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Priming *Chromobacterium violaceum* as a Gold Bioleacher in E-wastes: Advantages and Challenges

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ABSTRACT

With rapid technological advancement, the life span of electronic devices has become increasingly short, contributing to massive electronic waste (E-waste) and demanding sustainable management. E-waste contains precious metals like gold (Au) many fold higher than natural mining ores. However, the current techniques of recycling are not efficient. Methods like pyrometallurgy and hydrometallurgy not only require higher energy expenditure but also lead to environmental pollution, hence necessitating an alternative greener technology. In line with that, metal leaching using microorganisms is gaining popularity, and *Chromobacterium violaceum* has been considered a promising candidate. However, the heterogeneity of culture technique and abiotic condition variations have been a perceived challenge in priming *C. violaceum* as an Au bioleacher for upscaled industrial applications. Thus, the current review discusses what makes *C. violaceum* an excellent candidate for Au bioleaching and how to overcome the challenges associated with the application. This review will significantly enhance the current understanding of *C. violaceum* as an Au bioleaching agent, hence addressing the existing knowledge gaps.

1. INTRODUCTION

Electronic waste (E-waste) refers to electronic equipment and its components that are not used anymore or rejected. Due to rapid technological advancement, urbanisation, population growth, and lifetime consumption patterns, electric and electronic devices have become obsolete fast and deemed surplus necessary [1]. The source of E-waste usually comes from households, companies, hospitals, and the government, however, the most significant contribution comes from IT industries and consumer electronics. The United Nations (UN) reported that in 2020, E-waste generated more than 44 million metric tonnes (Mt), and without any contingency plans to curb it, such waste can accumulate to 78 million Mt by 2050 [2]; thus, E-waste management will be a critical global environment and sustainable challenges. On top of that, a typical E-waste contains more than 1,000 toxic substances, which can significantly impact human health, the environment, and the ecosystem [3].

On the flip side, E-waste components like printed circuit boards (PCBs) contain many precious substances like gold (Au), silver (Ag), and copper (Cu), hence, acting as a vast secondary reservoir for these metals compared to the natural primary source. Studies reported that approximately 200 g of Au could be recovered per ton of E-waste compared to Au ores containing just 5 – 30 g per ton [4, 5]. This presents a financial advantage to mining E-waste instead of ores from virgin sources. Ironically, only 20% of the metals in E-waste were being collected back, and due to a lack of selectivity for high-yield non-cyanide recycling practices, 80% were being dumped back into landfills [6-8]. The currently employed recycling methods are the pyrometallurgy and hydrometallurgy processes, both of which were shown to have considerable

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negative environmental effects. Metal recovery via pyrometallurgy demands significant energy via high-temperature processes, emitting harmful gases and heavy metals [9, 10]. On the other hand, hydrometallurgy is less harmful but costly, involving hazardous chemicals and releasing wastewater contaminants [10, 11].

The alternative and safe E-waste recycling method, compared to conventional pyrometallurgy and hydrometallurgy, uses microorganisms as bioleaching agents. Studies showed that numerous microorganisms, like *Acidithiobacillus* spp., *Bacillus* spp., *Sporosarcina* spp., *Pseudomonas* spp., and fungi were successfully employed as bioleachers, yielding better outputs [12-20]. However, there is one specific interest in *Chomobacterium violaceum*, a cyanogenic bacteria that is gaining a lot of acceptance as a prominent Au bioleaching agent. However, the literature search showed heterogeneity in the culture methods and abiotic conditions employed in various laboratories. This has prompted a comprehensive understanding and review of the advantages and challenges in priming *C. violaceum* as an Au bioleacher.

Therefore, the specific objectives of this review are to discuss (1) the current methods of metal recovery from E-waste, particularly discussing the disadvantages of the pyrometallurgy and hydrometallurgy processes, (2) the advantages of the bioleaching technique, (3) how *C. violaceum* holds more significant advantage compared to other bioleachers, (4) the Au bioleaching mechanism that take place in *C. violaceum,* (5) the challenges in utilising *C. violaceum* as a primary Au bioleacher, and (6) how to overcome those challenges while still priming *C. violaceum* as a first choice Au bioleacher. This review will significantly improve the basic understanding of why *C. violaceum* should be considered a primary microorganism in the Au bioleaching process, and significantly addressing the knowledge gaps in *C. violaceum* as a vital gold bioleacher in E-wastes.

2. METAL RECOVERY FROM E-WASTE

Metal recovery from E-waste can be accomplished via various physical, chemical, and biological processes. However, conventional E-waste recycling techniques such as pyrometallurgy and hydrometallurgy are the most employed in the metal recycling industry [21-23].

2.1 Pyrometallurgical Process

The pyrometallurgical processes are commonly used to extract non-ferrous and valuable metals from E-waste. The process starts with mechanical and physical pretreatment of E-waste followed by incineration, smelting, drossing, sintering, and melting. These processes primarily take place in a gas phase under a controlled high-temperature environment (Figure 1) [24, 25]. The heating process usually occurs in specialised incinerators such as blast or plasma arc furnaces, where metallic oxides combine with reducing agents such as charcoal or coke to release flue gases. Meanwhile, slag may form in the furnace when the non-metallic part of E-waste, known as gangue, is heated with flux materials such as charcoal, coke, lime, and borax. Slag is a molten mass that floats because it is lighter than the metallic portion (molten iron) and readily decanted away, leaving metallic residue behind (Figure 2) [9, 26].

Incongruously, the pyrometallurgical process is generally inefficient in metal recovery. This is mainly due to the metallic compound in the E-waste that contains alloy. The alloys' durability and poor melting separation behaviour usually restrict the recovery of pure metals and render pyrometallurgical an energy-demanding procedure [27, 28]. Thus, for efficient metal extraction, the melting process at smelters is usually employed [28].

