Biosynthesis of copper nanoparticles from copper tailings ore – An approach to the 'Bionanomining'

Liey-Si Wong-Pinto [a,*], Ana Mercado [b], Guillermo Chong [c], Pablo Salazar [c], Javier I. Ordóñez [a]

[a] *Departamento de Ingeniería Química y Procesos de Minerales, Universidad de Antofagasta, 1240000, Antofagasta, Chile*
[b] *Departamento de Biotecnología, Universidad de Antofagasta, 1240000, Antofagasta, Chile*
[c] *Departamento de Ciencias Geológicas, Universidad Católica del Norte, 1240000, Antofagasta, Chile*

ABSTRACT

Nanotechnology is a discipline that studies the behavior of materials on a nanometric scale. These materials are known to have a wide range of applications due to the outstanding optoelectronic, magnetic, and thermal properties that their scale provides. However, one of the current challenges associated with nanotechnology lies in how nanomaterials are generated. Within the new synthesis trends, biological routes have been proposed as an alternative that requires less polluting organic solvents and less energy consumption.

The biosynthesis of metallic nanoparticles is a Bottom-up method that requires precursors, which have mostly been described as pure solutions. However, in the present work and approaching the concept of 'Bionanomining', the use of a real effluent obtained from the leaching of sulfide ore tailings is studied for the biosynthesis of copper nanoparticles. For this purpose, the bacterial biomass of *Pseudomonas stutzeri* was used as a bioreducing agent.

The leaching of the chalcopyrite-bearing tailings achieved a copper recovery of 50% in 8 h, thanks to sodium nitrate that acted as an oxidizing agent. The copper concentration was increased by solvent extraction, allowing a solution of 700 mg/L of copper to be achieved.

In the biosynthesis of nanoparticles, the experiments were carried out at room temperature and pressure for 48 h, using two different copper precursor solutions. In the first stage, a pure solution was used to determine an adequate copper concentration and biomass dosage, which were 381 mg/L and 1050 mg, respectively. In an advanced approach, the solution obtained from the treated tailings was employed for the biosynthesis, obtaining nanoparticles in the two resulting streams: a solid dead biomass and sodium sulfate solution with 2.7% and 0.27% copper nanoparticles, respectively. These nanoparticles are spherical and with a size between 1 and 2 nm.

1. Introduction

Nanotechnology is a discipline considered by various authors as a frontier technological revolution, given that nanometric-sized materials have unique characteristics that may improve processes and features of other materials in a wide range of scopes (Fashandi and Leung, 2018). Among nanomaterials, nanoparticles have gained attention due to their small size that results in a large surface area, enhancing the reactivity, thermal, optical, electrical, and magnetic properties (Ali et al., 2019; Chen et al., 2019; Gogoi et al., 2019; Liu et al., 2012; Zhou and Coleman, 2016). Thus, nanoparticles' use is widespread in different fields such as mechanical industries, power generating plants, alloying processes, fabrication of superconductors, electronic factories, sewage treatment plants, pharmaceutical industries, and medical institutions, among

others (Anbia and Amirmahmoodi, 2016).

Nanoparticles are traditionally synthesized by two approaches: the first, based on a chemical method, is called Bottom-up, which synthesizes nanoparticles from precursors by reacting and precipitating them in a substrate. The second method, called Top-down, is a physical miniaturization to the nanometer scale of materials through high-energy grinding (Sanchez and Sobolev, 2010). Both techniques are expensive and use polluting substances (Wong-Pinto et al., 2020a).

In the search of cheaper and safer methods for the environment and health, recent studies have pointed out the potential of natural biomasses and biological substances to turn metal solutions into nanoparticles, known as biosynthesis (Kumar et al., 2008a; Rubilar et al., 2013). The biosynthesis of metal nanoparticles consists of a biochemical redox reaction where the ion is transformed to its solid phase with zero

---

* Corresponding author.

E-mail address: lieysi.wong@uantof.cl (L.-S. Wong-Pinto).

oxidation state (Wanarska and Maliszewska, 2019). This biotechnological route for nanoparticle synthesis has the advantages that reactions are spontaneous, the process does not negatively impact the environment, and it avoid a large energy consumption.

Two mechanisms could govern the nanoparticle biosynthesis process: i) a metabolism-dependent biosynthesis inside the living cell using as reactive agents enzymes and proteins (Ali et al., 2019), and ii) a metabolism-independent route, consisting of the reaction between metals and chemical groups found on the biomass cell wall or extracellular metabolites. In extracellular biosynthesis, dead biomass can carry out the process, which is an advantage under an operational point-of-view (Patil et al., 2019). Both mechanisms of biosynthesis have been described for a wide variety of biomasses, such as bacteria, fungi, plant, and algae, and for different metals, like gold, silver, and copper (David et al., 2019; Gahlawat and Choudhury, 2019; Shankar et al., 2016).

On the other hand, one of the most important activities since ancient times for supplying raw materials that develop cities and countries is mining, which has marked the evolution of humanity. However, within mineral extraction and physical and chemical ore treatment processes, large quantities of waste are generated, such as overburden, waste rock, and tailings, which are disposed of in landfills and dams (Diaby et al., 2007; SERNAGEOMIN, 2018). Although the conditions for protecting residual mineral deposits have evolved, such deposits are not exempt from climatic effects and natural threats. In recent history, many failures have been reported that have led to considerable environmental catastrophes in various parts of the world, such as breaking levees, roadblocks, the release of acid mine drainage, and the discharge of effluents containing heavy metals (Owen et al., 2020).

Treatment of mine tailings has focused on the stabilization of elements that are potentially toxic to the environment and human health due to the presence of heavy metals such as arsenic, copper, lead, and zinc and the acidity generation potential by acid mine drainage (Drahota et al., 2016; Klimkova et al., 2011; Srivastava et al., 2015). In more recent approaches, extraction of valuable elements from tailings has been increasingly considered; however, low recoveries and higher costs are the challenges to address in the next stage (Antonijević et al., 2008; Meschke et al., 2020). There is a growing need to promote circularity in mining processes in order to reduce negative environmental impacts and deal with the continuous decrease in mineral grades. Various initiatives are being carried out for the extraction of elements of economic interest in the field of the recovery of mining waste (Antonijević et al., 2008; Cortés et al., 2020).

Nanoparticle biosynthesis is a relatively new technology that dates back twenty years when the capacity of certain biomasses and extracts to synthesize nanoparticles from dissolved salts was described in the 2000s. For several years, biogenic nanomaterials were obtained through laboratory tests, under controlled conditions, and concentrations of biomass and precursors. In addition, gradually, the focus was including the type of biomass, from microbial cultures to incorporate bio-based materials, such as agro-industrial wastes (Pat-Espadas and Cervantes, 2018; Taghizadeh and Rad-Moghadam, 2018). In recent years, the studies have used a wide variety of biomasses and different secondary solutions as metal precursors, which are obtained mainly from industrial effluents. However, in most of the articles, the metal precursors are based on the usage of synthetic solutions and not on actual industrial solutions.

From the perspective of the biotechnological application of microorganisms or part of them to produce nanoparticles from mining wastes, it emerges the concept of 'Bionanomining' (Wong-Pinto et al., 2020a). Few articles have addressed the challenging issue of treating actual effluents to produce nanoparticles due to solutions are complex, often composed of several ions. Selenium nanoparticles and iron oxide nanoparticles have been obtained from anode slimes and coal tailings, respectively, by using fruit extracts and the bacterium *Rhodococcus opacus* (Maass et al., 2019; Shirmehenji et al., 2020).

This work aims to use mining tailings as a source of copper to synthesize copper nanoparticles (CuNPs) through a biotechnological approach using the non-pathogenic bacteria *Pseudomonas stutzeri* DSM 5190. The focus of this research is supported by the idea that the use of real effluents would give to biosynthesis a better position as part of the comprehensive treatments in mining waste valorization.

