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A Bioleaching Process for Sustainable Recycling of Complex Structures with Multi-Metal Layers

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Abstract: Industrial waste is accumulating, while primary metal resources are depleting. Bioleaching has been shown to be a cost-effective and environmentally friendly approach to metal recovery from waste, but improved designs are needed for large-scale recycling. Metal components that are manufactured by electrodeposition over a mandrel can be difficult to recycle using conventional techniques due to their complex geometry and inner Ag coating. A sustainable biotechnology for separating Cu and Ag from waste electrodeposited components is presented. Two-step bioleaching experiments were performed, during which Cu was solubilized by Fe^{3+} regenerated by *Acidithiobacillus* (*At.*) *ferrooxidans* CF3 and a consortium of ten acidophilic Fe^{2+}-oxidizers. High Cu recovery rates were achieved in agitated flasks (22 °C, pH 1.9), with *At. ferrooxidans* solubilizing 94.7% Cu in 78 days and the consortium 99.2% Cu in 59 days. Copper bio-solubilization was significantly accelerated in a laboratory-scale bioreactor (32 °C, 1 L air min^{-1}) using the bacterial consortium adapted to elevated Cu concentrations, reaching >99.6% Cu extraction in only 12 days. The bioreactor was dominated by *Leptospirillum* and *Acidithiobacillus*, with their proportions changing (from 83.2 to 59% of total reads and from 3.6 to 29.4%, respectively) during the leaching process. Dissolved Cu was recovered from the bioleachates (containing 14 to 22 g Cu L^{-1}) using electrowinning; >99% of the Cu was deposited (with Cu purity of 98.5 to 99.9%) in 3.33 h (at current efficiency between 80 and 92%). The findings emphasize the importance of a bioleaching system design to achieve economical separation of base and precious metals from industrial wastes. The presented technology minimizes waste generation and energy consumption. On a larger scale, it has the potential to contribute to the development of industrial recycling processes that will protect natural resources and contribute to the Net Zero target.

Keywords: bioleaching; acidophiles; iron oxidation; copper electrowinning; metal recycling

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1. Introduction

Electroforming is an electrolytic process that enables the manufacturing of complex, high-precision, and lightweight metallic technical components, such as radar waveguides, and precision medical, optical, and mechanical components [1]. The technical components are electroformed around a permanent (reusable) or expendable mandrel (which is destroyed in order to remove the component) [2]. The latter is widely used when the required final output has reentrant shapes and angles. Both conductive (e.g., stainless steel) and non-conductive (glass, plastics, wax, etc.) mandrels are used as cathodes in electroforming processes [2]. When a non-conductive mandrel is used, the cathode is made conductive by the application of a thin metallic film onto its surface, with Cu, Ag, and Ni being the most broadly used conductive paints in Cu electroforming [2]. After an electroformed component is removed from a non-conductive expendable mandrel treated with a conductive film, the inner surface of the component is coated with the mandrel coating material (e.g., Ag). Electroforming is generally widely used to recycle metals from technical components and wastes [3], but the presence of conductive layers described above makes it difficult to separate and selectively recover the base (e.g., Cu) and precious (Ag) metals using only electrochemical recycling methods.

Other types of industrial waste that contain base and precious metals are generated in large amounts, including e-waste, such as printed circuit boards (PCBs) and electronic chips [4]. Globally, over 50 million tonnes of e-waste are currently produced per year (53.6 million tonnes in 2019) [5], and based on current trends, it is predicted that global e-waste production will reach 120 million tonnes per year by 2050 [6]. Only 20% of e-waste is formally recycled, and the remaining 80% mostly ends up in developing countries in landfills or is informally recycled. These improper waste practices often lead to the release of hazardous metal(loid)s such as Hg, Pb, and Cd, which negatively affect the environment and human health [6]. Conversely, the world's natural resources are rapidly depleting, and the valuable metals and critical elements (such as rare earth elements) present in e-waste globally were valued at 57 billion USD for 2019, which highlights the need for sustainable metal recycling from e-waste [5]. The metal content in e-waste is much higher than that in primary ores; PCBs contain 30–40 times more Cu and 40–800 times more Au than US ores [7,8]. Urban mining of e-waste is, therefore, becoming more cost-effective than mining of primary resources [9].

Both base and precious metals are currently recovered from e-waste by techniques based on pyrometallurgy and hydrometallurgy [10]. However, there are multiple serious challenges associated with the conventional techniques for industrial metal recovery: pyrometallurgy generates hazardous emissions and lacks specificity, and hydrometallurgy uses toxic chemicals and is often costly [11]. Additionally, the products obtained by conventional methods are not suitable for recycling streams back into the supply chain, as required in a sustainable circular economy. Bioleaching (biohydrometallurgy), which uses non-pathogenic microorganisms to solubilize metals, has been successfully applied in the industrial recovery of base and precious metals from sulfide ores and concentrates [12,13]. Bioleaching-based technologies present a relatively simple, environmentally friendly approach that avoids using toxic chemicals and large amounts of energy. Despite the apparent advantages, bioleaching has only been demonstrated on a laboratory scale in metal recovery from e-waste [14], and improved designs need to be developed for industrial applications.

Most broadly used techniques for large-scale recovery of Cu from pregnant leach solutions (PLSs) in (bio)hydrometallurgical ore processing historically involve cementation (using zero-valent Fe or Zn to precipitate Cu) [15], and in modern processes, solvent extraction followed by electrowinning (SX-EW) [16,17]. The combination of Cu chemical leaching and subsequent electrowinning from PLS is also feasible in Cu recovery from e-waste and has been extensively applied in recent years in industrial e-waste recycling [18]. This approach shows a high efficiency of Cu recovery but faces several challenges, including high energy consumption and insufficient selectivity of Cu recovery from PLSs with complex chemistries. Generally, Cu electrowinning-based processes can be further improved to increase their scalability and sustainability.

The objective of this research was to develop a sustainable technology combining bioleaching and electrowinning for recycling electrodeposition waste components that are difficult to recycle by conventional mechanical and chemical techniques. The main aim was to improve the kinetics of Ag and Cu separation using sustainable technology. Two-step bioleaching of Cu from waste electroformed tubes was performed using the following mesophilic acidophilic Fe^{2+}-oxidizing bacteria in three different systems: (i) a pure culture of *Acidithiobacillus (At.) ferrooxidans* strain CF3 in an agitated flask; (ii) a consortium of ten acidophiles in an agitated flask; and (iii) the consortium from system 'ii' adapted to elevated Cu concentrations in a laboratory-scale bioreactor. Process kinetics in the above bioleaching systems were compared, and the results of microbiological analyses are presented. Bio-solubilized Cu was recovered from the bioleachates by electrowinning. Both Ag residues and Cu deposits were analyzed by scanning electron microscopy (SEM), and their suitability to return to the metal supply chains was assessed.

