Bioleaching of Gold from Obsolete Mobile SIM Cards

Project Report Submitted By

Name: Aditya Sahni

Roll Number: 123805

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WAKNAGHAT

Project Supervisor:
Dr. Sudhir Kumar
Associate Professor

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JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

WAKNAGHAT

CERTIFICATE

This is to certify that the project work entitled “**Bioleaching of Gold from Obsolete Mobile SIM Cards”** submitted by **Mr. Aditya Sahni (123805)**, a Bachelors student at Jaypee University of Information Technology, Solan is carried out in partial fulfilment of the award of the Degree of Bachelors of Technology in Biotechnology from Jaypee University of Information Technology and has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma. As a Final Year Project at Jaypee University of Information Technology, Solan, this work is a record of the candidate’s own work carried out by him under the supervision and guidance of the undersigned from July 2015 to June 2016 at this University.

Date-

Place- Waknaghat, Solan

Dr. Sudhir Kumar
Associate Professor
Department of Biotechnology and Bioinformatics
Jaypee University of Information Technology,
Solan - 173234

DECLARATION

I **Aditya Sahni,** student of Bachelors of Technology in Biotechnology with enrollment number **123805,** at Jaypee University of Information Technology, Solan, Himachal Pradesh hereby declare that the project entitled, "**Bioleaching of Gold from obsolete mobile SIM cards"** is an original work done during the period **July, 2015 – June, 2016** as a fourth year project, under the able guidance of **Dr. Sudhir Kumar,** Associate Professor, Department of Biotechnology and Bioinformatics, JUIT Solan to the best of my knowledge and has not been published anywhere in any institute or university.

Date-

(Aditya Sahni)

Place- JUIT Waknaghat, Solan

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Date-

Place- JUIT Waknaghat, Solan (Aditya Sahni)

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ABBREVIATIONS

OD	Optical Density
RT	Room Temperature
Gm	Grams
Kg	Kilograms
Cm	Centimeter
Gm	Grams
µm	Micro Mitre
ml	Mili litre
mM	Mili molar
nm	Nano meter
RPM	Rotations per minute
^{O}C	Degree celcius
AAS	Atomic Absorption Spectrophotometry
ESM	Electronic Scarp Material
RoHS	Restriction of Use of Certain Hazardous Substances
MoEF	Ministry of environment and Forests
EU	European Union
PPE	Personnel Protection Equipment
TRAI	Telecom Regulatory Authority of India
WEEE	Waste Electrical and Electronic Equipment
OECD	Organization for Economic Cooperation and Development
CPCB	Central Pollution Control Board
SIM	Subscriber Identity Module
PCB	Printed Circuit Boards
LB	Luria Bertani
e-waste	Electronic waste
Au	Gold
Ag	Silver
Cu	Copper

ABSTRACT

Electronic waste is the fastest growing municipality solid waste due to high obsolescence rate of electrical and electronic items. The exponential increase in the cellular networking and advancement in the related technology has also led to an increase in waste or obsolete SIM cards which are regarded as enriched source of precious metals, especially gold. With its significant gold content, e-waste is recognized as the emerging and the fastest growing waste stream and the recovery of gold from e-waste has not only economic benefits but can also serve as secondary source for gold, hence reducing the demand on gold mining operations. Bioleaching has been used for many years to recover metals from low grade ores or low grade mineral resources. Bioleaching of gold is done by cyanidation process, where cyanogenic bacteria produce hydrogen cyanide which helps to recover gold from the e-waste. This project focused on the bioleaching of gold from obsolete mobile SIM cards by cyanogenic bacteria (*Chromobacterium violaceum*) involving two step leaching process, evaluation of elemental composition of obsolete SIM cards and evaluating effect of pretreatment and spent medium leaching on metals recovery through bioleaching. The SIM metal was differentiated into 3 particle sizes varying from 355 µm to 700 µm or above. Elemental composition of obsolete SIM cards was found between 0.16% to 0.42% for Au, varying due to the particle size, where maximum was found in the particle size <355 µm. The concentration of Cu was found 75% with maximum found in the particle size >700 µm. The concentration of Ag was found between 0.003% to 0.22% and maximum was reported in size <355 µm. Two-step bioleaching process lead to maximum gold mobilization i.e. 2.36% of gold from obsolete mobile SIM card with particle size >700 µm followed by 2.09% gold mobilization for a particle size <355 µm and least gold mobilization was found with the particle size 355-700 µm i.e. 0.44%. Pretreatment of e-waste led to a 72.73% reduction of Cu from e-waste, with no effect on the chemical composition of other metals like Au and Ag. Two-step bioleaching of the pretreated SIM e-waste reported a gold mobilization i.e. 0.71% with a particle size 355-700 µm. Spent medium leaching at high temperature of SIM e-waste reported a gold mobilization i.e. 0.62% with a particle size 355-700 µm, whereas PCB e-waste reported a maximum gold mobilization i.e. 34.34% which was more than the gold mobilization in two-step bioleaching. Hence increase in temperature resulted in increased metal mobilization because of the increase in the reaction velocity.

Bioleaching did not found out to be an efficient way to leach out gold from the SIM e-waste due to the presence of other toxic metals like copper in very high proportions which even after pre-treatment and excessive removal turn out to be more affined towards cyanide complex formation at an alkaline pH. Also a hybrid technology, chemo-biohydrometallurgy may be effective to leach out significant amount of copper from the SIM e-waste, where even the residues left after pre-treatment was subjected to bioleaching and gave very significant results.

CHAPTER 1 INTRODUCTION

The present era of science and technology has made life much easier and comfortable. With the advancement of information and communication technology, the electronic sector is flourishing at the speed of light, leading to rapid product obsolescence and a new environmental challenge - the growing threat of electronic waste or e-waste comprises of discarded electronic appliances of which computers and mobile phones are disproportionately abundant because of their shorter life span [1]. This progress in technology has two faces: one is a boon, enhancing communication, strengthening economic structure, improving standards of living, progress in science and culture, whereas the other face of the same technology causes environmental and health problems [2].

Electronic waste or e-waste describes discarded electrical or electronic devices [3]. Rapid development in science and technology and changing life style has led to an enormous increase in the usage of electrical and electronic devices in every sphere of life possible, and simultaneously increasing the pressure on the land for its disposal. Informal processing of electronic waste in developing countries may cause serious health and pollution problems, as these countries have limited regulatory oversight of e-waste processing. The processes of dismantling and disposing of electronic waste in the third world lead to a number of environmental impacts. The developing countries are becoming a dumping site for the developed nation, since there is an illegal import of these hazardous electronic items in the name of free trade [1]. However most of these products can be recycled and reused, reducing the overall impacts on the environment. The recycling techniques used by these informal sectors are unsound since there is no proper infrastructure and no awareness among the people with respect to the hazardous effects of e-waste. The unsound practices followed by these informal sectors include incineration, open landfills and dumping into water bodies and such practices have a serious aftermath on the population living in the vicinity of these dumping or recycling sites. In addition to this, the working conditions in India are poor. In most of the cases there is no proper lighting, ventilation and workers are not provided with any kind of personnel protection equipment’s (PPE) while working. There are innumerable informal sectors which are difficult to regulate. They take the advantage of the widespread unemployment, low wage rate and lack of protest by the workers who think e-waste as a source of income for them and a step towards modernization. Liquid and atmospheric releases end up in bodies of water, groundwater, soil, and air and therefore in land and sea animals – both domesticated and wild, in crops eaten by both animals and human, and in drinking water [7].

According to Telecom Regulatory Authority of India (TRAI), India added 113.26 million new cellular customers in 2014, with an average 9.5million customers added every month. Cellular market grew from 168.11 million in 2003–2004 to 261.97 million in 2013–2014 (TRAI 2014–15). Sixty-five cities in India generate more than 60% of the total E-waste generated in India. Ten states are responsible for generating 70% of the total E-waste in India. In this list, Maharashtra ranks first followed by Tamil Nadu, Andhra Pradesh, Uttar Pradesh, West Bengal, Delhi, Karnataka, Gujarat, Madhya Pradesh and Punjab. Among the top ten cities generating e-waste, Mumbai ranks first followed by Delhi, Bangalore, Chennai, Kolkata, Ahmedabad, Hyderabad, Pune, Surat and Nagpur [2][3]. Despite a wide range of environmental legislation in India, there are no specific laws or guidelines for electronic waste. No attempts have been made to describe the fate of the emissions in the environment or to conduct a risk assessment for the affected population.

Electronic waste majorly is categorized into 6 types based on the general principle and application. They include monitors, televisions, computers, telephones, and their peripherals, DVD/VCR players, CD players, radios and hi-fi sets, refrigerators, washing machines, air conditioners, vacuum cleaners etc.

Most of the electronic waste is a complex mixture of precious metals (Ag, Au, and Pt); base metals (Cu, Al, Ni, Si, Zn and Fe); toxic metals (Hg, Be, Cd, Cr (VI), As, Sb and Bi) along with halogens and combustible substances such as plastics and flame retardants [4]. However, complex composition and increasing volumes of e-waste, along with difficulties in treating it, are causes of concern therefore, recycling of electronic waste is an important subject not only for waste management but also for the recovery aspect of valuable materials.

A subscriber identity module or subscriber identification module (SIM) card, which is an integral part of mobile telephony [19] has been considered as a potential electronic waste for the extraction of precious and valuable metals. The exponential increase in the cellular networking and advancement in the related technology has led to an increase in waste or obsolete SIM cards which are regarded as enriched source of precious metals, especially gold. The chemical composition of a SIM card reveals it as a metal enriched source, with a lot of precious metals like gold, copper etc. being used to manufacture a single SIM card [20].

Pure gold is used in abundance as due to its no reactive and non-corrosive nature, it is used to shield the metal surface of the SIM card.

There are various types of traditional ways and methods to leach out precious metals and to recycle the electronic waste, mostly used in extraction of metals in mineral ores like pyro metallurgy and hydro metallurgy. However, pyro metallurgical and Hydrometallurgical treatment raises concerns regarding possible formation of brominated and chlorinated di-benzo furans and dioxins in burning processes due to the presence of halogens in the plastic parts of electronic waste and also associated with risks of environmental impact due to the toxicity of the reagents used and the large amount of by-products generated respectively. These processes are also expensive because more investment is necessary to set up highly efficient facilities, there is a high consumption of energy and they are not regarded as an economical way to extract valuable components from electronic waste.

The bioleaching process is now emerging as commercial exploitable technology applicable for metal extraction from electronic waste and low grade ores [5]. Bioleaching using cyanogenic bacterial species has been used to recover metals such as zinc, copper and gold etc. from low grade ores. There are other metals and metalloids as well which form cyanide complexes which are water soluble and exhibit high chemical stability.

Electronic scrap material especially the PCBs and obsolete mobile SIM cards with its significant gold content, is recognized as a new emerging and fast growing waste stream and could be considered as a secondary ore for gold due to its high concentration. The bioleaching mechanism responsible for metal recovery in mining operations may be also applied in bio-mining of precious metals from such wastes. This project focused on the bioleaching of gold from obsolete mobile SIM cards. The bioleaching of this electronic waste is carried out using a cyanogenic bacteria, *Chromobacterium violaceum* which has been reported to have maximum bioleaching capability and also an affinity towards precious metals such as gold [5].

The present study was undertaken with following objectives:

1. Evaluation of elemental composition of obsolete SIM cards.
2. Two-step bioleaching for metals recovery using *Chromobacterium violaceum* from obsolete mobile SIM cards.
3. Evaluating effect of pretreatment and spent medium leaching on metals recovery through bioleaching.

Electronic waste may be defined as discarded computers, office electronic equipment, entertainment device electronics, mobile phones, television sets and refrigerators. Electronic waste is only a subset of Waste Electrical and Electronic Equipment (WEEE) [1]. According to the Organization for Economic Cooperation and Development (OECD), any appliance using an electric power supply that has reached its end-of-life would come under WEEE (EU 2002) [1, 2]. In simple terms, when electronic products are discarded, they become electronic waste (e-waste). These wastes are also called electronic scrap or electronic scrap material (ESM). ESM comprises of a variety of mixture of various metals especially copper, aluminum and steel attached to, covered with or mixed with various types of plastics and ceramics [6]. The composition of e-waste is very diverse and complex. E-waste consists of hazardous and toxic material that poses environmental risk if they are land filled and incinerated.

Rapid development in science and technology and changing life style has led to an enormous increase in the usage of electrical and electronic devices in every sphere of life possible, and simultaneously increasing the pressure on the land for its proper disposal. Although the global E-waste problem has been able to attract attention across the world, not much emphasis has been given to the e-waste engendered in developing countries. Developing countries like India, today, is burdened with the colossal problem of e-waste which is either locally generated or imported, causing serious menace to human health and environment [7]. Informal processing of electronic waste in developing countries may cause serious health and pollution problems, as these countries have limited regulatory oversight of e-waste processing. The processes of dismantling and disposing of electronic waste in the third world lead to a number of environmental impacts. Liquid and atmospheric releases end up in water bodies, soil, and air and therefore get entry in the food web.