2. Materials and methods

For CuNPs biosynthesis, two important procedures were performed: i) hydrometallurgical treatment of tailings ore and ii) culture and recovery of bacterial biomass. Both ways were carried out and complemented by physical and chemical characterization for the tailing, intermediate solutions, initial and final biomass, and for the generated CuNPs.

2.1. Sampling of mine tailings

Samples of chalcopyrite-bearing tailings were collected from an active deposit in a mining operation located in northern Chile. Approximately 1 kg of dried sample was collected and homogenized. Representative subsamples were taken to perform chemical, mineralogical, and granulometry analysis. The samples were milled before being analyzed, obtaining a material size with a D50 of 100 μm. Then, the milled solid was dried in an oven at 105 °C for 2 h. The chemical analysis of the solids was done by X-ray fluorescence (XRF, Siemens SRS3000) and listed in Table 1, whose values are expressed as the average of a duplicated dataset.

At the same time, the mineralogical composition was determined by X-ray diffraction (XRD, Siemens D5000), using the database of the ICDD (International Center for Diffraction Data). Copper is present as chalcopyrite. The main species are shown in Table 2.

The granulometry analysis of tailings was addressed by the advanced optical method of the Focused Beam Reflectance Measurement probe (FBRM, Metler Toledo ParticleTrack G400). This in-line technique provides a particle chord distribution related to the particle size comparable with conventional sizing techniques in the range of 50–400 μm. The solid was resuspended in a beaker with distilled water in a solid:liquid ratio of 1:200, and agitated with a mechanical stirrer at 170 rpm.

2.2. Dissolution and concentration of copper from tailings

In order to extract the copper from the solid matrix, the tailing was treated by curing with sulfuric acid, leached by agitation with the same acid solution, and then concentrated by solvent extraction. The curing was carried out with 120 g/L sulfuric acid solution (H_2SO_4) and 1 g/L sodium nitrate ($NaNO_3$) solution, which acts as an oxidizing agent that enhances the copper dissolution. The solution was stirred for five days, and the obtained pulp was then leached by diluting with 1 L of distilled water and kept under stirring at 250 rpm for three more days. The leaching kinetic curve was constructed by taking 10 mL liquid samples at 1, 5, 10, 40, 60, 1440, 2880, and 4320 min after leaching started. The obtaining solution from leaching was named as Pregnant Leaching Solution (PLS).

Subsequently, to extract the impurities and concentrate the copper content, the oxime-based extractant Acorga PT5050 (Solvay) diluted with Escaid 110 (ExxonMobil Chemicals) was mixed with the PLS in a volume ratio of 1:2 in a decantation funnel. Mixing was vigorously performed, and once the phases were differentiated, the aqueous fraction was eluted. This procedure was repeated fifteen times, using the obtained solution with fresh PLS additions in each cycle. Thus, the obtained organic phase was loaded. After, for the copper stripping, 120 g/L H_2SO_4 solution was used and mixed with the loaded organic phase in a volume ratio of 1:2. In this way, the aqueous fraction containing copper was recovered and named as tailing solution (TS).

Table 1

Chemical composition of main compound species and elements in tailing ore by XRF.

Major elements, %									
SiO_2	Al_2O_3	K_2O	CaO	Na_2O	Fe_2O_3	MgO	TiO_2	P_2O_5	MnO
68.11	16.60	5.29	2.23	1.77	1.74	0.51	0.25	0.09	0.06
Trace elements, mg/kg									
S	Cu	Sr	Zn	Rb	Zr	Pb	V	Cr	Y
4757	3762	473	251	122	117	95	41	47	19

Table 2

Main minerals composing the tailings analyzed by XRD. Copper is in the ore as Chalcopyrite.

Name	Formula	Content, %
Quartz	SiO_2	47.7
Albite	$NaAlSi_3O_8$	19.3
Orthoclase	$KAlSi_3O_8$	17.9
Illite	$K_{0.65}Al_2[Al_{0.65}Si_{3.35}O_{10}](OH)_2$	12.0
Anhydrite	$CaSO_4$	2.6
Chalcopyrite	$CuFeS_2$	0.4

2.3. Preparation of bacterial biomass

The bacterial strain *P. stutzeri* DSM5190 was acquired from the DSMZ catalog (*Deutsche Sammlung von Mikroorganismen und Zellkulturen* Gmbh). The inoculum of *P. stutzeri* was prepared by culturing overnight in nutrient broth (NB, 5 g/L peptone, 3 g/L meat extract, and 5 g/L NaCl) at pH 7, 37 °C, and 150 rpm. A solid culture was also performed in NB agar for macroscopic characterization, for control of contamination, and to keep the culture fresh for subsequent experiments. The grown biomass used for CuNPs biosynthesis was separated from the culture media by centrifugation at 5000 rpm for 5 min. The pellet was after washed with distilled water thrice to eliminate the residual broth.

2.4. Biosynthesis of copper nanoparticles (CuNPs)

For the CuNPs biosynthesis, pure synthetic and actual tailing solutions were used as copper precursors. Both experimental sets were carried out under the same agitation conditions at 150 rpm in a shaker for 48 h at 25 °C.

In the first stage, an adequate initial copper concentration and biomass dosage were determined, for which three initial concentrations of copper sulfate solution ($CuSO_4*5H_2O$) (Merck) were used at 127, 381, and 636 mg/L Cu. In addition, three dosages of fresh *P. stutzeri* biomass were tried: 100, 1050, and 2000 mg in 100 mL of solution. The best biomass dosage for biosynthesis was determined through this experiment by evaluating the weight percentage of CuNPs obtained from SEM-EDS spectra analysis.

Subsequently, in the second approach, 100 mL of TS was used as the copper source. According to results with synthetic solution, the selected condition for biomass dosage was 1050 mg. The copper concentration in TS was 719 mg/L. In this case, the initial concentration of copper was not fixed to avoid subsequent handling of the hydrometallurgical solution. The resulting concentration was close to the optimal concentration recently reported for the biosynthesis of copper oxide nanoparticles from $CuSO_4$ solution (Bukhari et al., 2021).

Because TS comes from acid leaching and solvent extraction, the pH of solution was very low. In order to provide an adequate acidity for biosynthesis, the pH was adjusted to 4 by neutralization with NaOH. In the case of the synthetic solution, no adjustment was required since the pH was naturally around 4.

Once biosynthesis finished, the pellet was separated from the solution by centrifugation at 3500 rpm for 5 min. Both the solid and the liquid fraction were recovered and analyzed to look for the presence of CuNPs attached to the biomass surface or detached from it. The pellet was washed, and the liquid, filtered in a 0.2 μm filter (Merck). Due to being liquid loaded in many soluble species from tailing, crystals began to appear once the liquid sample was dried for microscopic analysis. The salts were separated by precipitation with isopropanol and centrifugation. The crystals obtained were also studied.

2.5. Characterization of CuNPs

The analysis of CuNPs was performed both in the solution and in the pellet fractions. The pellet was analyzed by Fourier transform infrared spectroscopy (FTIR, Thermo Nicolet Avatar 360) and microfluorescence (Bruker M4 Tornado). The supernatant was characterized by XRD (Siemens D5000), field emission scanning electron microscopy (FE-SEM, Hitachi SU5000), and transmission electron microscopy (TEM, Hitachi HT7700). These techniques give complementary information on the chemical and physical state of the biomass and CuNPs.

FTIR was used to evaluate the changes in the functional groups on the biomass surface, particularly in biological molecules such as proteins, lipids, carbohydrates, and nucleic acids. The scanned range of infrared spectra was between 400 and 4000 cm^{-1}, and the vibrational analysis was performed on *P. stutzeri* biomass before and after biosynthesis.

Microfluorescence analysis detected the metal composition and distribution on the solid sample, and it was used to screen the samples with copper on the biomass surface. Once biosynthesis was performed, the washed *P. stutzeri* biomass was studied with a maximum excitation voltage of 20–50 kV.

The solution obtained after biosynthesis was dried and analyzed by XRD to determine copper and impurities. The ICDD (International Center for Diffraction Data) database was used. Crystals removed from the solution were analyzed by XRD, microfluorescence, and SEM-EDS.

