Biointerface Research in Applied Chemistry
Platinum Open Access Journal (ISSN: 2069-5837)

Article
Volume 12, Issue 5, 2022, 6723 - 6740
https://doi.org/10.33263/BRIAC125.67236740

Role of *Actinomycete sp.* in Bio-extraction of Copper from Electronic Waste

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Received: 3.09.2021; Revised: 29.09.2021; Accepted: 3.10.2021; Published: 20.11.2021

Abstract: Copper is bioleached from printed circuit boards (PCBs) using *Actinomycete sp*. It was identified by 16S rRNA, called *Streptomyces graminofaciens*. The tolerance of *S. graminofaciens towards copper* showed that it couldn't live in the presence of copper sulfate. The process was carried out by culturing 3.09×10^3 CFU of *S. graminofaciens* in 50 ml of modified starch nitrate medium for three days at 200 rpm and 30°C and then 0.5% e-waste was added for other 5 days. Glucose (0.01 g/ml) and ammonium sulfate (0.002g/ml) were the best carbon and nitrogen sources. At pH 5, the leached copper was 88.1%. The bio-dissolution mechanism was investigated via the production of enzymes of *S. graminofaciens* metabolites, which showed that the higher dissolution has occurred in an un-denatured sample (non-heating) than the denatured one (heating). FTIR spectra confirmed the action of *S. graminofaciens* through the disappearance and appearance of some peaks. SEM showed that the e-waste gained more pores as a result of bio-treatment, which refers to the liberation of metals in solution.

Keywords: bio-extraction; copper; printed circuit boards;*Streptomyces graminofaciens.*

1. Introduction

In recent years, electronic equipment production has been fast growing due to its increased use and the high cost of repair. It produces large amounts of electronic wastes (e-wastes). So, it needs recycling not only for environmental concerns but also for economic reasons. Many physical processes are used to classify the components of e-wastes, such as size, density, and magnetic separations, as well as flotation processes [1-3].

E-waste contains several organic pollutants and approximately 40% of base, precious and rare earth metals [4-6]. Printed circuit board (PCB) contains copper by 10-30%, which is the highest metallic content of e-waste [7]. Copper is an industrially significant metal, widely used in building construction, electrical and electronic products, transportation equipment, and industrial machinery [8]. So, the recovery of copper from e-waste is important to conserve the environment and metal resources. Its recovery is typically achieved by conventional methods as pyro-metallurgical and hydrometallurgical processes, which have a certain energy and environmental disadvantages [9-12].

The bio-hydrometallurgical route is an alternative method for conventional methods (mechanical and thermal) in which bioleaching of metals from e-waste. By this process, the efficiency of metals recovery can be increased as shown in copper and gold mining of low-

grade ores. These ores are treated by biological methods to obtain metal values, which are not accessible by conventional methods [13-15]. Mainly, three groups of microorganisms are classified in bioleaching: chemo-lithotrophic prokaryotes, including bacteria and archaea; heterotrophic bacteria and heterotrophic fungi [16].

The bacteria used for bioleaching of metals generally belong to the class of chemo-lithoautotrophs. Very little reported data is available on the use of heterotrophic organisms which could perform leaching of metals. Compared to acidophiles, heterotrophs adapt to a wider pH range and are used for treating moderately alkaline wastes. Research on the heterotrophic bioleaching of metals from waste materials focused on cyanide and organic acid-generating microorganisms. Heterotrophic bacteria and fungi are included in bioleaching with microbial production of organic acids. Organic acids are used as bioleaching agents. Also, other metabolites can be used as leaching agents for extracting metals from waste material. In most cases of heterotrophic bioleaching, organic acids directly dissolve metals [17].

Several strains belonging to the genus of *Acinetobacter* species have attracted growing interest from researchers for environmental and biotechnological applications. These bacteria are involved in the biodegradation of xenobiotic organic compounds [18]. *Acinetobacter* species (sp.) is widespread in water, soil, and living organisms. The primary characteristic of any bacteria to be used for bioleaching of metals is its high resistance to the metals which are present in e-waste [19].

This work aims to investigate the efficiency of Actinomycete sp.(*Streptomyces graminofaciens*)as a bioleachant of copper metal from e-waste.

2. Materials and Methods

2.1. Materials.

The scraps of PCBs were collected from the local market. All analytical grade chemicals used were supplied by Merck. *Actinomycete* was supplied by the microbiology department, Al-Azhar University. It was identified by 16S rRNA sequencing.

The composition of the used culture media is: Starch nitrate DSMZ [20], comprises of 20g starch, 2g KNO_3, 1g K_2HPO_4, 0.5g NaCl, 0.5g $MgSO_4.7H_2O$, 3g $CaCO_3$, 10mg $FeSO_4.7H_2O$, and 20g agar in distilled water up to 1 liter. Modified starch nitrate (HI media) is composed of 10g starch, 1g K_2HPO_4, 1g $MgSO_4.7H_2O$, 1g NaCl, 2g $(NH_4)_2SO_4$, 2g $CaCO_3$, 1mg $FeSO_4.7H_2O$, 1 mg $MnCl_2.7H_2O$, and 1mg $ZnSO_4$ in distilled water up to 1 liter. Modified malt-yeast extract medium [21] comprises 10g glucose, 5g peptone, 3g yeast extract, and 20g agar in distilled water up to 1 liter. The pH was adjusted at 6.8 before autoclaving at 121°C for 15 min. Also, It was solidified by adding 15g agar before pH adjusting. Bioleaching medium [21] comprises 10g glucose, 0.1g $CaCl_2$, 0.1g $MgSO_4.7H_2O$, 5g NH_4Cl, 0.5g NaCl, 3g KH_2PO_4, and 6g NaH_2PO_4 in distilled water up to 1 liter in deionized water. The pH was adjusted at 12 using 0.1N NaOH.

2.2. Methods.

2.2.1. Characterization of PCBs.

Firstly, the scraps of PCBs were cut into small pieces manually, and then they were crushed by the high-speed universal pulverizer for 2 min. XRF analysis was used for the

chemical analysis of the sample. Crystalline phases of PCBs samples were determined by XRD analysis.