In the context of India, recycling of Waste Electrical and Electronic Equipment is not undertaken to an adequate degree. While considering the problems related to e-waste in India, which includes five major components are main sources of e-waste, magnitude of the problem with respect to the Indian scenario, health and environmental implications of e-waste, current management practices of e-waste in India and policy level initiatives in the country. Realizing the growing concern over e-waste, the Government of India has been supporting several initiatives like restrictions on usage of hazardous substances as per global best-practices and to prevent e-waste dumping in the country is a subject which is being dealt by Ministry of

Environment and Forest (MoEF) [8]. Of particular importance is the assessment conducted by the Central Pollution Control Board (CPCB) on the management and handling of e-waste leading to the preparation of “Guidelines for environmentally sound management of e-waste” and “the e-waste (Management and Handling) Rules [9].

2.1 Categories of e-waste

The composition of E-waste consists of diverse items many of which contain hazardous elements. Electronic waste majorly is categorized into six types based on the general principle and application. They include (a) monitors (10%); (b) televisions (10%); (c) computers, telephones, and their peripherals (15%); (d) DVD/VCR players, CD players, radios and hi-fi sets (15%); (e) refrigerators (20%); and (f) washing machines, air conditioners, vacuum cleaners etc (30%). [10] These can also be categorized in the manner given in the following table as prescribed by the EU. [1][11]

Table 2.1: Categories of electronic waste.

Category	Examples	Label
Large household appliances	Refrigerator, freezer, washing machine, cooking appliances.	Large HH
Small household appliances	Vacuum cleaners, watches, grinders.	Small HH
IT & telecommunications equipment	PCs, printers, telephones, telephones.	ICT
Consumer equipment	TV, radio, video camera, Amplifiers.	CE
Lighting equipment	CFL, high intensity sodium lamp.	Lighting
Electrical and electronic tools (with the exception of large scale stationary industrial tools)	Drills, saws, sewing machine.	E&E tools
Toys, leisure and sports equipment	Computer, video games, electric trains.	Toys
Medical devices	With the exception of all implanted and infected products radiotherapy equipment, cardiology, dialysis, nuclear medicine.	Medical equipment
Monitoring and control instrument	Smoke detector, heating regulators, thermostat.	M&C

Table 2.2: Average weight, life span and composition of WEEE of EEE commonly used [1] [12].

Type of electronic equipment	Average Weight (kg)	Average useful life Span (Years)	Iron (Fe) % weight	Non Fe % metal weight	Glass % weight	Plastic % weight	Electronic component % weight	Others % weight
Refrigerators and freezers	40-48	10	64.4	6	14	13		15.1
Television	28-36	10	5.3	5.4	62	22.9	0.9	3.5
Personal computer(PC)	27.2-29.6	5	53.3	8.4	15	23.3	17.3	0.7
Cell Phone	.12	3	8	20	10.6	59.6		1.8
Washing Machine	40-47	12	59.8	4.6	2.6	1.5		35.1

2.2 Generation of e-waste

The growth in IT and electronic industry, changing lifestyle, low cost availability of electronic gadgets and peer pressure has led to increased consumption of electronic goods worldwide. Due to this high obsolescence rate the amount of e-waste is increasing all over the world. The developing countries are more vulnerable to this threat of e-waste since for the developed nations, these developing countries serve as the dumping site and millions tons of e-waste is imported from the developed countries despite the existence of Basal Convention, which is designed for the prevention of hazardous waste exportation from rich to poorer countries. However proper recycling of e-waste to extract out precious metals is a profitable business for the developed countries but the present recycling cost is not viable, therefore a large amount of e-waste is imported to the developing countries. Also the informal sector involved in recycling of the e-waste is add on to the problem of e-waste. Most of the electronic waste is a complex mixture of precious metals (Ag, Au, and Pt); base metals (Cu, Al, Ni, Si, Zn and Fe); toxic metals (Hg, Be, Cd, Cr (VI), As, Sb and Bi) along with halogens and combustible substances such as plastics and flame retardants [4].

Table 2.3: **Major hazardous components in WEEE and their health hazards [13].**

Substance	Applications	Health Hazards
Mercury	Thermostats, sensors, relays in switches and discharge lamps.	Damage to various organs including the brain and kidneys, the developing fetus is highly susceptible through maternal exposure to mercury.
Lead	Soldering of printed circuit boards, cathode ray tubes and light bulbs.	Damage to the central and peripheral nervous systems, blood systems, kidney and reproductive system in humans.
Cadmium	Switches, springs, connectors, housings and printed circuit boards.	Toxic and can bio-accumulate, and they pose a risk of irreversible effects on human health.
Hexavalent chromium	Metal coatings for corrosion protection and wear resistance.	Easily passes through cell membranes and is then absorbed producing various toxic effects in contaminated cells.
Polybrominated biphenyls (PBB) and Polybrominated diphenylethers (PBDE)	Flame retardants in printed circuit boards, connectors and plastic covers.	Thyroid hormone disruption, neurodevelopment deficits and cancer.

The unconventional recycling practices release a lot of amount of toxic substances like lead, mercury, cadmium, arsenic, chromium, etc. into the atmosphere which pose a great danger to human and the environment. Disposal of E-wastes is an unembellished problem faced by many regions across the globe. Electronic wastes that are land filled produces contaminated leachates which eventually pollute the groundwater. Acids and sludge obtained from melting computer chips, if disposed on the ground causes acidification of soil. Incineration of E-waste possesses another threat. It can emit toxic fumes and gases, thereby polluting the surrounding air [14].

The major approach to treat e-waste is to reduce the concentration of these hazardous chemicals and elements through recycling and recovery. In the process of recycling or recovery, certain e-waste fractions act as secondary raw material for recovery of valuable items. The recycle and recovery includes the unit operations like dismantling, segregation of ferrous metal, non-ferrous metal and plastic by shredder process, refurbishment and reuse, recycling / recovery of valuable materials and treatment/disposal of dangerous materials and waste.

2.3 Global e-waste production and mitigation

Globally many legal frameworks have been created to regulate e-waste management. The Basel Convention has played a significant role in curbing the E-waste trade from OECD countries to Non-OECD countries. The EU has formed two important directives: the WEEE directive and Restriction of Use of Certain Hazardous Substances (RoHS) in Electrical and Electronic Equipment, therefore taking a lead in protecting the environment from the hazards of e-waste. Many countries have implemented WEEE Directives with detailed guidelines to assist the producers and consumers in understanding their duty to handle e-waste in environmentally sound manner [1] but still most of the countries lack reliable data on E-waste generation. Comparison of the figures is difficult because there is no standard definition of e-waste, and the methods used to estimate e-waste are not compatible and varies among the various countries.

The WEEE directive was launched in 2005 with an aim to minimize the impact of electrical and electronic goods on the environment by increasing reuse and recycling, and reducing the amount of WEEE going to landfill by making producers responsible for financing the collection, treatment and recovery of waste electrical equipment and allowing consumers to return their waste equipment free of charge. The Restriction of Hazardous Substances (RoHS) Directive was created in 2003 which provides a ban on the use of certain substances in electrical and electronic equipment. It names six substances of immediate concern: lead, mercury, cadmium, hexavalent chromium, Polybrominated biphenyls (PBB) and Polybrominated diphenylethers (PBDE). The Basal Convention which came into force on 5 May 1992 aims to protect human health and the environment from adverse impacts resulting from the generation, management, transboundary movements and disposal of hazardous and toxic wastes [15].

Table 2.4: Total e-waste generated in different countries [7].

Country	Total e-waste generated (tonnes/year)	Year
Switzerland	66 042	2003
Germany	1,100,000	2005
UK	940,000	2003
USA	2,250,000	2007
Taiwan	14 036	2003
Thailand	60 000	2003
China	2,212,000	2007
Canada	86,000	2002
India	439,000	2007
Japan	860,000	2005
Kenya	7350	2007
South Africa	59,650	2007

2.4 Regulation of e-waste in India

In India, Ministry of Environment and Forests (MoEF) is the national authority responsible for legislation regarding waste management and environmental protection [1]. MoEF has laid out procedures for manufacturers under the Extended Producer Responsibility (EPR) which holds them responsible for recycling, reducing the level of toxic substances in electronic and setting up of collection centers. These rules apply to every producer, consumer and bulk consumer involved in manufacture, sale and purchase and processing of electronic equipment’s and components [16]. EPR rules also state that companies must strive to reduce toxic contents of their products. The Central Pollution Board keeps an eye and submits an annual report regarding implementation of these rules every year. Data from MoEF and CPCB shows that 10 Indian State generate nearly 70% of total e-waste in the country where Maharashtra ranks first, followed by Tamil Nadu, Andhra Pradesh and Uttar Pradesh [8].

2.5 Recycling of e-waste

Regardless of the fact that there is large amount of e-waste generated in India, there are few systematic or formal system for sound and proper disposal of e-waste. Large amount of e-waste is treated as junk and disposed off as municipal waste. E-waste, being rich in ferrous materials, nonferrous materials, plastic, and precious materials, has turned out as a major business opportunity for many unemployed. A versatile E-waste management technology package involving recovery of valuable metals, minimizing environmental and health impacts with demonstration on viable scale needs to be developed as no sophisticated technology is devised in India and recycling simply means manual dismantling of e-waste which certainly is a matter of concern [1]. The WEEE contains rarer and noble metals as compared to metals in the metal mines [16], so the proper recycling of e-waste not only contributes to environmental protection but also to the economic development.

2.6 Economic importance of e-waste

Most of the gold present in the electronic item comes from gold mining. Mining operations lead to many environmental problems, destruction of natural landscape and many health problems. Hence, recovery of gold from e-waste has not only economic benefits but can also serve as secondary source for gold, hence reducing the demand on gold mining operations. Recovery of metals from e-waste also helps to reduce the volume of waste for landfill or thermal destruction. Electronic is treated as a ‘secondary ore’ or ‘artificial ore’ [17] for the concentrations of precious metals richer than natural ores, which makes their recycling important from both economic and environmental perspectives. [5] Electronic scrap material with its significant gold content, is recognized as a new emerging and fast growing waste stream. Natrajan *et al.* in 2014 reported 17% higher gold value in e-waste as compared to the natural ores [18].

2.7 Obsolete Mobile SIM Cards: Metal enriched e-waste

With the growing telecom network and smartphone influence in the world, a large amount of mobile SIM card are being manufactured and marketed by telecom giants globally, nowadays the size of the SIM is reduced from macro level to latest nano SIM, depending upon the compatibility with the telecom device being used. Due to this, a huge volume of SIM cards is generated on daily basis globally as obsolete electronic waste. These little chips have been considered as a potential electronic waste for the extraction of precious and valuable metals.

A subscriber identity module or subscriber identification module (SIM) is an integrated circuit chip that is intended to securely store the international mobile subscriber identity (IMSI) number and its related key, which are used to identify and authenticate subscribers on mobile telephony devices such as mobile phones and pagers [19]. The chemical composition of a SIM card reveals it as a metal enriched source, with a lot of precious metals like gold, copper etc. being used to manufacture a single SIM card [20]. Pure gold is used in abundance as due to its no reactive and non-corrosive nature, it is used to shield the metal surface of the SIM card. Studies suggests us that, from about 1000 SIM Cards, 1 gm of Pure Gold can be leached out.

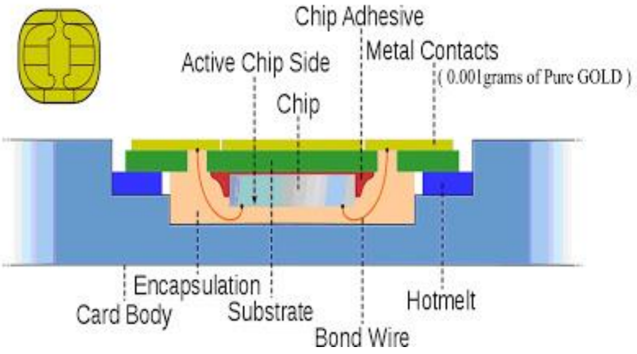


Figure 2.1: **Vertical Cross Section of a mobile SIM card.**

Image Courtesy: *www.metalminemedia.com*

The upper layer of the SIM card metal is coated with various precious metals like Gold and Silver, however gold is in majority due to its non-corrosive nature which helps in completing the electronic circuit without hampering the connection. The statistics provided suggest that, 1kg of SIM cards may have approximately 1.75 gm of pure gold which costs about INR 4400 [42] Therefore, recycling can be significant if the quantity of waste is in tonnes which can provide kilograms of gold [20].

2.8 Recovery of metals from e-waste

Large amount of e-waste is treated as junk and disposed off as municipal waste. E-waste, being rich in ferrous materials, nonferrous materials, plastic, and precious materials, has turned out as a major business opportunity for many. There are various types of traditional ways or methods to leach out precious metals from electronic waste, most commonly used is the recycling techniques and the ones used in extraction of metals in mineral ores are pyro metallurgy and hydro metallurgy. Specialized companies are responsible for the recycling and disposal of EEE, which is dismantled and sorted manually as shown in the figure 2.2.