2. Materials and Methods

2.1. Waste Electroformed Components

Technical tube-shaped Cu components with surface features (Figure 1A) were electroformed on a non-conductive mandrel (Supplemental Figure S1). First, a conductive Ag paint (52–54% Ag; density of 1.7 g mL^{-1}; Spa Plating, Bath, UK) was applied over wax mandrels using a dual-nozzle spray paint gun. The Ag-coated mandrels served as a cathode for Cu electrodeposition in a solution containing 250 g L^{-1} $CuSO_4 \times 5H_2O$, 50 g L^{-1} H_2SO_4, and 80 mg L^{-1} HCl. Soluble nuggets (99.9% Cu in a Ti basket; A.M.P.E.R.E., Birmingham, UK) were used as anodes for the electroforming process. The walls of the electroformed Cu tube had a thickness of ~500 µm (Figure 1B), and the Ag layer on the tube inner surface was ~24–30 µm thick. Waste electroformed tubes that showed defects (such as an incomplete Cu deposition or uneven wall thickness; Figure 1B,C) were used in this study to optimize bioleaching and electrowinning of Cu for sustainable recycling of electrodeposition waste.

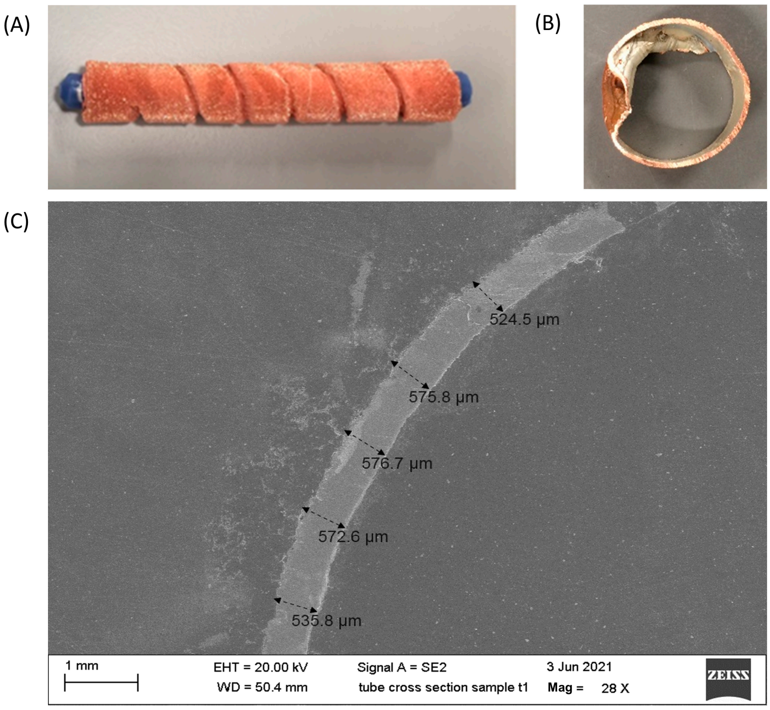

Figure 1. (**A**) Electroformed technical tube-shaped Cu component with surface features; (**B**) waste component cross section; (**C**) scanning electron microscopy (SEM) image of the component cross section (indicating the thickness of the Cu layer, with a purity of 100% Cu, determined by EDS).

2.2. Acidophilic Chemolithotrophs and Cultivation Conditions

All acidophilic chemolithotrophs used in this study (summarized in Table 1) were obtained from the Acidophile Culture Collection at Bangor University (Bangor, UK) and cultured at 30 °C and 130 rpm in a sterile pH 1.7 basal salts medium (121 °C, 15 min) supplemented with trace elements [19] and 20 mM Fe^{2+} (from a filter-sterilized 1 M stock solution of $FeSO_4 \times 7H_2O$; pH = 2.0).

2.3. Bioleaching of Cu from Waste Electroformed Components

Three bioleaching experiments were performed to investigate the bioleaching of Cu from the above-described waste electroformed tubes. Two-step direct bioleaching with mesophilic acidophilic Fe^{2+}-oxidizing bacteria was performed in agitated flasks and a stirred tank bioreactor (Supplemental Figure S2), as described below.

Table 1. Acidophilic chemolithotrophic bacteria used for direct bioleaching of Cu from waste electroformed components.

Bacterium	Temperature Optimum (Range) (°C)	pH Optimum (Range)	Fe^{2+} Oxidation (Fe^{3+} Reduction)	S Oxidation	References
Acidithiobacillus (At.) ferrooxidans T	28 to 30 (10 to 37)	2.0 to 3.0 (1.3 to 5.5)	+(+)	+	[20]
At. ferrooxidans (*'ferruginosis'*) strain CF3	28 to 30 (10 to 37)	~2.5 (min. 1.3)	+(+)	+	[21]
At. ferrivorans T	25 to 32 (min. 4)	2.5 (min. 1.9)	+(+)	+	[22]
At. ferridurans T	29 (10 to 37)	2.1 (1.4 to 3.0)	+(+)	+	[23]
At. ferriphilus T	30 (5 to 33)	2.0 (min. 1.5)	+(+)	+	[24]
At. ferrianus T	28 to 30 (20 to 32)	1.7 to 2.0 (1.5 to 3.0)	+(+)	+	[25]
Sulfobacillus (Sb.) thermosulfidooxidans T	50 (20 to 58)	1.9 to 2.4 (0.8 to 5.5)	+(+)	+	[26]
Leptospirillum (L.) ferrooxidans T	35 (10 to 45)	1.6 to 2.0 (min. ~1.0)	+(-)	-	[27]
L. ferriphilum T	30 to 37 (14 to 40)	1.4 to 1.8 (1.3 to 2.0)	+(-)	-	[28]
Sulfoacidibacillus (S.) ferrooxidans T	33 (max. 37.5)	1.7 (min. 0.9)	+(+)	+	[29,30]

'T' as superscipt signifies the bacterium is a type strain (=the first strain isolated and characterized within the species.

Two experiments were performed in a conical flask (0.7 L operating volume). A sterilized growth medium (121 °C, 15 min) containing basal salts and trace elements [19] of pH 1.7 (adjusted with 1 M H_2SO_4) was supplemented with 20 mM Fe^{2+} (from 1 M $FeSO_4 \times 7H_2O$). The first flask was inoculated with a pure culture of Fe^{2+}- and S-oxidizing acidophilic bacterium *At. ferrooxidans* strain CF3, and the second with a mesophilic consortium of Fe^{2+}-oxidizing acidophilic bacteria (most of which also oxidize sulfur). All bacterial species and their main physiological characteristics are shown in Table 1. When all Fe^{2+} was oxidized (after approximately 2 days), 19.64 and 18.98 g of waste electroformed tubes sterilized with 70% ethanol, respectively, were added to the flasks (corresponding to 2.8 and 2.7% pulp density, respectively). The flasks were cultivated at room temperature (~23 °C) using a magnetic plate and stirrer (at 220 rpm) and automated pH adjustment (set to pH 1.9 using 1 M H_2SO_4). To avoid inhibition of the bioleaching bacteria by an elevated concentration of dissolved Cu, the bioleaching was performed in two phases. The first bioleaching phase was initiated by the addition of the eletrodeposition waste and lasted approximately 30 days. At the end of the first phase, the leachate was partially drained from the flask, 70 mL of the leachate was left as inoculum, and a fresh medium was added (to the total volume of 0.7 L). This initiated the second phase, which lasted until the extraction of Cu ceased.