2.2.2. Characterization of the *actinomycete* strain.

DNA was extracted by using the protocol of Gene Jet genomic DNA purification Kit (Thermo K0721), then PCR by using Maxima Hot Start PCR Master Mix (Thermo K1051) with 16 S universal primer Forward primer:-5- AGA GTT TGA TCC TGG CTC AG-3 and Reverse R:- 5- GGT TAC CTT GTT ACG ACT T-3. The PCR cycle was carried out as the following initial denaturation 95°C for 10 min one cycle, denaturation 95°C for 30 sec, annealing 65°C for 1 min, extension 72°C for 1.30 min, number of cycles 35 cycle and final extension72°C for 10 min one cycle. PCR clean up to the PCR product using GeneJET™ PCR Purification Kit (Thermo K0701). Finally, the PCR product was sequenced by GATC Company by using ABI 3730×l DNA sequencer by using forward and reverse primers.

Combining traditional Sanger technology with the new 454 technology can genomes now be sequenced and analyzed in half the usual process time, with a considerable decrease in the number of recommended steps (coatings and gaps). In addition, considerable cost advantages now make genome sequencing with the 454 technology available to the research community.

2.2.3. Tolerance of the *actinomycete strain* to copper.

Modified starch nitrate agar medium with various concentrations of $CuSO_4$ ranging from (100-1000 ppm) was prepared, then inoculated with this actinomycete strain and incubated at 30°C. The growth of the actinomycete strain was followed on a plate with time to determine the ability of this actinomycete strain to survive in the presence of copper in addition to preparation control without copper [22].

2.2.4. Bioleaching procedure.

Different types of bioleaching methods one-step, two-step, and spent medium method were carried out to show bioleaching efficiency as the following: In one-step bioleaching method, 50ml of modified starch nitrate medium was prepared with inoculation 1.03×10^3 CFU and addition 0.25 g of PCBs at the same time then incubated at 30°C and180 rpm. In the two-step bioleaching method, culturing of 1.03×10^3 CFU/50 ml of modified starch nitrate medium was prepared for three days, then adding 0.25g PCBs and incubating at 30°C and 180 rpm. In a spent medium method, culturing of 1.03×10^3 CFU/50 ml medium was prepared for 7 days, then filtered, adding 0.25 g of PCBs to the filtrate, and incubated at 30°C and 180 rpm. In each type of bioleaching method, the concentration of copper was determined daily [23].

Different culture media, modified starch nitrate, starch nitrate, modified malt-yeast extract, bioleaching media were evaluated separately with 0.5% e-waste and inoculation with 1.03×10^3 CFU/50 ml medium and incubated at 30°C and 180 rpm. The amount of extracted copper was determined by titration method, measuring pH value and redox potential by pH meter. By applying predetermined conditions with the best medium, other factors were studied: incubation period, particle size, inoculum size, temperature, pulp density, carbon, nitrogen sources and their concentrations, initial pH, and shaking speed.

Mechanism of bio-extraction was investigated through producing various metabolites, including enzymes with structural complexity, and can decompose many of recalcitrant chemicals like hydrocarbons [24]. Detection of the effect of enzymes produced by this actinomycete strain for bioleaching of copper from e-waste was performed as follow, 50 ml of optimized bioleaching medium was prepared with addition 3.09×10^3 CFU of the actinomycete strain in duplicate then culturing for 3 days at 30°C and 200 rpm then filtered by centrifugation to remove any cells and two samples of supernatant which containing metabolites were taken. In the first sample of supernatant, the extracellular enzymes were removed by autoclaving at 115°C for 20 minutes (heat sterilization) to denature the enzymes. Whereas the second sample was left without sterilization. 0.25 g of e-waste was added for 50 ml of each sample then kept in an incubator for 5 days [25].

2.2.5. FTIR (Fourier Transform Infrared Spectrometer analysis.

The differences of spectra between control (50 ml of modified starch nitrate medium was prepared with adding 0.25g of e-waste in the absence of the actinomycete sp.*)* and sample (50ml of modified starch nitrate medium was prepared with adding 0.25g of e-waste in presence 1.03×10^3 CFU of the actinomycete strain at optimum conditions) were studied to show the appearance and disappearances of functional groups; these functional groups can be related to the dissolution process. The (5-8) ml of filtrate of leaching solution in bioleaching experiments were analyzed using FT-IR (Nicolet IS-10 FTIR) for the determination of active functional groups [26].

2.2.6. Field emission scanning electron microscopy(SEM) analysis.

The morphology changes in e-waste before and after bioleaching processes were determined by SEM analysis (a JEOL instrument QUANTAFEG 250, Netherlands).

3. Results and Discussion

3.1. Characterization.

3.1.1. Chemical composition of waste PCBs.

XRF analysis showed that the sample contains 21.96% copper, 20.49% bromine, and 16.15% tin. Other elements such as Pb, Zn, Fe, and Sb were found in small quantities ranging from 8.4% to 2.6%. In addition, other elements such as Ba, Si, Ca, Al, Ni, Sr, and Mg were detected, Table 1. XRD spectrum of PCBs sample (Figure 1) showed that copper, tin, lead, aluminum, and iron were the main phase constituents.

Table 1. XRF analysis of WPCBs sample.

Element	Cu	Zn	Sn	Fe	Pb	Sb	Ni	Br	Ba	Si	Sr	Mg	Ca	Al
%	21.96	2.62	16.15	2.32	8.40	2.67	0.47	20.49	1.41	0.35	0.14	0.10	0.50	0.16

3.1.2. Identification of actinomycete strain.

The actinomycete strain was applied to amplification of partial 16S rRNA gene. The produced sequence with the length of 1352 base pair was compared with available sequences in the NCBI database using BLASTN, as shown in Table 2. It was closely related to

Streptomyces graminofaciens with 99.15% sequence similarity, as in Figure 2. Hence it was designated as *Streptomyces graminofaciens (S. graminofaciens)*.

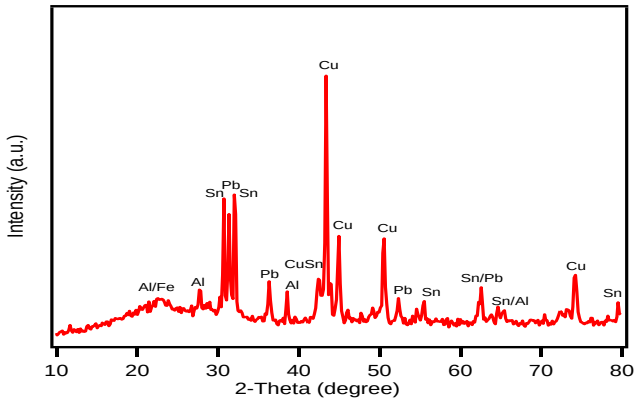


Figure 1. XRD patterns of powdered waste printed circuit boards sample (WPCBs).

