MINI-REVIEW ARTICLE



Bioleaching Extraction of Valuable Metal From E-Wastes: A Mini Review

Abstract: Electrical waste (E-waste) is a significant global issue, with an annual generation of over 50 million tons, causing detrimental effects on both the environment and human health. However, E-waste is also known as a secondary valuable metal resource. Alt-

hough hydrometallurgy and pyrometallurgy are still the preferred methods for metal recovery from E-wastes, bioleaching has gained significant attention as a promising alternative

for metal recovery from E-wastes due to its sustainability, environmental friendliness, and

cost efficiency. In this review, different chemical principles of bioleaching were first discussed. Major bacterial groups that can be applied in bioleaching were then introduced.

The bioleaching procedures and applications have been summarized and analyzed. Finally,



Mingjun Jiang¹, Siyu He² and Yongkui Zhang^{2,*}

¹Department of Biological Science, School of Science, Xi'an Jiaotong-Liverpool University, Suzhou, Suzhou, 215028, China; ²Department of Pharmaceutical & Biological Engineering, School of Chemical Engineering, Sichuan University, Chengdu, 610065, China

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a few perspectives were made on potential future research on bioleaching. **Keywords**: E-wastes, bioleaching, metal recovery, bioleaching process, bioleaching mechanism, bacterial groups.

1. INTRODUCTION

Electronic wastes (E-waste) refer to discarded electronic devices that are no longer economically viable for further use [1, 2]. These devices include various items such as electrical and electronic tools, consumer electronics, automatic dispensers, household appliances, leisure and sports equipment, information technology and computer equipment, and medical devices [3, 4]. The rapid increase in urbanization, industrialization, and population growth has led to a significant rise in the amounts of E-wastes generated globally [5-7]. In 2016, the globe generated 44.7 million tons of E-wastes, and the production increased to 52.8 million tons in 2021, showing a 17% increase [6]. Usually, the composition of E-wastes were metals (60.10%), plastics (15.20%), CRT and LCD screen

(11.90%), metal and plastic mixture (5.00%), pollutants (2.70%), wires and cables (2.00%), printed circuit boards (1.40%) and other materials (1.70%)

E-wastes generally contain both hazardous and nonhazardous substances in their components. The hazardous components include heavy metals such as cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), chlorofluorocarbons, such as polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), and polychlorinated dibenzo-p-dioxins, and di-benzofurans (PCDD/Fs), which pose health hazards to both environment and humans. Non-hazardous components of E-wastes include base metals (e.g., copper [Cu], Selenium [Se], zinc [Zn]) and precious metals (e.g., gold [Au], silver [Ag], and platinum [Pt]) [5, 8, 9]. Overproduction and improper management of E-wastes have created a challenge in terms of environmental pollution, and the safely dispose of E-wastes has become a global concern [10-13].

^{*}Address correspondence to this author at the Department of Pharmaceutical & Biological Engineering, School of Chemical Engineering, Sichuan University, Chengdu, 610065, China; Tel.: +86-28-85405221; Fax: +86-28-85405221; E-mail: zhangyongkui@scu.edu.cn

Table 1. Contents of valuable metals in E-wastes.

| E-wastes | Metals | Concentration (ppm) | Refs. |
|---------------------------|--------------------|---------------------|-------|
| Printed circuit boards | Au, Pd, V, Ga | 1-100 | [18] |
| | Al, Ag, Ni, Zn, Co | 100-100000 | |
| | Cu, Fe | >1000000 | |
| Lithium-ion batteries | Co, Li, Ni | 10000-100000 | [19] |
| Liquid crystal displays | In, Sr, Co | 100-2000 | [20] |
| Light emitting diode lamp | Au | 100-10000 | [21] |
| | Ga | 10-200 | |
| | Ag | 100-1000 | |
| HDD magnets | Nd, Pr, Dr | >10000 | [22] |

Despite the hazardous components, E-wastes could be viewed as a secondary metal resource, as they contain a variety of valuable metals, as shown in Table 1. For example, electronic devices such as computers and cell phones consist of ~48% Ferrum (Fe), 21% plastics, 7% Cu, and 6% other precious metals [6]. In addition, waste printed circuit boards (WPCBs) of computers contain a high content of metals, including 2.091x10⁵ g/ton Cu, 628.00 g/ton Ag, 220.82 g/ton Au, 23.63 g/ton scandium (Sc) [14], which are significantly higher than those found in natural deposits, making them a high-grade metal reservoir. Hence, appropriate technologies should be adopted to recycle valuable metals from E-wastes from economic and environmental perspectives [15-17].

Typically, conventional E-wastes recycling facilities employ two methods for metal recovery and purification, namely pyrometallurgy and hydrometallurgy. The former employs heat treatment, such as roasting and smelting, while the latter uses chemical leaching with acid or alkali wash [23, 24]. However, these traditional methods have several limitations, including environmental pollution, low metal recovery rates, time-consuming and high operational costs [25, 26]. Hence, a more sustainable and cost-effective technology is needed for E-wastes recycling.

Bioleaching is a process in which microorganisms are used to transform insoluble metal compounds into their water-soluble form [27-30], which is a promising technology for recovering valuable metals from E-wastes in an environmentallyfriendly and cost-effective way. Bioleaching

technology is primarily applied in recovering metals from low-grade metallic ores, mine tailings, and contaminated soils, having remarkable effects [31-36]. Owing to the scarcity of natural resources, bioleaching technology researchers have extended their focus toward the recovery of metals from secondary solid wastes such as E-wastes [3, 6], slag [37, 38], spent catalyst [39, 40], sludge [41, 42] and fly ash [43, 44]. In the past decades, many different microorganisms have been investigated for the bioleaching process. Among them, chemolithotrophs and organotrophs are the two most widely used microorganisms to mobilize metals from E-wastes based on different mechanisms [9]. Researchers have been devoted to studying the valuable metal recoveries and the efficacy of various organisms in mobilizing the metals in bioleaching the E-wastes by microbes [6, 45]. Advances have been made in bioleaching for extracting valuable metals from E-wastes. With the rapid development of bioleaching technology, there is a need for systematic reviews about bioleaching metals from E-wastes [10, 46].