In the third experiment, bioleaching of Cu from the electrodeposition waste was performed as described above, with several differences indicated below. Firstly, a stirred tank bioreactor (Fermac 360 system, 2 L vessel; Electrolab, Tewkesbury, UK) was used instead of a flask to investigate the effects of process optimization on the Cu bioleaching kinetics. The bioreactor was maintained at 32 °C and 130 rpm and connected to an air supply (~1 L min^{-1}; filter-sterilized). Secondly, the culture adapted to elevated Cu concentrations from the above-described flask experiment was used as inoculum. Thirdly, 19.83 g of waste electroformed tubes was added (corresponding to 2.8% pulp density). Finally, the leachate was replaced with a fresh medium after 7 days of bioleaching.

An abiotic (un-inoculated) control flask was set as described above for the two biotic flask experiments and cultivated for 75 days under the following conditions: 220 rpm, 25 to 28 °C (magnetic stirring), and pH 1.9 (automated adjustment). All leaching systems were regularly sampled to monitor pH, redox potential (E_H), and dissolved Cu and Fe concentrations, as well as for microbiological analyses (all analyses are described in detail below).

2.4. Electrowinning of Cu from Bioleachates

Dissolved Cu was recovered from two types of bioleachates (with Cu content ranging from 13.7 to 21.7 g L^{-1}), using electrowinning onto a Cu foil attached to a polystyrene sheet: (i) unamended leachates (pH 1.9; containing 20 mM Fe), and (ii) amended leachates, with pH adjusted to ~4.0 (using 1 M KOH) and filtered (CA; 0.22 µm; Corning Incorporated, USA) to remove precipitated Fe^{3+}. Electrowinning was performed in a total volume of 200 mL of bioleachate (serving as electrolyte) in a stirred beaker (magnetic plate; 300 rpm) at room temperature (~22 °C). The experimental setup is shown in Supplemental Figure S3. An insoluble Ti mesh coated with IrO_2-Ta_2O_5 was used as the anode, and a thin Cu foil (5 × 5 cm) was used as the cathode to recover the Cu. The current density used was 20 mA cm^{-2}, and the deposition was performed for 5 h. To monitor the Cu deposition rate, samples were regularly withdrawn, filtered, and stored at 4 °C until ICP analysis. The deposits were investigated using SEM.

2.5. Analytical Methods

All bioleaching systems were regularly sampled to monitor process kinetics. Volume changes due to sampling and H_2SO_4 automated additions were recorded. A combination Ag/AgCl pH and a redox electrode (both Thermo Scientific Orion; Thermo Fisher Scientific, USA) were used to monitor pH and E_H (relative to a standard hydrogen electrode), respectively, in leachate samples, both connected to a pH/conductivity meter (Orion Versa Star Pro; Thermo Fisher Scientific, Waltham, MA, USA). The concentration of dissolved Cu was measured in filtered (0.22 µm, MCE membrane) samples by ICP-OES (Optima 8300; PerkinElmer, Waltham, MA, USA). Concentrations of Fe^{2+} and total Fe (after reducing soluble Fe^{3+} to Fe^{2+} with ascorbic acid) were determined using the ferrozine colorimetric assay [31].

Solid samples (Ag residues, Cu deposits, and Cu/Ag residue from abiotic control) were washed in RO water, air dried, and examined by SEM (Carl Zeiss 1530 VP FEG SEM, Zeiss, Jena, Germany). Samples were sputtered with an Au/Pd coating (20 µm) for improved conductivity prior to SEM imaging. An Oxford Instruments X-Max 80 mm^2 EDX detector using an accelerating voltage of 5–20 kV was used to perform energy dispersive X-ray spectroscopy (EDS) of the solids, with magnification ranging from 1000–20,000×.

2.6. Microbiological Analyses

Planktonic cells in leachate samples were enumerated using a Thoma counting chamber and a Motic Panthera microscope (Motic Europe, Barcelona, Spain) at 400× magnification. The acidophilic consortia were investigated by plating onto selective solid media [32]. After a 7-day cultivation at 30 °C, isolates were identified by PCR using 27F/1492R primers, Sanger sequencing, and BLAST search.

Additionally, 16S rRNA gene amplicon sequencing was used to investigate the bioleaching consortia. Biomass was collected from leachates collected at the end of the experiments and during leachate replacements with fresh media. Leachate samples were filtered using 0.22 µm cellulose nitrate membranes (Whatman, UK), and total genomic DNA was extracted using the DNeasy PowerSoil Pro kit (Qiagen Inc., Hilden, Germany). DNA concentration and quality were determined using a nanodrop (Thermo Fisher Scientific, Waltham, MA, USA). Extracted DNA was stored at −20 °C prior to submission to the Centre for Environmental Biotechnology at Bangor University (Bangor, UK) for MiSeq Illumina, which was performed as described elsewhere [33]. In short, the V4 region was amplified using 515F/806R primers [34], and Illumina MiSeq paired-end sequencing was performed. The sequences were processed using a pipeline described by Fadrosh et al. [35] and the DADA2 plugin for Qiime2 (v2021.2) for denoising and ASV picking. Amplicon sequence variant (ASV) tables were generated, and taxonomy was assigned using the Silva database (v. 138).

3. Results

3.1. Bioleaching of Cu from Waste Electroformed Components Using At. ferrooxidans Strain CF3

The pure culture was pre-grown in the absence of electrodeposition waste, during which time all Fe^{2+} was oxidized to Fe^{3+}, E_{H} (Figure 2A) increased from +601 to +750 mV, and planktonic cells (Figure 2B) grew from 0.3×10^7 to 1.5×10^7 cells mL^{-1}. After 2 days, the waste electroformed tubes were added, and the first phase of Cu bioleaching commenced (Figure 2C). An increase in planktonic cell counts (to 2.8×10^8 cells mL^{-1}) and unchanging E_{H} (mean of +715 mV; $n = 12$) was observed until day 21, after which E_{H} and planktonic cells started to drop and the concentration of dissolved Cu plateaued at ~42% recovery. This was ascribed to the inhibition of *At. ferrooxidans* by elevated concentrations of dissolved Cu. The leachate was replaced on day 33 with a fresh medium (ending the first bioleaching phase), and Cu bioleaching resumed. During the second bioleaching phase, the E_H values ranged from +685 to +725 mV, and planktonic cells reached 3.4×10^8 cells mL^{-1}. In total, *At. ferrooxidans* strain CF3 recovered 95.2% Cu from the electrodeposition waste in 78 days (Figure 2C).

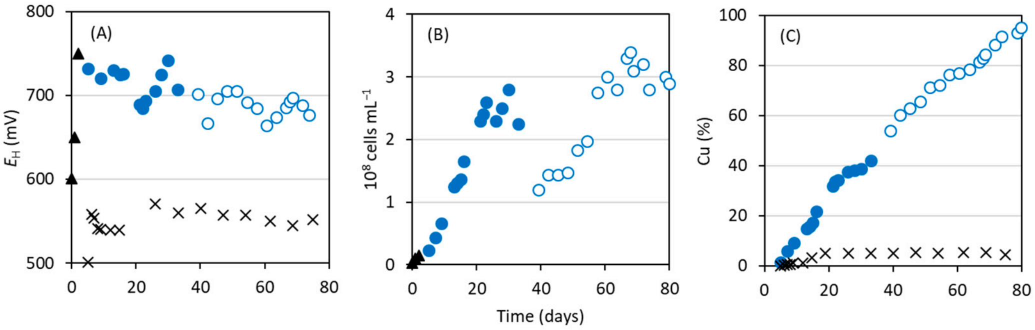

Figure 2. Changes in (**A**) redox potential, (**B**) planktonic cell counts, and (**C**) concentration of dissolved Cu during two-step direct bioleaching of electrodeposition waste (2.8% pulp density) in a flask (~23 °C; 130 rpm; pH maintained at 1.9), using *At. ferrooxidans* strain CF3. Legend: CF3 pre-grown without electrodeposition waste (▲), followed by Cu bioleaching from waste electroformed tubes performed in two phases, with (●) original and (○) fresh medium; (×) abiotic (un-inoculated) control.

