Isolation and characterization of copper leaching microbes from sanitary landfills for copper bioleaching of waste printed circuit boards

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Research

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Abstract

Electronic waste has been the fastest increasing waste generated globally and predicted to surpass 111 million tons per year by the end of 2050. The amount of e-waste is a concern not just due to its volume, but also due to its high composition of heavy metal elements, which has leads to increased development of urban mining in terms of heavy metal extraction. One common method of extraction, i.e., acid leaching, is known for its harmful residual leachate, in which can have a high impact on the environment. This focuses on the alternative leaching techniques known as bioleaching, which take advantages of microbial activity in mobilization of metal into a more soluble form. Strains from sanitary landfill soil were isolated in acidic media and identified as *Bacillus* sp. strain SE, *Lysinibacillus* sp. strain SE2, *Bacillus* sp. strain S1A, and *Oryzobacter* sp. strain SC. Among the isolated stains, the identified strain *Oryzobacter* sp. strain SC was able to extract up to 23.36 ppm copper from waste printed circuit boards using a two-step bioleaching process, confirming the ability of the strain to perform bioleaching of copper from e-waste.

Introduction

The global development of technology and reduced lifespan of equipment have resulted in an ever-increasing volume of end-of-life equipment, also known as waste electrical equipment or electric and electronic waste (e-waste). According to the Global E-waste Monitor 2020, 53.6 million tons of e-waste was dumped globally in 2019 (Forti et al. 2020), an increase of 21% from the previous year. Developed countries such as China, the United States of America and the European Union each produce almost three times the amount of e-waste as that of most developing countries (Li et al. 2015), approximately 23% of was exported both legally and illegally to other developing countries, especially in Asia and Africa (Breivik et al. 2014). Moreover, in the Asia region alone, it was reported that 24896 kilotons of e-waste are being generated while 42130 kilotons are being exported to Asia. In Malaysia alone, 364 kilotons of e-waste are estimated to have been generated amounting to nearly 11.1 kg generated per capita, while almost 547 kilotons are being exported to this country (The Global E-waste Statistic Partner 2020). E-waste is increasingly recognized as a serious, worldwide issue.

Previous studies have stated that one of the major issues in e-waste is the rate of recollection and recycling. Out of 53.6 million tons dumped, only 17.4% of that amount was recycled. This small percentage was due to low collection and recycling rates, even in countries that have formal e-waste management systems. A primary concern of the low rate of e-waste recollection and recycling is that most of the dumped e-waste made its way to landfills without proper management and recycling. This issue occurred due to a lack of understanding of the impact of e-waste on the environment without a proper management system. In developing countries such as Malaysia, the issue occurred mostly because of misunderstanding on how the disposal of e-waste has to be done and the reluctance to pay extra fees to properly discard e-waste (Agamuthu and Fauziah 2010). On the other hand, e-waste that was exported from developed countries to developing countries will most likely be dismantled and processed with informal methods, which bring hazards both to the environment and to the operator

handling the e-waste (Breivik et al. 2014; Golev et al. 2016). The major components of e-waste are metals usually found in capacitors and other electronic parts. Without a proper recycling procedure and with the e-waste being dumped in landfills, metal pollution is likely to occur considering the environment in a landfill.

Copper is the second most abundant metal in e-waste, usually in waste printed circuit boards (wPCB) and can easily seep into the soil and be easily absorbed into water bodies, which might be used as a source of drinking water and other household needs. Excess copper intake in the human body or copper toxicity, can introduce general illnesses such as nausea, vomiting and can also lead to fatal Wilson disease (Taylor et al. 2020). Such issues occur because copper itself is not being degradable in nature leading to accumulation in both plant and water sources (Masindi and Muedi 2018). In retrospect, as copper is being used in abundant amounts especially in generation of technological devices, the concept of urban-mining or recycling of copper is deemed to be the way forward because the sources of natural copper ore are running low globally (Ciacci et al. 2017; Dong et al. 2020). Copper extraction from wPCB has been performed through a few methods that involve mechanical and chemical processes (Shirodkar and Terkar 2017).