Table 2. Identification of actinomycete strain used in the dissolution of copper from e-waste.

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Streptomyces graminofaciens strain NBRC 13455 16S ribosomal RNA, partial sequence	1700	1700	100%	0.0	99.15%	NR_112404.1
Streptomyces luozhongensis strain TRM49605 16S ribosomal RNA, complete sequence	1694	1694	100%	0.0	99.05%	NR_164875.1
Streptomyces peucetius strain NBRC 100596 16S ribosomal RNA, partial sequence	1694	1694	100%	0.0	99.05%	NR_112574.1
Streptomyces pulveraceus strain NBRC 3855 16S ribosomal RNA, partial sequence	1694	1694	100%	0.0	99.05%	NR_041213.1
Streptomyces xantholiticus strain NBRC 13354 16S ribosomal RNA, partial sequence	1694	1694	100%	0.0	99.05%	NR_041123.1
Streptomyces kurssanovii strain NBRC 13192 16S ribosomal RNA, partial sequence	1694	1694	100%	0.0	99.05%	NR_041118.1
Streptomyces bottropensis ATCC 25435 16S ribosomal RNA, complete sequence	1688	1688	100%	0.0	98.94%	NR_115571.2
Streptomyces drozdowiczii strain NRRL B-24297 16S ribosomal RNA, partial sequence	1688	1688	100%	0.0	98.94%	NR_116093.1
Streptomyces gelaticus strain NRRL B-2928 16S ribosomal RNA, partial sequence	1688	1688	100%	0.0	98.94%	NR_043488.1
Streptomyces drozdowiczii strain NBRC 101007 16S ribosomal RNA, partial sequence	1688	1688	100%	0.0	98.94%	NR_041424.1
Streptomyces bottropensis strain NBRC 13023 16S ribosomal RNA, partial sequence	1688	1688	100%	0.0	98.94%	NR_041096.1
Streptomyces gelaticus strain NBRC 12866 16S ribosomal RNA, partial sequence	1688	1688	100%	0.0	98.94%	NR_112308.1
Streptomyces peucetius strain JCM 9920 16S ribosomal RNA, partial sequence	1688	1688	100%	0.0	98.94%	NR_024763.1
Streptomyces fulvissimus strain DSM 40593 16S ribosomal RNA, partial sequence	1683	1683	100%	0.0	98.83%	NR_103947.1
Streptomyces pratensis strain ch24 16S ribosomal RNA, partial sequence	1683	1683	100%	0.0	98.83%	NR_125616.1
Streptomyces pratensis strain ch24 16S ribosomal RNA, partial sequence	1683	1683	100%	0.0	98.83%	NR_125619.1
Streptomyces globisporus strain KCTC 9026 16S ribosomal RNA, partial sequence	1683	1683	100%	0.0	98.83%	NR_118107.1
Streptomyces rubiginosohelvolus strain NBRC 12912 16S ribosomal RNA, partial sequence	1683	1683	100%	0.0	98.83%	NR_041093.1
Streptomyces ederensis strain NBRC 15410 16S ribosomal RNA, partial sequence	1683	1683	100%	0.0	98.83%	NR_112457.1
Streptomyces ederensis strain NRRL B-8146 16S ribosomal RNA, partial sequence	1683	1683	100%	0.0	98.83%	NR_116384.1
Streptomyces phaeochromogenes strain NRRL B-1248 16S ribosomal RNA, partial sequence	1683	1683	100%	0.0	98.83%	NR_116382.1

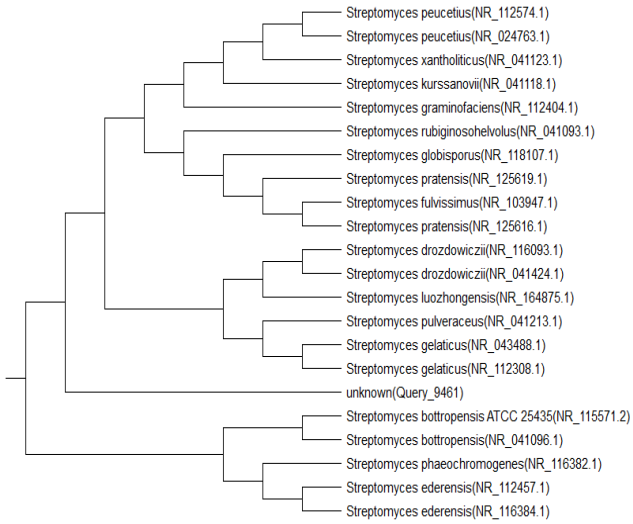


Figure 2. Phylogenetic tree for identification of actinomycete strain used.

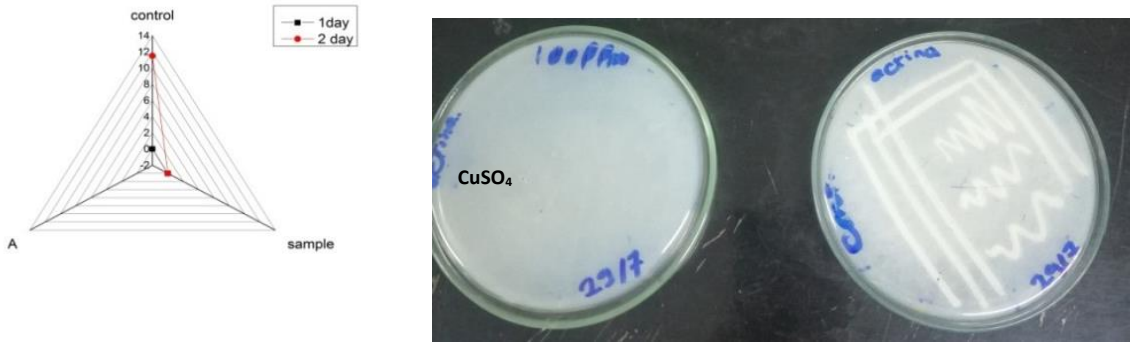
3.1.3. Tolerance of *Streptomyces graminofaciens* to copper.

It was observed that the optimum growth was occurred in the control plate(without copper sulfate) from the second day of incubation, but the plate with copper sulfate didn't have

any growth. This refers to the toxicity of copper to S. *graminofaciens*. These results were tabulated in Table 3 and drawn in Figure 3. This agrees with the literature. It was reported that the minimal concentration of copper caused the complete suppression of growth in a part of streptomycete cultures [27].