Only 5.3% Cu was solubilized in 75 days in an abiotic control (Figure 2C). A majority (70 to 100%) of the Fe present in the abiotic system was present as Fe^{2+}, and most E_{H} values ranged between +540 and +560 mV (Figure 2A).

The maximum concentrations of dissolved Cu in the leachates that were collected at the end of each bioleaching phase (defined by using a fresh medium) were 14.9 and 14.2 g L^{-1}, respectively. The Cu-rich leachates (from this and the below-described bioleaching experiments) were collected for subsequent Cu electrowinning (Section 3.4) to investigate routes for closed-loop recycling of electrodeposition waste. Solid Ag residues were collected, washed, dried, and stored for SEM analysis (Section 3.5).

3.2. Bioleaching of Cu Using Acidophilic Fe^{2+}-Oxidizing Consortium

A consortium of acidophilic Fe^{2+}-oxidizers (listed in Table 1) solubilized 99.2% of the Cu in 59 days, with 50.7 and 48.5% extracted in two subsequent bioleaching phases (Figure 3C). During the pre-growth phase, over 99% Fe^{2+} was oxidized, E_{H} (Figure 3A) increased from +622 to +744 mV, and planktonic cell counts (Figure 3B) increased from 1.0 to 4.4×10^7 cells mL^{-1}. Similarly to bioleaching with CF3, high E_{H} values were measured (mean of +713 mV; *n* = 27, disregarding the beginning of phase 2) during Cu bioleaching, and significant bacterial growth was observed due to Fe recycling in the system. The planktonic cell counts reached a maximum of 3.5 × 10^8 cells mL^{-1} at the end of each bioleaching phase.

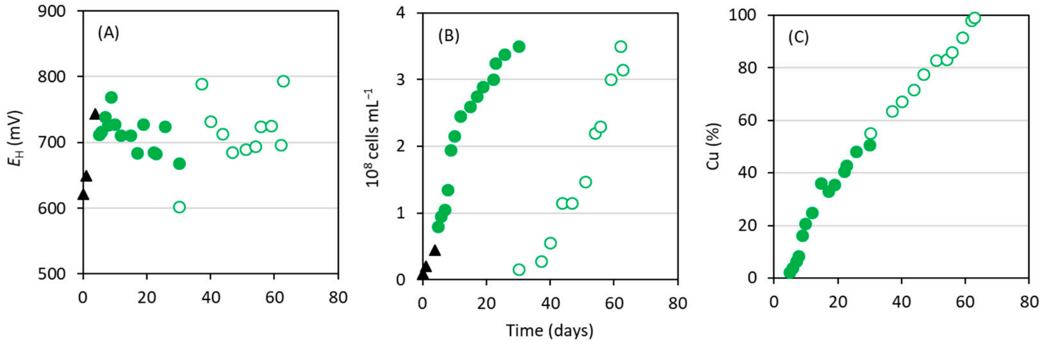

Figure 3. Changes in (**A**) redox potential, (**B**) planktonic cell counts, and (**C**) concentration of dissolved Cu during two-step direct bioleaching of electrodeposition waste (2.7% pulp density) in a flask (~23 °C; 130 rpm; pH maintained at 1.9), using an acidophilic consortium of Fe^{2+}-oxidizers (listed in Table 1). Legend: bacterial culture pre-grown without electrodeposition waste (▲), followed by Cu bioleaching from waste electroformed tubes performed in two phases, with (●) original and (○) fresh medium.

The Cu-rich leachates obtained during the electrodeposition waste bioleaching with the acidophilic consortium of Fe^{2+}-oxidizers contained Cu contents of 13.7 and 12.4 g L^{-1}, respectively.

Illumina amplicon sequencing of 16S rRNA genes identified three chemolithotrophic bacteria that oxidize Fe^{2+} in the bioleachate collected at the end of the flask experiment (on day 63); *Acidithiobacillus* accounted for 98.4% of total reads, while *Leptospirillum* and *Sulfoacidibacillus* (former *Acidibacillus*) [29,30] were present with low abundances (≤0.2%). Additionally, the human pathogen *Bordetella* was detected in the leachate with a low abundance (0.2%). Plating of viable chemolithotrophs onto selective solid media identified the presence of the Fe^{2+}- (and S-)oxidizing *At. ferridurands* (99.74% identity) and Fe^{2+}-oxidizing *L. ferriphilum* (98.83% identity) in the bioleachate collected at the end of the experiment.

3.3. Bioleaching of Cu in a Bioreactor Using Adapted Acidophilic Consortium

The leachate collected at the end of the flask experiment was used as inoculum for electrodeposition waste bioleaching in a stirred tank bioreactor, and a significantly greater rate of Cu solubilization (Figure 4C) was achieved under the strictly controlled conditions compared to the flask bioleaching. The consortium of acidophilic Fe^{2+}-oxidizers previously adapted to elevated Cu concentrations solubilized 99.6% Cu in only 12 days, with 78.1% of the Cu solubilized in the first bioleaching phase (lasting 7 days) and the remaining 21.5% in the following bioleaching phase (5 days long). The concentrations of dissolved Cu in the recovered leachates were 21.7 and 7.7 g L^{-1}, respectively.

Similar pre-growth kinetics were observed in the bioreactor as in the flasks described above (in Sections 3.1 and 3.2), with E_H (Figure 4A) increasing from +590 to +701 mV and planktonic cell counts (Figure 4B) increasing from 0.4 × 10^7 to 2.7 × 10^7 cells mL^{-1}. Again, significant bacterial growth was observed during Cu bioleaching, yielding a maximum of 3.0 × 10^8 cells mL^{-1} at the end of the first bioleaching phase. Interestingly, the changes in redox potential in the first bioleaching phase showed a similar trend to that observed in the pre-growth phase, before the waste electroformed tubes were added and Fe redox cycling was enabled. The second bioleaching phase showed a lower growth rate and constant E_H values (around +700 mV).

