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Self-assembly of supramolecular structure based on copper-lipopeptides isolated from e-waste bioleaching liquor

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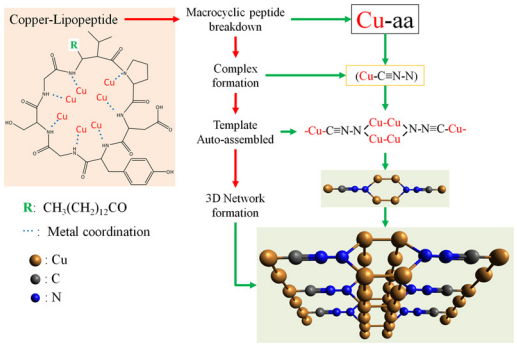
GRAPHICAL ABSTRACT



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ABSTRACT

Supramolecular structures were produced by auto-assembling CuCN blocks derived from copper-lipopeptides (CuLps) isolated from bioleaching liquor. Lipopeptides produced by *B. subtilis* Hyhel1 have been previously related as responsible by bioleaching and intracellular copper crystal production. However, there were no records relating CuLps to extracellular copper crystal production. To study this process, CuLps were isolated from bioleaching liquor and kept at 8 °C to facilitate the CuLps aggregation. After three months, blue spheres (BS) were observed in the CuLp fraction. These spheres were then analyzed by SEM-EDS, MALDI-TOF-MS/MS, GC–MS and FTIR. SEM-EDS analysis showed that they were formed by polycrystalline structures mainly composed by Cu (46.5% m/m) and positioned concentrically. MALDI-TOF-MS/MS and GCMS showed that peptide bonds of CuLp were broken, producing lipid chains and amino acids free. The FTIR of BS showed three nitro groups: C≡N, N═N and N−O, which were not found in the control. These data suggest that the CuLp amino acid produced a C≡N group linked to copper, as CuCN blocks, that auto-assembled in supramolecular structures. This phenomenon could be explored as a method to recover copper and to obtain supramolecular CuCN structures, which in turn may be used as template for superconductor or computing devices.

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1. Introduction

Supramolecular chemistry, defined as "chemistry of molecular assembles and of the intermolecular bond" was a term first applied by Jean-Marie Lehn, in 1978 [1]. This term can be described as blocks organized by molecular bonds, hydrogen bonding, electrostatic or hydrophobic interaction, to form supramolecular aggregates [2]. These supramolecular structures are self-assembled by the autonomous organization of components. The information regarding shape, charge and polarizability determine their interactions and sensibility to the external stimuli [3]. The understanding of these structuring features allows the usage of functional properties that could customize self-assembling of nanomaterials for biomedicine, informatics devices and environmental science application [4,5]. For example: (a) Supramolecular hydrogels of auto-assembling peptides can build a 3D structure to support cells, helping tissue regeneration [5]. (b) Hybrid [n]-rotaxanes supramolecular arrays can be used as molecular electron spin qubits to make devices that improve quantum information process [6]. (c) pH-switchable vesicles are self-assembling zwitterions that reversibly open and close by changing protonation, and can be used as a customized product delivery for soil remediation [7]. (d) Non-covalent interactions between macrocycle para-sulfonato-calix [4]arene (SC4) and C16 alkyl chain amino acid-based surfactants (16Ser) have built complex tubular structures and auto-assembled vesicles. In this case, the macrocycle SC4 concentration was the factor that determined the tubular morphology [8].

These applications are defined by the functional morphology of the material, using as templates biopolymer of DNA, collagen, polysaccharides alginate or starch. As result, nanowires, nanotapes or nanotubes are built [9]. In this sense, biosurfactants are interesting polymers capable of producing supramolecular structures with multiple chiral centers, controlling their morphology by changes on pH, temperature, surfactant concentration, as well as the ionic strength of solution [10].

In nature, bacteria from the *Bacillus* genus, such as *B. subtilis*, *B. cereus*, *B. pumilus* or *B. thuringiensis,* are known as lipopeptides producers, molecules composed by an amino acid macrocycle head linked to an alkyl chain tail [11]. These amphiphilic compounds are classified by their amino acid sequence and fatty acid branching in three families: fengycin, iturin and surfactin [12]. Fengycin and iturins are molecules containing a macrocycle of ten and seven amino acids, respectively, and an alkyl chain of 14–17 carbons. Differently, surfactins are molecules containing a macrocycle of seven amino acids and lipid chain of 12–16 carbons [13,14]. The amphiphilic nature of fengycins allows auto-assembling of molecules in spherical shell-like micelles shell-like, modifying its structure, thus becoming able to control the emergence of bacterial infection [15]. In turn, iturins and surfactins have been shown to be ion-conducting channels, due to their auto-assemble in lipid bilayer, permeabilizing the cell membrane to deliver drugs into the cells [16].