Therefore, in the current work, bioleaching approaches are pursued based on different chemical principles. Major groups of microorganisms used in bioleaching are introduced in the second section. The third section mainly focuses on bioleaching methods and procedures to extract metals from E-wastes. Finally, the perspective of future bioleaching development and efficient assessment of bioleaching metals from E-wastes have also been suggested.

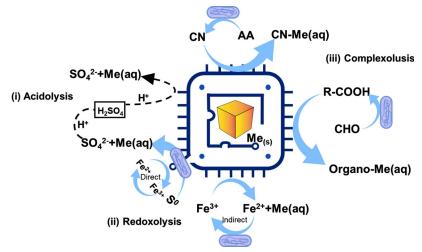


Fig. (1). Different bioleaching mechanisms. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

2. THE PRINCIPLE OF BIOLEACHING

In nature, a wide range of chemolithotrophic and organotrophic microorganisms could participate in the bioleaching of ores and solid wastes [31, 40, 47]. The chemical principles of various microbial leaching processes include acidolysis, redoxolysis, complexolysis, and their binary or ternary combinations (Fig. 1) [48-50].

2.1. Acidolysis

Acidolysis is a solubilization process in which the acidic compounds protonate and oxidize the oxygen atoms, coating the insoluble metal compound surface, and leading to its solubilization [50]. Microbes involved in this process are chemolithoautotrophic bacteria and fungi (2). The protonation of oxygen atoms occurs through organic acids (acetic, citric, formic, gluconic, and pyruvic acid) and inorganic acids (sulphonic acid) produced by microbes. Particularly, the sulphonic acids could be formed from externally added metal sulfate such as ferric sulfate and ferrous sulfate [48, 50].

The reactions involving the acidolysis process are presented below:

$$MeS_2+3.5O_2+H_2O \rightarrow Me^{2+}+2SO_4^{2-}+2H^+$$
 (1)

where 'MeS' represents metal sulfate.

Metal solubilization from oxidic ore by acidophiles through H₂SO₄ is given in Eqs. (2)

$$MeO+H_2SO_4 \rightarrow MeSO_4+H_2O$$
 (2)

where 'MeO' is the metal oxide.

Similarly, the solubilization of organically-bound metals by acidophilic microbes through H₂SO₄ is proposed in Eq. (3).

$$Me-R+H_2SO_4 \rightarrow MeSO_4 + RH_2$$
 (3)

where 'Me' and 'R' represent the organically attached metal and functional group or chain, respectively.

2.2. Redoxolysis

Redoxolysis is the oxidation-reduction process in which the insoluble metal compounds are converted to their corresponding ionic form (soluble form) by a mixed solvent of ferric ions and microbes, in which the ferric ions serve as oxidizing agents. Organic acids secreted by microbes are also involved in this process, which aids in the dissolution of the metal. Different organic acids may lead to different bioleaching mechanisms, producing various intermediates and influencing the leaching efficiency [51]. Heterotrophic bacteria, chemolithoautotrophic bacteria, and fungi mainly participate in this process [2]. In the redoxolysis process, ferric ion first serves as a strong oxidizing agent or lixiviant during the solubilization of metal sulfides. The formed ferrous iron (Fe²⁺) could then be oxidized back to Fe³⁺ through biochemical reactions by microbes and thus recycled for further metal solubilization [52].

Fe³⁺ dissolution of MeS is also shown below:

$$2Fe^{2+}+2H^{+}+0.5O_{2} \rightarrow 2Fe^{3+}+H_{2}O$$
 (4)

$$MeS+2Fe^{3+} \rightarrow Me^{2+} + S^0 + 2Fe^{2+}$$
 (5)

The type of metal and its oxidation state would influence its solubilisation. In fungal leaching of manganese, for instance, oxidised manganese (MnO₂) is mobilised through enzymatic reduction, as given in Eq. (6) [40]:

$$MnO_2 + 2e^- + 4H^+ \rightarrow Mn^{2+} + 2H_2O$$
 (6)

2.3. Complexolysis

Complexolysis is the process in which metalligand complexes and chelates are formed, leading to the solubilization of valuable metals [53]. Microbes involved in the complexolysis process are heterotrophic bacteria and fungi [2]. Complexolysis usually follows a chelation mechanism to stabilize the metal ions coming into the solution by acidolysis. Thus, metals are stabilized by complex formation between metal ions and organic acid. Some examples of combinations between metals and ligands are citric acid with Mg, tartaric acid, or oxalic acid with Fe, Mg, and Al [50].

A possible complexolysis reaction for Fe and Mg solubilization is as follows [51, 54]:

$$Mg^{2+}+2C_6H_8O_7 \rightarrow Mg(C_6H_7O_7)_2+2H^+$$
 (7)

$$Fe^{2+} + 2C_2H_2O_4 \rightarrow Fe(C_2HO_4)_2 + 2H^+$$
 (8)

After the above steps, metals in the E-wastes are present as metal-ligand complexes and chelates [55]. Several methods, including precipitation, ion exchange, solvent extraction, adsorption, and electrochemistry, are available for the separation and purification of metals from the leachate solution.

3. MICROORGANISMS

3.1. Chemolithotrophs

Chemolithotrophs are acidophilic (i.e., acidtolerant) microorganisms with a preferable growth temperature range between 28-80°C, which use inorganic compounds, such as ferrous iron (Fe²⁺), elemental sulfur (S), or reduced sulfur compounds as energy sources (47). Some of them could utilize atmospheric carbon dioxide (CO₂) as a carbon source, while others use organic carbons. Chemolithotrophs are classified into three categories, viz., mesophilic, moderately thermophilic, and thermophilic, based on growing-temperature preferences [56].

Mesophiles are the most frequently used bacteria in industrial bioleaching, with a preferable growth temperature range of 28-37°C. Acidithiobacillus and Leptospirillum are the two commonly used genera of mesophiles in bioleaching processes. Acidithiobacillus is a rod-shaped, non-sporeforming, and Gram-negative bacterium. Acidithiobacillus species such as A. ferrooxidans (iron and sulfur-oxidizing), A. thiooxidans, and A. caldus (sulfur-oxidizing) are commonly used in the bioleaching process [57-63]. Unlike Acidithiobacillus species, Leptospirillum ferriphilum could use zero-valent, reduced sulfur, and hydrogen to derive energy, inorganic setting with a pH between 1.3-2.0 provides the optimal conditions for it to grow [64-67].

