic acids production by Aspergillus niger in a bubble column bioreactor for V and Ni recovery enhancement from power plant residual ash in spent-medium bioleaching experiments." *Bioresour. Technol.*, 216, 729–736.

Rautela, R., Arya, S., Vishwakarma, S., Lee, J., Kim, K. H., and Kumar, S. (2021). "E-waste management and its effects on the environment and human health." *Sci. Total Environ.*, 773, 145623.

Rehm, H. . (2001). *Biotechnology: Special Processes*.

Rodrigues, M. L. M., Leão, V. A., Gomes, O., Lambert, F., Bastin, D., and

Gaydardzhiev, S. (2015). “Copper extraction from coarsely ground printed circuit boards using moderate thermophilic bacteria in a rotating-drum reactor.” *Waste Manag.*, 41, 148–158.

Rouchalova, D., Rouchalova, K., Janakova, I., Cablik, V., and Janstova, S. (2020). “Bioleaching of Iron, Copper, Lead, and Zinc from the Sludge Mining Sediment at Different Particle Sizes, pH, and Pulp Density Using Acidithiobacillus ferrooxidans.” *Minerals*.

Routledge, S. J. (2012). “Beyond de-foaming: The effects of antifoams on bioprocess productivity.” *Comput. Struct. Biotechnol. J.*, 3(4), e201210001.

Ruan, J., Zhu, X., Qian, Y., and Hu, J. (2014). “A new strain for recovering precious metals from waste printed circuit boards.” *Waste Manag.*, 34(5), 901–907.

Safar, C., Castro, C., and Donati, E. (2020). “Importance of initial interfacial steps during chalcopyrite bioleaching by a thermoacidophilic archaeon.” *Microorganisms*, 8(7), 1–13.

Samad, M. Y. A., Razak, C. N. A., Salleh, A. B., Zin Wan Yunus, W. M., Ampon, K., and Basri, M. (1989). “A plate assay for primary screening of lipase activity.” *J. Microbiol. Methods*, 9(1), 51–56.

Sand, W., Gehrke, T., Jozsa, P. G., and Schippers, A. (2001). “(Bio)chemistry of bacterial leaching - direct vs. indirect bioleaching.” *Hydrometallurgy*, 59(2–3), 159–175.

Santiago, C. D., Yagi, S., Ijima, M., Nashimoto, T., Sawada, M., Ikeda, S., Asano, K., Orikasa, Y., and Ohwada, T. (2017). “Bacterial Compatibility in Combined Inoculations Enhances the Growth of Potato Seedlings.” *Microbes Environ.*, 32(1), 14–23.

Shabani, M. A., Irannajad, M., Azadmehr, A. R., and Meshkini, M. (2013). “Bioleaching of copper oxide ore by Pseudomonas aeruginosa.” *Int. J. Miner. Metall. Mater.*, 20(12), 1130–1133.

Shahabuddin, M., Uddin, M. N., Chowdhury, J. I., Ahmed, S. F., Uddin, M. N., Mofijur, M., and Uddin, M. A. (2023). “A review of the recent development, challenges, and

opportunities of electronic waste (e-waste).” *Int. J. Environ. Sci. Technol.*, Springer Berlin Heidelberg.

Shi, L., Dong, H., Reguera, G., Beyenal, H., Lu, A., Liu, J., Yu, H. Q., and Fredrickson, J. K. (2016). “Extracellular electron transfer mechanisms between microorganisms and minerals.” *Nat. Rev. Microbiol.*, 14(10), 651–662.

Singh, S., Routroy, S., and Dasgupta, M. S. (2023). “Electrical and electronic equipment consumption pattern and e-waste disposal behaviour of individuals and households in India.” *J. Mater. Cycles Waste Manag.*, (0123456789).

Sodha, A. B., Tipre, D. R., and Dave, S. R. (2020). “Optimisation of biohydrometallurgical batch reactor process for copper extraction and recovery from non-pulverized waste printed circuit boards.” *Hydrometallurgy*, 191(February 2019), 105170.

Soleimani, M., Hosseini, S., Roostaazad, R., Petersen, J., Mousavi, S. M., and Vasiri, A. K. (2009). “Microbial leaching of a low-grade sphalerite ore using a draft tube fluidized bed bioreactor.” *Hydrometallurgy*, 99(3–4), 131–136.

Srichandan, H., Pathak, A., Singh, S., Blight, K., Kim, D. J., and Lee, S. W. (2014). “Sequential leaching of metals from spent refinery catalyst in bioleaching-bioleaching and bioleaching-chemical leaching reactor: Comparative study.” *Hydrometallurgy*, 150, 130–143.