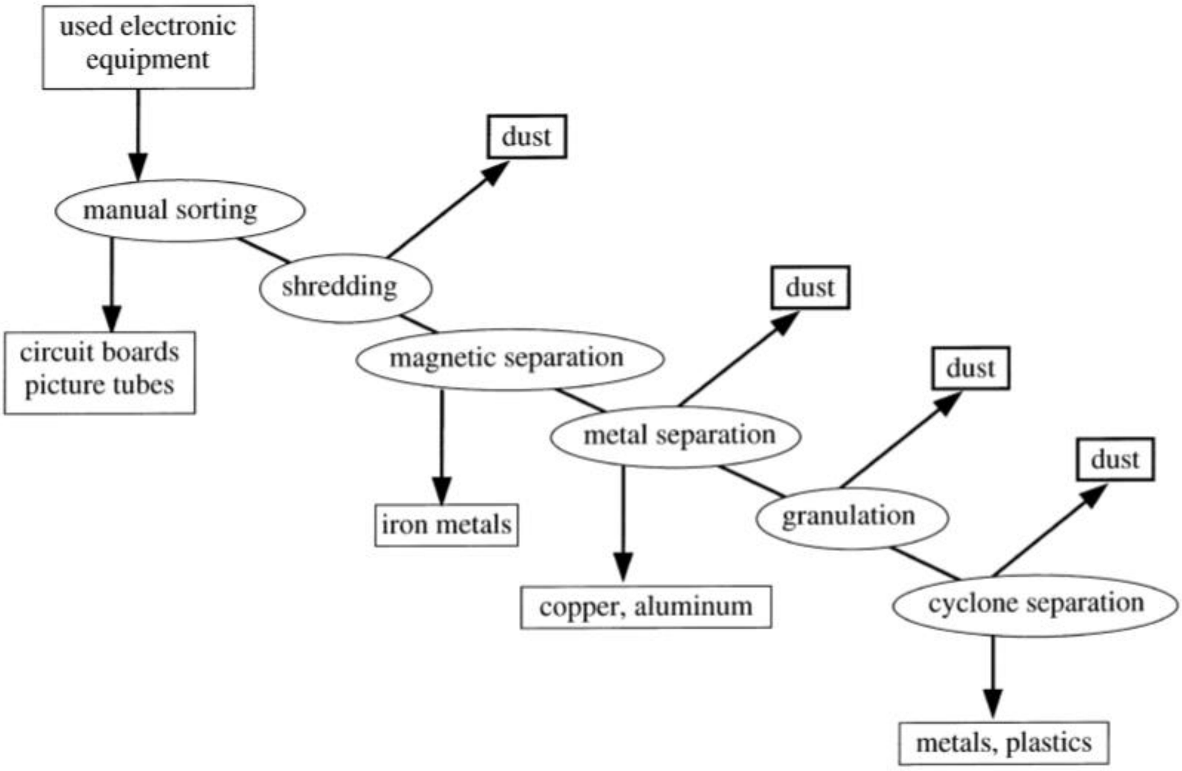
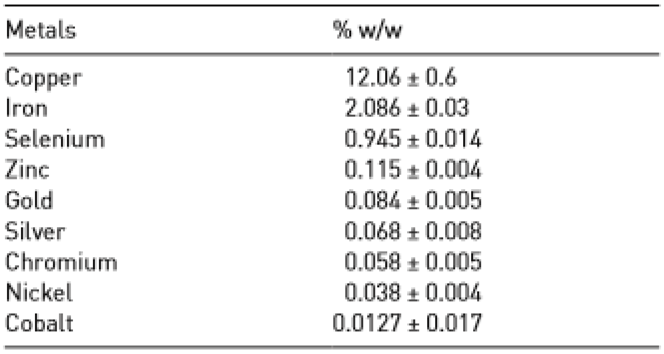


Figure 2.2: Schematic treatment process of used electronic equipment [6].

The resulting material is subjected to a mechanical separation process. Dust-like material is generated by shredding and other separation steps during mechanical recycling of electronic wastes [6]. The dust residues have to be disposed in landfills or incinerated. However, these residues can contain metals in concentrations, which might be of economic value [21]. Pradhan and Kumar, 2012 reported a fair concentration of various metals including precious metals that have a high economic value (Table 2.5).

There are some other metallurgical techniques on which the industries depend for recovering precious metals, most commonly used techniques are pyrometallurgy or thermal reclamation, heap leaching and hydrometallurgical techniques. Pyrometallurgy comprises of high temperature and melting of the material. Based on the temperature involved and nature of reactants pyrometallurgy is of two types: roasting and smelting [22].

Table 2.5: Metal analysis of electronic waste [5].



Metals	% w/w
Copper	12.06 ± 0.6
Iron	2.086 ± 0.03
Selenium	0.945 ± 0.014
Zinc	0.115 ± 0.004
Gold	0.084 ± 0.005
Silver	0.068 ± 0.008
Chromium	0.058 ± 0.005
Nickel	0.038 ± 0.004
Cobalt	0.0127 ± 0.017

Roasting is done at a high temperature of about 500-1200 ºC, involving reaction between solid and gaseous phases. Roasting is subdivided into calcinations, oxidation roasting, reduction roasting, chlorination and fluorination. Smelting is not just a melting process but comprises of complex reactions. The solid material interacts and reacts with the gaseous phase, giving rise to a number of liquid phases and altered gaseous phases. The liquid mixtures separate due to poor mutual solubility. Smelting comprises of ore smelting (reduction smelting, oxidation smelting, electrolyte smelting, etc.) and refining smelting (liquation refining, distillation refining, chlorine refining, etc.).

Heap leaching is an industrial mining process to extract precious metals, copper, uranium, and other compounds from ore via a series of chemical reactions that absorb specific minerals and then re-separates them after their division from other earth materials. similar to *in situ* mining, heap leach mining differs in that it places ore on a liner, then adds the chemicals via drip systems to the ore, whereas *in situ* mining lacks these liners and pulls pregnant solution up to obtain the minerals [23]. Hydrometallurgy or chemical reclamation takes place at comparatively at lower temperature 10-300°C on the interface between solid and liquid phases. There are different types of hydrometallurgy processes: leaching, refining, precipitation of metals from solution, etc. [22].

The conventional methods of pyrometallurgy and hydrometallurgy are still in practice despite their several disadvantages like high cost, high energy requirement, high consumption of leaching agents and release of toxic substances like dioxins, cyanide, chlorine, etc. [24].

These processes are also expensive because more investment is necessary to set up highly efficient facilities, there is a high consumption of energy and they are not regarded as an economical way to extract valuable components from electronic waste. Scientist now are more concerned to device new technologies to over these issues. Therefore, to overcome the disadvantages of these conventional practices, a new and promising technique called bioleaching has been successfully employed and is emerging as commercial exploitable technology applicable for metal extraction from electronic waste and low grade ores. Bioleaching is an environment friendly process without any emission of toxic substances. The leached and recovered metals can be recycled and re-used as raw material by metal manufacturing industries [25]. This process is more eco-friendly and economically feasible.

2.9 The bioleaching process

Bioleaching is a process that uses natural ability of the microorganisms to transform the metal present in waste in solid form to a dissolved form. It is the extraction of metals from their ores with the help of microorganisms. The microbes act on e-waste and solubilise the metals present in the same in an eco-friendly manner since it require less amount of energy and does not produce any harmful compounds as compared to physio-chemical processes. Bioleaching is an innovative, environmentally friendly, simple, economical and effective method, which has been adapted from the mining industry and used in various metal removal operations from soils, sediments, and sludge’s [26]. Bioleaching experiments can take place both in alkaline environment (involving cyanogenic bacteria) as well as acidic environment (including acidophilus species). The most commonly used bacterial strains are: mesophilic chemolithotrophic bacteria (*Acidithiobacillus ferroxidans* and *Acidithiobacillus thioxidans*), acidophilic moderately thermophilic bacteria (*Sulfobacillus thermosulfidooxidance* and *Thermoplasma acidophilum*), and cyanogenic bacteria (*Chromobacterium violaceum* and *Pseudomonas fluorescens*) [3]. Some fungal species have also been recorded and studied for the metal extraction like *Aspergillus niger* and *Penicillium simplicissimum.* [1].

There are numerous factors that affect the bioleaching process like temperature, pH of the medium, type of microorganisms, Quality and pulp density of e-waste, toxicity incorporated by e-waste and time. Advantages of bioleaching are mainly it being environmental, eco-friendly and harmless to the human and animal health. Also, no contamination of soil and water with toxic and hazardous substances, no gaseous emissions. This process is very economical as it is very cheap and requires less capital and there is a significant revenues through the recovery of contained metals -- Au, Ag, Cu, Ni, Zn, Co, Pb. It also provides local opportunities for work, training, development and the start of a new 'green' industry.

Table 2.6: Examples of industrial waste treated with bacterial leaching.

Waste	**Leached Metal**	**Used Microorganisms**
Lithium batteries	Li, Co	*Acidithiobacillus ferroxidans*
Fly ashes	Zn, Al, Cd, Cu, Ni, Cr, Pb, Mn, Fe	*Thiobacillus thiooxidans,* *Thiobacillus ferrooxidans,* *Aspergillus niger*
Tannery	Cr	Acidithiobacillus thiooxidans
Sewage sludge	Cu, Ni, Zn, Cr	Iron oxidizing bacteria
Used cracking catalyst, hydro processing catalyst	Al, Ni, Mo, V, Sb	*Aspergillus niger, Acidithiobacillus thiooxidans*
BOF slag from steel making, Slag from copper production	Zn, Fe, Cu, Ni	*Acidithiobacillus spp.,* *LeptospirillμM spp.*
Jewellery waste, automobile catalytic converter, electronic scrap	Ag, Au, Pt	*ChromobacteriμM violaceμM,* *Pseudomonas fluorescens, Pseudomonas plecoglossicida*
Electronic scrap	Cu, Ni, Al, Zn	*Acidithiobacillus ferrooxidans,* *Acidithiobacillus thiooxidans*

2.10 Cyanogenic Bacteria and mechanism of cyanide production

Cyanide is produced by a variety of bacteria and fungi, however, *Chromobacterium violaceum* is widely exploited for bioleaching of e-waste. *Chromobacterium violaceum* belongs to the genus *Chromobacterium*; it is rod-shaped with round ends, motile and Gram-negative. This genus has four important characteristics: indole metabolism and biosynthesis of violacein, production of cyanide, occurrence of unusual sugar compounds, and the production of extracellular polysaccharide. *C.violaceum* is rod-shaped, often cocco-bacillary. It is facultative anaerobic, although the oxidative strains grow slowly anaerobically. It is mesophilic and can grow in the temperature range from 10-40ºC, and the optimum temperature is at 30-37ºC. The optimum pH for its growth is 7-8. It produces a natural antibiotic called violacein, which may be useful for the treatment of colon and other cancers. The bacterium is soil and water organism common in the tropics and the subtropics, occasionally causing serious pyogenic or septicemic infection in mammals, including man.

The maximum cyanide production in *C.violaceum* occur in the onset of stationary phase. It is generally believed that the cyanide production in microorganism have any advantage to compete the inhibiting microorganisms [27].

Cyanide reacts with a number of metals forming highly water soluble complexes with a very high chemical stability. Cyanide has the ability to dissolve gold, the reaction is as follows:

$$2Au + 4CN- + H_2O + ½\ O_2 \rightarrow 2Au\ (CN)^{-2} + 2OH^-$$

Thus, cyanogenic organisms can be used in the bioleaching of precious metals like gold. Cyanogenic bacteria form biofilms on the surface of material [28] that helps cyanide to accumulate inside the biofilm and have close contact with the material, thus enhancing gold leaching efficiency even with low concentration of cyanides. Cyanide production by *C. Violaceum* is sufficient to dissolve gold while maintaining a high cyanide concentration which doesn't enhance gold dissolution [29].

There are various factors that governs the efficiency of gold bioleaching, like growth conditions, presence of enough oxygen as dissolved oxygen is the main factor affecting the efficiency of cyanide leaching of gold by bacteria. pH also plays and important role. HCN has a pKa of 9.2 and the concentration of cyanide of cyanide in solution is highly dependent upon pH [30].

$$H^+ + CN^- \leftrightarrow HCN$$

Thus, low pH shifts the equilibrium to the right, and thus promotes the production of HCN rather than CN^-. Temperature is also a crucial factor that influence gold bioleaching. Optimum temperature for the growth of *C. Violaceum* is 30-35ºC. Glycine is a precursor of cyanide which is formed by oxidative decarboxylation catalyse by HCN synthase which is membrane associated in *C.violaceum* [31]. It is first oxidized to iminoacetic acid. Then, the C-C bond is split, with a concomitant second dehydrogenase reaction which produces HCN and CO_2.

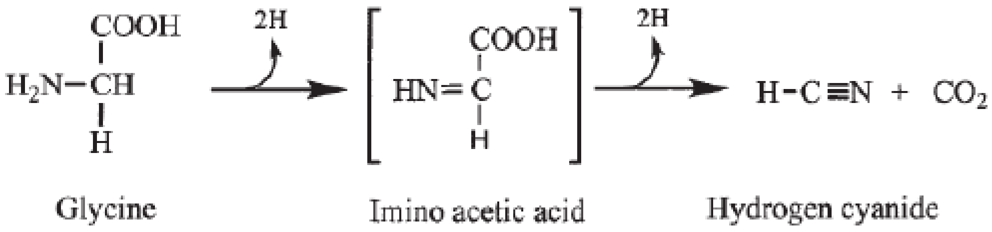


Figure 2.3: Formation of water soluble Cyanide complex, with Glycine as the precursor.