Even though the pyrometallurgical process is used widely in the metal recycling industry, the E-waste smelting process is reported to emit many harmful gases and compounds, including polybrominated dibenzofurans (PBDF), polybrominated dibenzodioxins (PBDD), biphenyl, tribromobenzene, phenol, anthracene or phenanthrene, naphthalene, dibenzo-p-dioxin, dibromobenzene, tetrabromobenzene, dibenzofuran, and many more, which leads to severe secondary pollution [29-31]. Besides that, in the presence of Cu as a catalyst, the low-temperature burning of brominated flame retardant produces hazardous dioxin vapours [9]. Thus, this requires further capital expenditures to manage gaseous emissions arising from this process. On top of that, metallic component processing and open burning were reported to release hazardous heavy metals such as Hg, Zn, dibenzofurans, and polychlorinated dibenzodioxins, which may elicit long-term environmental and health issues [9, 32, 33]. Therefore, enhancement in the traditional pyrometallurgical processes and their mechanical component is necessary to increase pure metal recovery besides minimising the secondary pollutants. For instance, a new and cleaner ultrasonic-aided pyrometallurgical metal recovery method with low waste emission and high recovery efficiency has been developed. This technique recovered Cu and Fe with 95.2 – 97.5 % and 97.1 – 98.5 % efficiency, respectively [34]. The advantage of the pyrometallurgical method in E-waste recycling is the use of a small number of chemicals besides being compatible with ore cracking and metal reduction.

2.2 Hydrometallurgical Process

The impediments associated with the pyrometallurgical processes necessitated the application of the hydrometallurgical processes as a potential alternative to recover metals from E-waste. Unlike the pyrometallurgical method, leaching is the first step in the recovery of metals in the traditional hydrometallurgical process, and it is carried out using appropriate chemical lixiviants to dissolve the metallic fractions in E-waste [9, 35, 36]. Acid or alkaline solutions such as sulfuric acid, nitric acid, and aqua regia are commonly used as lixiviants or leaching reagents [37-39]. These solutions can solubilise the solid matrix of E-waste and liberate the metals in the solution phase. Besides that, leaching reagents such as cyanide, thiourea, thiosulfate, and halides have also been widely utilised [40]. Thiosulfate and thiourea have been proposed as alternatives to cyanidation because of their rapid leaching rates. In the presence of ammonia and Cu catalysers for instants, thiosulfate creates a complex with metals such as Au and Ag, which speeds up the metal dissolution process. However, thiosulfate also possesses poor chemical stability, and as a result, it may quickly degrade into metallic sulphide [22]. In some cases, metal leaching is preceded by mechanical shredding to release the polymer or ceramic matrix-encapsulated metallic particles [9, 23, 41]. It is to ensure a better leaching process. Besides that, ligands such as ethylene diaminetetramaide (EDTA), diethylenetriaminepenta acetate (DTPA), and other chelators such as oxalate and citric acid also usually employed in the hydrometallurgical processes for efficient

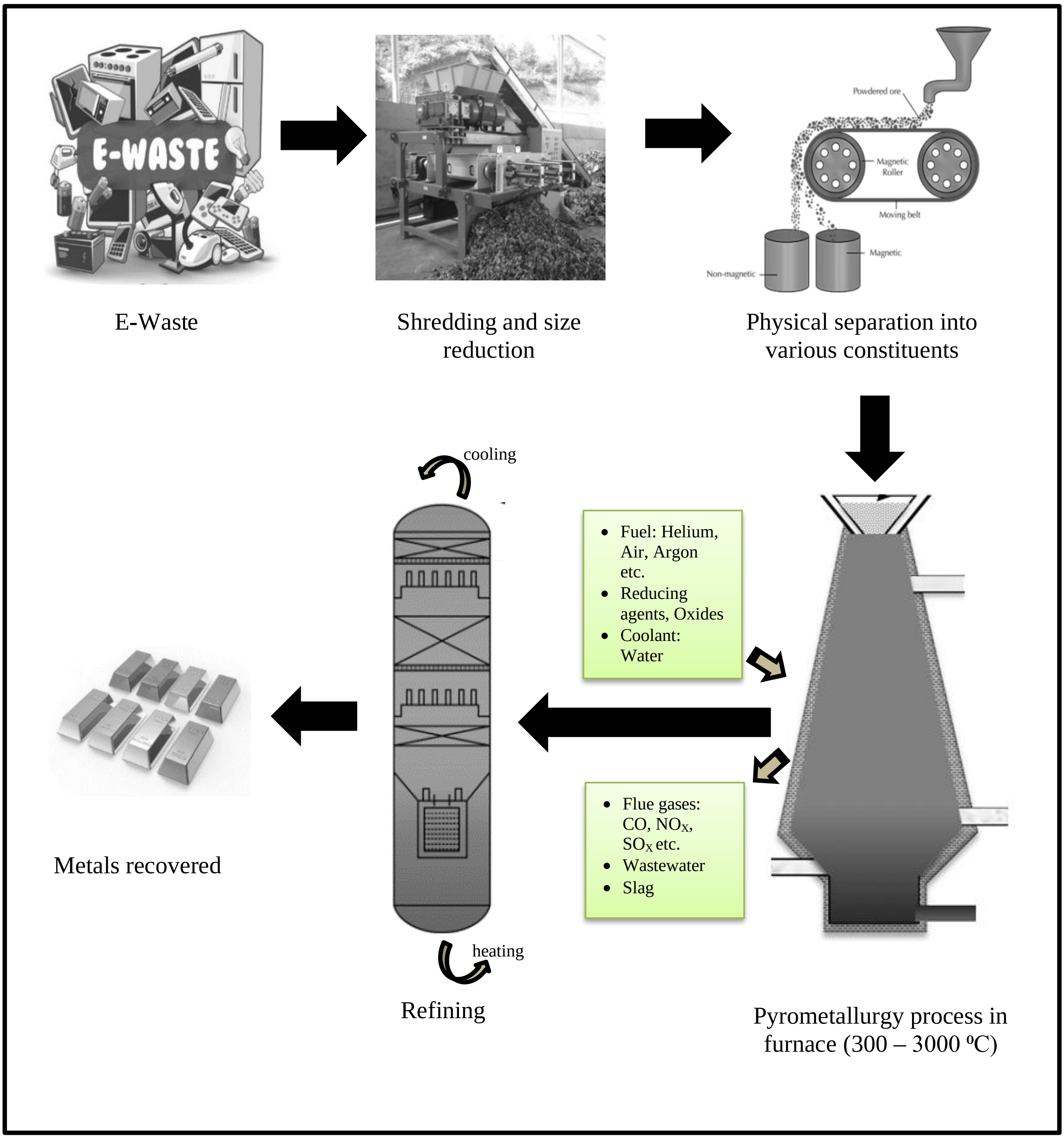

Figure 1. Metal recovery by Pyrometallurgical process. Mechanical-physical pretreatment is required for E-waste, including disassembling major components (e.g., Li batteries), shredding/ grinding operations for size reduction, component release, etc. The enhanced metals are then melted in furnaces to produce coarse metal ingots, followed by smelting. Flash smelting and bath smelting are the two common techniques used. Bath smelting relies on the roasting and smelting steps, and the reaction takes place in a molten pool containing both melts and slag phases. On the other hand, Flash smelting uses oxygenated gas to promote autogenous conditions.

metal recovery [39].