There are many ways, in which e-waste can be processed. This includes methods such as dismantling (Suja et al. 2014), and metallurgical processes (Debnath et al. 2018). The more prominent method in the leaching process, usually involves using strong acidic media that dissolve the e-waste to recover the metal component (Santos et al. 2011). This process requires the e-waste to be shredded into smaller pieces before being subjected to each leaching agent. However, the major problem with this kind of application is that the leaching agents, are usually strong acids such as aqua regia and sulfuric acid, which leave an acidic leachate after the process is completed. Similar to other waste treatment method, the concept of bioremediation can also be applied for e-waste handling and processing, in which contaminant is treated into its less toxic form using microorganisms by bioleaching process. Compared to common acid leaching, bioleaching uses microbial activity for metal solubilization, transforming the metals to their soluble, extractable and recoverable forms (Liu et al. 2008), avoiding the heavy usage of chemical reagents for the leaching process.

In this study, the novelty of bioprocess for leaching of copper concentrate from wPCB was performed as a standalone process with limited use of other preliminary processes, including mechanical grinding, acid leaching and pyrometallurgical treatment, was investigated. This approach will reduce the amount of secondary pollutants produced from the process. Besides, due to constant temperature throughout the year, bacterial strain from equatorial climate, like Malaysia, is hypothesized to be more resilient for bioleaching as compared to that from the seasonal countries. This could provide better bioleaching performance for e-waste. Thus, several acidophilic bacterial strains were isolated from a Malaysian sanitary landfill and was grown at room temperature, giving much room and variation in the bacteria that can be employed as bioleaching bacteria. Bioleaching on wPCB was performed using the isolated strains to evaluate the microbial activity of the strain to mobilize copper to an extractable form. As the mechanical grinding process was excluded from this study, the expected result will be much lower

compared than that of a previous study, but the physical traits of the wPCB used can be maintained, allowing only the copper to be mobilized through this metal solubilization concept.

Materials And Method

Evaluation of potential microbial sources for bioleaching from Sanitary landfill

Samples of soil, leachate and sludge were collected from the Jeram Sanitary landfill, Malaysia (3°11'24.1"N 101°21'49.5"E). Leachate and sludge samples were collected from the leachate treatment plant, while soil samples were collected from the topsoil of the landfill. One gram of each soil and sludge samples was added to 50 ml centrifuge tubes containing 20 ml sterile water and then vortexed vigorously and filtered. The mixture was centrifuged at 4000 rpm for 10 minutes and the supernatant was collected. The supernatant was initially grown in 15 ml Luria-Bertani (LB) media to enrich the microbial growth. Then, the sample was washed with 9K-Fe media (Wang et al. 2018) containing $(NH_4)_2SO_4$ (3 g/L), KCl (0.1 g/L), $MgSO_4$ (0.5 g/L), K_2HPO_4 (0.5 g/L), $CaNO_3$ (0.01 g/L) and $FeSO_4$ (15 g/L), resuspended in the same medium at pH 2.5 and growth by incubating at 37°C and 160 rpm for 7 days. The 9K-Fe was used to facilitate the growth of bacterial communities with potential for bioleaching.

Acquisition of waste printed circuit boards (wPCB)

Waste printed circuit boards (wPCB) were collected from an electronic recycler, UsedComputerMalaysia Sdn Bhd in Kuala Lumpur, Malaysia. Two types of wPCB were used in the experiment: 1. without any components and an exposed copper layer and 2. with components and a copper layer between the PCB structures. For the wPCB containing components, components such as capacitors, diodes and others were removed beforehand, leaving only the board. Both types of wPCB were then cut into smaller pieces (approximately 2 cm x 2 cm) using a mini saw. These wPCB were used as the material to evaluate the ability of the isolated bacteria to perform bioleaching.

Screening of bacterial sources for bioleaching using exposed copper wPCB as the target Samples that demonstrated growth in 9K-Fe media were used for copper bioleaching experiment prior to the isolation of the potential bacterial strains for bioleaching. 9K-Fe media was used as the bioleaching media with a pH of 2.50 at 37^{o}C at 160 rpm for seven days. The copper concentration in the media after the bioleaching experiment was then analyzed using atomic absorption spectroscopy (AAS, SHIMADZU AAS700). The standard was calibrated using an AAS Copper Standard. The standard was prepared at three different concentrations to obtain the standard calibration curve. The standard curve was calibrated to have a correlative coefficient of > 0.95. A characteristic concentration check value was determined to detect the sensitivity of the AAS.