2.11 One step and two step Bioleaching Process

One step bioleaching is a process where the microbial strain is inoculated in the medium and incubated along with e-waste from the beginning of the experiment itself [32]. The two step bioleaching process is divided into 2 steps, step one is pre-culturing of microorganism in absence of electronic scrap till the time it is grown to its full potential. Step two is when the Electronic scrap is added after the complete pre-culturing and is further incubated to several days [32]. Metabolites produced, are used to leach out metal ions. Advantages of two step bioleaching process is that, Biomass is not in the direct contact with metal containing wastes and is believed to be appropriate to increase the metal leaching efficiency of micro-organisms from electronic waste [5]. Waste material is not contaminated by microbial biomass. Acid formation can be optimized in the absence of waste material. Higher waste concentration can be applied. Therefore, for more efficient metal mobilization, direct growth of micro-organisms in the presence of electronic waste or one step bioleaching is not advisable due to its toxic effects. Gold leaching by cyanogenic microorganisms generally involves a direct process with microbial production of metabolites. These metabolites dissolve gold from minerals by the formation of soluble metallic complexes. The chemical reaction for the dissolution of gold, the "Elsner Equation", follows:

$$4\ Au + 8\ NaCN + O_2 + 2H_2O \rightarrow 4\ Na[Au(CN)_2] + 4\ NaOH$$

In this redox process, oxygen removes, via a two-step reaction, one electron from each gold atom to form the complex $Au(CN)^{-2}$ ion. [33]

2.12 Pre-treatment of e-waste

In the presence of cyanide, many metals and metalloids can form well-defined cyanide complexes [34]. Owing to its abundance in e-waste, copper might pose a major problem due to its cyanicidic properties. In the presence of copper higher amounts of cyanide are needed to dissolve gold. Copper cyanide is produced in the form of various complexes, such as $Cu(CN)_2^-$,$Cu(CN)_3^-$ and $Cu(CN)_4^-$,depending on the pH and other conditions, whereas gold mostly forms dicyanide anions $[Au(CN)_2^-]$. [35] Copper cyanide complexes, particularly tetra-cyanate, are more stable above pH 9.0 and tri-cyanate (stable in the pH range 7.0-12.0) can dissolve gold when the cyanide concentration is high. With low concentrations of free cyanide, the $Cu(CN)_2^-$ complex will be the most prevalent species, and it may not oxidize gold [16]. Thus, it is necessary to reduce the copper content of the sample by a suitable method like physical beneficiation of e-waste, using acidophilic microorganisms or by using certain chemicals prior to bacterial leaching.

3.1 Instrumentation and other facilities used

Perkin Elmer A Analyst 400 Atomic Absorption Spectrophotometer, Eutechnist's pH meter, Centrifuge, Vacuum pump, 0.45µm glass fiber filter (Pall life sciences), refluxing instrument.

3.2 Procurement of obsolete mobile SIM cards

Obsolete mobile SIM cards were majorly procured from M/S Vicky Communications, Sanjauli, Shimla, H.P. and also from JUIT Campus.

3.3 Physical treatment of the obsolete mobile SIM cards

Obsolete mobile SIM cards were manually sorted into metal parts and plastic covering. Then the metal parts were crushed and grinned up to three different pore size between 355 μm to 700 μm.

SIM particle size **~ 355** μm SIM particle size b/w 355-700 μm SIM particle size > 700 μm

Figure 3.1: Three different particle size of mobile SIM card metal used in this study.

Figure 3.2: Physical treatment of obsolete mobile SIM cards pre 2 step Bioleaching experiment.

3.4 Metal content analysis

The metal content of three different particle sizes (A: ≤ 355 µm, B: 355 -700 µm and C: >700 µm) were analyzed. One gram of waste SIM Card sample was dissolved in 100 mL of aqua regia (HNO_3: HCl = 1:3) by refluxing in a round-bottom flask for 1 h at 100 °C.

The solution was allowed to cool and the volume was made up to 100 mL with deionized water. After that, the solution was passed through glass fiber filters to guarantee particle-free suspensions and stored at 4 °C for further analysis. The concentrations of dissolved metal ions were determined by atomic absorption spectrometry (Perkin Elmer AAnalyst 400) at the following wavelengths (nm): Ag (328.1), Au (242.8), Co (240.7), Cu (324.8), Cr (357.9), Fe (248.3), Ni (232.0), Zn (213.9) [5].

Figure 3.3: Acid digestion of e-waste using aqua regia.

3.5 Pretreatment of e-waste

Owing to its abundance in SIM card metal, copper interfered in cyanide - gold ion complex formation and also increase the toxicity of the medium. Henceforth, it was removed using nitric acid. The metal particles were added to 6 M nitric acid at room temperature and was efficiently mixed. SIM card metal particles were added incrementally to the nitric acid to prevent excessive frothing due to production of nitric oxide gas. The mixture was centrifuged and the residue was washed with deionised water and re-centrifuged. The cycle was repeated until traces of blue-green copper nitrate were no longer visible. The pretreated SIM metal pellet were finally removed, dried and weighed to constant mass [36]. The leachate as well as dry pellet of e-waste were further subjected for metal content analysis using aqua regia.

3.6 Organisms and culture conditions

Chromobacterium violaceum was procured from microbial repository of the Department of Biotechnology and Bioinformatics at Jaypee University of Information Technology, Waknaghat. Organism was cultivated under sterile conditions in baffled 250-mL flasks and incubated at 30^{o}C on a rotary shaker at 150 rpm. The growth curve was analyzed by taking OD of the sample at 600 nm at regular interval to confirm the growth. The organism was also preserved on LB agar plates, LB agar slants and also in glycerol stocks stored at -20 ^{o}C.

3.7 Two-step bioleaching process

A two-step bioleaching process is believed to be appropriate to increase the metal leaching efficiency of micro-organisms from electronic waste. Microorganisms were grown in the absence of electronic waste to produce biomass followed by the addition of different concentrations of electronic waste for metal mobilization for an additional time period of 7 days [5].

50 ml of LB broth medium was prepared in a 150 ml flasks and glycine was added to it with the concentration of 5 gm/L. The pH was set to 9 with sodium hydroxide. The flasks were autoclaved at 121 °C and 15 psi pressure for 15 min.

Pure culture of *Chromobacterium violeceum* was inoculated into experimental flasks aseptically and incubated at 30 °C temperature and 150 rpm. After 2 days of incubation, 0.5 g of sterilized mobile SIM metal powder (1% w/v) was added to each flask under aseptic conditions. An un-inoculated control was run in parallel. Subsequently, the experimental flasks were incubated at 30 °C temperature and 150 rpm for another 7 day time period [5]. The leachate was analyzed for the presence of metal ions, final pH of the medium and microbial growth in terms of total protein content.

The pH was measured by digital pH meter (Name of the company). The samples were filtered through a Whatmann no. 1 filter paper to remove solid particles and centrifuged at 10 000 rpm for 10 - 12 minutes to remove bacterial cells. Further leachate was filtered through 0.45 micro meter glass fiber filter to ensure particle free suspension prior to AAS analysis.

Figure 3.4: Perkin Elmer A Analyst 400 Atomic Absorption Spectrophotometer used for Analysis.

3.8 Micro-biuret method for estimation of proteins

A standard curve was plot for Bovine Serum Albumin (BSA), taken as reference protein to determine the concentration of proteins in the medium. Two reagents were prepared:

(1) 0.21% $CuSO4.5H_20$ in 40% NaOH (to prevent precipitation of copper hydroxide, this should be prepared by adding dilute copper sulfate (e.g., 1%) to the alkali).

(2) 40% NaOH.

To proceed further with the method, the following mixtures were prepared:

(A1) 2 ml distilled water + 1 ml reagent 1.
(A2) 2 ml protein solution + 1 ml reagent 1.
(B1) 2 ml distilled water + 1 ml reagent 2.
(B2) 2 ml protein solution + 1 ml reagent 2.

All mixtures were shaken vigorously. The optical density at 310 nm of mixture (A1) is read against (A2) and of (B1) against (B2) giving values Da and Db, respectively. The difference, Da - Db, gave the optical density. Various Dilutions from the stock of 1gm/ml of BSA were prepared and a graph was plot between OD and the protein concentration in various dilutions. [37]

Now, to estimate the proteins concentration of unknown samples, the protein solution was centrifuged at 10,000 rpm for 10 minutes. The supernatant was discarded and pellet was suspended in 1 ml of 4M NaOH. Further, similar mixtures were prepared and similar methodology was followed to determine the OD of the sample, which could then correspond to a specific protein concentration on BSA standard curve.

3.9 Spent medium leaching

Following the similar methodology, 50 ml of LB broth medium was prepared in a 150 ml flasks and glycine was added to it with the concentration of 5 gm/L. The pH was set to 9 with sodium hydroxide. The flasks were autoclaved at 121 °C and 15 psi pressure for 15 min. these flasks were then inoculated with pure *Chromobacterium violeceum* culture aseptically and incubated at 30 °C temperature and 150 rpm for 2 days.

In spent medium leaching, after 2 days, cells were separated from the culture by centrifugation (at 4000 rpm) after it reached maximum cell density and cyanide production In the spent medium leaching only cell-free metabolites were used [38].

0.5 g of sterilized mobile SIM metal powder (1% w/v) was added to the flask containing medium with cell free metabolites under aseptic conditions. An un-inoculated control was run in parallel. Seeking for the effect of temperature on leaching, these experimental flasks were then incubated at 50 °C temperature and 150 rpm for another 7 day time period.

4.1 Revival of *Chromobacterium violaceum*

Chromobacterium violaceum is a gram negative facultative anaerobic, although the oxidative strains grow slowly anaerobically. It is mesophilic and grow in the temperature range from 10-40ºC, and the optimum temperature is at 30-37ºC. The optimum pH for its growth is 7-8. It readily grew on nutrient agar producing distinct, smooth, low convex colonies (Fig. 4.1). *C. Violaceum* produced violacein in the presence of tryptophan; it has antibiotic properties and is violet in colour. The bacterium is soil and water organism common in the tropics and the subtropics [41].

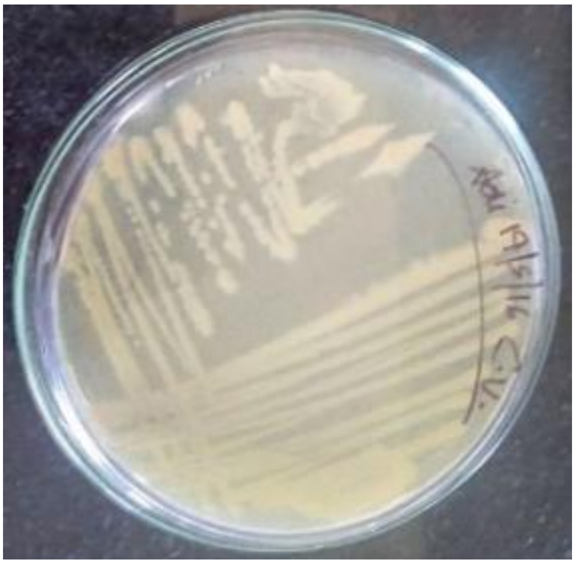


Figure 4.1: Culture plate showing colonies of *Chromobacterium violaceum*.

4.2 Growth Curve of *Chromobacterium violaceum*

Chromobacterium violaceum was grown in a LB medium with a pH of 9 and incubated at 30ºC and 150 rpm. Its growth was monitored according to its generation time. Sample were taken after a 4 hour interval and optical density was measured at 310 nm (Micro Biuret method was applied).

The growth curve thus obtained confirmed the capability of *Chromobacterium violaceum* to grow under alkaline conditions with a pH of 9. Microbial growth at alkaline pH favors higher cyanide production and increases stability of metal cyanide complexes thereby enhances metal bioleaching [40]. The growth curve is shown in figure 4.2.

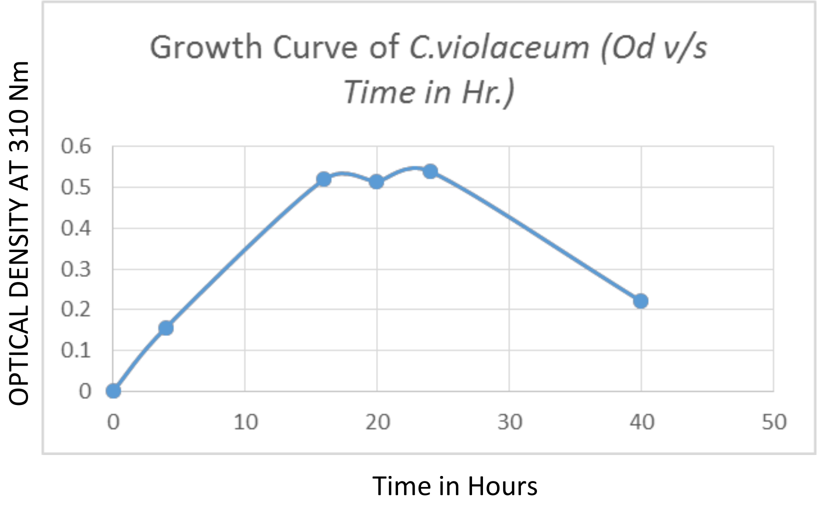


Figure 4.2: Growth Curve of *Chromobacterium violaceum* grown in a medium of pH 9.

4.4 Chemical content analysis for various particle size of SIM e-waste

The metal particles of three different sizes (> 355 µm, between 355 µm and 700 µm and <700 µm) were analyzed for metal analysis content by the chemical leaching method. One gram of SIM Card electronic waste sample was dissolved in 100 mL of aqua regia (HNO_3: HCl = 1:3) by refluxing in a round-bottom flask for 1 h at 100 °C.