In general, the transition of solid E-waste particles to a liquid phase in the hydrometallurgical processes occurs when a metal is transferred from the ore to the leaching solution. However, due to the selective dissolving ambiguities, most of the undesirable components in the ore are not impacted by the leaching process and thus remain solid. Hence, the metal component from this mixture must be separated from the solution, and the resulting solution is known as a pregnant leach solution. The pregnant leach solution is later purified using electro-refining, adsorption, and solvent extraction methods to concentrate and recover pure metals [9]. Figure 3 explains a typical metal recovery process in the hydrometallurgical process.

Although the hydrometallurgical process is technically viable and widely used in the metal recovery industries, the wastewater produced during the recovery process is highly toxic,

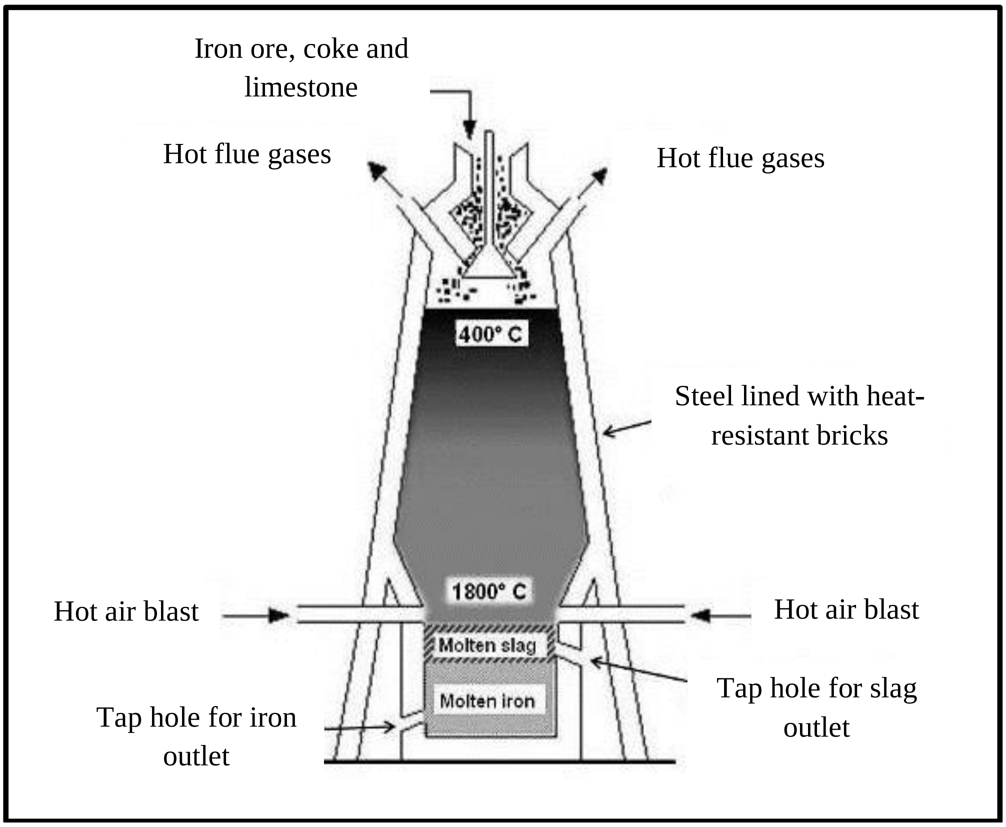

Figure 2. Blast furnace. Raw materials such as iron ore, coke, and limestone are added at the top of the furnace. Blasts of hot air are blown from the bottom of the furnace. Oxygen in the furnace reacts with coke (carbon) to form carbon monoxide [$2C + O_2 \rightarrow 2CO$]. This reaction is very exothermic, and the temperature in the furnace will reach 2000°C. As CO rises in the furnace, it reacts with iron ore (iron III) oxide to form iron [$Fe_2O_3 + 3CO \rightarrow 2Fe + 3CO_2$]. Molten iron runs to the bottom of the furnace. It is tapped off from time to time. The molten iron is used to make steel or poured into moulds to solidify. The large chunks of iron that are solidified are called 'pigs'.

difficult to recycle, and significantly restricts the recovery process [9, 42, 43]. It raises concerns about the potential formation of large amounts of toxic, highly acidic or alkaline, or flammable reagents with the generation of voluminous effluents, risks of exposure to acid fumes, acids in liquid form, and cleaning solvents, which may pose an acute to chronic exposure hazard to workers as well as a risk of environmental contamination [9, 22, 38].

2.3 Bioleaching

Bioleaching (also known as biohydrometallurgy) is a novel approach to recovering and recycling metals from the environment using microorganisms (Figure 4) [21, 44-46]. Unlike the conventional metallurgy processes, bioleaching enables sustainable development that contributes towards efficient environment and energy management. Microorganisms employed in the bioleaching process can convert metals into soluble and extractable molten that may subsequently be retrieved in a solution form [46, 47]. For instance, microbes, particularly iron (Fe) and sulfur-oxidising bacteria can oxidise metals directly or indirectly using their optimised biochemical corridors. They are capable of oxidising ferrous iron (Fe^{2+}) to a ferric state (Fe^{3+}), besides oxidising elemental sulfur and its compounds to produce necessary leaching reagents such as sulfuric acid and sulfur oxidation products [21, 44, 46, 48]. Examples are chemolithoautotrophic bacteria like *Thiobacillus ferrooxidans*, *T. thiooxidans*, *Sulfolobus* spp., and *Leptospirillum ferrooxidans* and fungi like *Aspergillus niger* and *Penicillium* spp. were frequently used in the bioleaching processes [49, 50]. In addition, heterotrophic microorganisms such as Heterotrophic *Bacillus* and some *Pseudomonas* bacteria were regularly used to extract metals mainly from non-sulfidic sources [51-53]. These bacteria obtain energy from organic sources like carbon to generate various metabolic by-products, including citric acid, oxalic acid, formic acid, and other biological materials that can be utilised as leaching cocktails in the metal recovery process [46, 54]. A summarised list of microorganisms commonly used in the bioleaching process is shown in Table 1 [12-20].