Moderate thermophiles grow at a temperature of 40-60°C with a pH range between 1.5-2.5 [47]. Dominant members of moderate thermophiles in bioleaching include Eubacteria, i.e., Acidimicrobium sp., Ferromicrobium sp., Sulfobacillus sp., and Acidithiobacillus caldus [68-71].

Thermophiles are desired in bioleaching to tolerate the high temperatures between 60-80°C, many of which belong to the domain archaea [71]. The use of thermophilic bacteria improves not only reaction rates but also yields of extracted metals from solid wastes with increased operating temperatures. Dominant thermophilic archaea under the order Sulfolobales include sulfolobus, metallosphaera, acidianus, and sulfurisphaera [72, 73].

Microbes in chemolithotrophs are predominant in acidic conditions with a pH of 2.0 or below during the metals bioleaching. Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans are known for recovering metals from E-wastes. For example, Chen et al. [61] studied column bioleaching of copper from waste printed circuit boards (WPCBs) using Acidithiobacillus ferrooxidans. They achieved a copper recovery of 94.8% after 28 days of column bioleaching, starting from materials containing 24.8% copper. Priya et al. [74] investigated the bioleaching of selected metals (i.e., Cu, Zn, Pb, Ni) from high-grade WPCBs using a pure culture of Acidithiobacillus ferrooxidans. They observed a maximum leaching rate of 89% Cu, 86% Zn, 52% Pb, and 69% Ni with bioleaching for 18 days at a pulp density of 7.5 g/L and the comminution fines in the size range of 0.075-1 mm. Roy et al. [19] used the cost-efficient

autotrophic bacterium *Acidithiobacillus ferrooxidans* to leach metals from the mixture of LiCoO₂-based lithium-ion batteries from mobile phones, laptops, and small gadgets from different manufacturers. After increasing the biogenic H₂SO₄ production in the culture media and replenishing the bacterial culture for three cycles, with a pulp density of 100 g/L and leaching time of 72 h, they achieved a recovery rate of 94% for cobalt and 60% for lithium. In the above bioleaching process, the metals in E-wastes are oxidized by Fe³⁺, while Fe³⁺ is then reduced to Fe²⁺. *Acidithiobacillus ferrooxidans* use Fe²⁺ as an energy source to support their growth and regenerate Fe³⁺ from Fe²⁺, maintaining continuous bioleaching with metal solubilization.

3.2. Organotrophs

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Organotrophs, also known as heterotrophic microorganisms, use organic carbon as their energy source. Fungi and cyanogenic microbes are the major groups of organotrophs [47]. Growing rely on an organic carbon source, fungi produce organic acids while cyanogenic microbes produce hydrogen cyanide, and both use these resulting products to leach metals from E-wastes [75].

Fungi are suitable for metal bioleaching from E-wastes because of the high level of oxides in these wastes, which could be the energy sources for chemolithotrophs [55]. Fungal bioleaching is usually performed at a pH of 3.0-7.0 and a temperature of 25-35°C [29, 76]. Penicillium Simplioissimum and Aspergillus Niger are majorly utilized fungi species in treating solid wastes [77-83]. Esmaeili et al. [84] conducted a study using Penicillium simplicissimum to leach Cu, Ni, and Al from computer-printed circuit boards (CPCBs). The results showed that acidolysis was the main mechanism for leaching Al and Ni, while complexoysis was the primary mechanism for Cu leaching. The optimal conditions of metal leaching were determined as follows: sucrose concentration of 60 g/L, pulp density of 16 g/L, and an initial pH of 6. Under these conditions, the leaching rate achieved was 100% for Cu, 70% for Ni, and 98% for Al. Nili et al. [51] used a pure culture of Penicillium simplicissimum in a bubble column bioreactor to extract Cu and Ni from mobile phone printed circuit boards (MPPCBs). The study showed that the maximum recovery rates of Cu were 96.94% under 8.8% (v/v) of molasses, aeration rate of 0.29 (l/min), and 10 g/L of the Ewastes powder, and the maximum recovery rates of Ni was 71.51% under 1.9% (v/v) of molasses, aeration rate of 0.37 (l/min), and 10 g/L of the Ewastes powder, while the maximum simultaneous leaching rate for both Cu and Ni was determined to be 40%. This may be due to the different effects of molasses concentration on the recoveries of Cu and Ni; with the increase of molasses concentration, the recoveries of copper increased, and with its decrease, the recoveries of nickel increased. Cui et al. [85] employed Aspergillus niger to produce low-concentration organic acids for dissolving In₃O₂ from waste liquid crystal display (LCD) panels with high toxicity. The main organic acids identified in the fermentation broth of A. niger were oxalic acid, gluconic acid, malic acid, and citric acid. The carboxy groups from organic acids and proteins were critical in releasing H⁺ for leaching indium. The result revealed that optimizing the initial pH, shaking speed and sucrose addition in the fermentation system could enhance the indium bioleaching efficiency from 12.3% to 100%. In a study by Kim et al. [86], bioleaching of spent Zn-Mn or Ni-Cd batteries was conducted using six Aspergillus strains: A. fumigatus KUC1520, A. flavipes KUC5033, A. japonicus KUC5035, A. tubingensis KUC5037, A. versicolor KUC5201, and A. niger KUC5254. Two different nutrients, malt extract (ME) and sucrose media, were used to produce various types of organic acids. Citric acid was identified as the major organic acid produced in sucrose media, while oxalic acid was dominant in ME media. The leaching results indicated that citric acid was a more suitable agent for metal extraction from both spent Zn-Mn or Ni-Cd batteries, as oxalic acid tended to form insoluble metal oxalates. More than 90% removal efficiency of Zn and Mn from the Zn-Mn battery by all species in sucrose, except A. niger KUC5254 after eight days, meanwhile more than 90% of Ni, Cd, and Zn were extracted by A. niger KUC5254 and A. tubingensis KUC5037 in sucrose for Ni-Cd battery after four days. This study highlighted that A. tubingensis as the most efficient fungal bioleacher because of its non-ochratoxigenic characteristics and ability to efficiently extract various metals, such as Ni, Cd, and Zn. Apart from cyanogenic microbes produce hydrogen cyanide (as a secondary metabolite) to be used for bioleaching metals from solid wastes such as spent refinery catalysts [87] and printed circuit boards [88]. These bioleaching processes