The results showed manifold increase in the concentration of precious metal, as previously reported in the studies conducted by Pradhan and Kumar in 2012 using printed circuit boards as the e-waste source and many similar studies conducted by Shin *et al.* in 2013, Chi *et al.* in 2011, Natrajan *et al.* in 2014, Brandl *et al.* in 2008 and Song Buck Tay in 2013 using printed circuit boards or other types of ESM.

Primarily talking about gold, the concentration in the SIM card was found between 0.16% to 0.42% varying due to the particle size, where maximum was found in the particle size <355 µm. This was nearly 6 times greater than that found in printed circuit boards as reported in the literature [5][6][18].

The concentration of Copper was found 75% with maximum found in the particle size >700 µm. This too was nearly 6 times greater than that found in the printed circuit boards as reported by Pradhan and Kumar in 2012 [5].

The concentration of copper was found between 0.003% - 0.22% and maximum was reported in size <355 µm. Even the concentration of silver was 4 times greater than the earlier reports with printed circuit boards [5].

All in all, mobile SIM cards are found to be a heavily metal enriched source and a potential category of e-waste to leach out precious metals. Although higher the metal content, more toxic it becomes for the microorganism to grow.

Table 4.1: Chemical content analysis for various particle size of SIM e-waste

Metal	Composition in % w/w
Size <355 µM	
Au	0.23±0.047
Ag	0.22±0.002
Cu	20.8±0.204
Size 355—700 µM	
Au	0.42±0.003
Ag	0.01±0.003
Cu	75.84±0.323
Size >700 µM	
Au	0.16±0.042
Ag	0.003±0.001
Cu	76.32±0.197

4.5 Bioleaching of SIM e-waste of different particle size

In order to reduce the toxic effects of e-waste on microorganisms, a two-step process was applied. In the first stage, biomass was produced in the absence of electronic waste. Subsequently, e-waste with pulp density 10 gm/L or 1% w/v was added and cultures were incubated for 7 days.

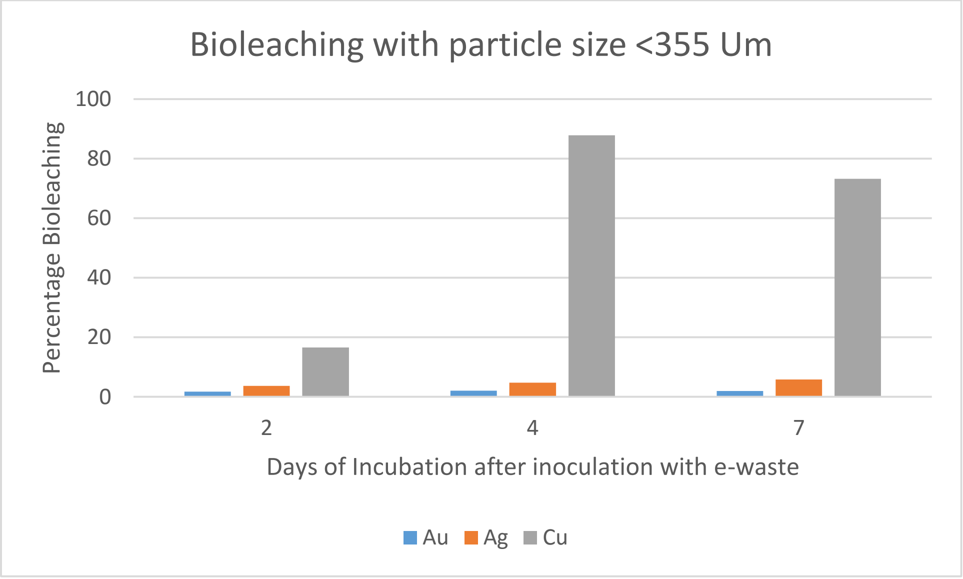
SIM e-waste with particle size <355 µm reported metal mobilization i.e. 2.06% of Au, 5.86% of Ag and 87.8% Cu after 7 days of incubation. SIM e-waste with particle size 355-700 µm reported metal mobilization i.e. 0.44% Au, 2.55% Ag and 13.79% Cu after 7 days of incubation. For particle size >700 µm, metal mobilization was found to be 2.36% for Au, 0.88% for Ag and 13.89% for Cu after 7 days of incubation.

This shows us that maxim metal mobilization for Au, Ag and Cu was found to be 2.06%, 5.86% and 87.8% respectively with particle size <355 µM. These results are significantly less than those obtained from two-step bioleaching process with PCB as e-waste where maximum metal mobilization reported as 11.31% of Au with similar operational parameters [39].

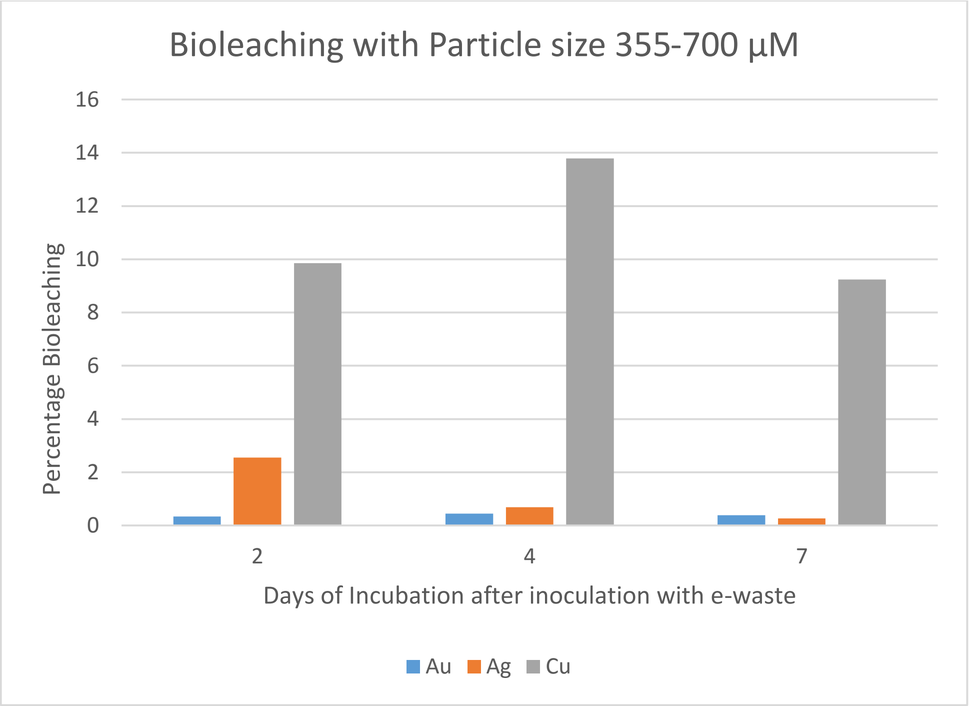
This is due to the manifold increased metal composition of SIM e-waste especially for gold and copper which results in higher level of metal toxicity during incubation [40]. Hence reduced level of cyanidation and metal mobilization.

A separate control was run in parallel i.e. a flask which was inoculated with only e-waste and not the bacteria was also incubated for a period of 7 days to see the effect of media on metal mobilization. Au and Ag did not show any metal mobilization with any particle size. Cu although, showed a significant metal mobilization i.e. 24.49% with particle size <355 µM, 6.39% with particle size 355-700 µM and 5.59% with particle size >700 µM. Similar findings were reported by Brandl *et al.* 2001; Natrajan *et al.* 2014 from e-waste of computer printed circuit board.

This tells us that the LB medium, with glycine as a precursor for cyanide formation also produces tetra-cyanate ions such as $Cu(CN)^{2-}$, $Cu(CN)^{3-}$ and $Cu(CN)^{4-}$ that mobilizes Cu into the medium[43].



(A)



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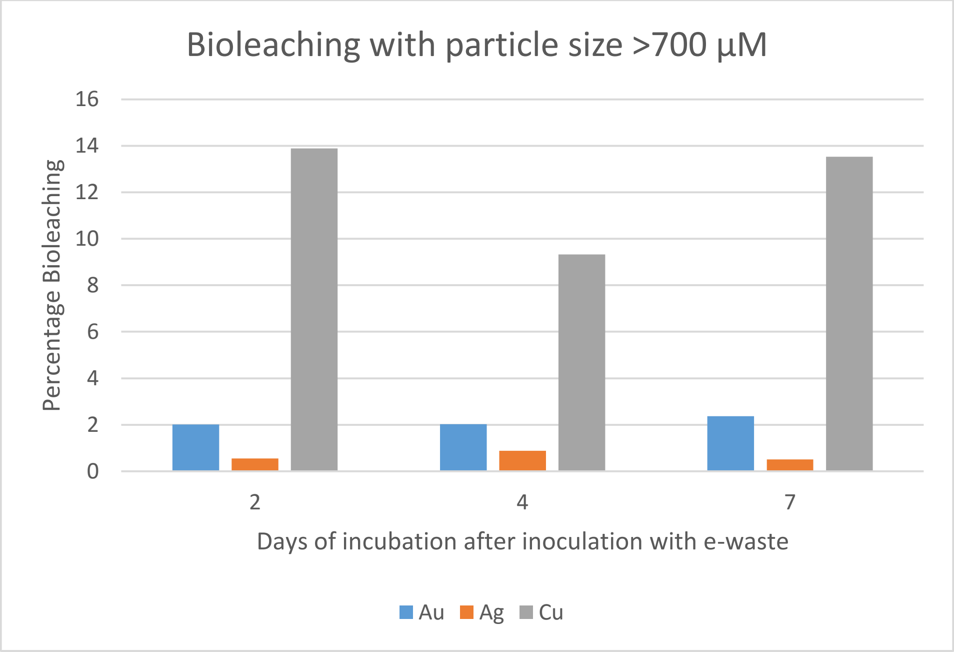


(C)

Figure 4.3: Percentage Bioleaching of precious metals from SIM e-waste of different particle size. (A) Bioleaching of Au, Ag and Cu with particle size <355 µm, (B) Bioleaching of Au, Ag and Cu with particle size 355-700 µm, (C) Bioleaching of Au, Ag and Cu with particle size >700 µm.

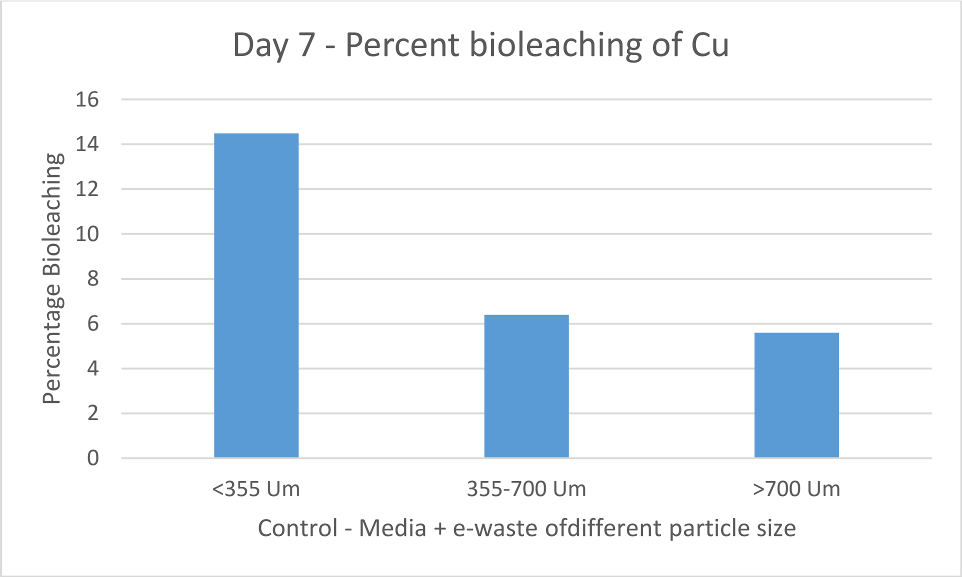


Figure 4.4: Percentage Bioleaching of Cu in control (Media + e-waste) with different particle size.

4.6 Bacterial growth estimation of two step bioleaching

The results in figure 4.5 show us that, the bacteria are able to grow at an alkaline pH as well as in a toxic environment due to the presence of electronic waste. The protein concentration is in proportion with the amount of bacterial biomass generated as the bacteria grow. The trend in the graph shows us that after growing for almost 48 hours to an optimal level before the inoculation of e-waste, the maximum protein concentration was found to be 800 mg/L to 1300 mg/L even after two days of inoculation with e-waste. The protein concentration progressively declines by the end of 4 days and reaches almost negligible by the end of 7th day of incubation. This was due to the high level of toxicity due to the e-waste which inhibited the growth of the microorganism. Although, *C. violaceum* was able to survive for 4 days in the presence of e-waste and also under alkaline pH conditions.