Isolation and identification of copper leaching bacteria

After bioleaching was performed, samples that showed the highest percentage of bioleaching were used as the inoculum for bacterial isolation. The selected sample was enriched in 9K-Fe liquid media with a pH of 2.50 at 37^{o}C, 160 rpm for seven days and streaked on LB plates to pick for single colonies. The pure cultures were then transferred to fresh 9K-Fe media and growth for 7 days at 37°C and 160 rpm . Then, the grown cultures were subjected for DNA extraction using DNA kit (QIAmp DNA Mini Kit). The genomic DNA obtained from the isolated strains were further used for PCR amplification of 16S rRNA gene using universal forward primer 27F (5 AGAGTTTGATCMTGGCTCAG 3,) with reverse primer 1492R (5' CGACGACCATGCANCACCT 3'). The PCR reaction mixture (25 µl) was performed using Promega, GoTaq® DNA Polymerase kit, which consisted of 5µL of 5x of Green GoTaq buffer, 1.5 µL of $MgCl_2$, 0.5 µL of dNTP mixture, 1.25 µL of each primer, 0.125 µL of DNA polymerase and 1.25 µL of template DNA. PCR thermocycler (ProFlex PCR System, Thermo Fisher Scientific) condition provided as 2 minutes and 30 seconds at 95 °C followed by 35 cycles of 15 seconds at 54 °C, followed by 7 minutes extension at 72 °C. Purified PCR product was sent for Sanger sequencing at First Base Laboratories Sdn Bhd (Malaysia) using an ABI Prism® 377 DNA Sequencer with the BigDye® Terminator 3.0 Cycle Sequencing Kit. The sequencing product was carried out both directions and the result based on the obtained sequence was aligned and compared with GenBank sequences by BLAST analysis to identify the isolated bacterial species.

Bioleaching of wPCB with an embedded copper layer

The isolated strains were used to perform bioleaching of wPCB with a copper layer embedded between the fiber of the wPCB. The two-stage, spent bioleaching method was used because it allows a higher rate of activity and bioleaching by the bacteria by mitigating the toxicity of the wPCB component to the bacterial colony (Işıldar et al. 2016), thus, allowing the bacterial colonies activities to be fully maximized for the bioleaching. Seven days of inoculation inside the 9K-Fe media with a pH of 2.50 was performed to maximize the growth of the colonies for each isolate without wPCB inside the leaching media followed by seven days of bioleaching activities with added wPCB. The condition of the bioleaching process was set at 37^{o} C at 160 rpm. The media was centrifuged at 4000 rpm for 10 minutes and the supernatant was extracted before being diluted for analysis using AAS to determine the copper concentration.

Mobilization of copper by an isolated strain from the landfill

Bacterial strain with highest copper recovery from the bioleaching experiment with wPCB was further tested for bioleaching experiment with a copper strip. A copper strip was chosen as a pure copper indicator to evaluate the interaction of the potential strain with copper. A 1 g copper strip was added to a 15 ml culture of the strain grown in the 9K-Fe media for seven days under the same conditions as the bioleaching that was performed previously. Samples were extracted at the end of the process and diluted

prior to AAS analysis. The media were analyzed using AAS to determine the mobilization of copper from the strip.

Results

Evaluation of copper leaching bacteria from sanitary landfills

Samples taken from the landfill in the form of soil, sludge and leachate were grown in LB and modified 9K-Fe medium under acidic conditions. The samples were collected from these sources because they are known to host a high microbial activity and were expected to contain multiple colonies of bacteria to be evaluated for the bioleaching. Both sludge and leachate samples showed growth in LB media but no growth in 9K-Fe media. On the contrary, soil samples showed growth in both media and therefore, were used as bacterial source for bioleaching.

Four samples of the soil from different sampling point, Soil 1, Soil 2, Soil 3 and Soil 4 were used directly for screening bioleaching using exposed copper wPCB. Bioleaching was conducted using wPCB with an exposed copper layer. Bioleaching was performed according to the one-step bioleaching process (Yang et al. 2008) for the bioleaching test. From the AAS analysis, bioleaching using Soil 1 extracted the highest amount of copper which is 37.7394 ppm in comparison to the other three soils, Soil 2 (2.9960 ppm), Soil 3 (2.7170 ppm) and Soil 4 (2.8395 ppm) as shown in Table 1.