Table 3. Effect of copper concentration on the growth of *Streptomyces graminofaciens*.

	Time(day)/diameter (cm)	
	1day	2 days
Control	--	11.5
Sample	--	--



(a) (b)

Figure 3. Effect of copper on the growth of *Streptomyces graminofaciens* (a), Inoculation of the actinomycete in aplate with and without copper sulfate (b).

3.2. Bioleaching process.

Different bioleaching methods (one-step, two-step, and spent medium) were studied by measuring copper dissolution with time. It was observed that the two-step method is preferred over one-step, and the insignificant difference between two-step and spent medium. The copper dissolution reached 51.5% using a modified starch nitrate medium, as illustrated in Table 4 and Figure 4. In one-step bioleaching, both microorganisms and e-waste were added to the medium at the same time. The growth of microorganisms was inhibitedm[28, 29]. Also, the swollen spores aggregated with e-waste particles after inoculation led to relatively large pellet nuclei formation.

In a two-step bioleaching process, *Streptomyces graminofaciens* was grown in a modified starch nitrate medium for three days, which entered the log phase. Then, e-waste was added and is thought to be appropriate to increase bioleaching of metals from e-waste. In which some metals can be coenzymes for enzymes produced by Streptomyces graminofaciensto increase leaching efficiency, and these metals can also cause stress for a microorganism that leads to increased production of metabolites that used in the leaching process [30].

Table 4. Effect of types of bioleaching methods on leaching of copper.

Bioleaching method	Cu%				
	1 day	2 days	3 days	4 days	5 days
One step	4.39	8	8.5	12	12.7
Two step	44.6	45.7	47.5	48.2	51.5
Spent medium	43.8	44.2	46.8	47.2	50.1

In the spent medium process, *Streptomyces graminofaciens* were cultured for 5 days then filtrated. E-waste was added to the filtrate, and copper dissolution was determined daily. The differences between the two-step bioleaching method and spent medium for copper dissolution were not significant which actinomycete produces metabolites that are necessary

for copper dissolution. The active secondary metabolism in streptomycetes may enable the future application of these organisms in biotechnologies for the remediation of natural environments and objects contaminated by heavy metals [31].

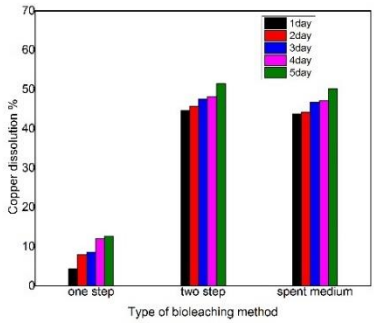


Figure 4. Effect of types of bioleaching methods on leaching of copper.

3.2.1. Effect of media types on bio-extraction of copper from PCBs.

The growth of any microorganism and production of metabolites can be affected by the composition of the growth medium, so different media compositions were studied (bioleaching medium, modified Starch nitrate, starch nitrate, and modified malt-yeast extract)to show the best medium for leaching of copper by *Streptomyces graminofaciens.* The 1.03x10^9 CFU of *S. graminofaciens* was cultured in 50 ml of modified starch nitrate medium and incubated for 3 days at 30°C and 180 rpm then 0.25 g of e-waste was added. Copper dissolution, pH, and redox potential were determined after 5 days of incubation. The results showed that the best medium for dissolution of copper was the modified starch nitrate medium by which 51.5% of copper dissolved with final pH 8.4 and redox potential -53.7, while the minimum copper dissolution occurred with the bioleaching medium, Figure 5. It is reported that the medium composed of 10g starch, 0.3g casein (Difco-vitamin-free), 0·3g KNO_3, 2g NaCl, 2g K_2HPO_4, 0.05g $MgSO_4.7H_2O$, 0.02g $CaCO_3$, 0·01g; $FeSO_4·7H_2O$, and 18g' "'Bacto, Agar'"Agar'(Difco) in 1 liter of distilled water at pH 7-7.2 allows the development of large numbers of *Streptomycetes*. So it is referred to use modified starch nitrate medium as the best medium for optimum growth of this microorganism [32].

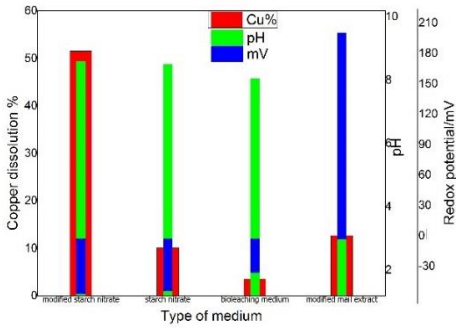


Figure 5. Effect of different media on bioleaching of copper by *S. Graminofaciens.*

3.2.2. Effect of incubation period on copper extraction by *S. graminofaciens.*

A 50 ml of the modified starch nitrate medium was prepared for culturing 1.03×10^9 CFU of *S. graminofaciens* and incubated for 3 days, then 0.25 g of +150 mesh of e-waste was added to the prepared culture and incubated at 30°C and 180 rpm. Copper dissolution, pH, and redox potential were determined daily. The results showed that the maximum biodissolution of copper (51.5%) occurred after 5 days of incubation at pH 8.3 and redox potential -52.1 mV,

Figure 6. Horeh, *et al*. [33] showed that counts of streptomycetes and fungi were made after 5 days of incubation.

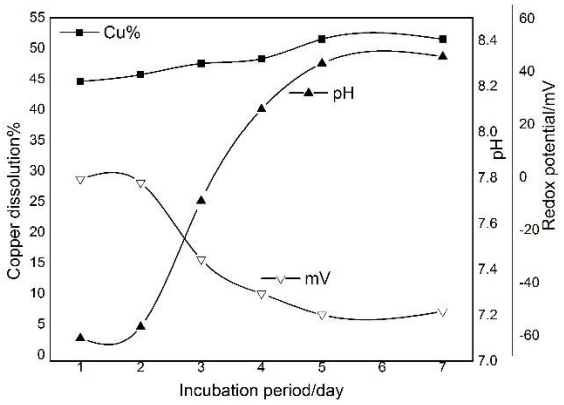


Figure 6. Effect of incubation period on copper dissolution by *S. graminofaciens*.