are generally performed at a pH between 7.0–11.0 and a temperature between 25-35°C [88, 89]. The major group of Cyanogenic bacteria used in bioleaching include Chromobacterium violaceum, Bacillus megaterium, and various Pseudomonas species such as P. fluorescens, P. aeruginosa, P. plecoglossicida, P. putida, and P. balearica [88, 90]. In the presence of cyanide, metal-cyanide complexes with higher water solubility and chemical stability could be formed. In a study by Merli et al. [90], Pseudomonas aeruginosa (belonging to cyanogenic bacteria) was used to mobilize Ag from the WPCBs obtained from discarded computers through a two-step process. In the first step, the bacteria were grown to produce cyanide in the optimized conditions (pH 8 and 1 g/L of glycine). After the maximum cyanide production was reached in 20h, WPCBs were added to the growth medium, and the pH was set to 9 in the second step. Through optimization, a remarkable 90% mobilization of Ag was achieved, while the bioleaching of Au displayed an efficiency of approximately 20%. Li et al. [91] investigated the leaching capability of Pseudomonas fluorescens for gold extraction from WPCBs. The results demonstrated a gold recovery rate of 42% under optimal conditions. During the bioleaching process, gold and Ag are dissolved in the alkaline cyanide solution, forming a gold cyanide complex, with cyanide being produced by P. fluorescens. The microbes thrive within a pH range between 8-9, generating cyanide ions (CN⁻) that are available for bioleaching in the alkaline environment. P. fluorescens exhibits strong adaptability, low pathogenicity, wide distribution, and cost-effectiveness, making it a more promising choice in bioleaching research. Pseudomonas spp. was also found to have differential capacity in biosorption and bioleaching of iron and copper. Kaur et al. [92] investigated the 20-day iron and copper desorption ability from E-wastes (PCBs) among seven kinds of microbial cultures (including Entinus edodes, Pleurotus florida, Ganoderma lucidum, Aspergillus niger, Trametes versicolor, Streptomyces spp., and Pseudomonas spp.) with suitable pH (bacterial cultures for 6.5 and fungal cultures for 7.2) under appropriate temperature (bacterial cultures for 27±2°C and fungal cultures for 25±2°C. The outcome demonstrated the exceptional capacity of Pseudomonas spp. for leaching iron and copper via bio-catalysis of laccase enzymes. In summary, combined the findings together, it could conclude that nearly all metals present in E-wastes can be bioleached by various microorganisms. However, the leaching efficiency of different metals varies significantly depending on the specific organism involved. Hence, it is essential to select appropriate microbial species based on the specific metals present in the E-wastes for optimal bioleaching outcomes.

3.3. Mixed Microorganisms

While single microbial strains are commonly used for metal recovery, using mixed culture comprising two or more bacteria appears to be more efficient in leaching a wide range of metals from E-wastes. Wang et al. [93] conducted a study comparing the bioleaching efficiency of Cu, Pb, and Zn from WPCBs using Acidithiobacillus ferrooxidans (A. ferrooxidans) and Acidithiobacillus thiooxidans (A. thiooxidans) and a mixture of both. The result showed that the mixture achieved a leaching percentage of 99.9% for Cu, which was higher than the individual strains of A. ferrooxidans (99.0%), and A. thiooxidans (74.9%). Brandl et al. [83] employed a mixed culture of Thiobacillus ferrooxidans and T. thiooxidans in a bacterial leaching process to mobilize metals from electronic waste materials. In the presence of 5-10 g/L of scrap concentrations, the Thiobacilli strains exhibited over 90% leaching of the available Cu, Zn, Ni, and Al. The findings suggest the potential for industrial applications in E-waste treatment, as bacteria, such as A. ferrooxidans and A. thiooxidans are widely present in nature and offer better leaching rates of metals that meet industrial requirements.

4. BIOLEACHING IN PRACTICES

4.1. Bioleaching Approaches

There are three approaches for bioleaching, which are one-step, two-step, and spent-medium, respectively. The mechanism for each method is shown in Fig. (2). For the one-step approach, microorganisms would be directly inoculated with solid E-wastes in the microbial culture where microbial culturing, growth, and metal leaching occur together [61, 74]. One-step leaching can reduce operating costs because fermentation and bioleaching are carried out simultaneously. However, the

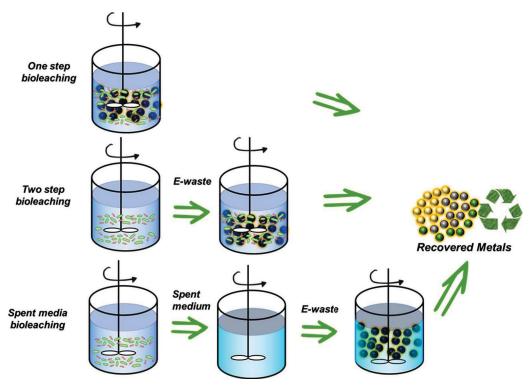


Fig. (2). Different bioleaching methods. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

efficiency of one-step bioleaching may be limited in the presence of poisonous materials in E-wastes due to the inhibiting effects on microbial growth. As a result, direct growth of microorganisms in the medium containing E-wastes limited bioleaching, may not be preferred.

The two-step approach is thus proposed to enhance the growth of the microorganisms without any hindrance or toxicity effect. In this approach, microbial culturing and metal leaching would be carried out separately. In the first step, the microorganisms are grown without the presence of electronic solid wastes. During this step, bacterial growing would enter into the logarithmic phase or active phase, which is considered to have maximum cell density. In the second step, the E-wastes are added to the medium, and the metabolites produced by the bacteria are used for metal solubilization. Compared to the one-step approach, the twostep bioleaching approach offers several advantages. For instance, higher production of microbial metabolites and reduced toxicity of heavy metal ions through complexation before the addition of solid wastes to the medium could cause a higher reaction rate and faster metal solubilization. Naseri et al. [94] proposed a two-step bioleaching method for metal recovery from spent coin cells by Acidithiobacillus thiooxidans. In this approach, the bacteria were allowed to enter the logarithmic phase before adding the spent coin cell powder for metal leaching. The highest recovery rates of Li, Co, and Mn were achieved with a pulp density of 30 g/L, resulting in recovery values of 99%, 60%, and 20%, respectively. The higher recovery of Li, compared to Co and Mn, can be attributed to its high reactivity in the aqueous medium. Heydarian et al. [95] developed the two-step bioleaching method for recovering Li, Co, and Ni from lithium-ion laptop batteries (LIBs) using a mixed culture of A. ferrooxidans and A. thiooxidans. They studied the best inoculum ratio and adaptation of the mixed bacteria to the waste before conducting bioleaching experiments under two-step conditions. The optimized conditions of 36.7 g L-1 iron sulfate concentration, 5.0 g L-1 sulfur concentration, and initial pH of 1.5 for the best inoculum ratio of 3:2 between A. ferrooxidans and A. thiooxidans, resulted in maximum metal recovery rates of 99.2% for Li, 50.4% for Co, and 89.4% for Ni.