Sundramurthy, V. P., Rajoo, B., Srinivasan, N. R., and Kavitha, R. (2020). “Bioleaching of Zn from sphalerite using Leptospirillum ferriphilum isolate: effect of temperature and kinetic aspects.” *Appl. Biol. Chem.*, 63(1).

Tay, S. B., Natarajan, G., Rahim, M. N. B. A., Tan, H. T., Chung, M. C. M., Ting, Y. P., and Yew, W. S. (2013). “Enhancing gold recovery from electronic waste via lixiviant metabolic engineering in Chromobacterium violaceum.” *Sci. Rep.*, 3, 2–8.

Tisa, F., Abdul Raman, A. A., and Wan Daud, W. M. A. (2014). “Applicability of fluidized bed reactor in recalcitrant compound degradation through advanced oxidation processes: A review.” *J. Environ. Manage.*, Elsevier Ltd.

Trivedi, A., Vishwakarma, A., Saawarn, B., Mahanty, B., and Hait, S. (2022). “Fungal biotechnology for urban mining of metals from waste printed circuit boards: A review.” *J. Environ. Manage.*, 323(September), 116133.

Vera, M., Schippers, A., and Sand, W. (2013). “Progress in bioleaching: Fundamentals and mechanisms of bacterial metal sulfide oxidation-part A.” *Appl. Microbiol. Biotechnol.*, 97(17), 7529–7541.

Vimalnath, S., and Subramanian, S. (2018). “Studies on the biosorption of Pb(II) ions from aqueous solution using extracellular polymeric substances (EPS) of Pseudomonas aeruginosa.” *Colloids Surfaces B Biointerfaces*, 172(August), 60–67.

Wei, X., Liu, D., Huang, W., Huang, W., and Lei, Z. (2019). “Simultaneously enhanced Cu bioleaching from E-wastes and recovered Cu ions by direct current electric field in a bioelectrical reactor.” *Bioresour. Technol.*, 298(December 2019), 122566.

White, C., Sayer, J. A., and Gadd, G. M. (1997). “Microbial solubilization and immobilization of toxic metals: Key biogeochemical processes for treatment of contamination.” *FEMS Microbiol. Rev.*, 20(3–4), 503–516.

Willner, J., and Fornalczyk, A. (2013). “Extraction of metals from electronic waste by bacterial leaching.” *Environ. Prot. Eng.*, 39(1), 197–208.

Wittsiepe, J., Fobil, J. N., Till, H., Burchard, G. D., Wilhelm, M., and Feldt, T. (2015). “Levels of polychlorinated dibenzo-p-dioxins, dibenzofurans (PCDD/Fs) and biphenyls (PCBs) in blood of informal e-waste recycling workers from Agbogbloshie, Ghana, and controls.” *Environ. Int.*, 79, 65–73.

Wu, W., Liu, X., Zhang, X., Li, X., Qiu, Y., Zhu, M., and Tan, W. (2019). “Mechanism underlying the bioleaching process of LiCoO2 by sulfur-oxidizing and iron-oxidizing bacteria.” *J. Biosci. Bioeng.*, 128(3), 344–354.

Wu, W., Liu, X., Zhang, X., Zhu, M., and Tan, W. (2018). “Bioleaching of copper from waste printed circuit boards by bacteria-free cultural supernatant of iron–sulfur-oxidizing bacteria.” *Bioresour. Bioprocess.*, 5(1).

Xia, M., Bao, P., Liu, A., Wang, M., Shen, L., Yu, R., Liu, Y., Chen, M., Li, J., Wu, X., Qiu, G., and Zeng, W. (2018a). "Bioleaching of low-grade waste printed circuit boards by mixed fungal culture and its community structure analysis." *Resour. Conserv. Recycl.*, 136(May), 267–275.

Xia, M. C., Bao, P., Liu, A. J., Zhang, S. S., Peng, T. J., Shen, L., Yu, R. L., Wu, X. L., Li, J. K., Liu, Y. D., Chen, M., Qiu, G. Z., and Zeng, W. M. (2018b). "Isolation and identification of Penicillium chrysogenum strain Y5 and its copper extraction characterization from waste printed circuit boards." *J. Biosci. Bioeng.*, 126(1), 78–87.