3.2.3. Effect of particle size factor on copper dissolution by *S. graminofaciens.*

The results showed that copper dissolution increases with decreasing size fraction to 150 mesh at which 52.5% copper has dissolved at pH 8.25 and redox potential -50.1 mV, Figure 7. Reducing the particle size to a certain limit increases the overall contact surface and thus better mass transfer. Also, the increased number of particles leads to cause tension between e-waste pulp and microorganisms, which results in microbe body damages or lyses. These two aspects must be balanced. These results were confirmed by Young and Veasey [34], who showed that large particle sizes lead to low metal extraction efficiency.

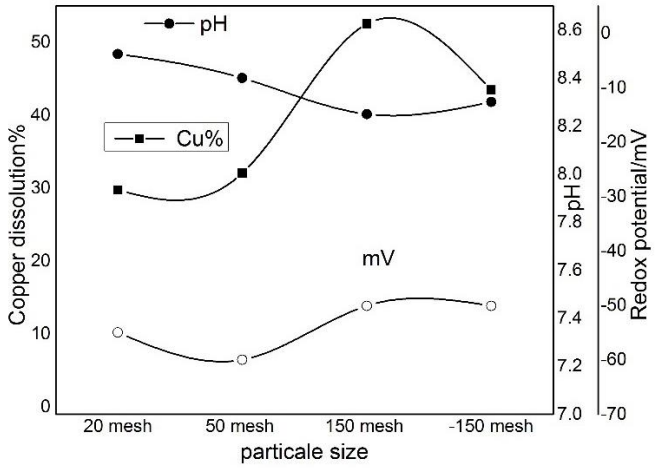


Figure 7. Effect of particle size factor on copper dissolution by *S. graminofaciens*.

3.2.4. Effect of inoculum size of *S. graminofaciens* on copper dissolution.

The results showed that copper dissolution increases with increasing inoculum size up to 3.09×10^3 CFU for 50 ml of medium, then it decreases with furthering increase inoculum size, at which copper dissolution reached 61.89% at pH 8.34 and redox potential -54.1mV, Figure 8. The increasing inoculum size above 3.09×10^3 CFU/50 ml leads to overgrowth of cells and adsorption of leached copper ions on a large amount of bacterial biomass surface. Also, the competition factor between the cells occurs at the large inoculum size, decreasing the necessary aeration for optimum growth of cells and producing metabolites necessary for copper dissolution [35].

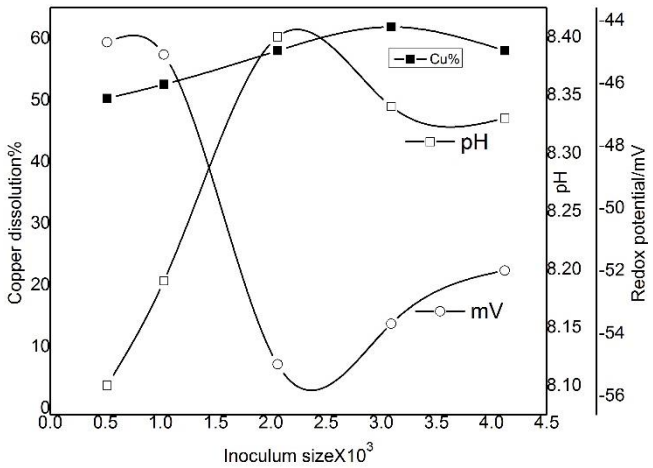


Figure 8. Effect of inoculum size of *S. graminofaciens* on copper dissolution.

3.2.5. Effect of incubation temperature on copper dissolution by *S. graminofaciens.*

The results showed that copper dissolution increases with increasing temperature up to 30°C, then it decreases. The copper dissolution reached 62% at pH 8.34 and redox potential -54.1 mV, Figure 9. The optimum activity and growth of *S. graminofaciens*for producing more efficient metabolites used in copper dissolution were at 30°C.

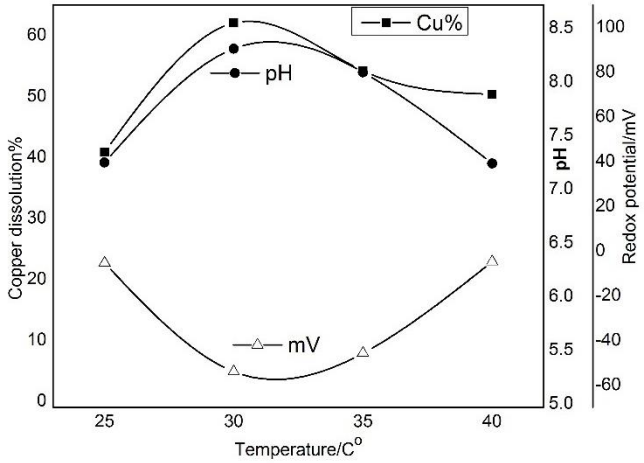


Figure 9. Effect of incubation temperature on copper dissolution by *S. Graminofaciens.*

The nature and amount of secondary metabolites produced by *Streptomyces* are high dependents on the temperature and growth rate [36]. It showed that the optimum growth was observed at 30°C, and above this temperature, the growth and production of metabolites decreased [37].

3.2.6. Effect of pulp density on copper dissolution by *S. graminofaciens.*

It was observed that copper dissolution increased with decreasing pulp density. The best pulp density was 0.1 g/50ml of a medium at which 100% Cu is dissolution at pH 7.9 and redox potential -34 mV. To reduce the economic value with lower pulp density, 0.25g of e-waste /50ml medium was selected as the optimum amount of pulp density at which 62.1% copper is dissolued at pH 8.4 and redox potential -55.4 mV, Figure10.

Obviously, the increase of the pulp density means high metals content, so they have inhibitory effects that reduce the bacterial growth and production of metabolites necessary for

copper dissolution. Also, the non-metallic fractions, i.e., epoxy-coated substrate and organic components of the PCB are toxic to the bacteria [38]. On the other hand, a low pulp density is not acceptable from an economic view.