One-step bioleaching was chosen for the screening of samples because it allows a faster process, provides enough data for identification, and neglects the toxicity of the wPCB toward the strain as the target used is a blank wPCB without components. Physical changes in the leaching media can also be observed from pale green (due to Fe (II)) to shade of blue (due to Cu (II)) with the formation of brown precipitate in the leaching media (Fig.S1).

Isolation of bacterial strain with potential bioleaching ability

Soil 1 was further inoculated to obtain individual colonies for bioleaching. Soil 1 was enriched in 9K-Fe liquid media with pH 2.50 and grown at 37^{o}C for seven days. The grown cultures were streaked on LB agar plates to ensure growth of single colonies (Fig.S2). Individual colonies obtained were then selected and identified by partial 16S rRNA gene sequencing. Four pure colonies were successfully isolated and named strain SE, SE2, S1A and SC. The four colonies were subjected for partial 16S rRNA identification and results are shown in Table 2. Both SE and SC were identified as *Bacillus* sp. with 99% similarity to *Bacillus bingmayongensis* for SE and 98.23% similarity to *Bacillus terrae* for SC. Meanwhile, SE2 was identified as *Lysinibacilllus* sp. with 98.81% similarity to *Lysinibacillus boronitolerans* and S1A was identified as *Oryzobacter* sp. with a similarity of 96.55% to *Oryzobacter terrae*.

Bioleaching of wPCB using the isolated strains

The isolated bacterial strains with bioleaching potential were subjected for two-step bioleaching mechanism in order to evaluate their ability to perform bioleaching. Two-step bioleaching mechanism was used to minimize the effect of toxicity from the wPCB, since the bacteria were cultured before introducing the wPCB to the 9K-Fe leaching media. The results after analysis using AAS are as follows: SE (1.04 ppm), SE2 (9.16 ppm), SC (23.36 ppm), and S1A (0.62 ppm). The physical changes of the leaching media can also be observed (Fig.S3) from pale green (due to Fe (II)) to shade of blue (due to Cu (II)) with the formation of brown precipitate in the leaching media.

Evaluation of the strain SC interaction with copper for the metal mobilization

The strain with the highest extraction of copper from wPCB was evaluated again using a copper strip to observe the actual copper extraction amount by the strain against. Since SC showed the highest bioleaching activity among the four isolates, it was chosen for evaluation by adding 1 g of copper into the culture medium to determine the activity against copper. Reading from AAS for the total copper concentration extracted. A total of 0.80 ±0.02 mg/g was extracted from the copper strip in the presence of the strain compared to the control, with only 0.03 ±0.08 mg/g of copper extracted.

Discussion

All soil, sludges and leachate samples showed growth in LB media, which confirmed the existence of bacterial colonies inside all the samples, however, only the soil samples exhibited growth in the acidic 9k-Fe media. This observation shows the tolerance of the bacterial colonies in the samples to the acidic conditions, which can be further inoculated for bioleaching. Soil 1 performed the best during the bioleaching screening using the exposed copper wPCB. The usage of this kind of wPCB allowed the one-step bioleaching mechanism to be applied, as the toxicity of wPCB due to its components can be neglected because it was a blank PCB. The significant amount of copper extracted from the wPCB using Soil 1 as the bacterial consortia might occur due to the presence of more colonies capable of copper bioleaching than the other three sources, thus, Soil 1 was used as the bacterial source to further isolate individual colonies for wPCB bioleaching.

Soil 1 was used as a source for bacterial isolation obtaining four individual colonies, SE, SE2, SC and S1A. The four colonies were submitted for partial 16S rRNA gene sequencing for identification. Strain SE was identified as *Bacillus* sp. with 99% similarity to *Bacillus bingmayongensis*. *B. bingmayongensis* is known to be able to grow in a wide range of conditions, including low pH conditions, as it was reported to be able to adapt to the environment in which it was found and grow at a pH of approximately 2–12 (Liu et al. 2014). The strain *B. bingmayongensis* is closely related to *B. cereus*, which has been reported to be able to perform bioleaching of mica from kaolin (Zaremba and Smoleński 2000) and participate in the

biosorption of Zn^{2+} (Joo et al. 2010). Despite the low copper extraction performance of this strain (1.04 ppm), no previous study was reported on the ability of this strain for copper bioleaching.