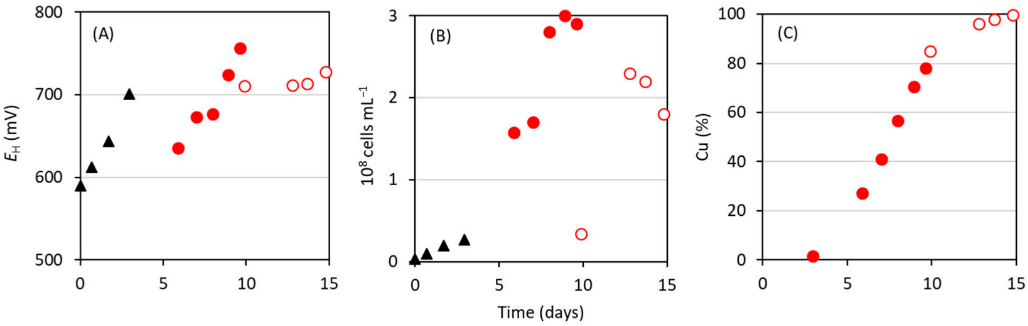

Figure 4. Changes in (**A**) redox potential, (**B**) planktonic cell counts, and (**C**) concentration of dissolved Cu during two-step direct bioleaching of electrodeposition waste (2.8% pulp density) in a bioreactor (32 °C; 130 rpm; 1 L air min^{-1}; pH maintained at 1.9), using an adapted acidophilic consortium of ten Fe^{2+}-oxidizers. Legend: bacterial culture pre-grown without electrodeposition waste (▲), followed by Cu bioleaching from waste electroformed tubes performed in two phases, with (●) original and (○) fresh medium.

The composition of microbial populations (Figure 5) was investigated in liquid samples collected throughout the course of the experiment. The inoculum was dominated by *Acidithiobacillus* spp. (with 98.4% of total reads), while a significantly lower proportion (3.6%) was detected at the end of the pre-growth phase (day 3) when the concentration of dissolved Cu was negligible. Instead, the culture pre-grown in the bioreactor was highly dominated by the genus *Leptospirillum* (accounting for 83.2%). The proportion of *Acidithiobacillus* gradually increased during Cu bioleaching, reaching 26.3 and 29.4% of total reads (and *Leptospirillum* accounting for 65.9 and 50.0%) at the end of each phase of the bioleaching step (i.e., on days 9 and 15, respectively). The only other genus known to contain species capable of Fe^{2+} oxidation was *Sulfoacidibacillus* (formerly *Acidibacillus*) [29,30], accounting for 0.1% of total reads in the inoculum and 3.6% at the end of the pre-growth phase. Other genera detected were minor contaminants (accounting for <5% of total reads). Plating onto solid media selective for chemolithotrophs identified the presence of *At. ferridurans* (99.63 to 99.82% identity) in liquid samples collected throughout the course of the experiment (days 3, 9 and 15) and *L. ferriphilum* (98.84% identity) in the final leachate (collected on day 15).

3.4. Cu Electrowinning from Bioleachates

Electrowinning was used to recover the bio-solubilized Cu, with the main goal of achieving the following: (i) high Cu recovery rates; (ii) high-quality products suitable for a wide range of applications; and (iii) high current efficiency. The Cu recovery rate from unamended bioleachtes (pH 1.9; containing 20 mM Fe) was 89% in 4 h, with a low current efficiency (ranging between 60 and 66%). To improve the Cu recovery process, the Fe present in the bioleachates was removed by selective precipitation at pH 4.0. The pH adjustment resulted in a complete Fe removal (>99.99%) as well as a partial loss of the Cu; 6 to 19% of the bio-solubilized Cu co-precipitated with the Fe (depending on the total content of dissolved Cu in bioleachate). Over 99.99% of the bio-solubilized Cu was then electrowon from the amended bioleachates in 3.3 h (Figure 6A). During this time, the pH of the leachate dropped from 4.1 to 1.0 (Figure 6A). The current efficiency improved compared to the Cu electrowinning in the presence of Fe, reaching values between 80 and 92% during the active Cu plating (Figure 6B). After 3.6 h of electrowinning, over 99% of the Cu in solution was recovered, and the current efficiency of the process started to drop (from 80 to 60%).

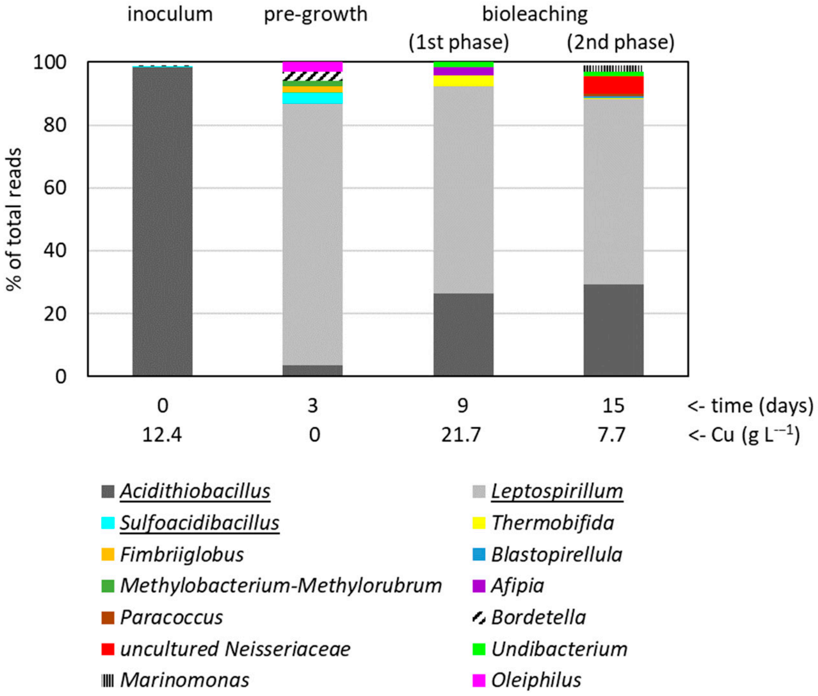

Figure 5. Variations in the composition of microbial consortia during different stages of two-step bioleaching of Cu from electrodeposition waste in a laboratory-scale stirred tank bioreactor (stages, collection times, and dissolved Cu contents are indicated in the chart). The consortia composition was determined (on the genus level) by 16S rRNA gene sequencing (with a cut-off of 0.5%). Legend: chemolithotrophs capable of Fe^{2+} oxidation are underlined.

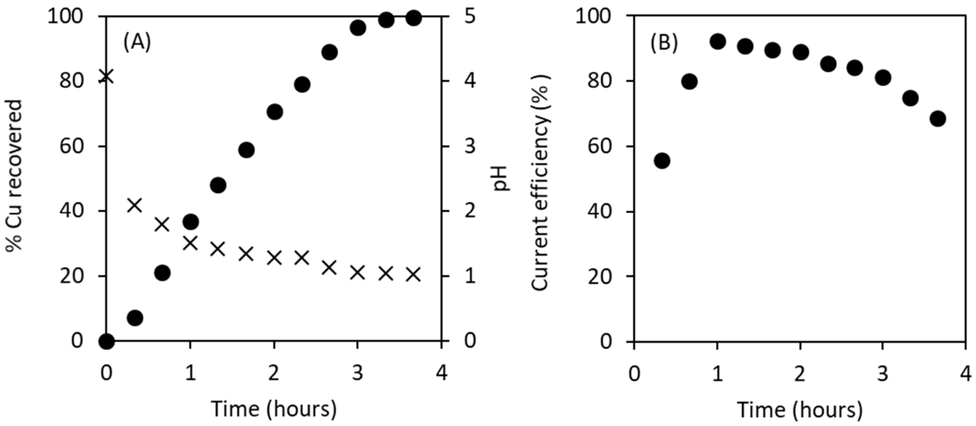

Figure 6. (**A**) Copper recovery rates (•) and changes in pH (x) during Cu electrowinning from amended (Fe-free) bioleachates (of initial pH 4.0); and (**B**) changes in current efficiency during the process.