The microbial metal leaching approach generally provides various benefits compared to conventional methods if the technique is optimised correctly. Some advantages of the bioleaching process are cheap operating costs, reduced amount of chemical sludge generation, environmental compatibility, low energy usage, and it envisages a simple metal extraction method. Compared to the pyrometallurgy and hydrometallurgy processes, bioleaching can recover sustainable metal from mineral deposits such as electronic E-waste. The advantages and disadvantages of various metal leaching processes were com-

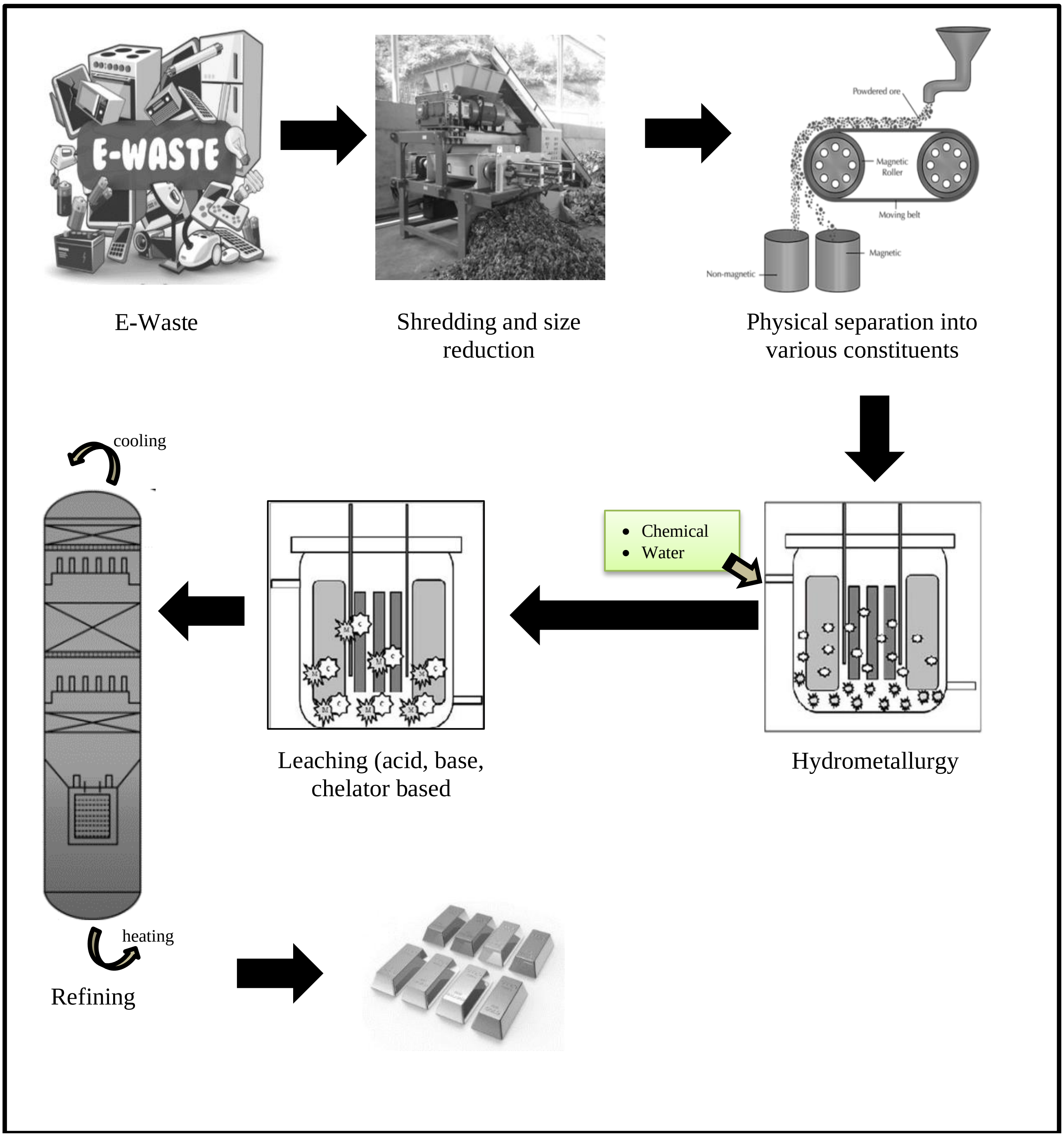

Figure 3. Metal extraction from E-waste via hydrometallurgy process. The hydrometallurgical process involves mechanical pretreatment and is followed by metal leaching using a suitable lixiviant. In some cases, the purification of pregnant leach solution is required to recover the metals. A suitable leaching process is usually developed based on the composition of metals in the E-waste, native, and alloys' form. In general, an oxidative leaching process is required to effectively extract base and precious metals of interest. The most common leaching agents tested for recovering precious metals include cyanide, halide, thiourea, and thiosulfate.

pared in Table 2.

Even though the bioleaching process holds an obvious advantage over the conventional metallurgy processes, the microbes' inefficiency in complete metal solubilisation remains a major hurdle. However, this shortcoming can be overcome by removing inhibitors that inhibit the development of microorganisms in the engaged process [46]. Enhancements are usually made by optimising the biotic factors, such as improving the characteristics and type of microorganisms used, besides adjusting their inoculum size. On the other hand, abiotic factors such as growth environment, pH, temperature, aeration rate, oxygen, and humidity access, the particle size of extractable E-waste, incubation time, and the nutritional composition of media were also shown to impact better bioleaching process [51, 55, 56].