Lipopeptides are biosurfactants known for removing heavy metals such as copper, cobalt, cadmium, nickel, iron, chromium and lead from contaminated soil and industrial wastewater [17]. The ability of chelating metal is provided by the macrocycle cationic head of lipopeptides, which offers CN groups that coordinates metal complexation [18]. The process involves the formation of CN^- ions by recombining monatomic C and N, which increases when these elements are bonded together in the same peptide, as observed in polyglycine molecules [19]. In this context, small macrocyclic peptides isolated from ascidians have been shown to form mono and dinuclear copper complexes, with the deprotonation of peptide amide nitrogen induced by the metal ion [20].

This copper complex is a copper cyanide (CuCN) binding structure present in 1-D polymer chain (−Cu−C≡N−Cu−C≡N−Cu−), which is also self-assembled into 2D-sheet and 3D structures formed by network cross-linked and cuprophilic interaction [21]. In turn, 2D-sheets are built by fused CuCN rings intersecting CuCN layers produced on the 3D network, which are the motif of polyhedral supramolecular structures, a limitless set of components to build up solid state materials [22]. Copper cyanide is a polymorphic material, composed of two forms: a poorly crystalline α-CuCN and a highly crystalline β-CuCN. It's structure depends on the Cu/CN ratio [23]. This substance can be used as copper catalyst or in the copper-mediated cyanation reactions to synthesize pharmaceuticals, agrochemicals, dyes, and herbicides [24]. The versatility of polymorphic copper cyanide structures allows the inclusion of ligands in the network to produce open framework material and superconducting materials [22,25].

In the present work, the extracellular formation of CuCN crystals was studied using copper-lipopeptides isolated from polymetallic solution. This solution was in turn produced by e-waste bioleaching using the bacteria *B. subtilis* Hyhel1 associated to the marine sponge *Hymeniacidon heliophila* [26]. The lipopeptides produced by Hyhel1 have been shown to be responsible for e-waste bioleaching at neutral pH, for carrying copper into bacteria and for producing intracellular copper crystals, which is a different process than the dissolution of acidic metal produced by chemolithotrophic bacteria [27]. In this case, the copper was specifically removed by linking atoms to lipopeptides amino acids, resulting in copper-lipopeptides. Furthermore, the bioleaching method did not require low pH or stable Fe concentration through biochar or S^o [28,29], that would interfere in the copper recovery.

To further understand extracellular production of copper crystals, CuLps were isolated from bioleaching liquor to facilitate the CuLps aggregation that formed blue spheres (BS). The SEM-EDS analysis showed that spherical crystalline formations were characterized by the presence of CuCN motif as the unique component of this structure. MALDI-TOF-MS/MS was carried-out to determine the presence of lipopeptides or its fragments in the supramolecular structure (SMS). GC–MS was used to determine which lipid chains were present in the SMS and FTIR was used to determine the group present in the SMS.

2. Material and methods

2.1. Supramolecular structures production

The structures were generated from e-waste bioleaching liquor by copper-lipopeptides isolated from a polymetallic solution (17 g/L of copper), previously produced by *Bacillus subtilis* strain Hyhel.1 [26]. The bioleaching liquor of six independent bioleaching experiment provided the copper-lipopeptides used to produce the supramolecular structures. For this, the liquor sample (60 ml) of each experiment, with the elemental composition mean as follows (% w/v): Cl (8.4), Cu (1.74), Ag (0.52), Si (0.37), S (0.08), Sn (0.02) and Zn, Fe, Ni, Br, Ca (< 0.006), was centrifuged (18,500 × *g*, 15 min) and filtered in 0.22 µm cellulose nitrite membrane. Then, the solution was loaded on Sep-Pack C18 12 cc column (Waters) and washed twice with 12 ml of MilliQ water. Following, the column was eluted with 5 ml of ethanol 50% and the retained copper-lipopeptides recovered with 5 ml of ethanol (HPLC grade). After each step, the solutions were removed from the column using a vacuum pump, to avoid the fraction mix. The *B. subtilis* Hyhel.1 control culture, without e-waste addition, was submitted to the same procedures to recover the copper-free lipopeptide (CuFLp). The eluted fractions, containing either CuLp or CuFLp, were kept at 8 °C and observed weekly under a light microscope until supramolecular structures were detected as blue spheres (BS).