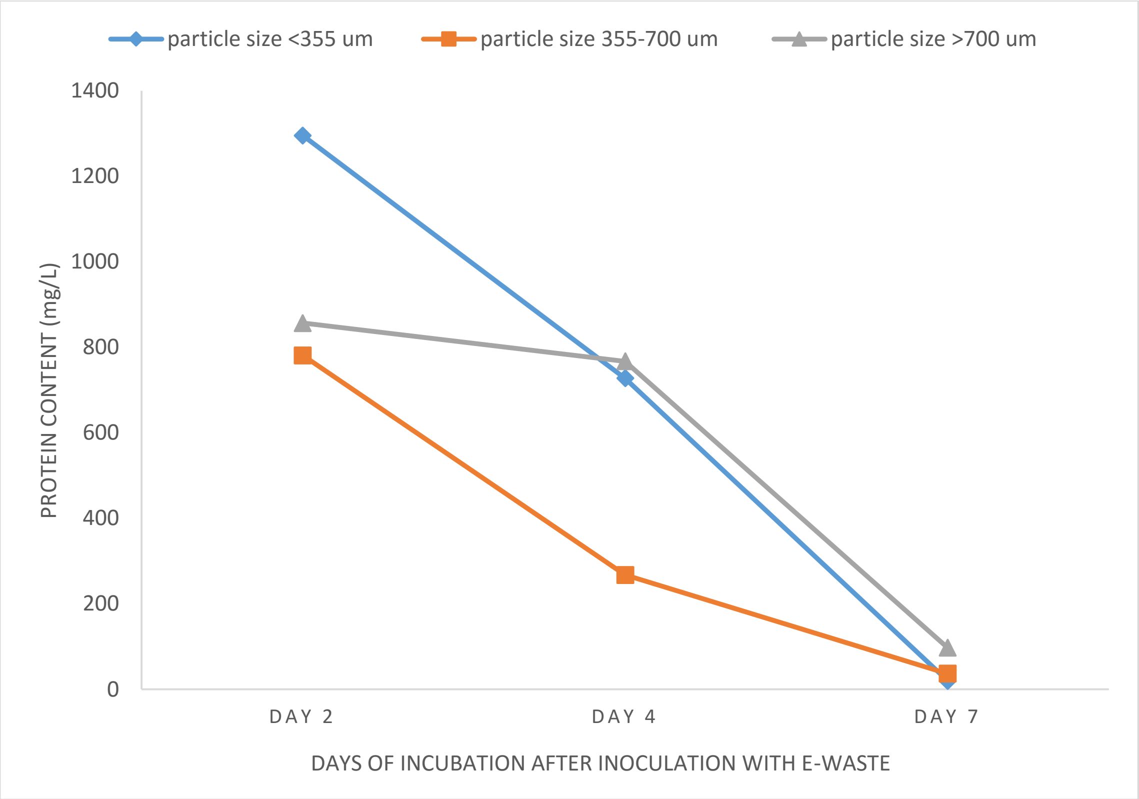


Figure 4.5: Bacterial growth estimation during two-step bioleaching experiment for different particle size of e-waste inoculated during the experiment.

4.7 pH Profile of Two step bioleaching using various particle size of SIM e-waste

The results are shown in Figure 4.6. Fig 4.6(A) gives us the trend of the pH profile for particle size > 355 µm. Initial pH was set to 9.0. After inoculation with *C. violaceum* incubation for two days, the pH fell to 8.9. Similar trend was seen with the particle size between 355-700 µm and >700 µm as shown in figure 4.8 (B and C). The decrease in the pH of the medium was attributed to the acidification process for the conversion of organic compounds, particularly glycine, into carboxylic acids such as glyoxylic, cyanoformic, and oxalic acids [39].

After inoculation with e-waste of different particle size, and incubation for further seven days, shows a common pH trend with all the particle size that was a sudden increase in pH to 9.4 during the course of incubation. The increase in pH after the point when e-waste was added was due to the increased generation of cyanide via the conversion of intermediate products such as carboxylic acids [40] also the pH of the system was near to the pKa value of the cyanide which added on to its accumulation in the medium so as to have maximum leaching. Although, by the end of 7th day, pH dropped to a level of 9.1 due to the unavailability of microorganism to produce cyanide, though the presence of e-waste added on to the alkalinity of the medium.

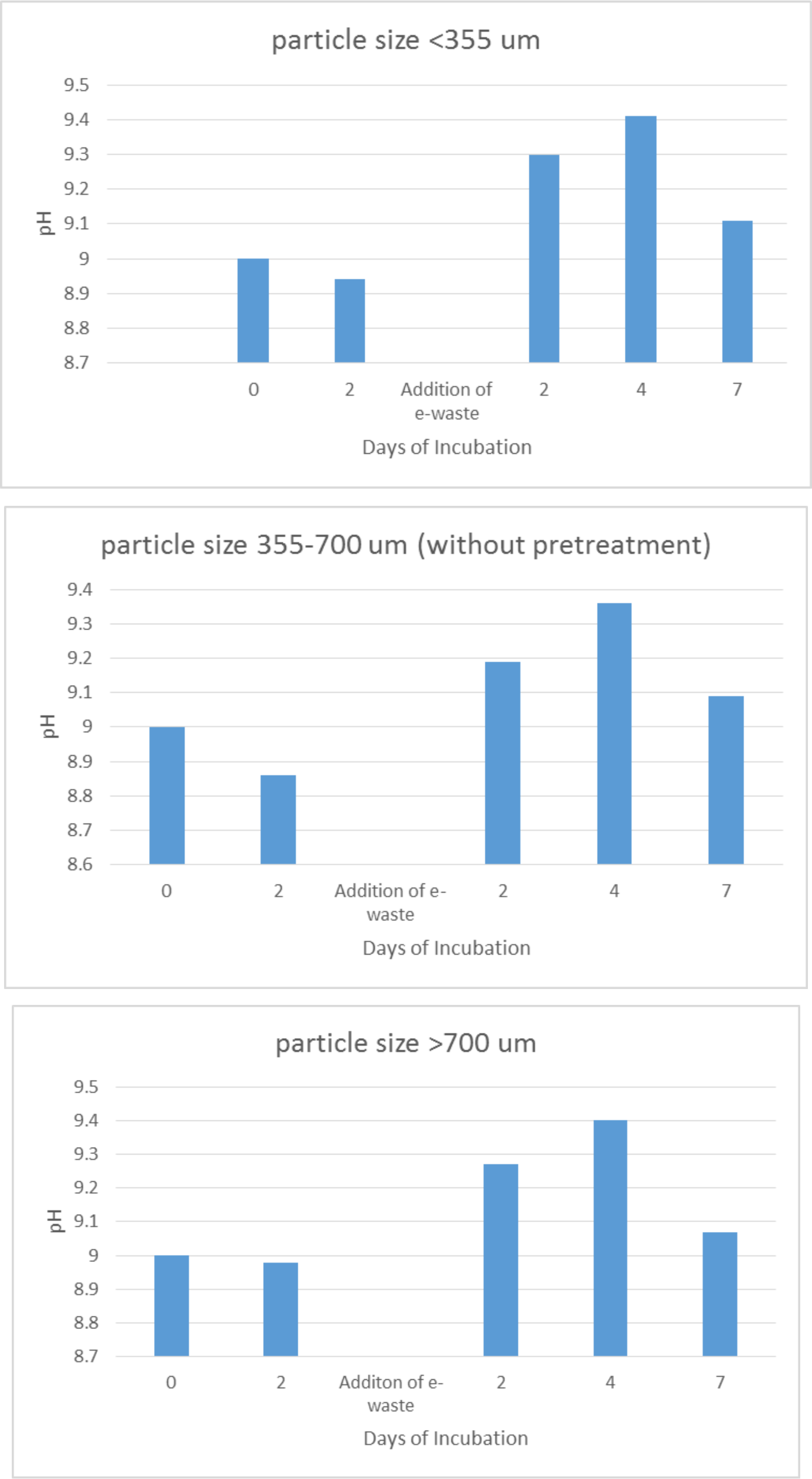


Figure 4.6: pH Profile of Two step bioleaching using various particle size of SIM e-waste. (A) SIM particle size <355 µm. (B) SIM particle size between 355 µm -700 µm. (C) SIM particle size > 700 µm

4.8 Chemical Content Analysis of Pretreated SIM e-waste

Owing to its abundance in SIM card metal, copper interfered in cyanide - gold ion complex formation and also increase the toxicity of the medium. Henceforth, it was removed using a pretreatment technique that involved nitric acid [36].

The pretreated SIM e-waste was subjected to refluxing using aqua regia at 100 °C. The resulting leachate after the analysis using AAS, showed a 72% reduction in Copper which was found to be only 20% as compared to 76% found in untreated SIM e-waste.

Gold and Silver concentration was found to be 0.4% and 0.01%, respectively. This was almost equal to chemical composition in the untreated SIM e-waste. This shows that Nitric acid possesses special affinity towards copper and thereby does not leach out gold or silver.

Table 4.2: Chemical Content analysis of pretreated SIM e-waste with particle size 355-700μm.

Metal	**Composition (%w/w)**
Au	0.40±0.003
Ag	0.01±0.0008
Cu	20.68±0.146
Cr	0.23±0.002
Ni	1.21±0.008
Co	0.02±0.0001
Zn	0.07±0.0005
Fe	0.58±0.004

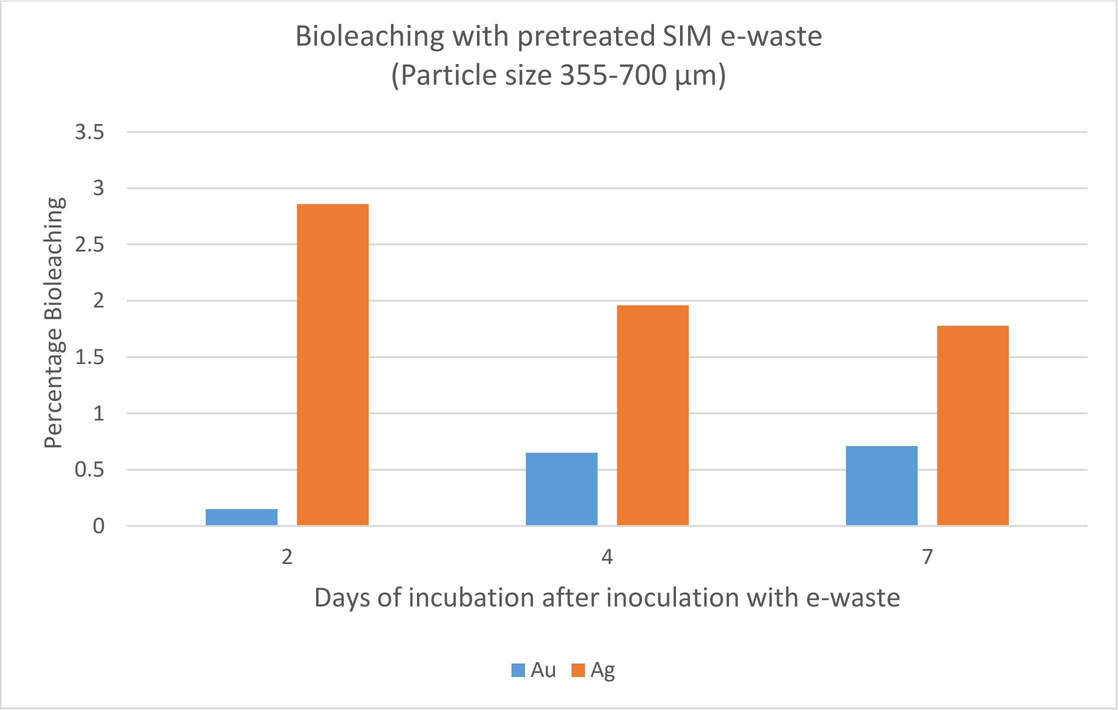
Table 4.3: Comparison between the chemical composition of e-waste of size 355-700 µM with and without the pretreatment and the percentage reduction in various metal content.

Metal	Composition % w/w		% Reduction
	Without treatment	With treatment	
Au	0.42	0.40	4.80
Ag	0.01	0.01	0.00
Cu	75.84	20.68	72.73
Cr	0.50	0.23	53.15
Ni	3.17	1.21	61.78
Co	0.02	0.02	16.67
Zn	0.05	0.07	0.00
Fe	1.28	0.58	54.83

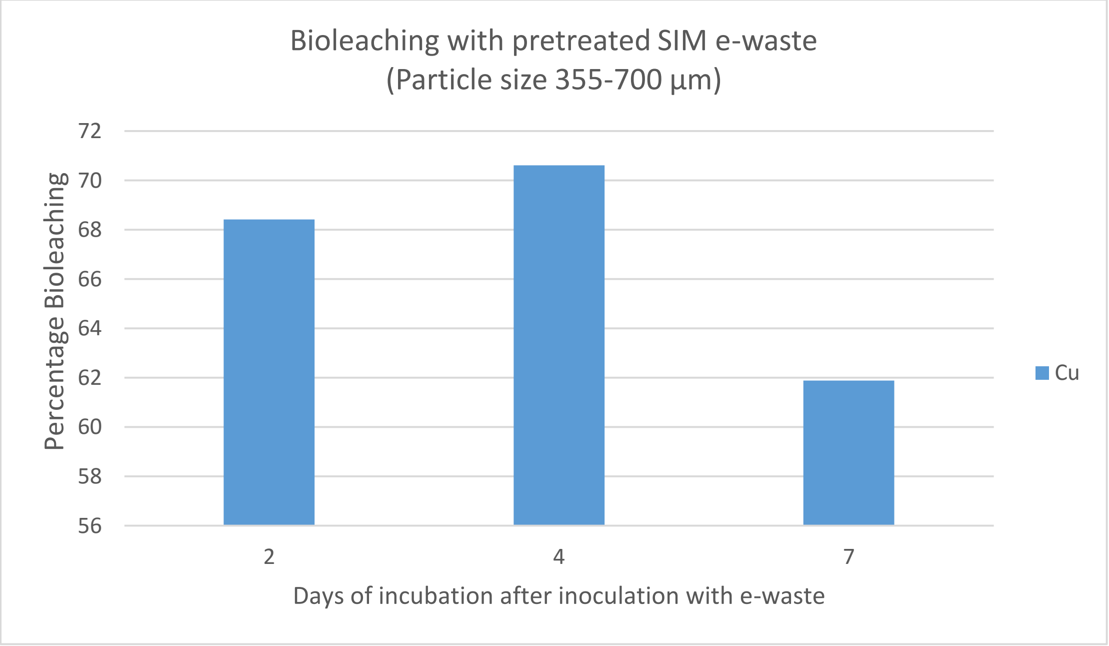
4.9 Bioleaching of pretreated SIM e-waste

Pretreatment of SIM e-waste with particle size 355-700 µm was done to remove excess of Cu from the e-waste so that it does not interfere with Au in making complexes with cyanide and hence maximum mobilization of Au takes place [40]. This was also done to reduce the metal toxicity of e-waste during incubation.