Both the pellet and the biosynthesis solution were observed in FE-SEM coupled with an elemental dispersive energy analyzer (EDS, Hitachi SU5000) with a resolution of up to 1 nm. The biomass was covered with a thin gold film before microscopy. FE-SEM-EDS determined the sizes, shapes, and elemental composition of the CuNPs.

A TEM with a full-throttle voltage of 120 kV was used to observe the distribution, shapes, and sizes of CuNPs. The samples were prepared by suspending in a microtube with ethanol and sonicating for 10 min. Ten microliters of suspension were placed on the TEM grid until dry. The composition of the CuNPs was determined by XRD (Siemens D5000).

3. Results and discussion

3.1. Tailing ore characterization

It was observed through the XRF analysis that the tailing ore contained mainly silica, alumina, potassium, calcium, and sodium, while copper content is 3.8 g/kg (Table 1). Other elements present in the waste are rubidium, vanadium, and zinc, which are relatively high and may open the eventual processing for other valorization approaches. Copper is as chalcopyrite, a refractory sulfide mineral for leaching; for this reason, nitrate was used as an oxidizing agent in the curing stage. The other species composing tailing are rock-forming minerals such as

quartz, albite, and orthoclase, while the alteration phase is represented by illite and anhydrite (Table 2).

Fig. 1 shows the granulometry analysis curve obtained by the FBRM probe, where D50 corresponds to 100 μm, indicating that the particle size is fine, and that the tailings are suitable to be leached by agitation. An adequate chemical and mineralogical characterization of the tailings helped to carry out an optimal leaching and copper recovery.

3.2. Hydrometallurgical processing of tailings

Considering the mineralogical and granulometric characteristics of the tailings, leaching was preceded by a curing stage to increase the oxidation of sulfide species. This pretreatment was critical for copper dissolution because almost 50% of the recovered copper was obtained after the acid attack during curing. The subsequent leaching for 72 h reached about 60% of recovery; however, after 24 h, the level was slightly greater than 50% (Fig. 2). Taking into account that chalcopyrite shows a low leaching kinetic at environmental conditions, the values obtained in this work were considered high, mainly because of the use of nitrate salt as an oxidizing agent and the concentration of acid.

In order to increase the copper concentration in the PLS solution, five cycles of leaching were performed. The dissolution agent was always the same, and the cured mineral was changed and refreshed in each cycle. The copper concentration increased about 100 mg/L in every cycle, reaching at the end of the fifth cycle, 450 mg/L. The use of solvent extraction rose even more, the copper levels, up to 700 mg/L. As a result of the intensive leaching and SX-concentrating, the acidity of the media was extremely low. The pH was adjusted to pH 4, adding NaOH, similar to the pH of the $CuSO_4$ solution, in order to avoid the degradation effect of the solution acidity on the biomass components during biosynthesis. Simultaneously, a fraction of the iron content was removed as iron hydroxide.

3.3. Biosynthesis of CuNPs

The condition that exhibited the highest biosynthesis of CuNPs for the synthetic solution was 1050 mg of biomass and an initial copper concentration between 381 and 636 mg/L. As observed in Table 3, the concentration of copper and the biomass dosage have optimal values that would be within the highest tested ranges, due to the nanoparticle formation notoriously increased at the first intervals, but in the second ones, the values decreased.

In the subsequent experiments of biosynthesis with TS, the previously obtained optimum biomass dosage was replicated. However, the copper concentration reached from the SX, i.e., 700 mg/L, was not
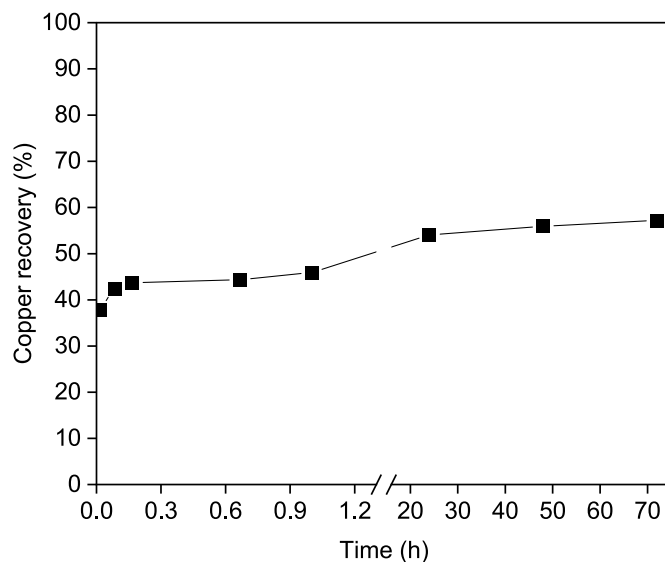
Fig. 2. Leaching kinetics of copper in a first cycle.

Table 3
Effect of biomass dosage and initial copper concentration on the CuNPs biosynthesis in synthetic solution.

Biomass dosage, mg	Copper concentration, mg/L		
	127	381	636
	Copper nanoparticles content (%)		
100	0.33	7.31	5.66
1050	3.02	26.99	22.35
2000	2.95	15.02	11.03

modified, considering that the level was close to the concentration limit evaluated and the preference to do not dilute the solution that was intensively concentrated.

The first observation that demonstrated bioreduction occurred was the color change of TS, which varied from a yellowish to a bluish coloration. Other authors have also made this observation that corresponds to the plasmon effect, where the collective oscillation of conductive electrons on the surface of the metal is excited by the electromagnetic field of light (Patel et al., 2016; Savchuk et al., 2019; Varshney et al., 2010).

In this work, the synthesis of copper nanoparticles was carried out using tailing leaching solutions and the *Pseudomonas stutzeri* biomass. Other bacteria have been reported for the biosynthesis of CuNPs or Cu oxides NPs, such as *Serratia* sp., *Escherichia coli*, and *Shewanella loihica* (Lv et al., 2018; Saif Hasan et al., 2008); however, all of them used pure solutions and ideal conditions of temperature and pressure for the biosynthesis. A few articles have addressed the biosynthesis of copper nanoparticles from industrial wastes (Salvadori et al., 2014; Schaffie and Hosseini, 2014; Varshney et al., 2011).

Nanoparticle biosynthesis is mediated by biomass, but these can be retained or in direct interaction with biomass or free in the process. Characterization was carried out for both the biomass and the solution to determine the retained or the free fraction.

3.4. Characterizations of CuNPs in biomass

Biosynthesis is a process that results from biosorption mechanisms, where the structural and chemical properties of the biomass surface change to uptake copper ions and give them electrons for their reduction. The cell wall of Gram-negative bacteria is complex and consists of two membrane layers (the outer and plasma membranes) that are
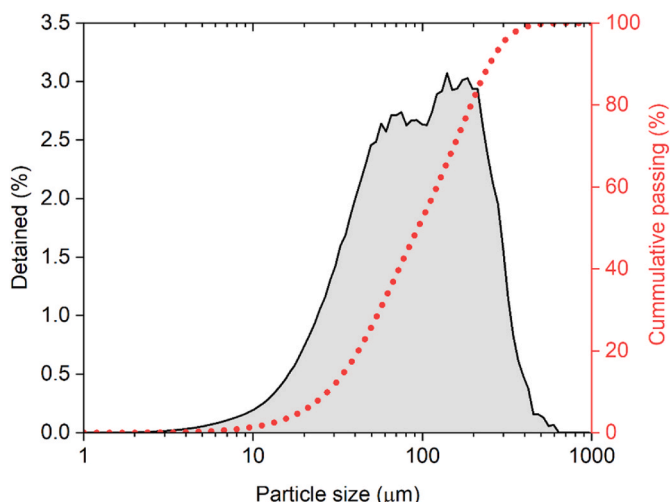
Fig. 1. Granulometry analysis of tailing by the in-line technique FBRM probe.

chemically and functionally different from each other and separated by a thin layer of peptidoglycan (Lalucat et al., 2006). The outer membrane and the peptidoglycan layer constitute the cell wall of this type of bacteria, which is negatively charged. The cell walls are formed mainly by lipopolysaccharides, phospholipids, proteins, lipoproteins, and peptidoglycan (Naik et al., 2002; Schleifer, 2009). In *P stutzeri*, as a Gram-negative bacterium, the interaction of the cell with copper would occur in the periplasmic region and the in the outer membrane.