Spent-medium leaching approach would reach even higher E-wastes bioleaching efficiency. For the spent-medium leaching, microorganisms are

cultured in a growth medium until they reach a fixed stage of metabolite production. Then, the microbes in suspension and liquid media would be centrifuged and removed, obtaining only microbial metabolites in the spent -medium for bioleaching. Finally, E-wastes are added to the medium, and performed metal leaching by the metabolitecontaining medium. Compared to the previous two bioleaching methods, the spent-medium bioleaching approach has the advantages of a simpler operation process and less processing time. This approach also allows independent optimization of chemical and biological processes, as the stages of the leaching process and the generation of metabolites are separate. Under such conditions, no microorganisms exist in the bioleaching medium, which could make it possible to apply more active leaching conditions (higher temperature and lower pH) to achieve higher metal recovery from Ewastes. However, this method needs high operating costs through extra bioreactor tanks due to the two-stage process of microbial fermentation and the use of microbial metabolites. Hubau et al. [18] reported the recovery of metals in a two-stage continuous bioreactor for acidic bioleaching of PCBs. In the first stage, an acidophilic consortium consisting of Leptospirillum ferriphilum and Sulfobacillus benefaciens was used to oxidise Fe²⁺ to Fe³⁺. In the second stage, the resulting liquor from the first stage was used to leach out metals from PCBs. This two-stage approach allowed the bacteria to adapt to the PCBs gradually, and high metal vields were achieved within 48 hours: 96% Cu, 85% Zn, 73% Ni, and 93% Co when 1% (w/v) PCB scraps were added into the bioleaching reac-

Various studies have been conducted to compare these three bioleaching processes. The research results indicate that the spent-medium approach often has the best bioleaching performance. In the research by Horeh et al. [54], one-step bioleaching, two-step bioleaching, and spentmedium bioleaching were compared for the recovery efficiency of Cu, Li, Mn, Al, Co, and Ni metals from spent lithium-ion phone mobile batteries using Aspergillus niger. The one-step approach was carried out by inoculating spore suspension into the source medium (containing battery powder) within 30 days of incubation. Li, Al, Cu, and Mn were leached respectively 100%, 58%, 11%, and 8% with insignificant bioleaching of Ni and

Co. The two-step bioleaching approach was performed through the pre-culturing of fungi and obtaining organic acids produced by microbes in the logarithmic phase, which happened in 3 days. Subsequently, the battery powder was added to the source medium for the bioleaching process. 100%Li, 61%Mn, 10%Cu, 6%Co, and 1%Ni were leached after 27 days of incubation. For the spentmedium method, culturing fungi was done in the culture medium until the growth of microbes entered into the logarithmic phase in 14 days. The suspension of mycelia was filtered to reach a microbe-free medium. Then, the addition of battery powder was applied to the filtrate medium containing bio-generated acids for leaching purposes in 16 days. Co, Li, Mn, Al, Co, and Ni were bioleached with the efficiency of 100%, 95%, 70%, 65%, 45%, and 38%, respectively. The result indicated that the recovery of metals in spent-medium bioleaching was the highest among all three approaches.

In general, E-wastes often contain a variety of valuable metals, and to selectively leach metals, a two-stage or multi-stage bioleaching process can be designed using different microorganisms and operation conditions in each stage. Marra et al. [96] developed a two-stage bioleaching process for recovering base metals, precious metals, and rare earth elements from dust clouds generated during the shredding of waste electrical and electronic equipment. In the first step, Acidithiobacillus thiooxidans was used to leach almost all of the base metals from the dust within 8 days. Cerium, europium, and neodymium showed high leaching percentages (>99%), while lanthanum and yttrium exhibited an extraction yield of 80%. In the second step, Pseudomonas putida WSC361, which produces cyanide, was introduced to mobilize gold from the shredding dust previously leached by A. thiooxidans. Within 3 hours and at a pH of 12.0, P. putida WSC361 achieved a 48% mobilization of gold. This stage specifically focused on the recovery of precious metals. The study demonstrates that a two or multi-stage bioleaching process involving a series of bioreactors with different microorganisms and operating conditions can selectively leach different metals from E-wastes. By tailoring each stage to specific metals, higher recovery rates and efficiency can be achieved in the bioleaching process.

4.2. Enhanced Metal Bioleaching

4.2.1. Bioleaching with Pretreatment

To enhance the efficiency of bioleaching for the recovery of valuable metals from E-wastes, pretreatment steps are often necessary to remove protective and harmful or inhibitory substances that can hinder microbial activity. For instance, the surface of E-wastes is typically covered by epoxy resin, which impedes bioleaching [46]. Sodha et al. [97] employed NaOH treatment to remove the epoxy coating from WPCBs before bioleaching. In their pretreatment study, 22 WPCB plates were treated in triplicate They observed that treating the WPCBs with 7L, 2M NaOH resulted in an average weight loss of 2.6 g for each plate, representing the removal of the epoxy color layer and other alkalisoluble materials. Subsequently, a two-step bioleaching process was conducted, where ferric sulfate was generated by Leptospirillum ferriphilum from ferrous sulfate in the first step, followed by copper extraction using the resulting ferric iron in the second step. This approach yielded an extraction efficiency of nearly 99% for copper. Using NaOH as a cleaning agent efficiently removes surface coverings like epoxy resin, ensuring the proper growth of microorganisms and achieving a high metal leaching rate.