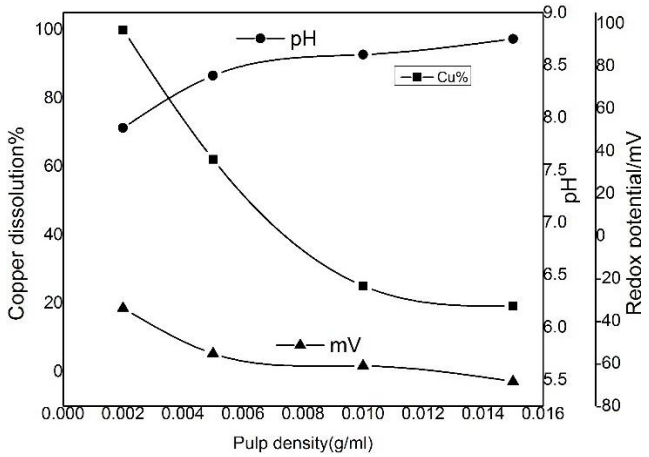


Figure 10. Effect of pulp density on copper dissolution by *S. graminofaciens.*

3.2.7. Effect of carbon sources on copper dissolution by*S. Graminofaciens.*

Microorganisms can use a variety of carbon sources and adapt to the altering in the osmotic strength, nutrients, oxygen limitation, and stress conditions. The carbon source is important, which is easily metabolized and is necessary for the active proliferation of microorganisms. Also, it affects metabolic function in many bacteria [39]. Different carbon sources (starch, sucrose, glucose, dextrose, lactose) were tested with predetermined conditions.

The results showed that the best carbon source was glucose, at which copper dissolution is reached 68.8% at pH 8.06 and redox potential 14.3 mV, as drawn in Figure 11. The worst carbon source was sucrose. This is due to the simplest of glucose sugar and eases to be intake by *Streptomyces*, in addition to glucose providing carbon atoms which are important for the mycelium and generations production. It showed the vital effect of glucose as a carbon source on metabolic function in many bacteria. It is reported that the maximum cell growth in the medium is amended with glucose, fructose, and mannose [40].

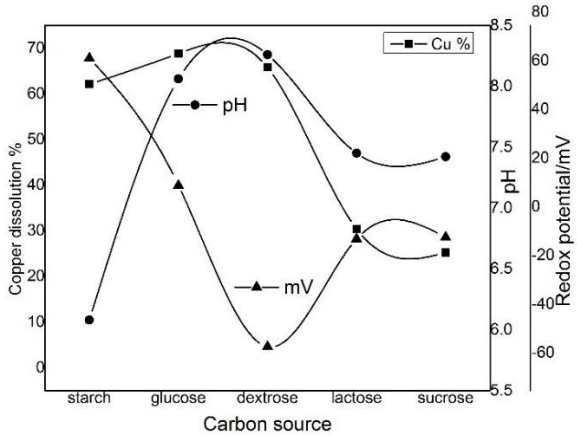


Figure 11. Effect of carbon sources on copper dissolution by*S. graminofaciens.*

3.2.8. Effect of carbon concentration on copper dissolution by *S. graminofaciens.*

The results revealed that the bioleaching of copper increases with increasing glucose concentration up to 0.015 g/ml, which reached 76.98% at pH 8.06 and redox potential 14.3

mV, then it decreases with increasing glucose concentration, Figure 12. It was reported that the further increase in the glucose concentration led to a gradual decrease in the production of metabolites and the growth of the mycelium. Also, the glucose concentration in the medium has got a significant effect on the growth of *Streptomyces* and bioactive metabolite production [40].

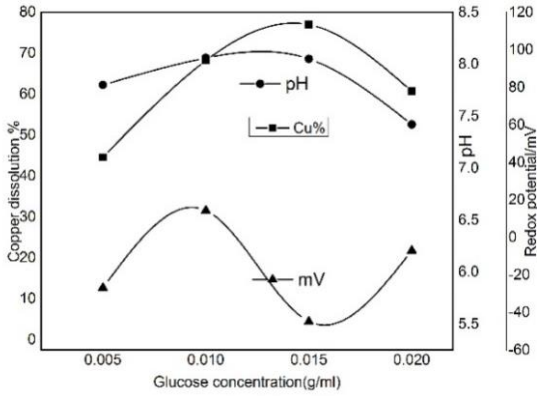


Figure 12. Effect of carbon concentrations on copper dissolution by *S.graminofaciens*.

3.2.9. Effect of nitrogen sources on copper dissolution by *S.graminofaciens.*

Nitrogen metabolism and nitrogen scavenging are vital processes for saprophytic organisms such as Streptomyces and other actinobacteria. Nitrogen is also found in many secondary metabolites, whose function may be related to cell interactions with their surroundings [41]. Nitrogen sources in the medium can affect the growth and activity of microorganisms and the nature of the produced metabolites. The nitrogen source was used to regulate secondary metabolism, so various nitrogen sources (ammonium sulfate, ammonium chloride, ammonium phosphate, asparagine) were tested with applied predetermined conditions.

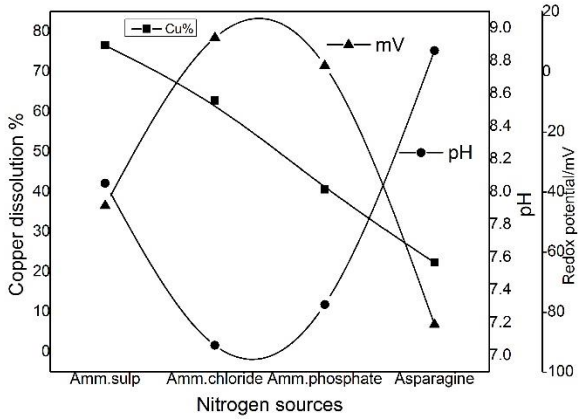


Figure 13. Effect of nitrogen sources on copper dissolution by *S. graminofaciens.*

The results showed that the best nitrogen source was ammonium sulfate, at which the copper dissolution is reached 76.5% at pH 8.05 and redox potential -44.8 mV, Figure 13. The wrest nitrogen source was asparagines that inhibit the growth of *S. graminofaciens*.

The maximum activity of Streptomyces sp. 201 was obtained in culture filtrates supplemented with asparagine, followed by potassium nitrate, tyrosine, and valine [42]. The ammonium source is preferred than nitrate because the ammonium enter the cell directly by a specific transporter (AmtB) [43], but a nitrate enters through a nitrate transporter (NarK) then

reduced to nitrite by an assimilatory nitrate reductase (NasA), and finally to ammonium by a nitrite reductase (NirB) [44].

3.2.10. Effect of nitrogen concentration on copper dissolution by *S. graminofaciens.*

The results showed that copper dissolution increases with increasing ammonium sulfate concentration up to 2mg/ml then decreases. At 0.002g/ml of ammonium sulfate, the copper dissolution is reached 76.2% at pH 8.06 and redox potential -40.7 mV. The optimum growth of this strain and production of more metabolites necessary for the dissolution process occurred at this concentration of ammonium sulfate, Figure14.