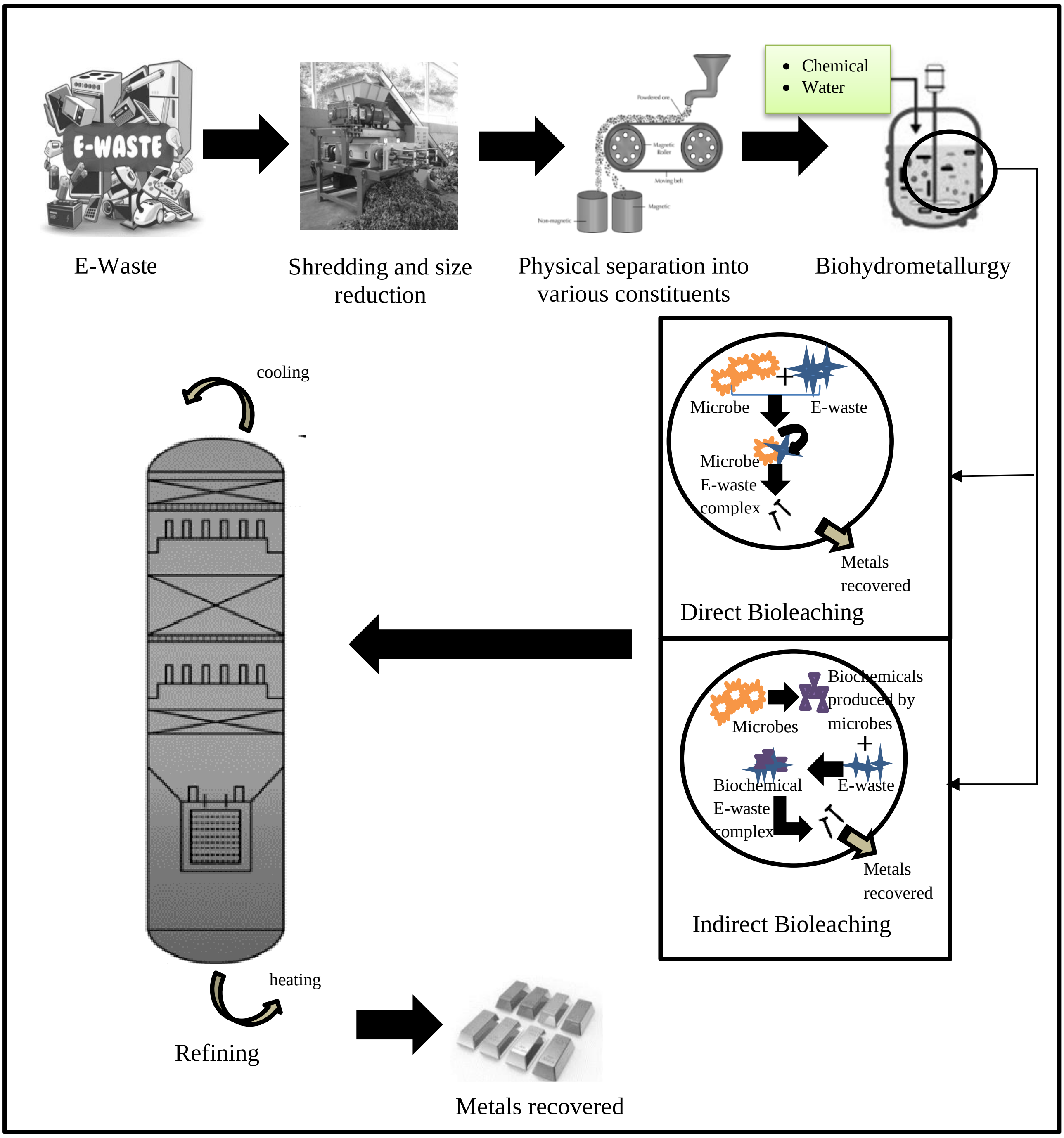

Figure 4. Bioleaching of E-waste. Bioleaching of metals using microbes can take place either directly or indirectly. In the direct method, bacteria will be physically attached to a solid matrix, and metals are oxidised enzymatically. In contrast, the bacteria in the indirect method usually generate leaching agents such as ferric ions and sulfuric acid to oxidise the metals. Because E-waste does not include energy sources, iron and sulphur (ferrous sulphate and elemental sulphur) are often added to the medium to promote bacterial growth throughout the bioleaching process.

Metal recovery using microbes generally can take place either directly or indirectly. In the direct process, microorganisms oxidise metal sulphides by obtaining electrons from the reduced minerals, and the cells must be tightly attached to the mineral surfaces. The adsorption of microbes to suspended mineral particles occurs in a few minutes or hours. On the other hand, in the indirect process of microbial metal recovery, reduced metal oxidation is enabled by the presence of ferric (III) ions produced by microbes during the oxidation of ferrous iron contained in the minerals. Ferric iron is an oxidant that can oxidise metal sulphides before being reduced to ferrous iron, which microorganisms can oxidise. The electron carrier in this instance, is iron. It has been suggested that the oxidation of iron does not need direct physical contact [57].

Table 1. Microorganisms commonly used in the bioleaching process.

Type/Temperature range (°C)		Bioleaching microorganisms	Material	Metal	Reference
Mesophilic	28–37	*Acidithiobacillus ferrooxidans* *Acidithiobacillus thiooxidans*	Spent processing catalyst from an Iranian oil refinery	Al, Co, Mo and Ni	[12]
		Acidithiobacillus thiooxidans	Copper-rich electronic waste material	Cu	[13]
		Acidithiobacillus ferrooxidans	Waste-printed circuit boards	Cu	[14]
Moderate thermophiles	40–60	*Bacillus* spp. *Sporosarcina* spp. *Pseudomonas* spp	Pb/Zn smelting slag	Al, As, Cu, Mn, Fe and Zn	[15]
Thermophiles	60–80	*Sulfolobus metallicus*	Spent petroleum refinery catalyst	Ni, Al, Mo, and V	[16]
		Acidianus brierleyi	Spent hydrotreating catalyst	Al, Fe, Ni, and Mo	[17]
Fungal bioleachers	25–35	*Aspergillus niger*	Sulphide ore with a low concentration of arsenic	Fe, Zn, As, Cu, and Al	[18]
Cyanogenic bioleachers	25–35	*Chromobacterium violacein* *Chromobacterium* sp. Dyh27s2016 strain	E-waste	Cu, Fe, Zn and Ag	[19]
		Chromobacterium violacein	E-waste	Au	[20]