Strain SE2 was identified to be similar to *Lysinibacillus boronitolerans*, with a similarity of 98.81%. This species is known to be widely available in soil with no mention of its ability to grow under low pH conditions (Nam et al. 2012). The present study, however, was able to isolate the strain under low pH (pH = 2.50) conditions. The strain was reported to show metal-binding properties and remediation toward contaminated matrices (Bustos et al. 2018). Similar to strain SE, strain SE2 also shows low copper bioleaching ability, with copper recovery of 9.16 ppm. This is also the first report on the use of this strain as a bacterial strain for copper bioleaching.

Strain S1A was identified to be similar to *Oryzobacter terrae*, with a 96.55% similarity. There is no previous report on the ability of this strain to perform bioleaching, and this strain performed the worst during the bioleaching procedure with only 0.62 ppm of copper extracted.

Strain SC was identified to be in the same genus as strain SE, *Bacillus* sp., but yielded a better bioleaching result, with a 182.95% difference. Besides, this strain was identified to have 98.23% similarity to *B. terrae*. Similar to strain SE, this strain has never been reported to be involved or to have any abilities to perform bioleaching of copper or any other metal. The present research, however, was able to analyze and identify that the bioleaching of copper can be performed using this strain. Compared to a previous study, we were able to grow this strain at a low pH rather than at pH 5 to 8 and optimum at pH 7 (Díez-Méndez et al. 2017).

All strains displayed clear physical changes in the leaching media during bioleaching. Brown precipitates were formed due to the oxidation of Fe (II) to Fe (III), which was present inside the media. Similar to the proposed ionic exchange in the bioleaching mechanism, these changes were reported in a previous study (Hansford and Vargas 1999; Zhang et al. 2013). The color of the media also changed from a pale shade of green (due to Fe (II) initially in the media) to a shade of blue, indicating the presence of Cu (II) inside the media.

The solubilization of copper by strain SC was evaluated by using copper strips to determine the interaction of the strain during copper solubilization. From the 1g copper strip used, 0.80 mg/g (± 0.02) was solubilized, as analyzed using AAS in comparison to the set control with only 0.03 mg/g (± 0.08) solubilized in the absence of strain SC. By comparison, a previous study reported, the total amount of copper extracted through bioleaching to be approximately 80–90% within a similar timeframe used in the current study (Rodrigues et al. 2015). This result indicates that strain SC has the ability to solubilize copper, thus confirming the activity of the strain shown during the bioleaching process with wPCB.

As this study focused more on the novelty of bioleaching as a standalone process to limit other procedures that involve either heavy mechanical or pyrometallurgical processes, the present research managed to minimize the use of mechanical methods, such as grinding down the wPCB to a size smaller than 0.55 mm (Zhang et al. 2013; Shirodkar and Terkar 2017; Xia et al. 2017), which produces microdust

as the byproduct of the mechanical grinding process and generates secondary pollutants from the process itself. Minimal mechanical treatment helps to retain the wPCB in the original shape after bioleaching and allows the copper to be extracted via solubilization of the metal itself, producing less toxic leachate than the previous study. In fact, copper bioleaching using the standalone process have successfully been performed by all the isolated bacterial strains, though the performance of each strains were varied.

Conclusion

Isolates acquired from landfill soil show the ability to grow under low pH conditions in comparison with isolates retrieve from landfill sludge and leachate, which show no growth. This feature fulfills one of the criteria as a leaching bacterium. The oxidation of Fe (II) to Fe (III) in the leaching media indicated by the brown precipitation also indicates that the bacteria isolated were able to act as iron oxidizers, which fulfill the mechanism of bioleaching proposed from the previous study, although isolates SC and S1A have never been previously mentioned in terms of bioleaching activity. Physical changes in the leaching media after bioleaching also support the proposed hypothesis in which the bacterial strain acquired from the sanitary landfill was able to perform bioleaching because the leaching 9K-Fe media turned blue from colorless, most likely due to the presence of Cu (II) ions. The present study evaluated that copper bioleaching of wPCB is viable as a standalone process. However, the significant difference of performance with bioleaching with pre-treated wPCB by mechanical processes should be further reduced. Further optimization of the bioleaching process including re-evaluating the common factors such as the duration for bioleaching to maximize the overall performance of the process need to be performed.