Xia, M. C., Wang, Y. P., Peng, T. J., Shen, L., Yu, R. L., Liu, Y. D., Chen, M., Li, J. K., Wu, X. L., and Zeng, W. M. (2017). "Recycling of metals from pretreated waste printed circuit boards effectively in stirred tank reactor by a moderately thermophilic culture." *J. Biosci. Bioeng.*, 123(6), 714–721.

Xin, B., Zhang, D., Zhang, X., Xia, Y., Wu, F., Chen, S., and Li, L. (2009). "Bioleaching mechanism of Co and Li from spent lithium-ion battery by the mixed culture of acidophilic sulfur-oxidizing and iron-oxidizing bacteria." *Bioresour. Technol.*, 2, 6164–6169.

Yang, J., Wang, Q., Wang, Q., and Wu, T. (2008). "Comparisons of one-step and two-step bioleaching for heavy metals removed from municipal solid waste incineration fly ash." *Environ. Eng. Sci.*, 25(5), 783–789.

Yang, Y., Chen, S., Li, S., Chen, M., Chen, H., and Liu, B. (2014). "Bioleaching waste printed circuit boards by Acidithiobacillus ferrooxidans and its kinetics aspect." *J. Biotechnol.*, 173(1), 24–30.

Yang, Y., Diao, M., Liu, K., Qian, L., Nguyen, A. V., and Qiu, G. (2013). "Column bioleaching of low-grade copper ore by Acidithiobacillus ferrooxidans in pure and mixed cultures with a heterotrophic acidophile Acidiphilium sp." *Hydrometallurgy*, 131–132, 93–98.

Zhou, G., Zhang, H., Yang, W., Wu, Z., Liu, W., and Yang, C. (2020). "Bioleaching

assisted foam fractionation for recovery of gold from the printed circuit boards of discarded cellphone." *Waste Manag.*, 101, 200–209.

Zhou, H. B., Zeng, W. M., Yang, Z. F., Xie, Y. J., and Qiu, G. Z. (2009). "Bioleaching of chalcopyrite concentrate by a moderately thermophilic culture in a stirred tank reactor." *Bioresour. Technol.*, 100(2), 515–520.

Zhou, Y., and Qiu, K. (2010). "A new technology for recycling materials from waste printed circuit boards." *J. Hazard. Mater.*, 175(1–3), 823–828.

Zhou, Y., Wu, W., and Qiu, K. (2010). "Recovery of materials from waste printed circuit boards by vacuum pyrolysis and vacuum centrifugal separation." *Waste Manag.*, 30(11), 2299–2304.

Zhu, J., Jiao, W., Li, Q., Liu, X., Qin, W., Qiu, G., Hu, Y., and Chai, L. (2012). "Investigation of energy gene expressions and community structures of free and attached acidophilic bacteria in chalcopyrite bioleaching." *J. Ind. Microbiol. Biotechnol.*, 39(12), 1833–1840.

Zhu, N., Xiang, Y., Zhang, T., Wu, P., Dang, Z., Li, P., and Wu, J. (2011). "Bioleaching of metal concentrates of waste printed circuit boards by mixed culture of acidophilic bacteria." *J. Hazard. Mater.*, 192(2), 614–619.

PUBLICATIONS & CO-CURRICULAR ACTIVITIES

Research Articles

- Minimol Madhavan., Vidya Shetty K., and Saidutta, M. B. (2023). "Performance of Fluidized-Bed Bioreactor in Cu Bioleaching from Printed Circuit Boards using *Alcaligenes aquatilis*." *Waste and Biomass Valorization*.
- Minimol, M., Vidya Shetty, K., and Saidutta, M. B. (2023). "Bioleaching of zinc from e-waste by A. aquatilis in fluidised bed bioreactor." *Indian Chem. Eng.*, 1–13.
- Minimol Madhavan., Vidya Shetty K., and Saidutta, M. B. (2023). "A mechanistic study on One-step bioleaching of metals from Waste Printed Circuit Boards in a Fluidized-bed bioreactor by heterotrophic bacteria as individual and co-cultures." – Under Preparation.

Book Chapters

- Minimol, M., Shetty K, V., and Saidutta, M. B. (2020). "Process engineering aspects in bioleaching of metals from electronic waste." *Handb. Environ. Chem.*, Springer Science and Business Media Deutschland GmbH, 27–44.
- Minimol, M., Shetty K, V., and Saidutta, M. B. (2021). "Biohydrometallurgical methods and the processes involved in the bioleaching of WEEE." *Environ. Manag. Waste Electr. Electron. Equip.*, 89–107.