3. CHOMOBACTERIUM VIOLACEUM

Chromobacterium spp. are opportunistic gram-negative bacteria commonly isolated from tropical and subtropical regions [58-60]. The bacteria are easily distinguished from other species as they appear as an eminent violet colony on the growth medium (Nutrient agar), and this unique colour is the reflection of the violacein compound it produces [61]. The first species identified from this genus was the *Chromobacterium violaceum* in 1881 [62]. However, from 2007 onwards, new species within the genus have been identified from various locations [59, 60, 63-70]. Despite many new *Chromobacterium* species being discovered lately, the *C. violaceum* is the only species extensively studied and recommended for potential industrial and medical applications, including its synthesised violacein compound.

The *Chromobacterium* spp. as a whole organism is mainly utilised for the quorum sensing (QS) model and as a bioleaching agent. The QS defines the mode and adaptation of how bacterial colonies communicate with each other within a dynamic environment for survival. For instance, the network established by the bacteria enables them to communicate among themselves via secreted QS molecules to synthesise hydrolytic enzymes (e.g., chitinases, esterases, proteases, and pectinases), biosurfactants, biofilms, or various virulence factors as defence mechanisms to protect themselves from harmful threats [71]. In fact, violacein is a QS molecule synthesised by *Chromobacterium* spp. to protect itself against competing bacteria [72, 73], fungi [74], and viruses [75]. Besides, violacein has also been demonstrated to be an effective anticancer [76] and immunomodulatory compound [77], two important characteristics for successful cancer treatment. Hence, it may be advantageous to use violacein derived from *Chromobacterum* spp. compared to conventional drugs or cell-based immunomodulatory therapies like mesenchymal stem cells for cancer treatment [78, 79], the latter were often shown to have adverse effects [79, 80].

3.1 Gold Bioleaching by Chromobacterium violaceum

Chromobactarium violaceum has been shown to be capable of immobilising metals like gold (Au), copper (Cu), iron (Fe), zinc (Zn), and silver (Ag) from E-waste [19, 20]. Since this review aims to analyse the suitability of *C. violaceum* in recovering Au from E-waste, we confine the points within the discussion topic.

Cyanide (in the form of NaCN) has been widely used in Au mining for over 100 years due to its high gold recovery, low cost, and robustness. On the other hand, *C. violaceum* is a cyanogenic bioleacher shown to have superior ability in Au recovery due to bacterial hydrogen cyanide (HCN) production [81, 82]. Hence, the *C. violaceum* holds apparent advantages compared to other microorganisms, as listed in Table I. The gene for HCN synthase is found within the bacterial genome, and under low O_2 levels, *C.* violaceum can naturally synthesise HCN as a secondary metabolite [81, 83], hence, it can be utilised for the Au dissolution from E-waste. Usually, cyanide

Table 2. Advantages and disadvantages of pyrometallurgical, hydrometallurgical, and bioleaching processes

Method	Advantages	Disadvantages
Pyrometallurgical process	Good compatibility for ore cracking and metal reduction.	Require high-temperature operation.
	Require small chemical consumption.	Limited selectivity of metal refining.
	Applicable to complex electronic scrap.	Generation of hazardous fumes (dioxins, Hg, Pb, Cd) and emission of great volumes of toxic gases (E.g., SO_2)
		Precious metals are obtained at the very end of the process (stay for a long time).
		High energy consumption.
		High operation cost.
Hydrometallurgical process	Pure products and a high recovery rate.	Not applicable to complex E-wastes
	High treatment capacity.	High volumes of solutions are used (can be corrosive or toxic).
	High removal efficiency of other impurities.	Causes secondary pollution with wastewater or sludges.
	Selective leaching of various metals.	
	Low temperature of operation and low energy consumption.	
	Low or no gas emission.	
	No or low dust generation.	
	Easy working conditions and require simple equipment.	
Bioleaching process	Low temperature of operation and energy consumption.	Slower and less efficient than hydrometallurgy.
	Greener than pyro- and hydrometallurgy.	Limited efficiency of metal leaching due to the sensitivity of microorganisms.
	Selective for specific metals.	
	Low investment cost.	

lixiviants are derived from these HCN, which then react with solid Au and complete the bioleaching process. The HCN synthase in this process will yield four electrons, which the terminal electron acceptor, the oxygen molecule, will accept through the respiratory (electron transport) chain. The reaction process is summarised in equations (1) and (2) [81].

$$4Au + 8CN^- \rightarrow 4Au(CN)_2^- + 4e^- \quad (1)$$

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \quad (2)$$

The balance equation for Au dissolution using cyanide lixiviant and HCN synthase is shown in equation (3).

$$4Au + 8CN^- + O_2 + 2H_2O \rightarrow 4Au(CN)_2^- + 4OH^- \quad (3)$$

It is to be noted that the HCN and cyanide lixiviant that formed during this process is often limited (20 mg of cyanide per liter of bacteria culture, or approximately 1×10^{16} CFU). Thus, the widespread use of cyanogenic bacteria for metal leaching (especially Au) in industrial applications is often limited. Furthermore, the metal-cyanide complex that forms during the bioleaching process can be toxic to the bacteria, and it usually affects bacterial growth, further affecting cyanide production. Pretreatment of E-waste and removal of non-targeted metals may enhance the Au recovery using *C. violaceum* [81]. The other notable cyanogenic bacteria that can be used in the Au bioleaching are *Pseudomonas fluorescens* [84], *Pseudomonas aeruginosa* and *Bacillus megaterium* [85], *Pseudomonas putida* [86], and *Pseudomonas balearica* [87].