FTIR spectra of biomass before and after biosynthesis are shown in Fig. 3 and give a qualitative analysis of those functional groups of the cell wall that may be related to the copper bioreduction. The absorption bands for the untreated *P. stutzeri* biomass revealed the presence of hydroxyl, carboxyl, and amino groups, which are related to polysaccharides, (glyco)proteins, and peptidoglycan. A broad absorption zone can be recognized at 3200–3400 cm^{-1} corresponding to the stretching vibration of the group O–H, at 1650 cm^{-1} associated with carbonyl residues of amide bond I (Baker et al., 2015). The absorption band at 1540 cm^{-1} corresponds to the bending of N–H and the stretching of C–N, both related to amide II bonds (Wanarska and Maliszewska, 2019). The absorption band at 1408 cm^{-1} is related to the symmetric stretching of C–O of phosphate group. The range between 1028 and 1400 cm^{-1} is identified as stretching of P=O in the phosphate group.

Changes in the O–H, C–O, and P=O groups demonstrate that cell wall constituents and free biopolymers forming exopolymers may be implied in the copper reduction during CuNPs biosynthesis by *P. stutzeri*, supporting the basis that CuNPs biogenesis occurred extracellularly (Z. Li et al., 2020b). FTIR spectrum for the post-treatment biomass shows that changes are in the intensity of bands, and three were the most noticeable. The absorption bands at 1650, 1408, and 1190 cm^{-1}. The overall decrease of peak intensity suggests that functional groups' presence was affected by the interaction between copper and biomass.

Microfluorescence analysis in *P. stutzeri* biomass showed that after biosynthesis, peaks for copper appeared. A semi-quantitative microfluorescence analysis indicated a copper weight percentage of 2.83%. Both Microfluorescence and FTIR suggest a preferential interaction of the biomass envelope with the tailing solution.

SEM-EDS analysis of biomass treated with TS showed that CuNPs were formed and dispersed in the surroundings and on the bacteria surface with a homogeneous spherical shape. The CuNPs sizes were preliminary determined and valued between 5 and 80 nm. EDS profile indicated 2.6% of copper and 19.1% of oxygen. Potassium may come from media components and other biomolecules produced by biomass,
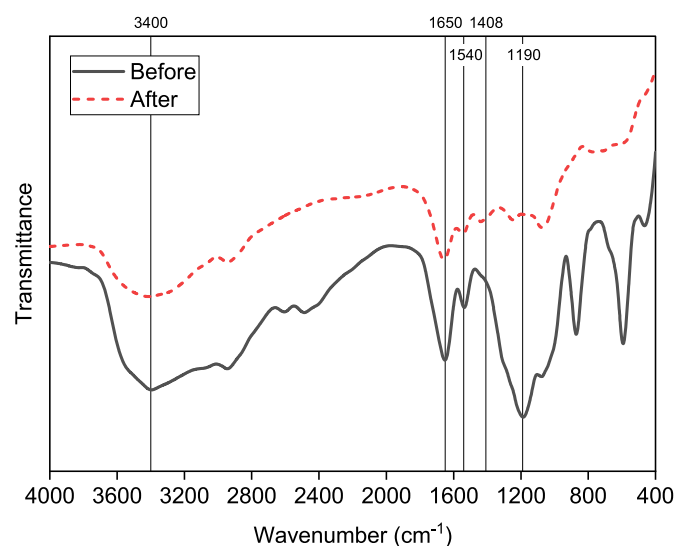
and gold corresponded to the coating for SEM visualization (Fig. 4).

The definitive sizes of CuNPs were determined by TEM analysis, which is shown in Fig. 5. After 48 h of biosynthesis with TS, CuNPs were deposited in the exopolysaccharides of *P. stutzeri*, and sizes between 1 and 5 nm with an average size of 1–2 nm. The sizes were smaller than those observed by SEM images, maybe due to clustering that made a differentiation between grouped nanoparticles difficult. The shape was confirmed as spheres. XRD analysis of the biomass demonstrated the biogenesis of native copper, since the peak positions of the diffractograms coincide with metallic copper at 43.6°, 50.7°, and 74.45°, which are equivalent to the crystalline planes of metallic copper of the FCC structure (111), (200) and (220) (Bogdanović et al., 2014).

The distribution of the CuNPs diameters has also been observed by other researchers with a normal distribution (Fabrega et al., 2009); however, in this work, a skewed distribution to the left (smaller particles) was recognized, which may be due to the low concentrations of Cu^{2+} ions in the TS.

3.5. Characterizations of CuNPs in biosynthesized solution

It was observed that CuNPs were present both in the biomass and in the liquid fraction. This solution contains many dissolved species that some precipitated when the solution was dried for microscopy analysis. The obtained greenish-yellow crystals were analyzed by microfluorescence, detecting the presence of Na and S, which indicates that salts are mainly sodium sulfate, the derivative salt from the sulfuric acid used in the hydrometallurgical processing of tailing. Cu was also detected in a lower proportion, about 0.24% (Fig. 6).

SEM analysis of the solution treated with isopropanol to remove salts detected that finer crystals remained in the sample, with sizes of 1 μm. CuNPs were found among sulfate salts in clusters of sizes between 45 and 250 nm, with an average size of 150 nm. Polydisperse and agglomerated nanoparticles were obtained, probably due to the presence of sulfate salts that may act as binding agents (Mulfinger et al., 2007). The elemental composition of the synthesized nanoparticles was detected by the EDS spectrum (Fig. 7). The strong peak of EDS at 1 keV and the signal at 8 keV reveal the presence of Cu. On the other side, the presence of sulfate salts was strongly endorsed by the peak of S at 2.4 keV (Nikonow and Rammlmair, 2016). The weight fraction detected for copper by microfluorescence, 0.24%, was ratified by the quantification of EDS, 0.3%.

The dark field TEM image strongly differentiates between copper nanoparticles and sodium sulfate salts. CuNPs were spherical, with size ranges from less than 1 nm to above 5 nm. Particle size distribution revealed that 70% of CuNPs have very small diameters (less than 1 nm), which is explained that this sample originally came from the liquid fraction after biosynthesis. XRD analysis indicated the formation of sodium sulfate as salt and as thenardite, and metallic copper (Fig. 8).
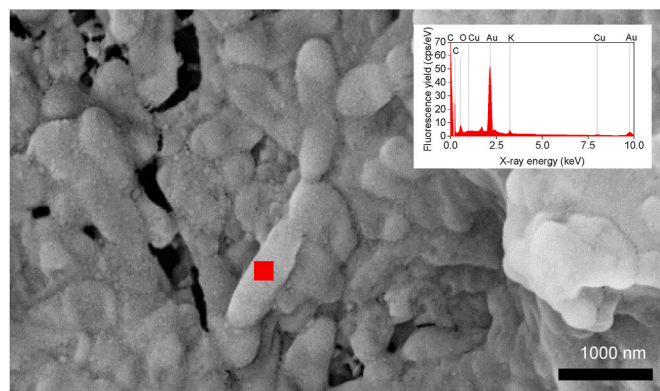

Fig. 3. Characterization of the biomass surface by FTIR before and after biosynthesis with TS.