Abbreviations

e-waste : electric and electronic reference

kg : kilogram

wPCB : waste printed circuit board

mL : milliliter

rpm : revolution per minute

LB : Luria Bertani

g : gram

$(NH_4)_2SO_4$: Ammonium sulfate

KCl : Potassium chloride

$MgSO_4$: Magnesium sulfate

K_2HPO_4 : dipotassium hydrogen phosphate

$CaNO_3$: Calcium carbonate

$FeSO_4$: Iron (II) sulfate

ppm : part per million

Declarations

Ethics approval and consent to participate

Not applicable

Consent for Publication

All authors gave consent to publish this paper

Availability of data and material

All data generated or analyses during this study are included in this article.

Competing interest

Author declared that they have no competing interest

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Contribution

MSR and FAR contributed equally to the manuscript and experiment. MSR, FAR, FNMD, MAMY, HH contributed to writing, reading and approved the final manuscript.

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References

1. Agamuthu P, Fauziah SH (2010) Heavy metal pollution in landfill environment A malaysian case study. 2010 4th Int Conf Bioinforma Biomed Eng iCBBE 2010. https://doi.org/10.1109/ICBBE.2010.5516886
2. Breivik K, Armitage JM, Wania F, Jones KC (2014) Tracking the global generation and exports of e-waste. Do existing estimates add up? Environ Sci Technol 48:8735–8743. https://doi.org/10.1021/es5021313
3. Bustos MC, Ibarra H, Dussán J (2018) The golden activity of Lysinibacillus sphaericus: New insights on gold accumulation and possible nanoparticles biosynthesis. Materials (Basel) 11:. https://doi.org/10.3390/ma11091587
4. Ciacci L, Vassura I, Passarini F (2017) Urban mines of copper: Size and potential for recycling in the EU. Resources 6:. https://doi.org/10.3390/resources6010006
5. Debnath B, Chowdhury R, Ghosh SK (2018) Sustainability of metal recovery from E-waste. Front Environ Sci Eng 12:1–12. https://doi.org/10.1007/s11783-018-1044-9
6. Díez-Méndez A, Rivas R, Mateos PF et al (2017) Bacillus terrae sp. nov. isolated from Cistus ladanifer rhizosphere soil. Int J Syst Evol Microbiol 67:1478–1481. https://doi.org/10.1099/ijsem.0.001742
7. Dong D, van Oers L, Tukker A, van der Voet E (2020) Assessing the future environmental impacts of copper production in China: Implications of the energy transition. J Clean Prod 274:122825. https://doi.org/10.1016/j.jclepro.2020.122825
8. Forti V, Baldé CP, Kuehr R, Bel G (2020) The Global E-waste Monitor 2020
9. Golev A, Schmeda-lopez DR, Smart SK et al (2016) Where next on e-waste in Australia ? Waste Manag 58:348–358. https://doi.org/10.1016/j.wasman.2016.09.025
10. Hansford GS, Vargas T (1999) Chemical and electrochemical basis of bioleaching processes. Process Metall 9:13–26
11. Işıldar A, van de Vossenberg J, Rene ER et al (2016) Two-step bioleaching of copper and gold from discarded printed circuit boards (PCB). Waste Manag 57:149–157. https://doi.org/10.1016/j.wasman.2015.11.033
12. Joo JH, Hassan SHA, Oh SE (2010) Comparative study of biosorption of Zn2 + by Pseudomonas aeruginosa and Bacillus cereus. Int Biodeterior Biodegrad 64:734–741. https://doi.org/10.1016/j.ibiod.2010.08.007
13. Li J, Zeng X, Chen M et al (2015) “control-Alt-Delete”: Rebooting Solutions for the E-Waste Problem. Environ Sci Technol 49:7095–7108. https://doi.org/10.1021/acs.est.5b00449