Conferences attended

- The 3R International Scientific Conference on Material Cycles & Waste Management in Bangkok, Thailand from 27th Feb – 1st Mar 2019. **Oral Presentation - Bioleaching for the Recovery of Au and Ag from Electronic Waste Facilitated by Metal Resistant Heterotrophic Bacteria.**
- International Conference on Affordable Strategies for Health & Environment at NMAMIT, Udupi from May 23rd – 24th, 2019. **Oral Presentation –Pulsed plate Bioreactor for bioleaching of Fe & Zn by *Acinetobacter* sp. CR B2 from Printed Circuit Boards.**

CHEMCON 2021, 74th Annual session of Indian Institute of Chemical Engineers in Hybrid mode from 26th – 30th, Dec 2021. **Oral Presentation– Bioleaching of Zn from E-waste.**

BIO-DATA

Minimol M

Email ID: immini.91@gmail.com

Contact no.: +91-9443284042

PERSONAL INFORMATION

Date of birth	09/02/1991
Gender	Female
Marital status	Married
Spouse name	Ashwin K
Addess	13/277, Chandu Colony Jagathala Road, Aruvankadu The Nilgiris – 643202 Tamil Nadu, India

SCHOLASTICS (FULL-TIME)

- **PhD** in the Department of Chemical Engineering, National Institute of Technology Karnataka (NITK), Surathkal.
- **Master of Science (Microbiology)** from the Department of Microbiology, Pondicherry University, Puducherry with Cumulative CGPA – 9.31/10 in 2015
- **Bachelor of Science (Microbiology)** from Sri Krishna Arts and Science College, Coimbatore with 80.86% in 2012
- **Higher Secondary** from St. Josephs' Convent Anglo-Indian Girls Higher Secondary School, Coonoor with 67% in 2009
- **ASLC from** St. Josephs' Convent Anglo-Indian Girls Higher Secondary School, Coonoor with 84.3% in 2007

PUBLICATIONS

Book Chapters

- Minimol, M., Shetty K, V., Saidutta, M.B., 2021. **Biohydrometallurgical methods and the processes involved in the bioleaching of WEEE**, in: Environmental Management of Waste Electrical and Electronic Equipment. pp. 89–107. https://doi.org/10.1016/b978-0-12-822474-8.00005-2

- Minimol, M., Shetty K, V., Saidutta, M.B., 2020. **Process engineering aspects in bioleaching of metals from electronic waste**, in: Handbook of Environmental Chemistry. Springer Science and Business Media Deutschland GmbH, pp. 27–44. https://doi.org/10.1007/698_2020_575

Research Papers

- Minimol, M., Shetty K, V., Saidutta, M.B., 2023. **Bioleaching of Zinc from E-waste by *A. aquatilis* in Fluidized bed Bioreactor**, Indian Chemical Engineer. https://doi.org/10.1080/00194506.2023.2196558

- Minimol Madhavan., Vidya Shetty K., and Saidutta, M. B. (2023). **Performance of Fluidized-Bed Bioreactor in Cu Bioleaching from Printed Circuit Boards using *Alcaligenes aquatilis*.** Waste and Biomass Valorization. https://doi.org/10.1007/s12649-023-02202-8

TECHNICAL EXPERIENCE:

- Doctoral Research on **"Bioleaching of metals from electronic waste by heterotrophic bacteria in Fluidized-bed Bioreactor"** from July 2017 under the guidance of Prof. M. B. Saidutta & Prof. Vidya Shetty K at the Department of Chemical Engineering, National Institute of Technology Karnataka, Mangaluru.
- **Volunteer Biotechnology Resource Person** for outreach activities of **Virtual Labs (Faculty Development Programmes)**, Centre for System Design, the interdisciplinary R&D centre of National Institute of Technology Karnataka
- **Senior research fellow (from January 2017 – July 2017)** in the Project **"Evaluation of Bioinoculants of Novozyme on Paddy"** at the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore.
- **Junior research fellow (from September 2015 – September 2016)** in a Project entitled **"Developing newer methods of mass multiplication of Arbuscular Mycorrhizal fungi for sustainable sugarcane production"** at the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore.
- **M. Sc Dissertation** entitled **"Isolation and molecular characterization of PPFMs from the phyllosphere, a comparative study on their plant growth promoting activities"** for six months under the guidance of Dr. Busi Siddhardha at the Department of Microbiology, Pondicherry University, Puducherry.