3.5. Solid Product Analysis

All solid products obtained during the electrodeposition waste recycling were analyzed using SEM. The Ag residues (Figure 7A) collected from the bioleaching systems after Cu bio-solubilization had a smooth surface, as shown in Figure 7B. The elemental composition obtained through the EDS analysis was (in %): 92.6 Ag, 7.2 Cu, and 0.2 Fe (Figure 7C), indicating a presence of residual Cu on the residue surface, together with minor amounts of Fe.

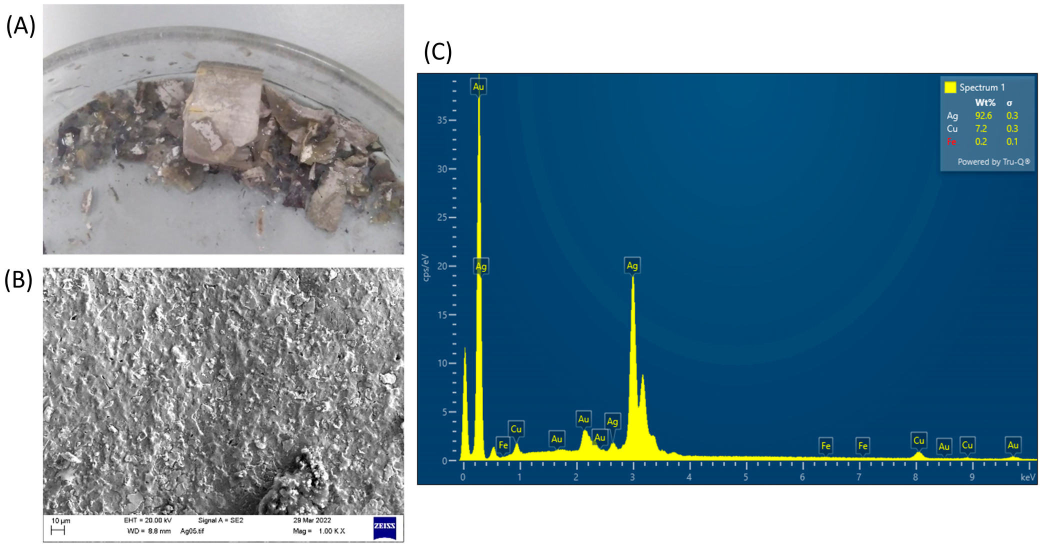

Figure 7. (**A**) Silver residue collected after bioleaching of waste electroformed tubes; (**B**) SEM image (1000× magnification) of the residue surface; and (**C**) EDS spectrum showing the residue elemental composition.

Copper electrowon from the amended (Fe-free) bioleachates formed a thick Cu foil (Figure 8A) with a rough surface morphology (Figure 8B). An EDS analysis performed on different areas of the electrowon sample showed the purity of the electrowon Cu ranged from 98.5 to 99.9% (with minor Fe and/or Ag impurities; Figure 8C).

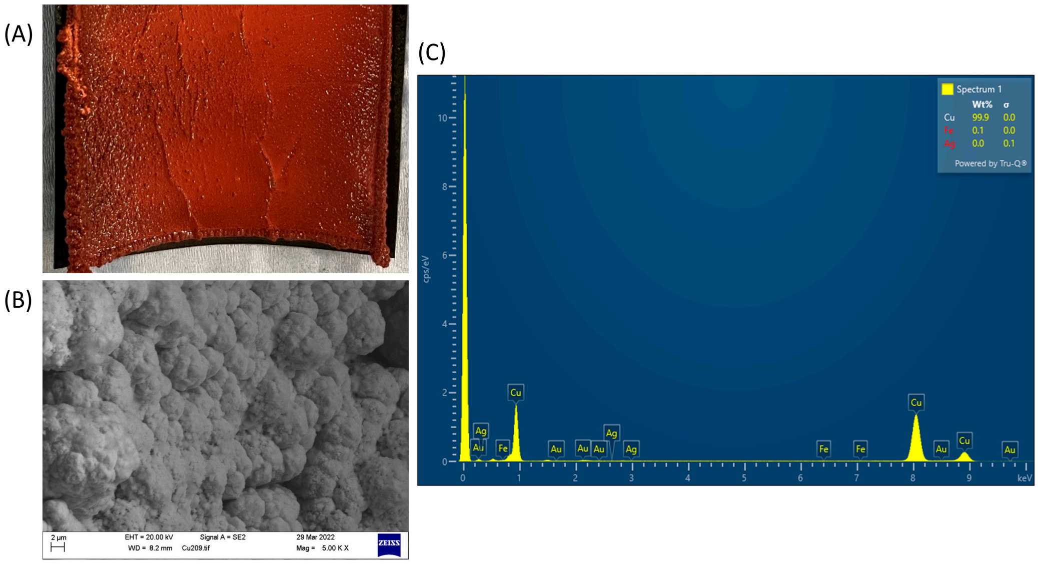

Figure 8. (**A**) Copper sheet (25 cm^2; 1 mm thickness) recovered from bioleachates by electrowinning; (**B**) SEM image (5000× magnification) of Cu deposit surface; (**C**) EDS spectrum showing the elemental composition of the Cu sheet.

4. Discussion

The global demand for Cu is predicted to reach 25.5 million tonnes per year by 2030 [36], and the demand for Ag achieved record highs (35.2 thousand tonnes) in 2022 [37]. The global depletion of high-quality deposits leads to the need to extract metals from low-grade ores as well as from secondary resources such as e-waste. Bioleaching has been successfully used for decades in industrial metal extraction from low-grade Cu sulfides [38,39], and in recent years, the application of bioleaching in e-waste recycling has gained increasing attention from both researchers and industry.

This study investigates the separation and recovery of Cu and Ag from electroformed components to achieve their efficient and sustainable recycling. A number of studies have been published on the selective recovery of base and precious metals from other types of e-waste, such as PCBs, using direct bioleaching to solubilize base metals. Generally, relatively high to high base metal recovery rates (75 to >99%) have been achieved in the laboratory using Fe^{2+}- and/or S-oxidizing prokaryotes [40–53]. However, particularly when multiple base metals were targeted for extraction, the metal recovery rates could be significantly lower (mostly 50–60%) [51,53,54]. Importantly, the time required for the base metal extraction varied greatly in the literature, ranging from 1–3 days [43–45,50] (in all reports, this was the duration of a metal leaching phase within two-step bioleaching) to 60 days [51]. The kinetics of base metal leaching greatly depend on the pulp density, leaching microorganisms used, and other leaching conditions, while also indicating the importance of the right design and optimized bioleaching conditions for improved metal recovery.