The complex composition of electronic Ewastes may contain substances that inhibit the growth of microorganisms, such as Lead and Tin, which can restrict the microbial viability during bioleaching of the WPCBs [16]. To improve the biological leaching efficiency, pretreatment is necessary to eliminate these harmful substances. Ilyas et al. [98] proposed using HNO₃ to effectively remove toxic metals (lead and tin) from WPCBs. They found that treating the WPCBs with 4 mol/L HNO₃ at a temperature of 90°C for 180 minutes resulted in approximately 96% dissolution of lead and 91% dissolution of tin. Following the pretreatment, bioleaching was conducted using Sulfobacillus thermosulfidooxidans (strain RDB) to recover the metal from the ground WPCBs. Optimal bioleaching conditions, including an aeration rate of 0.5 L/min, an O₂ enrichment dosage of 30%, an external CO₂ supply of 0.1%, a temperature of 55°C, and a time period of 18 days, led to maximum extraction yields of 93% Cu, 91% Ni, and 94% Zn. This sequential approach combining pretreatment and bioleaching proved effective in

achieving high metal recovery rates. Regarding gold recovery from electronic scrap material (ESM), Natarajan *et al.* [99] employed pretreatment with nitric acid before bioleaching using *Chromobacterium violaceum*. The pretreatment aimed to dissolve interfering metals such as copper, aluminum, iron, zinc, and lead, thus reducing competition for the cyanide ion involved in forming the gold-cyanide complex. The pretreated ESM exhibited higher gold recovery than untreated ESM across various pulp densities.

In addition, pretreatment could also be carried out to eliminate conventional metals that may compete with the target rare and precious metals during bioleaching. Pourhossein et al. [100] developed a novel green hybrid acidic-cyanide bioleaching process for the efficient recovery of valuable metals from spent LED lamps. Before bioleaching, a bio-pretreatment step was employed using biogenic ferric produced by Acidithiobacillus ferrooxidans to remove all base and heavy metals from the spent LEDs. This bio-pretreatment resulted in the leaching of metals such as Sn, Ni, Cu, Al, Ga, Pb, Cr, and Fe. The residue after biopretreatment was then subjected to bioleaching using Bacillus megaterium (B. megaterium). The hybrid direct bioleaching approach yielded significantly higher amounts of metals than single cyanide bioleaching without pretreatment. Notably, 93% Au, 91% Ag, 98% Ni, 87% Cu, and 84% Ga were leached after four days.

4.2.2. Bioleaching with Oxidants

In bioleaching, the presence of oxidants plays a crucial role in enhancing metal leaching efficiency. The primary oxidants are ferric iron, sulfuric acid, and organic acid. Ferric iron, in particular, acts as a potent oxidizing agent and facilitates the oxidation of metals through redoxolysis. On the other hand, sulfuric acid and organic acid promote metal leaching by converting them into their ionic state through acidolysis.

Yken *et al.* [101] conducted a study on the biohydrometallurgical extraction of metals from PCBs. They employed a non-contact leaching method using ferric iron (Fe³⁺) generated by *Acidithiobacillus ferrooxidans* to leach Cu, Ni, Zn, and Al from milled high-grade PCBs. The experimental conditions included a pulp density of 1%, Fe³⁺ concentration ranging from 5 to 20 g/L, and

pH levels between 0.6 and 1.2. The results indicated that increasing the concentration of ferric iron and decreasing the pH significantly improved the leaching efficiency. In the presence of 20 g/L ferric iron, the optimal recovery rate of 87% for Cu and 100% for Al, Zn, and Ni was achieved. This study demonstrated the effectiveness of ferric iron and its ability, in conjunction with sulfuric acid, to enhance metal leaching in the context of E-wastes. Velgosová et al. [102] performed experiments using A. ferrooxidans in three different media: a bioleaching media (9 K medium), an H₂SO₄ solution, and a Fe₂(SO₄)₃ solution. Their objective was to investigate the influence of acids and ferric ions on the bioleaching efficiency of Cd from spent Ni-Cd batteries. In the bioleaching process, Fe²⁺ ions served as an energy source for bacterial growth, and they were oxidized to Fe³⁺ ions, which, as strong oxidation agents, led to the solubilization of Cd. The study revealed that sulfuric acid was important in Cd leaching. Moreover, Fe³⁺ was more efficient than sulfuric acid in extracting Cd during the bioleaching process.

In the process of bioleaching, the addition of dilute sulfuric acid as an oxidant serves multiple purposes. Firstly, it reduces the pH of the leaching systems, which enhances the ability of the microbes to oxidize Fe²⁺ to Fe³⁺. This oxidation process is important for the overall leaching efficiency, as illustrated previously. Secondly, sulfuric acid promotes the oxidation of metal compounds present in E-wastes by Fe³+, increasing the likelihood of metal dissolution into the liquid phase. Xin et al. [103] conducted a study on the recovery of valuable metals, including Li, Co, Mn, and Ni, from spent LiNi_xCo_yMn_{1-x-y}O₂ using mixed cultures of sulfur-oxidizing bacteria (SOB) and iron (II)-oxidizing bacteria (IOB). In the bioleaching process, a mixed energy source (elemental sulfur: pyrite at 1:1 by weight) and a mixed culture system (Acidithiobacillus thiooxidans and Leptospirillum ferriphilum) referred to as MS-MC was applied. To improve the metal leaching efficiency, the leaching system was maintained at a pH value of 1.0 by adding exogenous H₂SO₄. The result showed an average extraction efficiency of over 95% for the four valuable metals from the resistant LiNi_xCo_yMn_{1-x-y}O₂, with significant improvements in the release efficiencies of Co and Ni, increasing from 43.5% to 96% and from 38.3% to 97%, respectively. Chen et al. [61] investigated column

bioleaching of Cu from waste PCBs using Acidithiobacillus ferrooxidans. The key reaction in this leaching process is the oxidation of copper by Fe³⁺ in the solution, while Fe³⁺ itself is reduced to Fe²⁺. A. ferrooxidans facilitates the oxidation of ferrous ions to ferric ions by consuming H⁺, establishing a Fe²⁺-Fe³⁺ cycle within the leaching process. However, the hydrolysis of Fe³⁺ and the formation of jarosite precipitate reduce the available Fe³⁺ concentration. To prevent the formation of jarosite precipitate and maintain an acidic condition in the leaching medium, dilute sulfuric acid can be added during the bioleaching process. This ensures that the Fe²⁺-Fe³⁺ cycle continues, creating a favorable environment for Cu bioleaching. After 28 days of column bioleaching, a recovery rate of 94.8% for copper was achieved from the raw materials with a Cu content of 24.8%.