FELLOWSHIPS:

- Awarded **AABS Travel Fellowship** at the International Symposium at Fortune Resort Sullivan Court, Ooty.
- Qualified **Tamil Nadu State Eligibility Test – 2016** for Lectureship in Life Sciences.
- Qualified **GATE 2017** with a score of 398 and All India Rank – 1298 in Life Sciences.
- Qualified **ARS-NET 2017** with 54.67% in the subject Agricultural Microbiology.

INTERNSHIPS

- On- the-job Training in **Microbiology laboratory** of Paalana Institute of Medical Sciences, Palghat for 19 days
- In-house Training on **Wheat crop improvement and pathology** in Indian Agricultural Research Institute, Wellington for 15 days
- Implant Training on the **Quality Control of Milk and Milk Products** in the Nilgiris District Co-operative Milk Producers Union Limited (aavin), Ooty for 15 days

WORKSHOP

- **Microbial and Molecular techniques** in PSGR Krishnammal College for Women, Coimbatore
- Completed one-day training programme on **Mushroom Cultivation** in Tamil Nadu Agricultural University, Coimbatore
- **"Know the Act & Regulations" Series one-day workshop on Food safety Act on** (14th March, 2014) conducted by the Department of Food Science & Technology, Pondicherry University& Association of Food Scientists and Technologists, Puducherry Chapter
- **Author workshop** (6th February,2014) conducted by Springer Publications at the Pondicherry University
- A three – day National Level Workshop on **Strategies for Recombinant Protein Production** (from 25th-27th February, 2016) organized by JSS College of Arts and Science in association with the Association of Microbiologists of India, Mysore Chapter.
- A short term course on **"Bioremediation for Environmental Sustainability"** under the Global Initiative for Academic Networks (GIAN) Programme of Ministry of Human Resources Development, Govt. of India (11 – 15th August, 2018) at the Department of Chemical Engineering, NITK, Surathkal.
- **Winter Workshop on Bioprocess Engineering – Lectures & Laboratory 2018** (17-21 December, 2018) organized by the Department of Biotechnology, Centre for Continuing Education, Indian Institute of Technology Madras, Chennai.

CONFERENCES

Title of the Paper	Award	Symposium/Conference/ Seminar Title & Venue	International/ National/State Level	Date
Bioleaching for the Recovery of Au & Ag from Electronic waste Facilitated by Metal Resistant Heterotrophic Bacteria	-	The 3R International Scientific Conference on Material Cycles & Waste Management at Pullman Bangkok King Power, Thailand	International Conference	27/02/2019 - 01/03/2019
Pulsed-plate Bioreactor for Bleaching of Fe & Zn by *Acinetobacter* sp. CR B2 from Printed Circuit Boards	-	International Conference on Affordable Strategies for Health & Environment	International Conference	23/05/2019 - 24/05/2019
Bioleaching of Zn From E-waste	**Best Oral Presentation Award - Mineral Processing**	Indian Chemical Engineering Congress & 74th Annual Session of Indian Institute of Chemical Engineers		26/12/2021 - 30/12/2021
Isolation and Molecular Characterization of PPFMs from the Phyllosphere, a Comparative Study on their Plant Growth Promoting Activities	**Best Paper Presentation Award**	International Symposium on Biodiversity, Agriculture, Environment & Forestry held at Fortune Hotel Sullivan Court, Ooty	International Symposium	11/12/2015 12/12/2015
Construction of Biogas Plant	**Second prize for Oral Presentation**	Biorevolution – A Promising Strategy (2012) held at Sri Krishna Arts & Science College, Coimbatore	National Conference	08/02/2012

Study on the Antibacterial activity of fruits	**Second prize for Oral Presentation**	"Biovision 2011" at Mercy College, Palakkad	National level Seminar	19/08/2011
A Comparative Study on the Bioremediation of Retting Effluent by Plant, Bacteria and Fungi	-	MICROMEET'10 "Innovative Approaches in Biosciences" at Sree Narayana Guru College, Coimbatore	National Conference	26/02/2010
The Effect of VAM Spores on Medicinal Plants in Pot Culture Experiments	-	Recent Scenario in Biological Innovations held at Hindustan College of Arts and Science, Coimbatore	National Conference	03/02/2011 04/02/2011
Isolation of Total Heterotrophic Bacteria from Coastal regions after Tsunami	-	PSGR Krishnammal College for Women, Coimbatore	National level Seminar	28/01/2011 29/01/2011
Conservation of Water	-	Emerging Trends in Physical and Biological Sciences at Nanda Arts and Science College, Erode	State level Conference	17/02/2012

DECLARATION

I hereby declare that the above mentioned details are true to the best of knowledge.

Place: Surathkal, Karnataka

Date: 08.06.2023