In this study, high Cu recovery rates (95 to >99%) were achieved. Fe^{2+}-oxidizing bacteria generated Fe^{3+} from Fe^{2+}, using oxygen as an electron acceptor (Equation (1)), and the formed Fe^{3+} oxidized zero-valent Cu present in the electrodeposition waste, producing soluble Cu^{2+} (Equation (2)). The oxidant was then regenerated by the action of the Fe^{2+}-oxidizers.

$$2\,Fe^{2+} + \frac{1}{2}\,O_2 + 2\,H^+ \rightarrow 2\,Fe^{3+} + H_2O \tag{1}$$

$$Cu^0 + 2\,Fe^{3+} \rightarrow Cu^{2+} + 2\,Fe^{2+} \tag{2}$$

Fe^{2+} oxidation is a proton-consuming reaction, which is why an automated pH adjustment was used to maintain a constant pH during the Cu leaching process. Acid consumption varied between 31 and 39 g H_2SO_4 in the three experiments, with the time course of acid consumption following that of Cu extraction in each experiment. The variation in acid consumption among experiments was ascribed to experimental error, mainly caused by acid tubing changes and priming. Inorganic acid consumption could be lowered by the addition of a sulfur substrate. Bas et al. [55] reported a 62% reduction in acid consumption by adding pyrite to a mixed culture of mesophilic chemolithotrophs leaching Cu from TV circuit boards.

Chemical oxidation of Fe^{2+} is slow at $pH < 4$ [56]. Therefore, Cu solubilization in the abiotic control was low (reaching ~5% in 75 days) and can be ascribed to acidic dissolution (Equation (3)) by protons present in the growth medium and supplied in pH adjustments. The results of the SEM-EDS analysis of the Cu residue collected from the abiotic control are shown in Supplemental Figure S4.

$$Cu^0 + \frac{1}{2}\,O_2 + 2\,H^+ \rightarrow Cu^{2+} + H_2O \tag{3}$$

Significantly greater amounts of Cu were solubilized in the biotic assays in the current work compared to data reported in the literature, considering the following: (i) a greater pulp density (~2.8%) was used compared to 1% used in most studies, and (ii) the source material comprised >94 wt.% Cu, while a majority of studies have investigated the extraction of base metals from shredded or crushed waste PCBs with low metal contents (typically 8–20% Cu, 1–9% Fe, 0–8% Zn, 1–5% Pb, 1–3% Ni, and 0.1–0.4% precious metals [7,40,57–59] or PCB metal concentrates with slightly elevated metal contents [42,44–46]. Additionally, Cu bio-recovery has been reported by many researchers to decrease gradually with increasing PCB contents [42,45,50,60]. To achieve high-performance leaching systems, two-step bioleaching was performed in all three experiments, and the inhibitory effect of elevated concentrations of dissolved Cu was removed by replacing leachates with fresh media. In total, ~19 g of Cu was solubilized in each experiment, and the Fe oxidation rates obtained in active leaching phases were 25, 36, and 379 mg $L^{-1}\,h^{-1}$ in the flask with CF3, flask with consortium, and bioreactor with consortium, respectively, based on the rates of Cu solubilization (described by Equation (2)). A high redox potential was achieved in all experiments during active Cu bioleaching, as >98% of the Fe in the systems was present in the form of Fe^{3+} (determined by a colorimetric assay). The strictly controlled process in the bioreactor was significantly more performant than a continuous bioleaching reported by Ilyas et al. [57], who leached 2 kg of base metals from scrap PCBs in 280 days (295.4 mg h^{-1}) using moderate thermophiles, but with a high flow rate (50 mL min^{-1}), lowering the recovery of base metals to 98.5 mg L^{-1} h^{-1}.

The two flask bioleaching experiments in this study were designed to resemble heap bioleaching [13]; an ambient temperature was used for Cu extraction without additional aeration. This approach reduced costs for maintaining an elevated temperature (for mesophiles, usually 32 °C) and providing air supply, but on the other hand, it resulted in relatively long Cu solubilization times (78 and 59 days using a pure and mixed mesophilic culture, respectively). The third experiment was performed in a strictly controlled bioreactor (stirred tank). Such an approach is used on an industrial scale to extract gold from refractory sulfide concentrates [13]. The use of stirred tanks is significantly more energy-demanding compared to heaps, but it also considerably reduces the time required for metal extraction, as observed in the third experiment in this study (only 12 days were required to solubilize all of the Cu). The choice of a bioleaching system is generally defined by the metal value and its content in the source material.

As described above, the results presented in the current study showed a great effect of process parameters (temperature and aeration) on Cu bio-solubilization, but no great difference was observed between the rates of Cu extraction by a pure culture of *At. ferrooxidans* and a consortium of mesophilic Fe^{2+}-oxidizers in the flask experiments. However, the advantages of using leaching consortia of chemolithotrophs rather than individual species in biomining industrial processes have been conclusively described previously [61]. Bacterial consortia are generally more resilient and robust than pure cultures, and the use of a consortium allows for efficient metal leaching within a broader range of conditions such as varying metal concentrations, redox potentials, etc. For example, Ilyas et al. [41] demonstrated a measurable difference in the extraction rates of base metals from waste PCBs between two different mixed cultures (each of which comprised *Sb. thermosulfidooxidans* and one other chemolithotrophic prokaryote). Based on the above, it can be concluded that both the choice of leaching microorganisms and the optimization of the oxidation process are important to achieve a maximum metal extraction rate. Initial adaptation of the leaching culture can also improve the process kinetics; prokaryotic cultures adapted to a source material and elevated concentrations of dissolved metals have been used in other studies [41,42,47] as well as in the third experiment presented here.

As in other studies [62,63], the Fe-oxidizing bioreactor was dominated by *Leptospirillum* and *Acidithiobacillus* spp., with *L. ferriphilum* and *At. ferridurans* playing a major role in Fe^{2+} reoxidation and, therefore, in metal bioleaching. *Leptospirillum* was highly dominant in the Cu-free system, but the changes in relative proportion of the two major genera throughout the bioleaching process indicate that Fe^{2+}-oxidizing *Acidithiobacillus* spp. had a higher tolerance to elevated dissolved Cu concentrations in comparison to *Leptospirillum* spp. The high tolerance of *Acidithiobacillus* spp. to Cu has been postulated by others, with differences described between species as well as strains [64,65]. Tolerance of Cu >100 mM (16 g L^{-1}) has been reported for *At. ferrooxi*dans [64], and the data obtained in this study show that the mixed culture could resist concentrations up to 22 g L^{-1} Cu under the experimental conditions. It is worth noting that the proportion of *Leptospirillum* spp. in the bioleachates might have been overestimated due to sequencing not distinguishing between viable and dead cells, particularly when the high dominance of *Acidithiobacillus* spp. in the flask bioleachate is considered. The leaching phases in the flask took significantly longer (~30 days) compared to those in the bioreactor (which took 6 days), allowing dead cells to lyse.