Metal mobilization was found out to be 0.71%, 2.86% and 70.61% for Au, Ag and Cu, respectively. The experiment was setup so as to increase the metal mobilization of Au, which although increased by 35% as compared to the Au mobilization for untreated SIM e-waste with similar particle size.



(A)



(B)

Figure 4.7: Percentage Bioleaching of precious metals from pretreated SIM e-waste with a particle size of 355—700 µm. (A) Percentage Bioleaching of Au and Ag and (B) Percentage Bioleaching of Cu.

4.10 Bacterial growth estimation of pretreated SIM e-waste

The results show us that, the bacteria are able to grow at an alkaline pH as well as in a toxic environment due to the presence of electronic waste. The protein concentration is in proportion with the amount of bacterial biomass generated as the bacteria grow. The trend in the graph shows us that after growing for almost 48 hours to an optimal level before the inoculation of e-waste, the maximum protein concentration for the pretreated SIM e-waste was found to be 900 mg/L which was slightly more than the maximum protein concentration of the medium containing untreated SIM e-waste, even after two days of inoculation with e-waste. This shows that the pretreatment reduces the toxicity level of the e-waste as most of the copper was leached out initially.

The protein concentration progressively declines by the end of 4 days and reaches almost negligible by the end of 7th day of incubation. This was due to the high level of toxicity due to the e-waste which inhibited the growth of the microorganism. Although, *C. violaceum* was able to survive for 4 days in the presence of e-waste and also under alkaline pH conditions.

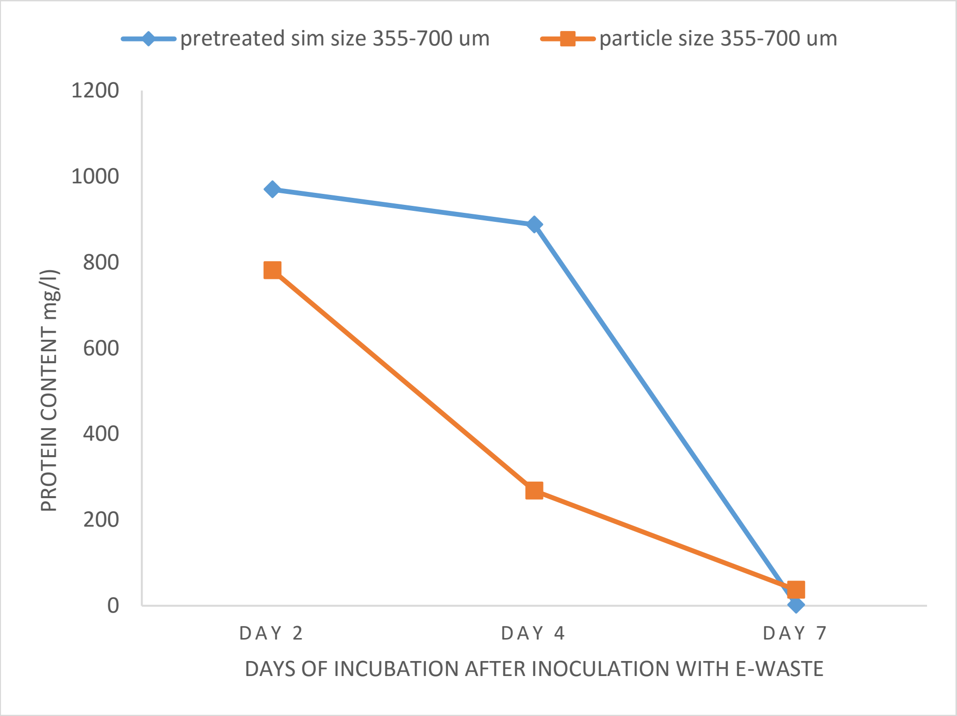


Figure 4.8: Bacterial growth estimation with and without pretreatment.

4.11 pH profile of pretreated SIM e-waste

The results are shown in Figure 4.9 gives us the trend of the pH profile of two step bioleaching with pretreated SIM e-waste of particle size between 355-700 µm. Initial pH was set to 9.0. After inoculation with *C. violaceum* incubation for two days, the pH fell to 8.8. The decrease in the pH of the medium was attributed to the acidification process for the conversion of organic compounds, particularly glycine, into carboxylic acids such as glyoxylic, cyanoformic, and oxamic acids [39].

After inoculation with e-waste and incubation for further seven days, shows a pH trend with a sudden increase in pH to 9.4 during the course of incubation. The increase in pH after the point when e-waste was added was due to the increased generation of cyanide via the conversion of intermediate products such as carboxylic acids [40] also the pH of the system was near to the pKa value of the cyanide which added on to its accumulation in the medium so as to have maximum leaching. Although, by the end of 7th day, pH dropped to a level of 9.1 due to the unavailability of microorganism to produce cyanide because of toxicity, though the presence of e-waste added on to the alkalinity of the medium.

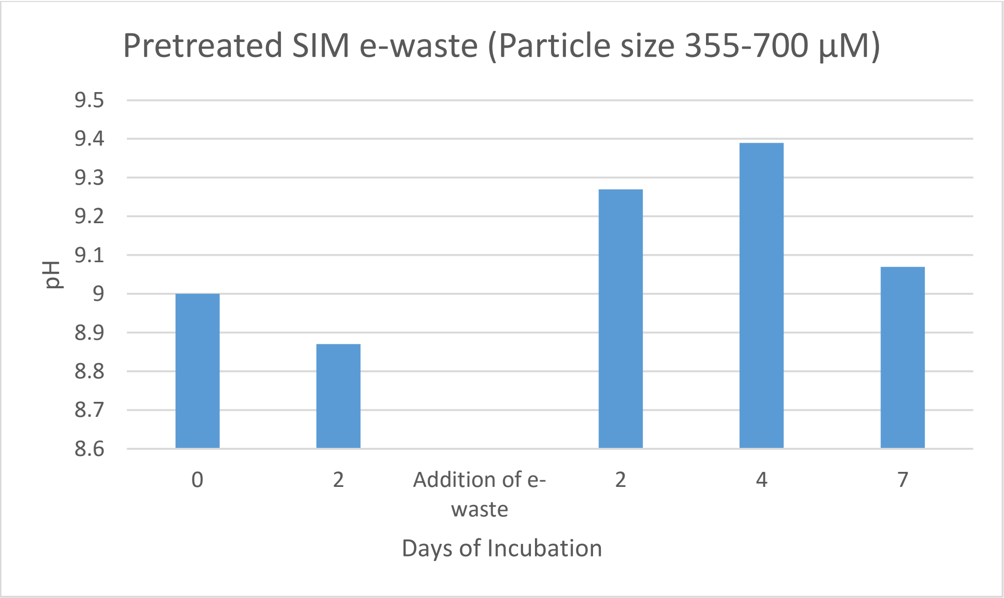


Figure 4.9: pH Profile of Two step bioleaching with pretreated SIM e-waste.

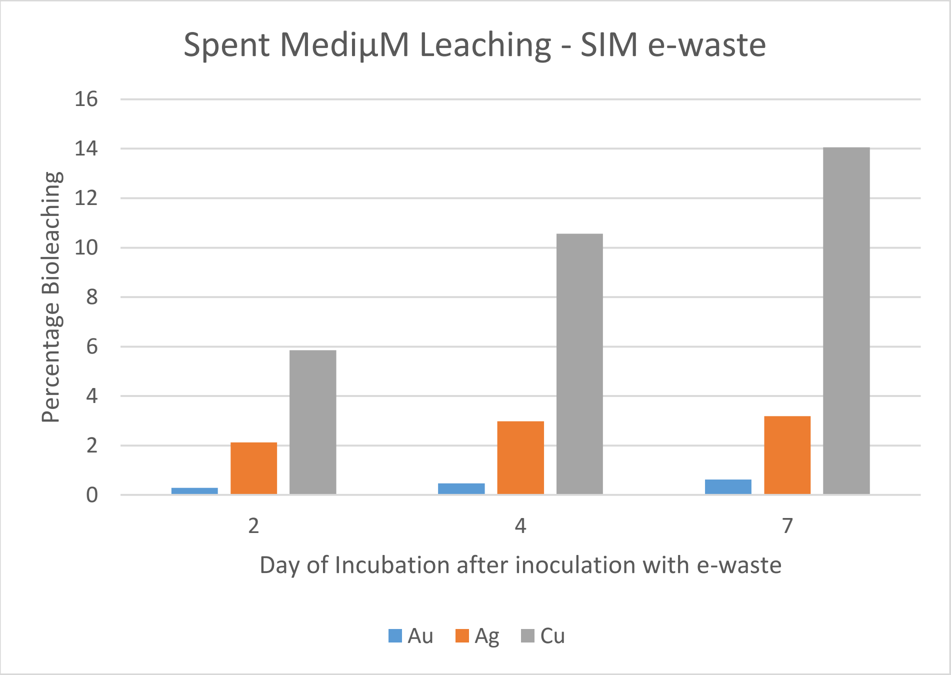
4.12 Spent medium leaching – Percentage Bioleaching

Spent medium leaching experiment was setup to check reduce the effect of metal toxicity on the bacterial growth and to check the effect of temperature on leaching process. Incubation was done at 55°C and only the cell free metabolites were subjected to the bioleaching.

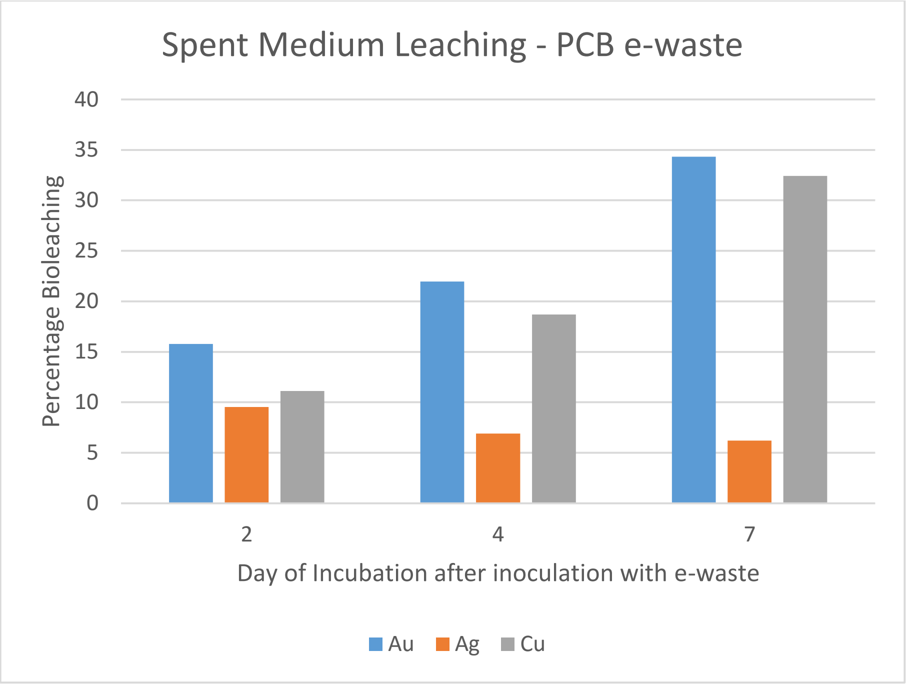
The metal mobilization from SIM e-waste and PCB e-waste undergone spent medium leaching was compared. Percentage mobilization for Au was reported to be 0.62% from SIM e-waste and 34.34% from PCB e-waste. Ag reported a percentage mobilization of 3.19% from SIM e-waste and 9.52% from PCB e-waste. Percentage mobilization for Cu came out to be 14.05% from SIM e-waste and 32.41% from PCB e-waste.

A set of controls were also incubated in parallel with the experiment. Au and Ag did not show any metal mobilization in the control medium. Although Cu reported some significant results.

Percentage mobilization of Cu from SIM e-waste of particle size 355-700 µm with control 1, i.e. medium + e-waste and with control 2, i.e. water + e-waste came out to be 9.76% and 3.12% respectively. Percentage mobilization of Cu from PCB e-waste with control 1, i.e. medium + e-waste and with control 2, i.e. water + e-waste came out to be 25.07% and 6.25%, respectively.