Fig. 4. SEM image of *P. stutzeri* biomass after biosynthesis of CuNPs with TS. It is observed that nanoparticles are dispersed over the cells.
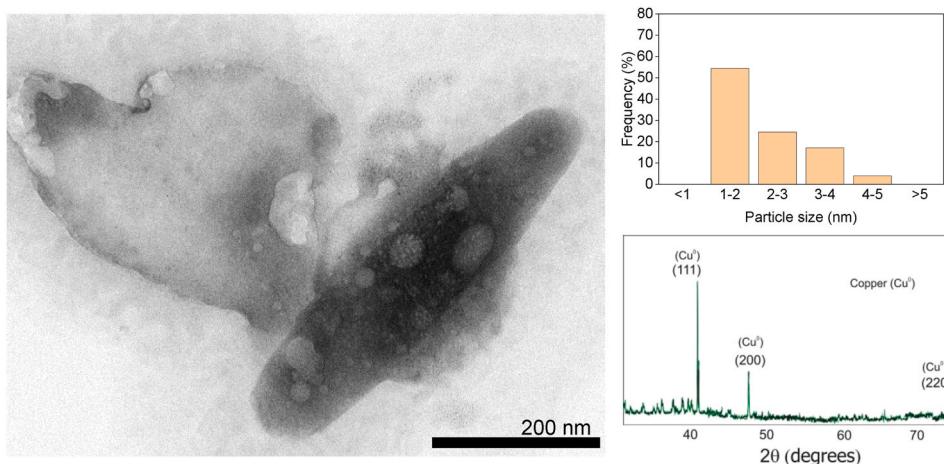
Fig. 5. TEM image of CuNPs in the *P. stutzeri* biomass with the particle size distribution of nanoparticles.
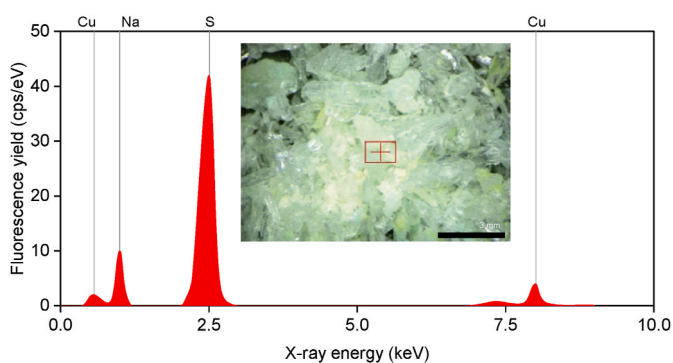

Fig. 6. Microfluorescence analysis of crystalline fraction obtained from dried solution after biosynthesis with TS.

SEM-EDS analysis overestimates the sizes of CuNPs, due to nanoparticles were grouped, and the used resolution could not distinguish free units (Table 4). Although in the literature, the natural capacity of biomolecules to protect nanoparticles from clustering is mentioned as an advantage of biosynthesis, in this work, it was not observed. The use of real solutions as metal precursors would lead to the degradation of the protecting biomolecules, so it would be necessary for this type of protocol to add capping agents. These agents should be from natural and innocuous origin, in line with the biotechnological approach of biosynthesis, such as the bovine serum albumin.

More CuNPs were obtained in the biomass than in the solution, which indicates that the biosynthesis is preferably carried out under the direct interaction between the copper ions and the functional groups of the biomass. The CuNPs released would be a consequence of the separation processes after biosynthesis and not of a bioreduction produced by biopolymers released or generated by biomass during biosynthesis.

In general, the biosynthesis produced small CuNPs that as range was not different for biomass and solution fractions, even though those obtained in the solution had a noticeable smaller average size. On the other hand, more nanoparticles were observed in the solution since the formation of sodium sulfate crystals would produce a capture of CuNPs, acting as a binding agent.

Finally, in this work, fundamental results are provided that have great potential for projection, based on two fields: i) studying the biosynthesis of other metal nanoparticles by using the same approach, and ii) use of the salts precipitated in the liquid fraction after biosynthesis as raw material to produce molten salts for energy storage; the CuNPs content may improve the thermal properties, being an attractive alternative for the traditional sodium sulfate used (Wong-Pinto et al.,
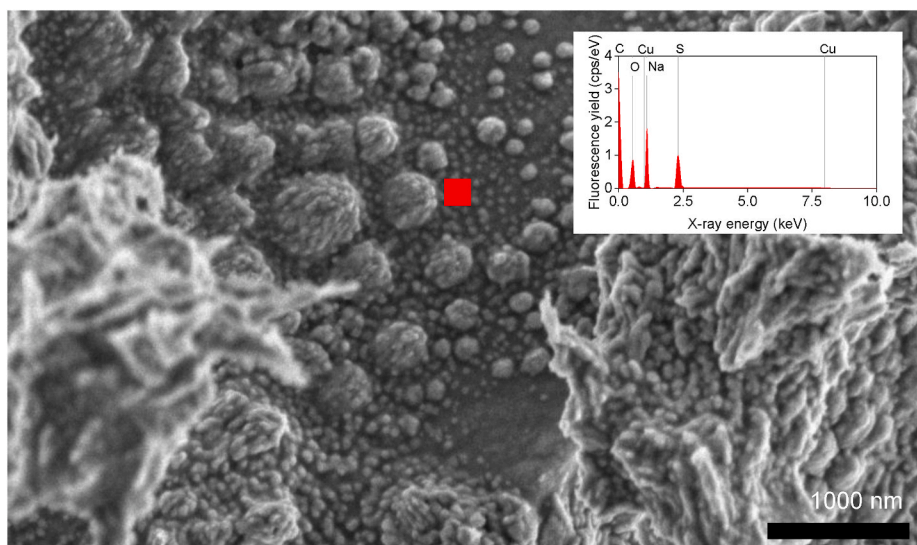

Fig. 7. SEM image of isopropanol-treated solution after biosynthesis with TS. CuNPs and sodium sulfate salts were identified.
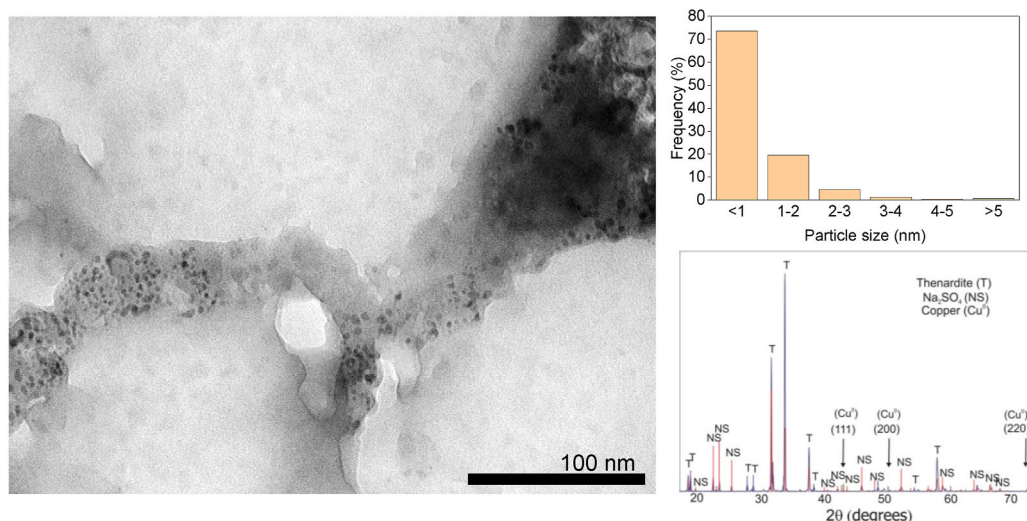
Fig. 8. TEM image of isopropanol-treated solution after biosynthesis with TS. CuNPs are distributed throughout the sodium sulfate saline matrix.

Table 4

Characteristics of CuNPs biosynthesized by *P. stutzeri* using TS as copper precursor. The Sizing techniques were used for the particle size range, and the average particle size. Quantification techniques calculate Cu composition (%).

Fraction	Particle size range (nm)	Average particle size (nm)	Sizing technique	Cu composition (%)	Quantification technique
Biomass	5–80		SEM	2.83	µFRX
	1–4	1–2	TEM	2.6	EDS
Solution	42–250		SEM	0.24	µFRX
	1–5	<1	TEM	0.3	EDS

2020b).