3.2 Optimal Au Boleaching Condition for Chromobacterium violaceum

Figure 5 shows the abiotic and biotic factors influencing metal yield during bioleaching in general. Factors like temperature, pH, bacterial nutrients, aeration, and substrate concentration have been shown to affect the outcome [88]. Though different microorganism types may also influence the outcome in bioleaching, the current review only focuses on the ability of *C. violaceum* in Au bioleaching from E-waste.

The optimal growth temperature of *C. violaceum* is 30 – 37°C, and the pH ranges from 6 – 9 [58, 89]. However, for Au bioleaching with *C. violaceum*, pH 9 – 11 is often found to yield maximum output [90]. This contradicts the most reported study that mentioned the utilisation of low pH values during bioleaching strains other than *C. violaceum*. For instance, Zhang et al. (2020) reported that at pH below 2.5, microbial leaching was performed better [91]. At higher pH (pH> 2.5), it is inferred that the metal ions could form precipitation, thus interfering with the biospecies and ion transportations. On top of that, the acidic conditions allow protons to prevent metal precipitation and enhance targeted metal release [91]. Nevertheless, Tran et al. (2011) reported that Au leaching at pH 11 by *C. violaceum* increased the yield from 7.78% (0.225 ppm) to 10.8% (0.46 ppm) on day 8 [90]. The study showed that at higher pH (pH 9 – 11), cyanide generation by *C. violaceum* was found to be highest compared to pH lower than 9 [90]. As cyanide is a major component required for Au leaching, higher cyanide production at pH >9 can significantly improve the bioleaching yield.

In terms of nutrients, supplementing glycine, H_2O_2, suitable pulp density/concentration, and higher dissolved oxygen (DO) were reported to yield better Au from E-waste using *C. violaceum*. The addition of H_2O_2 (0.005%) in the nutrient was shown to increase DO, which increases the *C. violaceum* population during leaching, hence contributing to better Au leaching [90]. During the inoculation of bacteria with E-waste, the DO is often depleted after 24 h. Thus, improving the DO is necessary to maintain bacterial growth. Bioleaching metals from E-waste often take longer in culture (8 – 14 days); therefore, higher DO must be maintained. However, supplementing H_2O_2 must be careful, as too high concentrations can inhibit bacterial growth altogether, yielding low Au during bioleaching [90]. On the other hand, glycine is an essential substrate that is often added to the *C. violaceum* culture. Glycine acts as a precursor for HCN production, utilising the HCN synthase enzyme produced by the bacteria [92, 93]. Abdol Jani et al. (2023) showed that using the Response Surface Met-

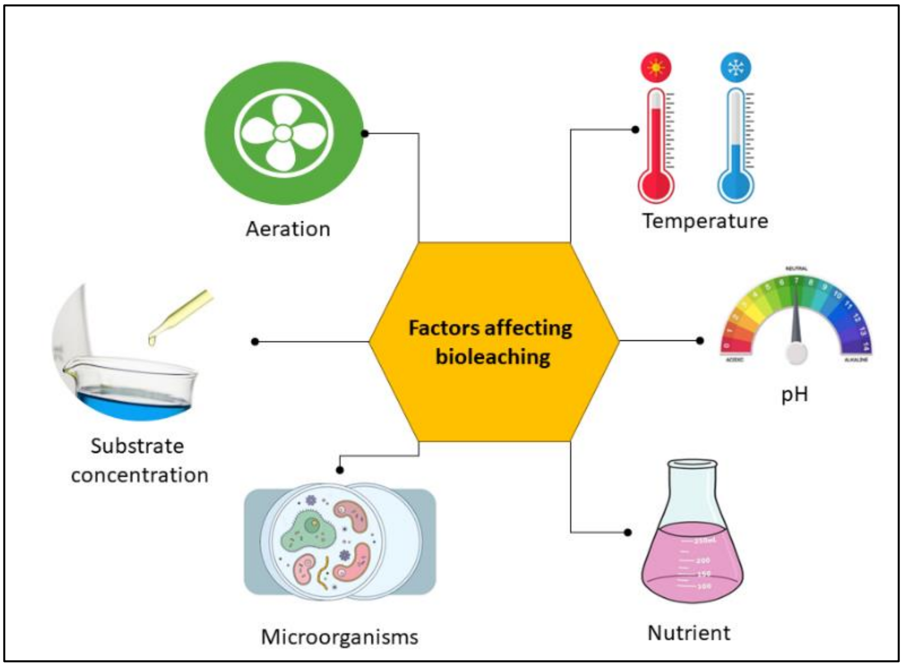

Figure 5. Factors affecting the bioleaching process.

hodology, the glycine concentration at 2.49 mgL^{-1} could recover 62.40% of Au from E-wastes. Lastly, the substrate, especially the metal concentration plays a vital role in bioleaching. E-waste contains precious metals like Au and Ag and other substances like plastics, ceramics, glasses, etc. Hence, the higher E-waste dosage (pulp density/concentration) can be hazardous to *C. violaceum,* thus, limiting its bioleaching ability. The optimal pulp concentration of metal bioleaching is generally 10 – 20 g/L, and the particle size ranges from 40 – 200 µm [88]. Abdol Jani et al. (2023) showed that the pulp density at 1.95% was optimal for Au recovery by *C. violaceum*, hence, both glycine and pulp density can significantly impact the Au bioleaching by *C. violaceum* [94].

3.3 Challenges in the Bioleaching Process using Chromobacterium violaceum

The *Chromobacterium* spp. are opportunistic bacteria often considered harmless and saprophytic. However, over 150 cases of *C. violaceum* infections have been reported worldwide till-to-date [95], causing urinary tract infections, pneumonia, localised cutaneous infections, severe sepsis with metastatic abscesses, and septic shock, which may lead to multiorgan failure [96, 97]. Even though the number of cases recorded may seem insignificant since the first human infection of *C. violaceum* was reported in Malaysia in 1927 [98], the fatality rate among the infected patients reached up to 60% in some countries [99]. Hence, *C. violaceum* infection is often regarded as a deadly global pathogen, and utilising such strains as a bioleaching agent may have severe public health consequences.