(A)



(B)

Figure 4.10: Percentage bioleaching of Au, Ag and Cu from spent medium leaching (A) from SIM e-waste and (B) from PCB e-waste.

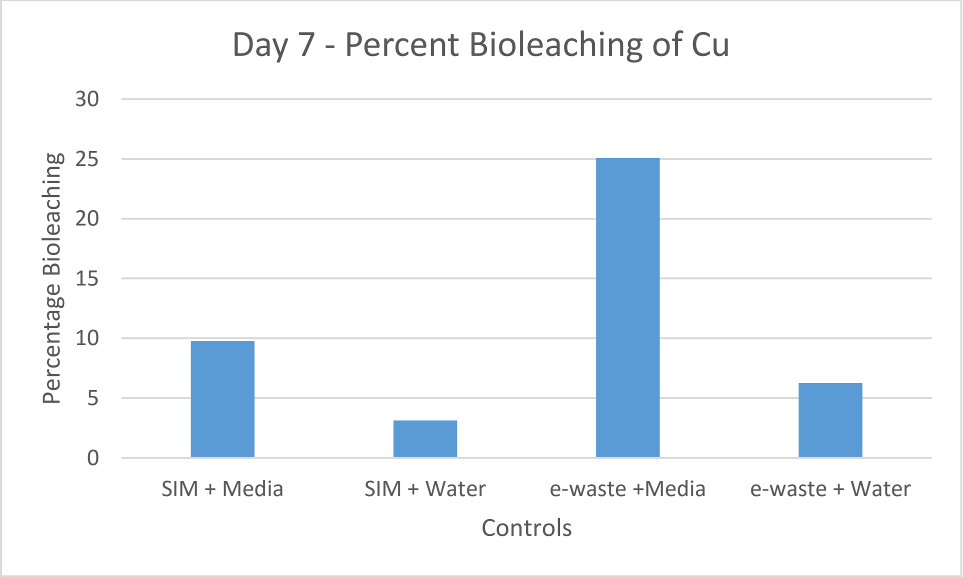
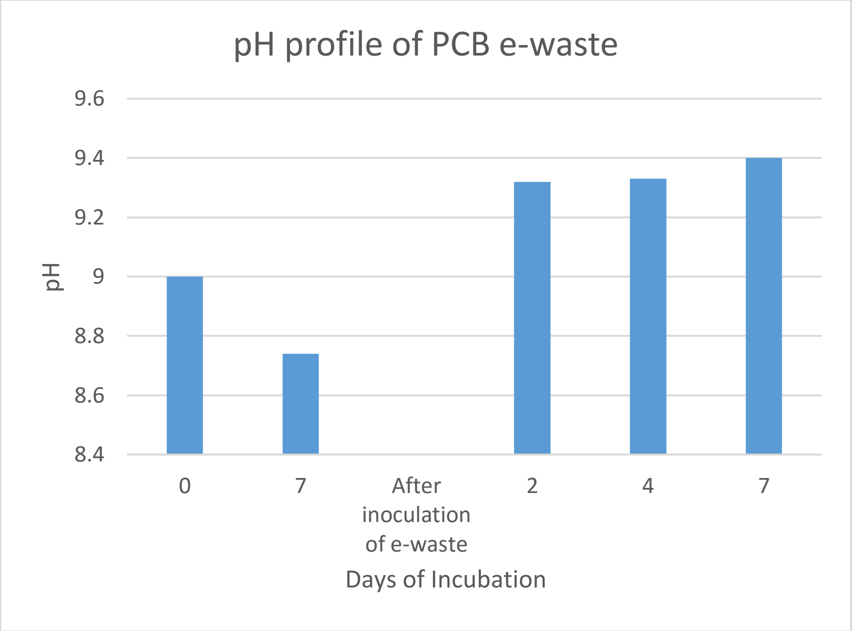


Figure 4.11: Percentage Bioleaching of Cu in various controls set up in spent medium leaching.

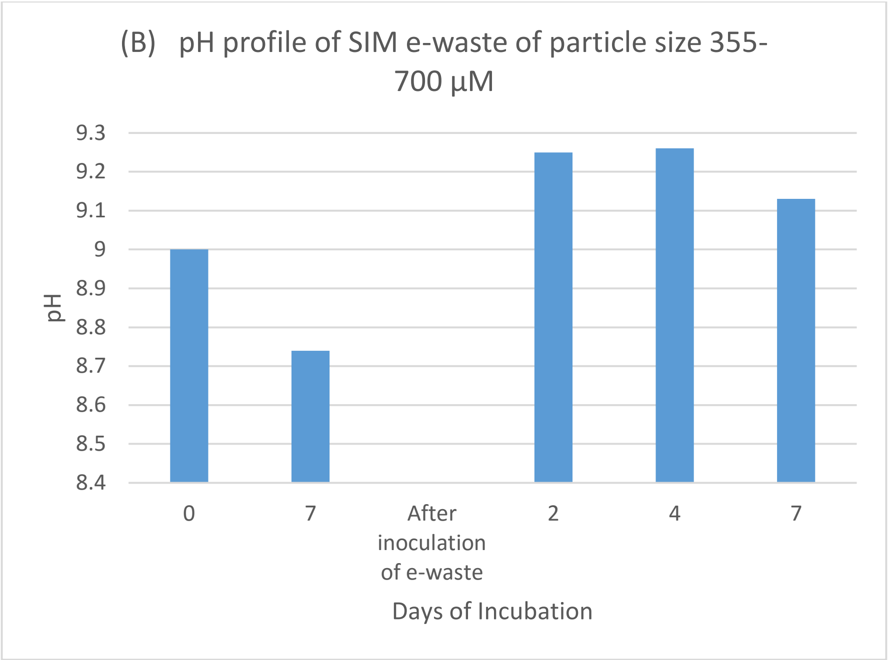
4.13 Spent Medium Leaching – pH Analysis

The results are shown in Figure 4.12. Fig 4.12(A) gives us the trend of the pH profile for medium with PCB e-waste. Initial pH was set to 9.0. After inoculation with *C. violaceum* incubation for two days, the pH fell to 8.7. Similar trend was seen for medium SIM e-waste with the particle size between 355-700 µm as shown in figure 4.14 (B). The decrease in the pH of the medium was attributed to the acidification process for the conversion of organic compounds, particularly glycine, into carboxylic acids such as glyoxylic, cyanoformic, and oxamic acids [39].

After inoculation with e-waste of different particle size, and incubation for further seven days, shows a common pH trend for both type of e-waste that was a sudden increase in pH to 9.3 during the course of incubation. The increase in pH after the point when e-waste was added was due to the increased generation of cyanide via the conversion of intermediate products such as carboxylic acids [40] also the pH of the system was near to the pKa value of the cyanide which added on to its accumulation in the medium so as to have maximum leaching. Although, by the end of 7th day, pH dropped to a level of ~9.1 in case of medium with SIM e-waste due to the unavailability of microorganism to produce cyanide due to high level of toxicity, though the presence of e-waste added on to the alkalinity of the medium. But, for the medium containing PCB e-waste, the pH further increased to ~9.4. This implies that PCB e-waste is less toxic as compared to SIM e-waste as the bacteria is able to survive for 7 days.



(A)



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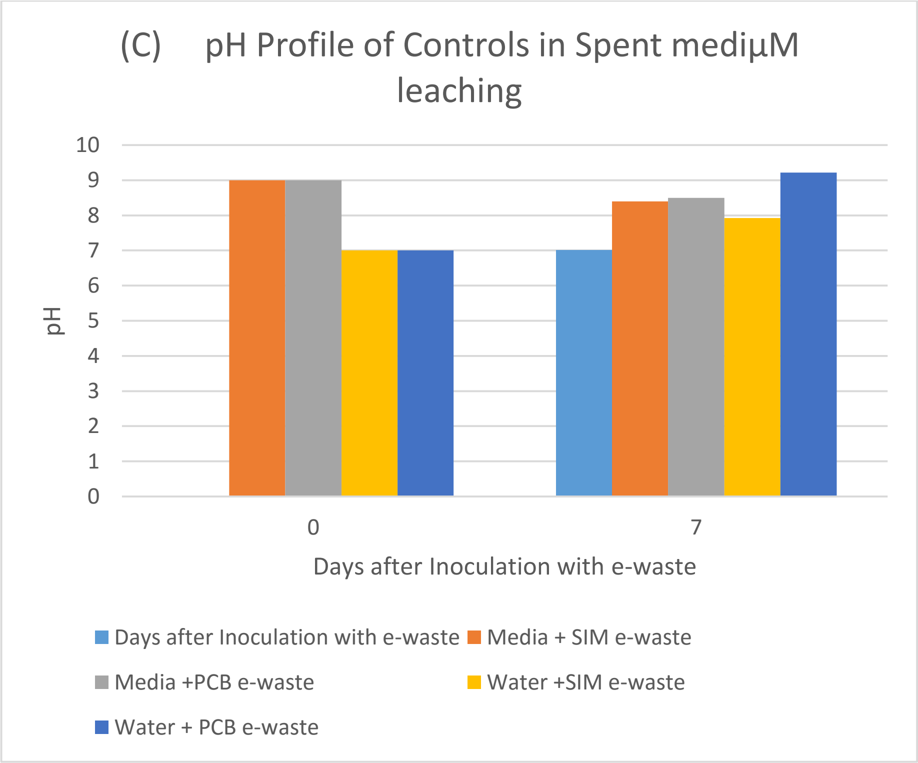

Figure 4.12: pH Profile of spent medium bioleaching using PCB e-waste and SIM e-waste. (A) pH profile of medium with PCB e-waste, (B) pH profile of medium SIM e-waste and (C) pH profile of various controls.

Electronic waste is the fastest growing municipality solid waste due to high obsolescence rate of electrical and electronic items. The growing quantity of e-waste is beginning to reach disastrous proportions. E-waste contains many hazardous constituents that may negatively impact the environment and affect human health if not properly managed. The problem of e-waste is growing in alarming proportions in India and also in the whole world. E-waste has become an important issue worldwide because of high use of rare earth elements and safe management. The WEEE contains rarer and noble metals as compared to metals in the metal mines, therefore recycling of e-waste not only contributes to environmental protection but also to the economic development. A subscriber identity module or subscriber identification module (SIM) card, which is an integral part of mobile telephony has been considered as a potential electronic waste for the extraction of precious and valuable metals. The exponential increase in the cellular networking and advancement in the related technology has led to an increase in waste or obsolete SIM cards which are regarded as enriched source of precious metals, especially gold. The chemical composition of a SIM card reveals it as a metal enriched source, with a lot of precious metals like gold, copper etc. being used to manufacture a single SIM card. Pure gold is used in abundance as due to its no reactive and non-corrosive nature, it is used to shield the metal surface of the SIM card.

Leaching is a process of extracting metals from solid by dissolving them into liquid. The most commonly used leaching agents are mineral acids, acid solutions, mixture of solutions, alkaline liquors, cyanide and chlorine. However the traditional methods of extracting metals like pyrometallurgy and hydrometallurgy are quite expensive and produce toxic compounds leading to health as well as environmental hazards. Therefore, the major challenge is to develop sustainable recycling technologies to address the problem of e-waste using cost effective and environment friendly methods. The most promising technology that has evolved is the use of naturally occurring microorganism and their metabolic products in extracting the valuable metals from the e-waste.

The bioleaching of this electronic waste is carried out using a cyanogenic bacteria, *Chromobacterium violaceum* which has been reported to have maximum bioleaching capability and also an affinity towards precious metals such as gold.

Gold from obsolete mobile SIM cards was dissolved using a two-step bioleaching process with

Chromobacterium violaceum as the bacterial strain. *Chromobacterium violaceum* has an affinity towards gold leaching and leach out significant amount of gold. Choosing the right microorganism, and providing optimum conditions for the sufficient growth of the bacteria are essential for the bioleaching medium to achieve satisfactory solubilisation.

The present study revealed that the chemical composition of the metals especially gold and copper in SIM e-waste is manifold greater than in the e-waste of PCB. This poses a higher level of toxicity for the bacteria to grow. Also the results indicate a very significant increase in gold mobilization from the chemical reflux treatment using aqua regia, but no significant leaching is predicted in the bioleaching process using *Chromobacterium violaceum.* Very profound levels of copper in the SIM turned out to be a limitation for gold mobilization using *C. violaceum.* Pre-treatment of SIM e-waste using nitric acid resulted in a huge reduction in the concentration of copper in the e-waste, with no effect on the concentration of gold.

Bioleaching of the pre-treated SIM e-waste using *C. violaceum* did not result in significant gold mobilization. While, copper mobilization was found to be very significant from chemical as well as biological treatment. Even after pretreatment of the e-waste, a very significant level of copper was leached out.

Finally it is concluded that, bioleaching did not found out to be an efficient way to leach out gold from the SIM e-waste due to the presence of other toxic metals like copper in very high proportions which even after pre-treatment and excessive removal turn out to be more affined towards cyanide complex formation at an alkaline pH. Therefore, a hybrid technology like chemo-biohydrometallurgy may be effective to leach out significant amount of copper from SIM e-waste, where even the residues left after pre-treatment was subjected to bioleaching and gave very significant results. For gold mobilization, chemical treatment found to be efficient and more suitable. Further soluble metals can be subjected to proper precipitation processes like electrolysis or by chemical precipitation using a suitable solute or by hydroxide precipitation methods to obtain purest forms of the metals for industrial applications.

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