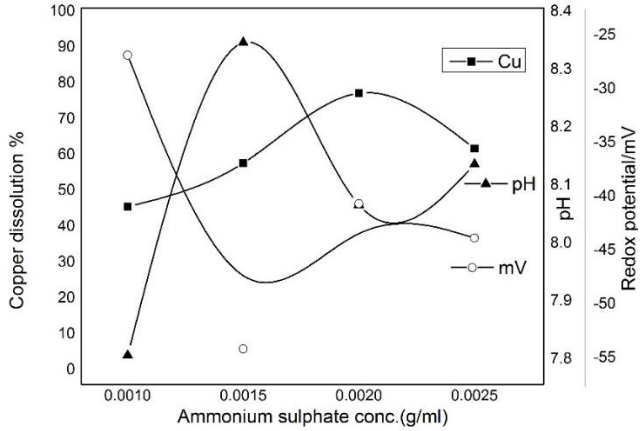


Figure 14. Effect of nitrogen source concentration on copper dissolution by *S. graminofaciens*.

3.2.11. Effect of initial pH on copper dissolution by *S.graminofaciens.*

The pH of the culture medium is one of the most vital environmental factors. It has an effect on the activity of several enzymes that are necessary for catalyzing metabolic reactions and has a significant effect on complex physiological phenomena such as membrane permeability and cell morphology. The initial pH of the medium affects many cellular processes, such as the regulation and production of secondary metabolites [45]. Hence, the medium's different initial pH (5, 7, 8, 9) were tested with applied predetermined conditions. The results showed that the optimum initial pH of the modified starch nitrate medium was pH 5, at which the copper dissolution is reached 85.08% at final pH 6.7 and redox potential 33.1 mV, Figure 15. It was reported that the maximum metal bioleaching was observed at pH 6, and most streptomyces species showed an optimum pH range from 7-8 [46].

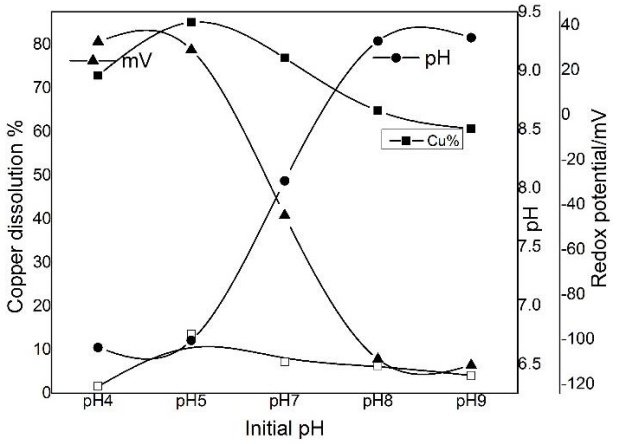


Figure 15. Effect of initial pH on copper dissolution by *S.graminofaciens.*

3.2.12. Effect of Aeration on Copper Dissolution by *S.graminofaciens.*

The agitation speed of microorganisms in the growth medium is responsible for the aeration of microorganisms. It can affect the growth rate, metabolism by transporting nutrients and enzyme activities, cell morphology, mass transfer characteristics, and the shear stress on microbial cells. The results showed that the copper dissolution increases with increasing shaking speed up to 200 rpm then decreases. At 200 rpm, copper dissolution is reached 88.1% at pH 8.14 and redox potential -47.3 mV. It refers to medium agitation required for optimum growth of Streptomyces strain for production maximum metabolites, Figure 16. It is reported that the agitation rate increases the mass transfer and shear stress to bacterial cells. Above certain limit of shaking speed leads to damage of cells by shear stress [25].

3.3. Mechanism of copper bioleaching by S. graminofaciens.

For surviving in an environment contaminated by metals, streptomycetes produce a wide range of metal ion chelators, such as siderophores, to protect from the negative effects of heavy metals of e-waste or metal uptake for specialized metabolic processes [47]. Also, Streptomycetes produce extracellular polymeric substances (EPS), which are, together with siderophores, of interest in removing heavy metals from the environment.

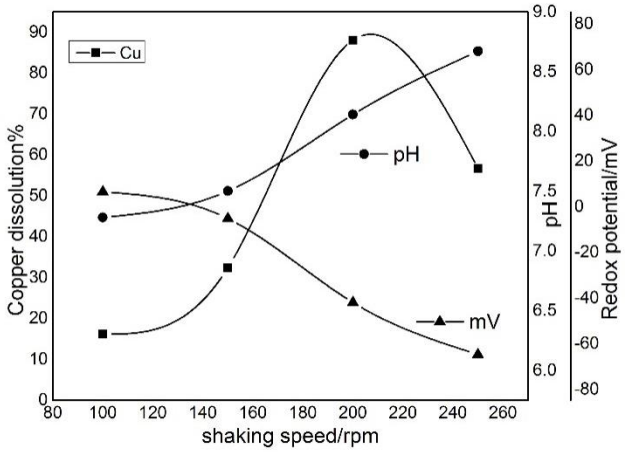


Figure 16. Effect of aeration on copper dissolution by *S. graminofaciens.*

3.3.1. Detection enzymes produced by *S. graminofaciens.*

To ensure the production of enzymes in metabolites of *S. graminofaciens* that had a role in the bioleaching of copper was occurred by preparing two samples of culturing 3.09×10^3 CFU of Streptomyces in 50 ml of optimized bioleaching medium for 3 days at 200 rpm and 30°C, then they were centrifuged at 15000 rpm and collect the supernatant. The protein content of the supernatant is denatured by heat sterilization, autoclaved at 110°C for 20 min., and another supernatant was not treated. A 0.25 g of e-waste was added to both samples and incubated at 30°C and 200 rpm [48].

The results showed that the copper dissolution in a sterilized sample reached 48.63%, while it was 88% in an unsterilized sample. This refers to the effect of both enzymes and metabolites produced by *S. graminofaciens*. In the absence of enzymes (denatured sample), metabolites are produced by *S. graminofaciens* causes bioleaching. In the presence of enzymes and metabolites, the bioleaching is almost doubled. It was reported that the leaching efficiency

in the heat-treated sample is lower than that in the untreated one for bioleaching of metals from fly ash using *Thiobacillus*sp [49].