14. Liu B, Liu GH, Hu GP et al (2014) Bacillus bingmayongensis sp. nov., isolated from the pit soil of Emperor Qin's Terra-cotta warriors in China. Antonie van Leeuwenhoek. Int J Gen Mol Microbiol 105:501–510. https://doi.org/10.1007/s10482-013-0102-3
15. Liu YG, Zhou M, Zeng GM et al (2008) Bioleaching of heavy metals from mine tailings by indigenous sulfur-oxidizing bacteria: Effects of substrate concentration. Bioresour Technol 99:4124–4129. https://doi.org/10.1016/j.biortech.2007.08.064
16. Masindi V, Muedi KL (2018) Environmental Contamination by Heavy Metals. Heavy Met. https://doi.org/10.5772/intechopen.76082
17. Nam Y, Do, Seo MJ, Lim S, Il, Lee SY (2012) Genome sequence of Lysinibacillus boronitolerans F1182, isolated from a traditional Korean fermented soybean product. J Bacteriol 194:5988–5988. https://doi.org/10.1128/JB.01485-12
18. Rodrigues MLM, Leão VA, Gomes O et al (2015) Copper extraction from coarsely ground printed circuit boards using moderate thermophilic bacteria in a rotating-drum reactor. Waste Manag 41:148–158. https://doi.org/10.1016/j.wasman.2015.04.001
19. Santos F, Souza C, De, Peixoto R, Rocha P (2011) Copper extraction from electronic scraps by an oxidative acid leaching process. 1–8
20. Shirodkar N, Terkar R (2017) ScienceDirect Stepped Recycling: The Solution for E-waste Management and Sustainable Manufacturing in India. Mater Today Proc 4:8911–8917. https://doi.org/10.1016/j.matpr.2017.07.242
21. Suja F, Abdul Rahman R, Yusof A, Masdar MS (2014) e-Waste Management Scenarios in Malaysia. J Waste Manag 2014:1–7. https://doi.org/10.1155/2014/609169
22. Taylor AA, Tsuji JS, Garry MR et al (2020) Critical Review of Exposure and Effects: Implications for Setting Regulatory Health Criteria for Ingested Copper. Environ Manage 65:131–159. https://doi.org/10.1007/s00267-019-01234-y
23. The Global E-waste Statistic Partner (2020) E-waste Statistic Sheet. https://globalewaste.org/. Accessed 6 Jan 2021
24. Wang S, Chen L, Zhou X et al (2018) Hydrometallurgy Enhanced bioleaching e ffi ciency of copper from printed circuit boards without iron loss. Hydrometallurgy 180:65–71. https://doi.org/10.1016/j.hydromet.2018.07.010
25. Xia MC, Wang YP, Peng TJ et al (2017) Recycling of metals from pretreated waste printed circuit boards effectively in stirred tank reactor by a moderately thermophilic culture. J Biosci Bioeng 123:714–721. https://doi.org/10.1016/j.jbiosc.2016.12.017
26. Yang J, Wang Q, Wang Q, Wu T (2008) Comparisons of one-step and two-step bioleaching for heavy metals removed from municipal solid waste incineration fly ash. Environ Eng Sci 25:783–789. https://doi.org/10.1089/ees.2007.0211
27. Zaremba LS, Smoleński WH (2000) Bacterial destruction of mica during bioleaching of kaolin and quartz sands by Bacillus cereus. Ann Oper Res 97:131–141.

28. Zhang Y, Yang Y, Liu J, Qiu G (2013) Hydrometallurgy Isolation and characterization of Acidithiobacillus ferrooxidans strain QXS-1 capable of unusual ferrous iron and sulfur utilization. Hydrometallurgy 136:51–57. https://doi.org/10.1016/j.hydromet.2013.03.005

Tables

Table 1 Evaluation for bacterial source via one-step bioleaching

Samples	Weight of wPCB (g)	Concetration of copper (ppm)
Soil 1	3	37.7394
Soil 2	3	2.9960
Soil 3	3	2.7170
Soil 4	3	2.8395

Table 2 16s rRNA gene sequence and ascencion number

Isolate name	Most probable BLAST hits with 16s rRNA gene	% Sequence identity	Ascension number (16s rRNA sequence)
SE	*Bacillus bingmayongensis* (NCBI: txid1150157)	99.00	MW426374
SE2	*Lysinibacillus boronitolerans* (NCBI: txid1294264)	98.81	MW426350
SC	*Bacillus terrae* (NCBI: txid1914933)	98.23	MW426370
S1A	*Oryzobacter terrae* (NCBI: txid1620385)	96.55	MW426372