3.6. Perspectives

The findings obtained in this work can be analyzed from different perspectives, which deserve attention to evaluate the real implications of its application and discuss cleaner processes in mining effluent treatment operations.

As the first article on the recovery of metallic nanoparticles from real mining effluents, it is positive to verify the feasibility of using biomass to recover valuables from mining tailings. Nevertheless, research on more effective pretreatment techniques and optimizing the bioreduction process during the biomass-solution interaction are fields of development that may arise from this research whose approach has not been described previously.

Biosynthesis with tailing solution obtained significantly fewer CuNPs than with the synthetic solution due to the greater competition between the ions for the functional groups of the biomass. On the other hand, the concentration of CuNPs obtained in the biomass, 2.7%, is a concentration that enables the application of this material as an antibacterial agent since various authors in this field have demonstrated the effectivity of CuNPs in concentrations between 8 and 150 mg/L, which are less than the fraction obtained in this work attached to biomass (Bogdanović et al., 2014; P. Li et al., 2020a; Velsankar et al., 2020)

It is important to mention that, from a biosafety approach, the proposed process is safe, because the bacterium *Pseudomonas stutzeri* is a species originally isolated from mining environments, which is not pathogenic and, that under the conditions of low pH and high metal concentration in solution, it is expected that biomass is stabilized as a result of its heterotrophic and non-acidophilic metabolism (Lalucat et al., 2006).

Under a technical view of the proposed process, we observe that a rapid leaching was performed, but with high acid consumption due to the mineralogical nature of the tailings; recoveries close to 50% are considered good. During solvent extraction, it was used an organic solution whose use in mining is widely extended. The ability of commercial solvents to be recycled once the metal has been released makes it possible to reduce the amount of waste at this stage. On the other hand, the acidic aqueous solutions obtained are linked to the leaching stage, where a low pH is necessary for the dissolution of the mineral species (Fig. 9).

The CuNPs biosynthesis is understood as a subsequent stage to the hydrometallurgical treatment of tailings. The acid solutions generated in the solvent extraction can be recirculated to dissolve metallic species present in new solid matrices. Water recovery rates are high in copper mineral processes, as in the Chilean mining context, given the abundant operations in desert areas.

Biosynthesis does not generate dangerous solutions for the environment compared to traditional bottom-up methods, which employ toxic and corrosive reagents such as hydrazine hydrate and sodium borohydride used as reducing agents (Srivastava et al., 2021; Sudhasree et al., 2014; Wong-Pinto et al., 2020a). In a circular economy context, the proposed biosynthesis process is part of the terminal value retention strategy, 'Re-mine', since it consists of recovering valuable species from already discarded waste (Reike et al., 2018). In the future, the alternative of integrating this methodology before tailings collection can be evaluated, which could be even more beneficial from an energy and environmental point of view since some solvent use could be significantly reduced.

After biosynthesis and drying solution (evaporation), two products were obtained: partially degraded biomass with nanoparticles (2.7%) and Na_2SO_4 salts with nanoparticles (0.27%). Although the scope of this work was to obtain streams with reduced copper, it should be noted that, from the point of view of the application, the products are intermediate and would require a subsequent separation stage. According to different authors, the separation of CuNPs from biomasses can be performed by using washing-vacuum filtration, density gradient centrifugation, sonication, cationic and anionic detergents, among others, which are clean and relatively cheap methods (Kumar et al., 2008b; Salvadori et al., 2015).

As mentioned before, due to its copper content biomass-CuNPs can give rise to antimicrobial additives. On the other side, Na_2SO_4-CuNPs is a salt whose presence of nanoparticles improves its thermal conductivity, which can be used as a salt for storing energy in phase change materials at high temperature (Abdollahzadeh Jamalabadi, 2021).
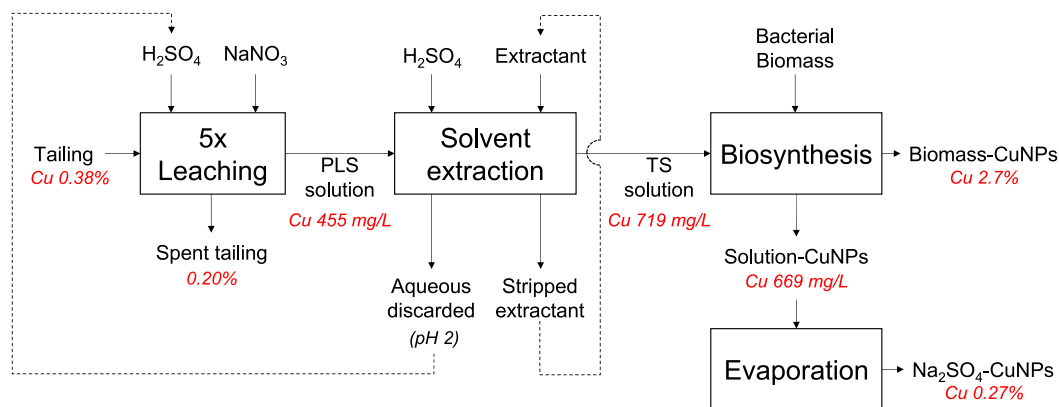
Fig. 9. Diagram of the experimental procedure used in this work for the CuNPs biosynthesis by *P. stutzeri* biomass from tailing ore. In addition, a balance of copper is shown in each step.

From an economic perspective, two main factors that determine the feasibility of a nanoparticle biosynthesis operation are operating costs and the price of NPs. According to preliminary observations made on the proposed methodology, the cost of bioproduction of CuNPs, in terms of supplies and energy on a laboratory scale, was approximately 60 US$/g. Although it seems high, considering that CuNPs have commercial prices from 1.1 to 5 US$/g, depending on the purity, particle size, and presentation, the specific costs would decrease as the process is scaled up to superior dimensions. On the other hand, the costs associated with the pretreatment of the tailings concentrate almost 70% of the total cost.

There is no information available on the specific costs of traditional synthesis at the laboratory level that allows comparison under similar scales. However, based on the particular analysis, energy consumption in biosynthesis weighted approximately 20% of the total cost, which is expected to be higher for traditional bottom-up techniques, since carrying out the process under ambient conditions is a relevant factor in controlling operating expenses.

Given the experimental approach of this work, the biosynthesis process was mainly addressed from the technical validation; however, a more in-depth consideration of the economic elements would allow this type of mining waste treatment technology to be made viable.

4. Conclusions

This work addressed two important challenges: the leaching of sulfide mining tailings with a high level of refractoriness and low-grade ore and the biosynthesis and characterization of copper nanoparticles. At room temperature, the leaching of chalcopyrite-bearing tailing was possible using an oxidizing agent, reaching a recovery of about 50%. Copper nanoparticles from mine waste were obtained using bacterial biomass of *P. stutzeri*, which were spheres with an average size of about 1–2 nm.

The observed biosynthesis mechanism corresponds to an extracellular pathway, where exopolysaccharides had an essential role in the prior biosorption and subsequent reduction of copper ions to metallic nanoparticles. In this sense, the chemical groups O–H, C–O, and P=O, present in the most abundant biomolecules of the cell wall, are associated with the initial interaction metal-biomass.

Bionanomining may turn out to be a cutting-edge tool to produce copper or even other metal nanoparticles obtained from mine waste since generating valuable products cheaper, faster, and under more environmentally friendly methods than traditional techniques. From this experimental study, it is feasible to project the application of harmless bacterial biomasses to produce nanomaterials in a small scale, which should be complemented in the future with economic evaluations.

References

Abdollahzadeh Jamalabadi, M.Y., 2021. Use of nanoparticle enhanced phase change material for cooling of surface acoustic wave sensor. Fluid 6, 31. https://doi.org/10.3390/fluids6010031.

Ali, J., Ali, N., Wang, L., Waseem, H., Pan, G., 2019. Revisiting the mechanistic pathways for bacterial mediated synthesis of noble metal nanoparticles. J. Microbiol. Methods 159, 18–25. https://doi.org/10.1016/j.mimet.2019.02.010.