2.2. Scanning electron microscope (SEM) analysis

The BS was mounted on adhesives carbon strips attached to stubs, and dried in vacuum at 60 °C. The samples were analyzed in a SEM with energy-dispersive X-ray spectroscopy (EDS) to determine the elemental composition of structures. To follow, the same samples were platinum

coated and SEM-FEG analyzed. The elemental composition of 73 spheres were obtained and analyzed. The spheres were produced by copper-lipopeptides isolated from bioleaching liquor of six independent experiments.

2.3. Mass spectrometric analysis

The MALDI-TOF-MS/MS analysis were performed with CuLp fraction collected immediately after Sep-Pack column separation and after BS formation. Simultaneously, BS washed twice in Milli-Q water, were extracted with 100 µl of formic acid (P.A.), diluted with 100 µl of acetonitrile, centrifuged at 18,500 × *g* and supernatant sequencing by mass spectrometry. Aliquots (0.6 µl) of CuLp fractions or formic extract were placed in a MALDI-TOF target, air dried and covered with 0.6 µl of α-Cyano-4-hydroxycinnamic acid matrix. The spectra were recorded using a LIFT mode in Ultra-flex II TOF/TOF mass spectrometer (Bruker Daltonics) by selecting appropriate precursor ions within a window range of ± 3 Da. The Flex analysis software (Bruker Daltonics, ver. 3.4) was used for the MALDI-MS data analysis.

After this procedure, fatty acids in BS formic acid extract were determined by Gas chromatography–mass spectrometry (GC–MS). For this analysis, samples were vacuum dried, dissolved in methanol (300 µl), shaked for 2 min and dried again. Following, samples were derivatized by adding a mixture (150 µl) of bis-(trimethylsilyl) trifluoroacetamide, acetonitrile, dichloromethane, cyclohexane and triethylamine (10:5:4:1 v/v). The mixture was again shaked for 2 min, incubated in dry bath at 60 °C for one hour and centrifuged at 11,300 × *g* for 2 min at room temperature. Supernatant was transferred to vial and analyzed in a GC–MS with a capillary column of 30 m length, 0.25 mm i.d. and 0.25 µm film thickness. The GC oven temperature was linearly ramped from 50 °C to 280 °C at 5 °C/min and kept at this temperature for 10 min, for column bake out. The sample injector and GC/MS transfer line were kept at 280 °C and 300 °C, respectively. Electron impact spectra in positive ionization mode were acquired between 40 and 600 *m/z*.

2.4. Fourier transform infrared spectroscopy

Blue spheres that were preserved after the formic acid extraction (method described in Section 2.3) were washed in ethanol P.A. (1 ml) and centrifuged for 3 min at 18,500 × *g*. Ethanol was removed and BS were shaken in hexane (1 ml) for 2 min. To follow, hexane was removed, and BS were dried at 80 °C. After, BS were mixed with KBr powder in a proportion of 1:100, the samples were manually grinded and pressed powder into pellets. The pellet was put into the samples holder and analyzed in a FTIR spectrophotometer IRPrestige-21. Spectra were collected in the range of 700–4000 cm^{-1} range and 32 scans were integrated for each analysis. The CuFLp isolated from a culture of *Bacillus subtilis* Hyhel.1 without e-waste addition was used as control.

3. Results

3.1. SEM analysis of supramolecular structure

After three months of incubation at 8 °C, blue spheres (BS) (Fig. 1A) were observed in the copper-lipopeptides fraction (CuLp) eluted with ethanol from the Sep-pack column. The SEM-EDS analysis showed that the BS were an array of organic copper crystals composed by 46.5 ± 2.3% (m/m) of Cu, 3.6 ± 0.5% (m/m) of C and 1.9 ± 0.1% (m/m) of N, present in all samples. Other elements were recorded in approximately 32% of the samples, such as Cl (3.6 ± 0.7% m/m), Fe (1.5 ± 0.3% m/m), Al (3.2 ± 0.9% m/m), P (1.9 ± 0.7% m/m) and Si (0.8 ± 0.1% m/m). In addition, Ca (1.60 ± 0.7% m/m) and S (0.6 ± 0.1% m/m) were recorded in 6% of the samples (Fig. 1B).