In recent days, *C. violacein* infections have been reported in countries like Australia, Bangladesh, the US, Japan, and Africa [95, 99-101]. Ironically, the bacterium is often reportedly resistant to common clinically prescribed antibiotics. Hence, treating infected patients is often challenging and leads to mortality, especially in children and older patients. For instance, a fatal case was reported in Japan in 2021 (the first incident in the country), linked to *C. violaceum* infection in a 49-year-old man. The patient contacted the bacteria when he met in an accident and was thrown into a contaminated muddy rice field. After hospitalisation, the patient's condition remained stable with persistent fever for seven days. However, he had a sudden collapse on the eighth day and died. The cause of death was confirmed due to *C. violaceum* infection, which led to a rare combination of fatal sepsis on dissemination to the iliopsoas abscess [102]. The report also admitted that the bacteria was challenging to detect by the medical staff due to a lack of knowledge on the pathogenesis and the optimal antibiotic therapy, since only negligible cases were reported worldwide. Despite cefotiam administration as a perioperative antibiotic to prevent surgical site infections, it failed to restrain the *C. violaceum* infection [102]. Another *C. violaceum* infection case was reported in Bangladesh hospital (the first incident in the country), linking to a 40-year-old older woman with persistent fever for four weeks, dry cough for two weeks, and yellow discolouration urine and sclera for three days. She was diagnosed with pneumonia, acute respiratory distress syndrome, disseminated intravascular coagulation, and *C. violacein* infection was confirmed from the patient swab sample. Despite prescribing antibiotics like ceftriaxone, piperacillin, and cotrimoxazole, no clinical improvement was noticed, and multiorgan failure progressively happened until the patient died [95]. Incidences from both cases in Japan and Bangladesh can be noticed that despite vigorous antimicrobial treatment to the patients, the bacterial infections failed to curb, hence, there is a high risk of utilising *C. violacein* as a bioleaching agent. Moreover, Sandrasaigaran et al. (2021, 2022) confirmed that *Chromobacterium* spp. is a good bioleacher, however, the bacteria expressing extended-spectrum β-lactamase genes [19] and being resistant to multiple antibiotics [58] could be a major public health concern.

4. FUTURE DIRECTION

Despite possessing great ability and potential application in Au bioleaching, *C. violacein*'s pathogenicity and virulence factors generate great concerns. If such drawbacks are addressed, the sustainability of Au recovery can be ascertained, bringing a new revolution to the gold mining industry. Hence, eliminating the virulence gene in *C. violacein* may enable the safer application of the bacteria. This can be achieved by employing the clustered regularly interspaced short palindromic repeats (CRISPRs) and their associates (CRISPR-associated = Cas); capable of gene editing in bacteria and many organisms. The CRISPRs-Cas systems are frequently detected in the genomes of both archaea and bacteria, and mainly function as an acquired defence system of the host immunity [103, 104]. However, their role in gene editing, especially in virulence genes, has recently been explored. Hou et al. (2020) showed that using a two-plasmid system of CRISPRs-Cas, the virulence gene of pathogenic *E. coli* can effectively edited and deleted [105], thus laying a foundation for virulence gene editing and removal in *C. violacein* in future. Besides targeting the virulence factor, CRISPRs-Cas systems can also be targeted to modify the antimicrobial-resistant genes in microbes; moreover, the CRISPRs-Cas antimicrobials hold greater advantages than conventional antimicrobials. The antimicrobial-resistant genes in bacteria are commonly found in the plasmids and gene cassettes, which can be transferred between bacteria via horizontal gene transfer. Hence, targeting and eliminating such vectors can restore bacterial sensitivity towards antibiotics [106]. Knowing *C. violacein* for its virulence and antimicrobial resistance, the CRISPRs-Cas system may optimise the bacteria in bioleaching industries without posing further harm to people.

Conversely, the CRISPR-Cas system is also a relatively a new technology, and developing a mechanism specifically targeting the virulence and antimicrobial resistance gene in *C. violacein* can be challenging. This includes eliminating or modifying off-target genes in *C. violacein*, which could lead to unintended mutations and consequences. Besides, there is a growing concern that bacteria escaping CRISPR-Cas double-strand break point due to mutation in target sequences or CRISPR-Cas effector proteins, thus resulting in the bacteria developing an intrinsic resistance or tolerance towards CRISPR-Cas antimicrobials [107, 108]. The accidental release or non-regulation of such strains can further contribute to the emergence of multi-drug resistant ‘superbugs’, which are harder to treat. To address these problems, continued research and innovation in the field of CRISPR-Cas gene editing are required, focusing on enhancing accuracy, limiting off-target effects, and taking into account the safety implications of deleting virulence genes in bacteria.

On the other hand, bioleaching of Au and other metals is focused chiefly on the laboratory scales. Hence, industrial-scale applications require increasing the size of equipment and processes. Small reaction equipment in laboratories allows for complete reaction execution, but large reaction containers used in industrial production make it difficult to maintain the solution's pH, dissolved oxygen concentration, microbial distribution, and pulp density, which may lower the bioleaching rate and efficiency. Such conditions may incur extra operational costs and be deemed inefficient resource management. Hence, researching and upscaling Au bioleaching using *C. violaceum* is required, especially in the bioreactor design, automation, and an optimal real-time monitoring system. A multidisciplinary collaboration, including microbiologists, chemical engineers, and environmental scientists, must work together to overcome these obstacles. Furthermore, dedication to sustainable practices and resource-efficient technology is necessary to make industrial-scale Au bioleaching a workable and ecologically responsible with *C. violaceum*.

5. CONCLUSION

In conclusion, because of its rapid buildup, environmental risks, and probable loss of valuable metals, E-waste poses a serious worldwide problem. Pyrometallurgy and hydrometallurgy are two conventional techniques for recovering metal from E-waste, but they have a number of limitations, including energy-intensive procedures and the emission of hazardous materials. A possible substitute is the employment of microbes, notably *Chromobacterium violaceum*, as a bioleaching agent. Despite obstacles linked to its pathogenicity and antibiotic resistance, *C. violaceum* can effectively extract gold from E-waste. Hence, careful scrutinisation and genetic modification of *C. violaceum* using CRISPR-Cas technology, especially on its virulence and antibiotic-resistant genes may provide extra cushion for the safe recovery of Au from E-waste. Besides, addressing abiotic factors and culture conditions of *C. violaceum* during the bioleaching process may enhance scalable industrial procedures for sustainable metal recovery in the future.

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