Anbia, M., Amirmahmoodi, S., 2016. Removal of Hg (II) and Mn (II) from aqueous solution using nanoporous carbon impregnated with surfactants. Arabian Journal of Chemistry 9, S319–S325. https://doi.org/10.1016/j.arabjc.2011.04.004.

Antonijević, M.M., Dimitrijević, M.D., Stevanović, Z.O., Serbula, S.M., Bogdanovic, G.D., 2008. Investigation of the possibility of copper recovery from the flotation tailings by acid leaching. J. Hazard Mater. 158, 23–34. https://doi.org/10.1016/j.jhazmat.2008.01.063.

Baker, S., Mohan Kumar, K., Santosh, P., Rakshith, D., Satish, S., 2015. Extracellular synthesis of silver nanoparticles by novel Pseudomonas veronii AS41G inhabiting *Annona squamosa* L. and their bactericidal activity. Spectrochim. Acta Mol. Biomol. Spectrosc. 136, 1434–1440. https://doi.org/10.1016/j.saa.2014.10.033.

Bogdanović, U., Lazić, V., Vodnik, V., Budimir, M., Marković, Z., Dimitrijević, S., 2014. Copper nanoparticles with high antimicrobial activity. Mater. Lett. 128, 75–78. https://doi.org/10.1016/j.matlet.2014.04.106.

Bukhari, S.I., Hamed, M.M., Al-Agamy, M.H., Gazwi, H.S.S., Radwan, H.H., Youssif, A. M., 2021. Biosynthesis of copper oxide nanoparticles using *streptomyces MHM38* and its biological applications. J. Nanomater. 2021, 1–16. https://doi.org/10.1155/2021/6693302.

Chen, M., He, Y., Ye, Q., Zhang, Z., Hu, Y., 2019. Solar thermal conversion and thermal energy storage of CuO/Paraffin phase change composites. Int. J. Heat Mass Tran. 130, 1133–1140. https://doi.org/10.1016/j.ijheatmasstransfer.2018.11.026.

Cortés, S., Soto, E.E., Ordóñez, J.I., 2020. Recovery of copper from leached tailing solutions by biosorption. Minerals 10, 158. https://doi.org/10.3390/min10020158.

David, S.A., Veeraputhiran, V., Vedhi, C., 2019. Biosynthesis of cobalt oxide nanoparticles - a short review. J. Nanosci. Technol 5, 734–737. https://doi.org/10.30799/jnst.s01.19050308.

Diaby, N., Dold, B., Pfeifer, H.-R., Holliger, C., Johnson, D.B., Hallberg, K.B., 2007. Microbial communities in a porphyry copper tailings impoundment and their impact on the geochemical dynamics of the mine waste. Environ. Microbiol. 9, 298–307. https://doi.org/10.1111/j.1462-2920.2006.01138.x.

Drahota, P., Knappová, M., Kindlová, H., Culka, A., Majzlan, J., Mihaljevič, M., Rohovec, J., Veselovský, F., Fridrichová, M., Jehlička, J., 2016. Mobility and attenuation of arsenic in sulfide-rich mining wastes from the Czech Republic. Sci. Total Environ. 557 (558), 192–203. https://doi.org/10.1016/j.scitotenv.2016.03.079.

Fabrega, J., Fawcett, S.R., Renshaw, J.C., Lead, J.R., 2009. Silver nanoparticle impact on bacterial growth: effect of pH, concentration, and organic matter. Environ. Sci. Technol. 43, 7285–7290. https://doi.org/10.1021/es803259g.

Fashandi, M., Leung, S.N., 2018. Sodium acetate trihydrate-chitin nanowhisker nanocomposites with enhanced phase change performance for thermal energy storage. Sol. Energy Mater. Sol. Cell. 178, 259–265. https://doi.org/10.1016/j.solmat.2018.01.037.

Gahlawat, G., Choudhury, A.R., 2019. A review on the biosynthesis of metal and metal salt nanoparticles by microbes. RSC Adv. 9, 12944–12967. https://doi.org/10.1039/C8RA10483B.

Gogoi, M., Boruah, P., Sengupta, P., Saikia, L., 2019. Separation of ultrafine chalcogenide particles using Fe3O4 magnetic nanoparticles and ligands with metal selectivity. Miner. Eng. 137, 147–156. https://doi.org/10.1016/j.mineng.2019.04.004.

Klimkova, S., Cernik, M., Lacinova, L., Filip, J., Jancik, D., Zboril, R., 2011. Zero-valent iron nanoparticles in treatment of acid mine water from in situ uranium leaching. Chemosphere 82, 1178–1184. https://doi.org/10.1016/j.chemosphere.2010.11.075.

Kumar, A., Vemula, P.K., Ajayan, P.M., John, G., 2008a. Silver-nanoparticle-embedded antimicrobial paints based on vegetable oil. Nat. Mater. 7, 236–241. https://doi.org/10.1038/nmat2099.

Kumar, S.A., Peter, Y.-A., Nadeau, J.L., 2008b. Facile biosynthesis, separation and conjugation of gold nanoparticles to doxorubicin. Nanotechnology 19, 495101. https://doi.org/10.1088/0957-4484/19/49/495101.

Lalucat, J., Bennasar, A., Bosch, R., Garcia-Valdes, E., Palleroni, N.J., 2006. Biology of *Pseudomonas stutzeri*. Microbiol. Mol. Biol. Rev. 70, 510–547. https://doi.org/10.1128/MMBR.00047-05.

Li, P., Liang, J., Su, D., Huang, Y., Pan, J., Peng, M., Li, G., Shan, Y., 2020a. Green and efficient biosynthesis of pectin-based copper nanoparticles and their antimicrobial activities. Bioproc. Biosyst. Eng. 43, 2017–2026. https://doi.org/10.1007/s00449-020-02390-w.

Li, Z., Lin, L., Liu, X., Wan, C., Lee, D.-J., 2020b. Understanding the role of extracellular polymeric substances in the rheological properties of aerobic granular sludge. Sci. Total Environ. 705, 135948. https://doi.org/10.1016/j.scitotenv.2019.135948.

Liu, X., Wang, D., Li, Y., 2012. Synthesis and catalytic properties of bimetallic nanomaterials with various architectures. Nano Today 7, 448–466. https://doi.org/10.1016/j.nantod.2012.08.003.

Lv, Q., Zhang, B., Xing, X., Zhao, Y., Cai, R., Wang, W., Gu, Q., 2018. Biosynthesis of copper nanoparticles using *Shewanella loihica* PV-4 with antibacterial activity: novel approach and mechanisms investigation. J. Hazard Mater. 347, 141–149. https://doi.org/10.1016/j.jhazmat.2017.12.070.

Maass, D., de Medeiros Machado, M., Rovaris, B.C., Bernardin, A.M., de Oliveira, D., Hotza, D., 2019. Biomining of iron-containing nanoparticles from coal tailings. Appl. Microbiol. Biotechnol. 103, 7231–7240. https://doi.org/10.1007/s00253-019-10001-2.

Meschke, K., Hofmann, R., Haseneder, R., Repke, J.U., 2020. Membrane treatment of leached mining waste – a potential process chain for the separation of the strategic elements germanium and rhenium. Chem. Eng. J. 380, 122476. https://doi.org/10.1016/j.cej.2019.122476.

Mulfinger, L., Solomon, S.D., Bahadory, M., Jeyarajasingam, A.V., Rutkowsky, S.A., Boritz, C., 2007. Synthesis and study of silver nanoparticles. J. Chem. Educ. 84, 322. https://doi.org/10.1021/ed084p322.

Naik, R.R., Stringer, S.J., Agarwal, G., Jones, S.E., Stone, M.O., 2002. Biomimetic synthesis and patterning of silver nanoparticles. Nat. Mater. 1, 169–172. https://doi.org/10.1038/nmat758.