The spheres were composed of crystals positioned in a concentric

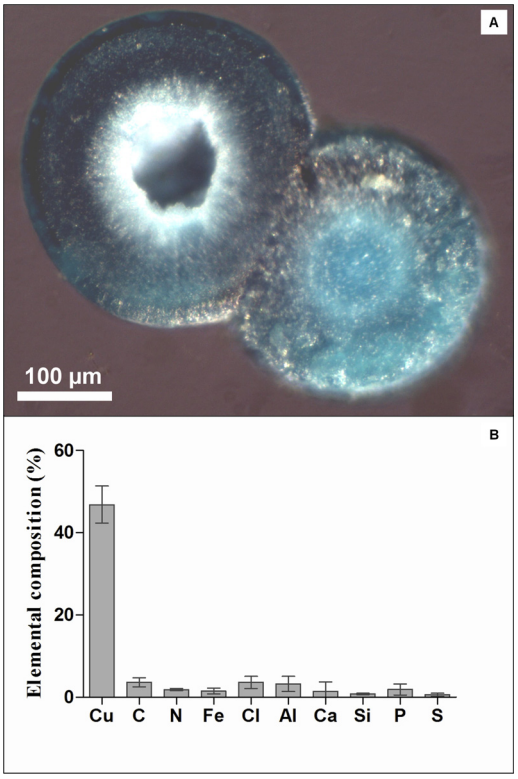

Fig. 1. Blue spheres produced by auto-assembling of copper-lipopeptides isolated from e-waste bioleaching liquor. (A) Blue sphere observed in optical microscope. (B) Elemental composition of blue spheres determined by SEM-EDS. The error bars represent the standard error. N = 73. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

organization (Fig. 2A and E). These crystals were disposed like flower petals and oriented perpendicularly to the center of sphere, fusing crystals and shaping microtubes (Fig. 2B and C). The transition zone (between the center and the border) was exactly where the crystals formed the "petals" shapes (Fig. 2D). These crystals were morphologically different than crystals present in the border zone, characterized by stacked polygons (Fig. 2E). The formation of unitary crystals were not observed. However, material aggregating was observed in the center of some crystals (Fig. 2F). Elemental composition of each zone showed that the concentration of copper changed with the position, with 55.2 ± 3.1% (m/m), 72.9 ± 5.5% (m/m) and 42.9 ± 6.4%(m/m) in the center, middle and border zone, respectively.

Different structural arrangements of the crystal and wide variation of copper concentrations were observed during the sphere's formation (Fig. 3). The spheres showed their core containing 35.5 ± 3.3% of copper (Fig. 3A), surrounded by structures containing 87.9 ± 5.1% of copper (Fig.3D) and a membrane with different structures attached (Cu: 47 ± 4.3%) (Fig. 3B). The structures attached to the membrane were shaped by aggregation like the structure of a druse (Fig. 3C) and different to the layer covering the core, which showed structures formed by strata (Fig. 3E). Although these membranes were initially observed without any druse attached to them (Fig. 3A and F), they showed copper concentration of 11.5 ± 3.1% m/m, lower than membrane with drusen formation.

The starting process to build BS showed a sunflower-like structure conformation (Fig.4A and B), whereas the ending process showed compacts structures (Fig. 4E and F). These processes, after folding and fusing the crystal that formed the second layer, were covered by an amorphous substance (Fig. 4C and D) that finally "cimented" the crystals like a compact sphere.