The dissolved Cu was recovered from the bioleachates using electrowinning. When the electrolyte is a Cu sulfate solution, the overall reaction of Cu deposition is as shown in

Equation (4). Protons are generated at the anode during the electrowinning process, and H_2SO_4 is generated.

$$CuSO_4\ (aq) + H_2O \rightarrow Cu\ (s) + H_2SO_4\ (aq) + \frac{1}{2}\,O_2\ (g) \tag{4}$$

Electrochemical approaches for metal recovery from industrial and electronic wastes have been reviewed [66], and electrowinning has been used to recover base metals from chemical leachates. For example, Veglio et al. [67] reported >95% current efficiency for Cu and Ni recovery from leachates containing 10 g L^{-1} of each metal, which were obtained by H_2SO_4 leaching of mixed electronic waste. Low current efficiency (63.84%) was reported by Fogarasi et al. [59], who recovered a Cu deposit of high purity (99.04%) from PCB chemical leachates. Electrowinning has also been used to recover bio-solubilized Cu from PCB bioleachates. Erust et al. [53] improved a low current efficiency (66.1% over 4 h) by Fe removal, achieving a current efficiency of ~88%. Sinha et al. [68] recovered 92.7% Cu in 2.5 h, achieving a 95.2% purity Cu product, but the authors did not report current efficiency. Baniasadi et al. [54] recovered 75.7% of Cu present in PCB using bioleaching and electrowinning, obtaining a Cu foil of >99% purity at a current efficiency of >80% (in a 3 h electrowinning process). Compared to the studies above, an improved recovery rate (>99.9% Cu in 3.3 h) was achieved in this study. Also, the product purity (98.5 to 99.9%) and current efficiency (80 to 92%) obtained were high. A great advantage of electrowinning is that solubilized Cu is recovered in the form of sheets suitable for a wide range of applications (e.g., as anodes for further electroforming) as well as for the general Cu supply chain. The recovered Cu sheets had a rougher surface morphology compared to components manufactured via industrial electroforming. It is worth noting, however, that industrial electroforming uses a variety of additives, brighteners, and levelers to obtain a smooth deposition surface [69]. Direct remanufacturing of the tube-shaped components by electroforming would be possible, but the bioleachates contained 3 to 4.5 times lower Cu concentrations than the Cu solutions used for electroforming high-quality components (14 to 22 versus 64 g L^{-1}) and would need to be enriched. Additionally, electroforming requires a constant Cu concentration in solution to maintain the deposition rate and the material properties and is, therefore, used for component manufacturing, while electrowinning is more suitable as a metal recovery technique.

In systems with complex chemical compositions, such as (bio)leachates from waste PCBs, solvent extraction can be used to remove impurities prior to electrowinning. The combination of solvent extraction and electrowinning (SX-EW) has been used for decades in industrial Cu recovery from ores [70], and such an approach could be suitable for metal recovery from PCB leachates, both chemical and biological. Zhang et al. [71] extracted 97.45% of Cu from a bioleachate using an organic solvent, and the purity of the final Cu plate was 99.83% (deposited from solutions containing 30–35 g L^{-1}, with a current efficiency of 93%). A Life Cycle Assessment of the biohydrometallurgical way to extract Cu from waste PCBs involving bioleaching-SX-EW was shown to have a lesser environmental impact than pyrometallurgical processing [72]. An even more environmentally friendly approach could be a fully microbiological approach that would involve a combination of sequential bioleaching of base and precious metals using acidophilic chemolitrotrophs and fungi producing organic acids or cyanogenic bacteria, respectively [49,73], followed by metal biorecovery such as bioprecipitation [74,75], biosorption [76], or chelation [77]. The latter has been used by Sinha et al. [68] to concentrate dissolved Cu in bioleachates before recovery by electrowinning. Extensive research is still needed to select, develop, and optimize the most suitable approaches for industrial waste processing on a large scale. Importantly, these approaches will inadvertently vary according to the waste type and composition. Nonetheless, bioleaching-based technologies have been recognized as more environmentally friendly than conventional hydro- and pyro-metallurgical methods, and they should be further explored for large-scale electronic and urban waste recycling.

It is important to emphasize that the economic feasibility of recycling (bio)technologies should be carefully assessed prior to their translation into industry. Apart from the initial cost of a bioleaching plant, the major operating costs in biohydrometallurgy are associated with aeration, stirring, temperature control, and chemicals. However, the cost-effectiveness of all metal extraction processes is affected primarily by metal prices, which are subject to significant fluctuations. The price of Cu (currently, 8.42 USD per kg [78]) has been particularly volatile in recent years (due to fears of a global economic recession, weaker demand from Chinese industries, a higher US dollar, and the Russia–Ukraine conflict), and long-term price predictions are uncertain. Precious metals have a significantly higher value than base metals (with Ag's current market price reaching 767 USD per kg [79]). As mentioned above, e-waste is much higher-grade than primary ores [7,8], and urban mining should, therefore, be more cost-effective than primary mining [9]. The metal waste investigated in this study was particularly high-grade (comprising 94.4 wt.% Cu and 5.6 wt.% Ag) and, thus, high value (50.7 USD per kg, with 84% of the value associated with Ag). Recent forecasts predict a stable increase in Ag prices (from the current 767 USD per kg to 2822 USD in 2035 [79]), which should secure the financial feasibility of the presented biotechnology.

5. Conclusions

Electroformed Cu tubes are challenging to recycle due to their complex shape and Ag coating on their inner surface. Efficient selective separation of Cu and Ag was achieved via bioleaching using acidophilic chemolithotrophs, followed by electrowinning of biosolubilized Cu. The waste tubes contained five times more Cu than average waste PCBs, and a high pulp density (2.8%) was used in the experimental work. The findings emphasize the importance of a well-designed and optimized system to achieve a high-performance bioleaching system. The optimal process parameters (temperature and aeration) showed a greater effect on the Cu extraction rate than the composition of the acidophilic chemolithotrophic culture. A high Cu solubilization rate (379 mg L^{-1} h^{-1}) was obtained with an adapted consortium of Fe^{2+}-oxidizing bacteria in a strictly controlled bioreactor, with 99.6% of the Cu solubilized in 12 days. Iron was removed from bioleachates as Fe hydroxide prior to Cu recovery by electrowinning. Over 99.9% of Cu was deposited within 3.3 h at a high current efficiency. The deposited Cu was of high purity and suitable for the metal supply chain, including the manufacturing of a variety of technical components and its use in electroforming processes.

The presented results show that bioleaching-based technologies are suitable for separation of base and precious metals from industrial wastes, and acidophilic chemolithotrophs can be designed and adapted to tolerate high concentrations of dissolved base metals (up to 22 g L^{-1} Cu in this study). Results of efficient electrowinning of dissolved Cu from bioleachates are presented. On a broader scale, the findings can contribute to the development of other bioleaching-based technologies for cost-efficient and environmentally friendly recycling of base and precious metals from a range of electronic and urban wastes.

Supplementary Materials: The supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su151914068/s1. Figure S1: (A) Designed Cu technical component (tubes with special surface features), and their manufacturing: silicone resin moulds (B) for the injection of wax mandrels (C) coated with a thin conductive layer of Ag (D); (E) manufactured Cu components (electroformed over the Ag-coated mandrels); Figure S2: Bioleaching of Cu from waste electroformed components using mesophilic acidophilic chemolithotrophs was performed in (A) agitated flasks (magnetic stirring 220 rpm; room temperature ~23 °C; automated pH adjustment set to 1.9), and (B) a stirred tank bioreactor (130 rpm; 32 °C; 1 L air min^{-1}; automated pH adjustment set to 1.9); Figure S3: Experimental design for electrowinning of Cu from bioleachates; Figure S4: (A) Flat-sheet residue collected from an abiotic control flask; (B) SEM image (1000 × magnification) of the residue surface, (C) compared to that of an intact component; (D) EDS spectrum showing elemental composition of the abiotic residue.

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