Nikonow, W., Rammlmair, D., 2016. Risk and benefit of diffraction in Energy Dispersive X-ray fluorescence mapping. Spectrochim. Acta B Atom Spectrosc. 125, 120–126. https://doi.org/10.1016/j.sab.2016.09.018.

Owen, J.R., Kemp, D., Lèbre, É., Svobodova, K., Pérez Murillo, G., 2020. Catastrophic tailings dam failures and disaster risk disclosure. Int. J.Disaster Risk .Reduct. 42, 101361. https://doi.org/10.1016/j.ijdrr.2019.101361.

Pat-Espadas, A.M., Cervantes, F.J., 2018. Microbial recovery of metallic nanoparticles from industrial wastes and their environmental applications. J. Chem. Technol. Biotechnol. 93, 3091–3112. https://doi.org/10.1002/jctb.5681.

Patel, B.H., Channiwala, M.Z., Chaudhari, S.B., Mandot, A.A., 2016. Biosynthesis of copper nanoparticles; its characterization and efficacy against human pathogenic bacterium. J Environ Chem Eng 4, 2163–2169. https://doi.org/10.1016/j.jece.2016.03.046.

Patil, Y.M., Rajpathak, S.N., Deobagkar, D.D., 2019. Characterization and DNA methylation modulatory activity of gold nanoparticles synthesized by Pseudoalteromonas strain. J. Biosci. 44, 15. https://doi.org/10.1007/s12038-018-9842-6.

Reike, D., Vermeulen, W.J.V., Witjes, S., 2018. The circular economy: new or refurbished as CE 3.0? — exploring controversies in the conceptualization of the circular economy through a focus on history and resource value retention options. Resour. Conserv. Recycl. 135, 246–264. https://doi.org/10.1016/j.resconrec.2017.08.027.

Rubilar, O., Rai, M., Tortella, G., Diez, M.C., Seabra, A.B., Durán, N., 2013. Biogenic nanoparticles: copper, copper oxides, copper sulphides, complex copper nanostructures and their applications. Biotechnol. Lett. 35, 1365–1375. https://doi.org/10.1007/s10529-013-1239-x.

Saif Hasan, S., Singh, S., Parikh, R.Y., Dharne, M.S., Patole, M.S., Prasad, B.L.V., Shouche, Y.S., 2008. Bacterial synthesis of copper/copper oxide nanoparticles. J. Nanosci. Nanotechnol. 8, 3191–3196. https://doi.org/10.1166/jnn.2008.095.

Salvadori, M.R., Ando, R.A., Oller Do Nascimento, C.A., Corrêa, B., 2015. Extra and intracellular synthesis of nickel oxide nanoparticles mediated by dead fungal biomass. PloS One 10, e0129799. https://doi.org/10.1371/journal.pone.0129799.

Salvadori, M.R., Ando, R.A., Oller Do Nascimento, C.A., Corrêa, B., 2014. Intracellular biosynthesis and removal of copper nanoparticles by dead biomass of yeast isolated from the wastewater of a mine in the Brazilian Amazonia. PloS One 9, 1–9. https://doi.org/10.1371/journal.pone.0087968.

Sanchez, F., Sobolev, K., 2010. Nanotechnology in concrete – a review. Construct. Build. Mater. 24, 2060–2071. https://doi.org/10.1016/j.conbuildmat.2010.03.014.

Savchuk, V.V., Gamernyk, R.V., Virt, I.S., Malynych, S.Z., Pinchuk, A.O., 2019. Plasmon-exciton coupling in nanostructured metal-semiconductor composite films. AIP Adv. 9, 45021. https://doi.org/10.1063/1.5090900.

Schaffie, M., Hosseini, M.R., 2014. Biological process for synthesis of semiconductor copper sulfide nanoparticle from mine wastewaters. J Environ Chem Eng 2, 386–391. https://doi.org/10.1016/j.jece.2014.01.006.

Schleifer, K.H., 2009. Classification of bacteria and archaea: past, present and future. Syst. Appl. Microbiol. 32, 533–542. https://doi.org/10.1016/j.syapm.2009.09.002.

SERNAGEOMIN, 2018. Catastro de depósitos de relaves en Chile (Santiago, Chile).

Shankar, P.D., Shobana, S., Karuppusamy, I., Pugazhendhi, A., Ramkumar, V.S., Arvindnarayan, S., Kumar, G., 2016. A review on the biosynthesis of metallic nanoparticles (gold and silver) using bio-components of microalgae: formation mechanism and applications. Enzym. Microb. Technol. 95, 28–44. https://doi.org/10.1016/j.enzmictec.2016.10.015.

Shirmehenji, R., Javanshir, S., Honarmand, M., 2020. A green approach to the bio-based synthesis of selenium nanoparticles from mining waste. J. Cluster Sci. https://doi.org/10.1007/s10876-020-01892-7.

Srivastava, S., Agrawal, S.B., Mondal, M.K., 2015. A review on progress of heavy metal removal using adsorbents of microbial and plant origin. Environ. Sci. Pollut. Control Ser. 22, 15386–15415. https://doi.org/10.1007/s11356-015-5278-9.

Srivastava, S., Usmani, Z., Atanasov, A.G., Singh, V.K., Singh, N.P., Abdel-Azeem, A.M., Prasad, R., Gupta, G., Sharma, M., Bhargava, A., 2021. Biological nanofactories: using living forms for metal nanoparticle synthesis. Mini Rev. Med. Chem. 21, 245–265. https://doi.org/10.2174/1389557520999201116163012.

Sudhasree, S., Shakila Banu, A., Brindha, P., Kurian, G.A., 2014. Synthesis of nickel nanoparticles by chemical and green route and their comparison in respect to biological effect and toxicity. Toxicol. Environ. Chem. 96, 743–754. https://doi.org/10.1080/02772248.2014.923148.

Taghizadeh, A., Rad-Moghadam, K., 2018. Green fabrication of Cu/pistachio shell nanocomposite using Pistacia Vera L. hull: an efficient catalyst for expedient reduction of 4-nitrophenol and organic dyes. J. Clean. Prod. 198, 1105–1119. https://doi.org/10.1016/j.jclepro.2018.07.042.

Varshney, R., Bhadauria, S., Gaur, M.S., Pasricha, R., 2011. Copper nanoparticles synthesis from electroplating industry effluent. Nano Biomedicine and Engineering 3, 115–119. https://doi.org/10.5101/nbe.v3i2.p115-119.

Varshney, R., Bhadauria, S., Gaur, M.S., Pasricha, R., 2010. Characterization of copper nanoparticles synthesized by a novel microbiological method. JOM 62, 102–104. https://doi.org/10.1007/s11837-010-0171-y.

Velsankar, K., Aswin Kumar, R.M, P, R., Muthulakshmi, V., Sudhahar, S., 2020. Green synthesis of CuO nanoparticles via *Allium sativum* extract and its characterizations on antimicrobial, antioxidant, antilarvicidal activities. J Environ Chem Eng 8, 104123. https://doi.org/10.1016/j.jece.2020.104123.

Wanarska, E., Maliszewska, I., 2019. The possible mechanism of the formation of silver nanoparticles by *Penicillium cyclopium*. Bioorg. Chem. 93, 1–9. https://doi.org/10.1016/j.bioorg.2019.02.028.

Wong-Pinto, L., Menzies, A., Ordóñez, J.I., 2020a. Bionanomining: biotechnological synthesis of metal nanoparticles from mining waste—opportunity for sustainable management of mining environmental liabilities. Appl. Microbiol. Biotechnol. 104, 1859–1869. https://doi.org/10.1007/s00253-020-10353-0.

Wong-Pinto, L., Milian, Y., Ushak, S., 2020b. Progress on use of nanoparticles in salt hydrates as phase change materials. Renew. Sustain. Energy Rev. 122, 109727. https://doi.org/10.1016/j.rser.2020.109727.

Zhou, W., Coleman, J.J., 2016. Semiconductor quantum dots. Curr. Opin. Solid State Mater. Sci. 20, 352–360. https://doi.org/10.1016/j.cossms.2016.06.006.