3.3.2. FTIR analysis.

FTIR analysis was used to investigate the effect of bioleaching of e-waste on the functional groups, material composition, and variation between molecular phases. The filtrate spectrum of modified starch nitrate in the presence of e-waste with or without of *S. graminofaciens* at optimum conditions, Fig.17, showed that a peak shift from 1037.76 cm^{-1} to 1091.70 cm^{-1} and 1083.00 cm^{-1} to 1131.39 cm^{-1} which is related to C–N stretching amine. The peak shift from 1400.68 cm^{-1} to 1399.61 cm^{-1} which is related to O–H bending alcohol. The peak shift from 1637.05 cm^{-1} to 1636.26 cm^{-1} which is related to C=C stretching conjugated alkene or N-H bending amine. Also, the peak shift from 3429.74 cm^{-1} to 3435.22 cm^{-1} which is related to alcohol. While, the spectrum of the filtrate with *S. graminofaciens,* showed a peak at 2088.09 cm^{-1} which is related to N=C=S isothiocyanate. The difference between the two spectra is related to the dissolution of copper from e-waste [50].

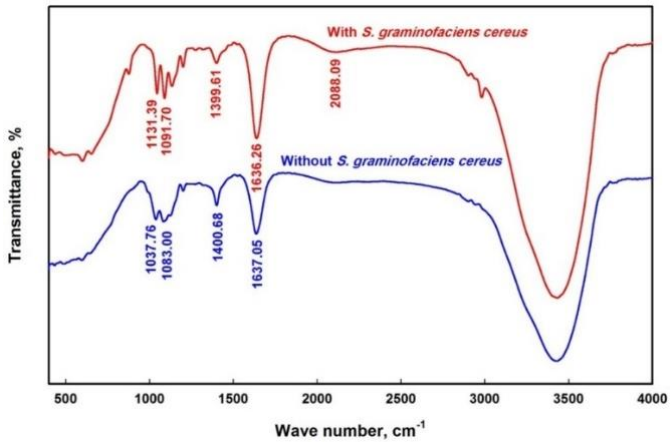


Figure 17. FTIR spectra of the filtrate with and without *Streptomyces Graminofaciens*.

Through FTIR, it was showed the presence of amine and alcohol groups that participate in the leaching of the copper process, as shown in equation (1-5). The reaction of copper with amine groups to form copper ammine complex than in the presence of molecular oxygen dissolved in the solution as the oxidant, achieving the separation of copper and other metal components[51].

$$Cu+2O_2+4NH_3\cdot H_2O=Cu(NH_3)_4^{2+}+4H_2O \quad (1)$$

$$Cu+ 2O_2+4NH_4^{+}= Cu(NH_3)_4^{2+}+2H_2O \quad (2)$$

$$Cu+ Cu(NH_3)_4^{2+}=2Cu(NH_3)^{2+} (3)$$

$$2Cu(NH_3)^{2+}+ 2O_2+4NH_3\cdot H_2O=2Cu(NH_3)_4^{2+}+6H_2O \quad (4)$$

O-H bending alcohol+ Cu-[e-waste]===soluble copper complex (5)

Also, the presence of hydroxy bending alcohols interact with copper of e-waste by direct displacement of hydrogen ions to form of soluble copper complex and chalets, [52].

3.3.3. SEM analysis.

SEM image of e-waste before treatment showed the heterogeneity and variety of species of particles in the sample, Figure 18(A). SEM images of e-waste after the bioleaching process showed an interaction with *S. graminofaciens*, Figure 18 (B & C). The increasing holes in the surface are due to the large destruction of the structure by metabolites and enzymes secreted by *S. graminofaciens*. It was reported that the structural changes in the PCB after the bioleaching process had been determined by scanning electronic microscope analysis [53].

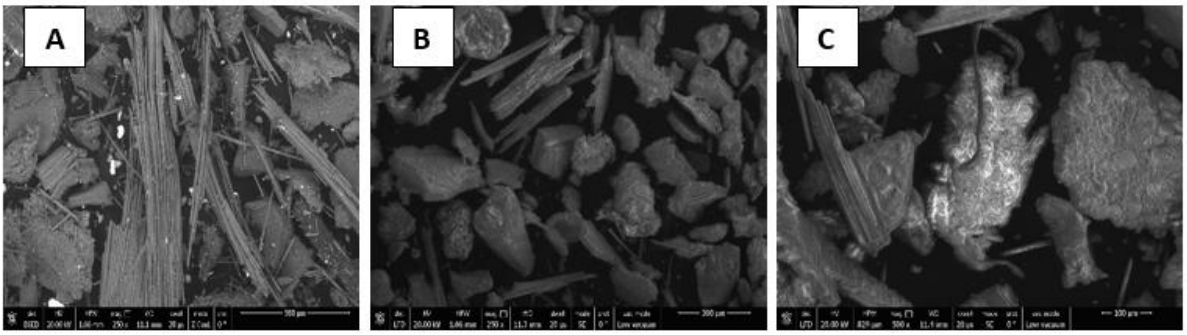


Figure 18. SEM analysis of e-waste before (A) and after (B,C) bioleaching process.

4. Conclusions

This work is interested in the dissolution of copper from e-waste by using *Actinomycete sp*. These studies can be summarized as follows: XRF analysis showed the presence of high content of copper (21.96%). Also, other elements such as Br, Sn, Pb, Zn, Fe, and Sb were present. XRD analysis showed that metallic copper, tin, lead, aluminum, and iron were the main phase constituents; Viability of *S. graminofaciens* in the presence of copper was studied and found that *S. graminofaciens* didn't grow in the presence of copper; Different types of bioleaching methods (one-step, two-step, spent medium) were studied and found that two-step bioleaching method was the preferred method for extraction of copper; The maximum dissolution of copper (88.1%) was achieved using modified starch nitrate medium at initial pH 5 with inoculation 3.09×10^3 CFU of *S. graminofaciens* in 50 ml medium for 5 days at 30°C and shaking speed 200 rpm. Also, the best pulp density was 0.5% with a size fraction +150 mesh. Both 0.01 g/ml glucose and 0.002 g/ml ammonium sulfate were the best carbon and nitrogen sources, respectively; The mechanism of bioleaching was studied by ensuring the production of enzymes in metabolites of *S. graminofaciens* and found that high dissolution occurred in the un-denatured sample (not-heating) than denatured one (heating). The effect of bioleaching was confirmed by the peaks shifting of FTIR spectra of e-waste with and without *S. graminofaciens* and the new peak of isothiocyanate in the presence of *S. Graminofaciens*; SEM images showed that the e-waste after treatment contained more pores than before treatment, and this refers to the liberation of metals from e-waste to solution during treatment.

Funding

This research received no external funding.

Acknowledgments

This research has no acknowledgment.

Conflicts of Interest

The authors declare no conflict of interest.

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