In addition to dilute sulfuric acid, organic acid can also be added during the bioleaching process. Zhao et al. [104] conducted a study on the bioleaching of waste computer motherboards using a mixed culture called HO0211, consisting of bacteria such as Acidithiobacillus ferrooxidans, Ferroplasma acidiphilum, Leptospirillum ferriphilum, and Thermoplasma acidophilum. In their research, they focused on the effect of humic acid (HA), a complex macromolecular organic matter with redox functional groups, such as hydroxyl, carboxyl, and quinoid structures. The presence of hydroxyl and carboxyl groups in HA contributes to creating a new active surface on WCMBs after the action of bacteria corrosion, acid medium, and oxidationreduction processes. This new active surface promotes metal exposure and accelerates the electron conversion between bacteria and Fe³⁺, facilitating the release of metals from the solid phase. As a result, the addition of HA not only improves the leaching percentage of copper but also shortens the leaching time. Under optimum conditions, including an initial pH of 1.53, a pulp density of 1.35% (w/v), and HA addition of 0.31 g/L, complete leaching of copper was achieved. This research demonstrated that HA enhances copper extraction during bioleaching in conjunction with chemical leaching methods.

4.2.3. Bioleaching with Catalysts

The addition of catalysts has been reported to enhance the bioleaching process for metal recovery from E-wastes. Usually, carbon materials and certain metal ions are used as catalysts [105, 106].

Graphene, a form of carbon material, has been widely used as a catalyst in bioleaching. Gu *et al*. [107] investigated the catalytic effect of graphene in the bioleaching of copper from WPCBs using *A. ferrooxidans*. They observed that graphene, along with *A. ferrooxidans*, facilitated the conversion between Fe²⁺ and Fe³⁺ during the bioleaching reactions, resulting in the generation of FeCl₃·6H₂O and KFe(SO₄)₂(OH)₆ (Fe³⁺ could enhance copper leaching). The optimal dosage of graphene was determined to be 0.04 g in a 50 mL culture solution, leading to an extraction efficiency of 84% for Cu from PCBs, compared to 74% extraction without the catalyst.

Biochar, another carbon material with redox activity, could enhance the bioleaching efficiency of metals from E-wastes. In the previous study, oak wood biochar was used as the catalyst to improve the copper and nickel bioleaching efficiency from spent mobile phone PCBs [108]. The sulfuric acid solution generated by A. thiooxidans (a kind of mesophilic bacterium) was used with biochar during the experiment for metal bioleaching. It was found that 98% of copper and 82% of nickel were leached by indirect bioleaching under optimum conditions with 1.6 g/L biochar and 16 g/L pulp density. Wang et al. [109] revealed the role of biochar through three basic pathways: carbonmediated (through the reaction between E-wastes and microbial metabolites produced by microbes), sulfur-mediated (based on reduced sulfuroxidizing microbes to produce sulfuric acid), and iron-mediated bioleaching pathways (based on a cycle of Fe³⁺/Fe²⁺). The result showed that Cu bioleaching rates were approximately 0.2% for the carbon-mediated process, 20% for the sulfurmediated process, and 80% for the iron-mediated process. Biochar strongly enhanced the bioleaching efficiency of Cu in the Fe-mediated system, which resulted from the behavior of redox-active biochar by transferring electrons to speed up the redox reaction of Fe²⁺ and Fe³⁺, especially in the presence of microbes.

Metal ions have also been investigated as catalysts in the bioleaching process to improve extraction yield. For example, the addition of Ag⁺ as a catalyst during the bioleaching of spent Li-ion batteries using *A. ferrooxidans* resulted in improved

extraction yield of Co [110]. The best effect was observed at an Ag⁺ concentration of 0.02 g/L, leading to a leaching efficiency of 98.4% for Co in 7 days. In the absence of Ag⁺, only 43.1% of the Co was leached. Ag⁺ in the bioleaching medium reacted with Co in the spent Li-ion batteries, forming AgCoO2 as an intermediate product, which was subsequently oxidized by Fe³⁺ to yield a Co²⁺rich bioleaching solution. Additionally, Niu et al. [111] studied metallic ions catalytic bioleaching using a consortium of A. thiooxidans and L. ferriphilum to release Zn and Mn from spent Zn-Mn batteries. Four metallic ions, Co²⁺, Ni²⁺, Cu²⁺, and Ag⁺, were tested as catalysts, and only Cu²⁺ significantly accelerated the mobilization of Zn and Mn from the spent batteries. As the Cu²⁺ concentration increased from 0 to 0.8 g/L, the maximum release efficiency increased from 47.7% to 62.5% for Zn and 30.9% to 62.4% for Mn, respectively. The release of Zn and Mn from different components of the spent Zn-Mn batteries (ZMBs), such as hetaerolite (ZnMn₂O₄), manganese oxide (MnO₂), siomkolleite (Zn₅(OH)₈Cl₂H₂O) and zinc ammine chloride (Zn (NH₃)₂Cl₂) involved various dissolution and attack mechanisms. On the one hand, the mobilization of Zn²⁺ from zinc ammine chloride could be facilitated due to its soluble salt nature. On the other hand, the extraction of Zn²⁺ from siomkolleite could also be achieved by the dissolution obtained by biogenic acid (H2SO4). Mn from manganese oxide was attained by a combined reaction of acidic dissolution and Fe³⁺ attack based on a cycle of Fe³⁺/Fe²⁺. Cu²⁺ catalysis greatly enhanced the release of both Zn and Mn from the hetaerolite by forming a CuMn₂O₄ intermediate complex which enable subsequent Fe³⁺ attack based on the Fe³⁺/Fe²⁺ cycle. These materials promote the leaching of Cu, which is the hardest part to recycle compared to other parts.

4.3. Optimization of Bioleaching Conditions

The efficiency of bioleaching in E-wastes is influenced by various factors, including the interaction between microorganisms and metals. Several other factors, such as the type of bacteria, E-wastes composition, dissolution temperature, and pH (as shown in Fig. 3) [46, 50, 112], also play a significant role and require optimization to increase metal recovery efficiency.

In a study by Yang et al. [20], Acidithiobacillus ferrooxidans, a pure mesophile bacteria culture,

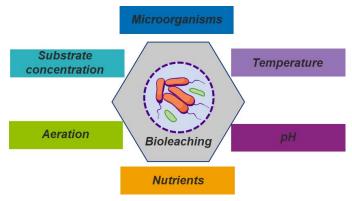


Fig. (3). Factors affecting bioleaching and need optimization. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

was used to investigate the effects of different factors on the bioleaching of PCBs. The study focused on varying the additional quantities of stock culture, the initial concentration of Fe³⁺, and pH. The results showed that Fe³⁺ and pH played significant roles in leaching copper. The highest leaching rate of copper was achieved at a pH of 1.5, with 6.66 g/L of Fe³⁺, 100% addition quantities of stock culture. Xiang et al. [113] examined the impact of initial pH, initial Fe²⁺ concentration, PCB dosage, and time for adding PCB powder on copper solubility in WPCBs using a bacterial consortium enriched from acid mine drainage. The extraction of copper was primarily achieved indirectly through oxidation by ferric ions generated from ferrous ion oxidation bacteria. The optimal conditions for copper leaching were found to be an initial pH of 1.5, an initial Fe²⁺ concentration of 9 g/L, and 20 g/L of printed circuit board powder after five days, resulting in a maximum leaching rate of 95%. Liang et al. [114] optimized a mixed culture of Acidithiobacillus thiooxidans and Acidithiobacillus ferrooxidans to maximize the additional amounts of PCBs and copper leaching percentage simultaneously. They found that an initial inoculums ratio of 1:2 between Acidithiobacillus thiooxidans and Acidithiobacillus ferrooxidans, a pH of 1.56, FeSO₄·7H₂O and S⁰ of 16.88 and 5.44 g/L, respectively, and a PCBs addition of 28.8 g/L resulted in a copper recovery rate of 92.6% after 240 hours of cultivation. These findings highlight the importance of optimizing microbial operating conditions to improve metal leaching efficiency, recognizing that the optimal conditions may vary depending on the specific microorganisms and metals involved. Hence, targeted optimization should be carried out in practical work based on Ewastes sources and metals of interest.

D-optimal response surface methodology (RSM) is often employed to optimize operating conditions of metal leaching from E-wastes. Faraji et al. [53] used RSM to optimize fungal bioleaching parameters, including the bioleaching method (one-step, two-step, and spent medium) and pulp densities, to maximize the recovery of Zn, Ni, and Cu from WPCBs. Under the optimized conditions, A. niger could dissolve 98.57% of Zn, 43.95% of Ni, and 64.03% of Cu within 21 days. In another study by Jowkar et al. [21], adapted Acidithiobacillus thiooxidans was employed for bioleaching In from discarded LCDs. RSM was applied to evaluate the effects of initial pH, pulp density, and initial sulfur concentration on the recovery of In and Sr. The optimized conditions, including an initial pH of 2.6, a pulp density of 1.6% (w/v), and an initial sulfur concentration of 8.6 (g/l), resulted in the recovery of 100% of In and 10% of Sr were recovered.

CONCLUSION

Recovering valuable metals from E-wastes through bioleaching is considered a promising and environmentally friendly alternative to conventional metal leaching methods. It helps reduce the need for resource extraction and protects the environment by utilizing metals from E-wastes instead of low-grade natural metallic mineral resources. Over the past few decades, microbial hydrometallurgy, specifically bioleaching, has been successfully applied to recover metals such as copper, gold, uranium, and others from various sources, including E-wastes. In bioleaching, autotrophic or heterotrophic microorganisms have been intensively studied as the primary agents for metal leaching. The mechanisms involved in bioleaching, such as acidolysis, redoxolysis, and complexolysis, have been discussed in detail to understand the processes. Different applications of bioleaching processes, including single-step bioleaching, and two- or multi-step bioleaching, have been explored, highlighting their advantages and limitations. Additionally, the use of oxidants, catalysts, and various pretreatment techniques in bioleaching has also been examined and analyzed.

However, current bioleaching techniques still have some disadvantages. To begin with, the production efficiency is relatively low. Bioleaching process requires the cultivation and growth of microorganisms, which would take a relatively long time. Besides, the output and quality of bioleaching are relatively low. These may reduce the production efficiency of bioleaching. Secondly, the bioleaching products may be unstable. Some products could not be stored for a long time, and the composition of products is susceptible to environmental factors during the leaching process. This instability may limit the wide application of bioleaching technology. Thirdly, bioleaching techniques have high standards for environmental requirements. Bioleaching technology has high requirements for environmental factors such as temperature, pH, oxidation-reduction, which may increase the production costs and technical difficulty. To deal with these issues, improvements could be made in the following aspects. For improving microbial cultivation and growing efficiency, optimizing the medium, fermentation equipment, and fermentation conditions could be considered. Additionally, the stability of product composition could be obtained through improved leaching processes, which include decreasing the temperature and pH value and the use of antioxidants. Finally, the development of new optimum microbial strains for different environments such as high metal tolerant or pH tolerant microbial strains could decrease the bioleaching external requirements, reducing the producing costs and difficulty.

Moreover, most of the current bioleaching research has been done on a laboratory scale, focusing on leaching metals with known microorganisms. Therefore, it is crucial to conduct pilot-scale and large-scale research to verify the feasibility of further development and ultimately achieve industrial applications. Combining biological and chemical processes shows promise in improving the ex-

traction efficiency and recovery level of metal from E-wastes. The optimization of process parameters such as pH, temperature, pulp density, and particle size has been discussed to enhance bioleaching performance.

In conclusion, bioleaching holds great potential as an eco-friendly, cost-effective method for recovering valuable metals for E-wastes. Further research and development, including pilot-scale/large-scale studies, the isolation of new microorganisms, and the integration of biological and chemical processes, will contribute to advancing the field and achieving sustainable metal recovery from E-waste in the future.

LIST OF ABBREVIATIONS

E-wastes = Electronic Wastes

PCBs = Printed Circuit Boards

WPCBs = Waste-Printed Circuit Boards

CPCBs = Computer Printed Circuit Boards

MPPCBs = Mobile Phone Printed Circuit

Boards

LCD = Liquid Crystal Display

LIBs = Lithium-ion Laptop Batteries

SOB = Sulfur-Oxidizing Bacteria

IOB = Iron (II)-Oxidizing Bacteria

HA = Humic Acid

ESM = Electronic Scrap Material

RSM = Response Surface Methodology

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