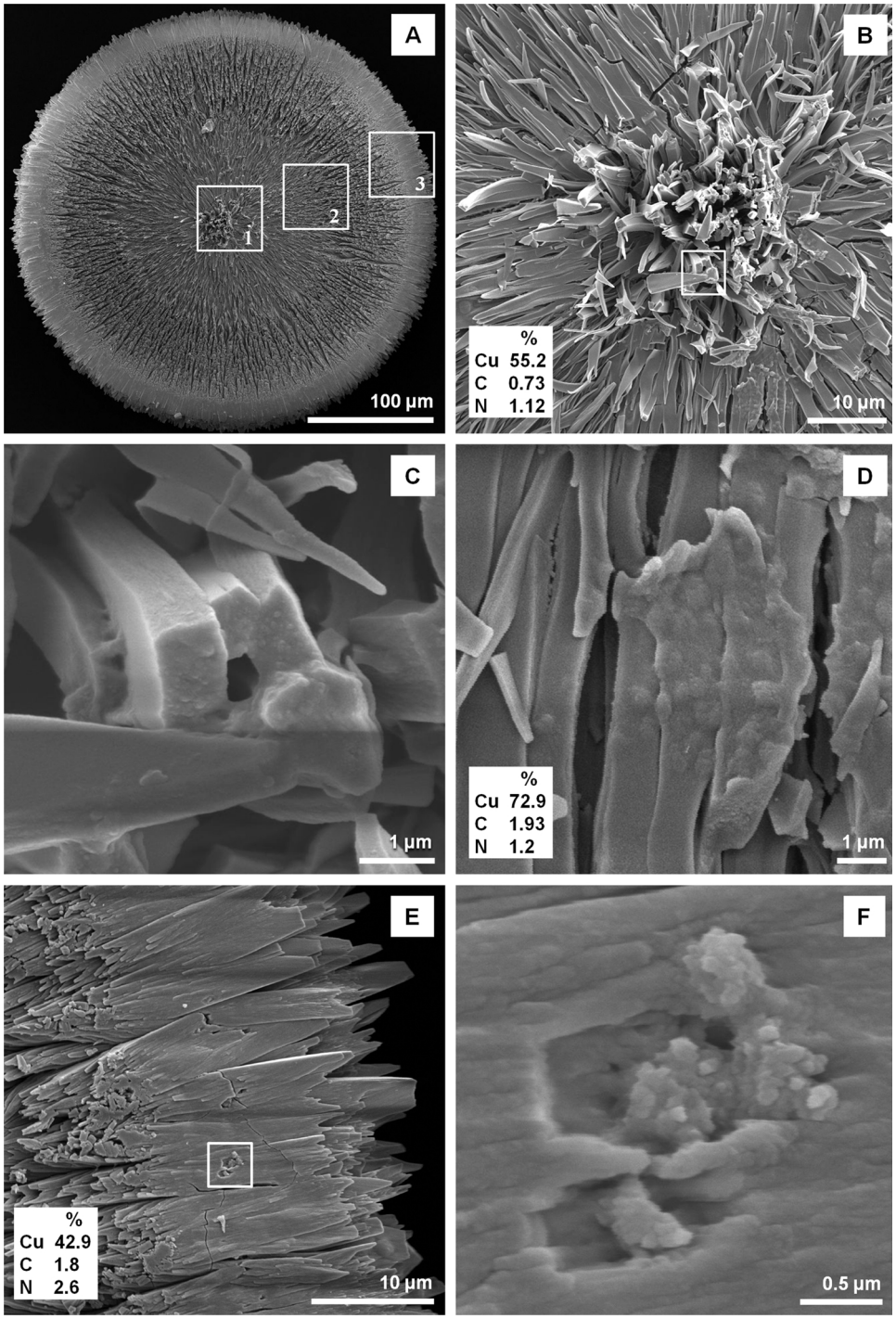

Fig. 2. Crystal morphology of blue spheres (BS). (A) SEM image of whole BS: Rectangles enclosing three zones of different crystal morphology. (B) Zone 1: Crystal disposed like flower petal and crystal fusion in the center of the BS. Rectangle enclosing microtube formation (C) Detail of crystals fusion for microtube formation. (D) Zone 2: Formation of crystals like petals without crystal fusion. (E) Zone 3: Alignment of crystals layers oriented to the center. Rectangle enclosing a failure. (F) Detail of failure in the crystal. The tables enclosed in the image B, D and E showed the mean concentration of the elemental composition within each zone.

3.2. Mass spectrometric analysis

MALDI-TOF-MS profile of CuLp fraction was analyzed immediately after elution showing presence of Iturin-like surfactants (1107 *m/z*) (Fig. 5A). This lipopeptide was not observed in the same fraction after spheres formation. In addition, ions with mass lower than 950 *m/z* were observed in the formic acid extract of theses spheres (Fig. 5B). The mass fragmentation spectrum of precursor ions *m/z* 523.7 (Fig. 5C) and *m/z* 550.6 (Fig. 5D) showed that Iturin-like was broken resulting in fatty acid linked to 3 amino acid. The sequencing showed that ion *m/z* 523.7 was constituted by a fatty acid chain of 17 carbons ($CH_3(CH_2)_{15}COOH$) linked to -G-S-G- amino acid sequence and ion *m/z* 550.6 was constituted by a fatty acid chain of 14 carbons ($CH_3(CH_2)_{12}COOH$) linked to D-P-V-amino acid sequence. These sequences were corroborated by the presence of ions b_1, b_2 and b_3, whose values were *m/z* 275.688, 332.672 and 420.196 from precursor ion *m/z* 523.7 and *m/z* 238.956, 354.884 and 451.568 from precursor ion *m/z* 550.6. On the other hand, the GCMS analysis of formic acid extract of BS showed presence of fatty

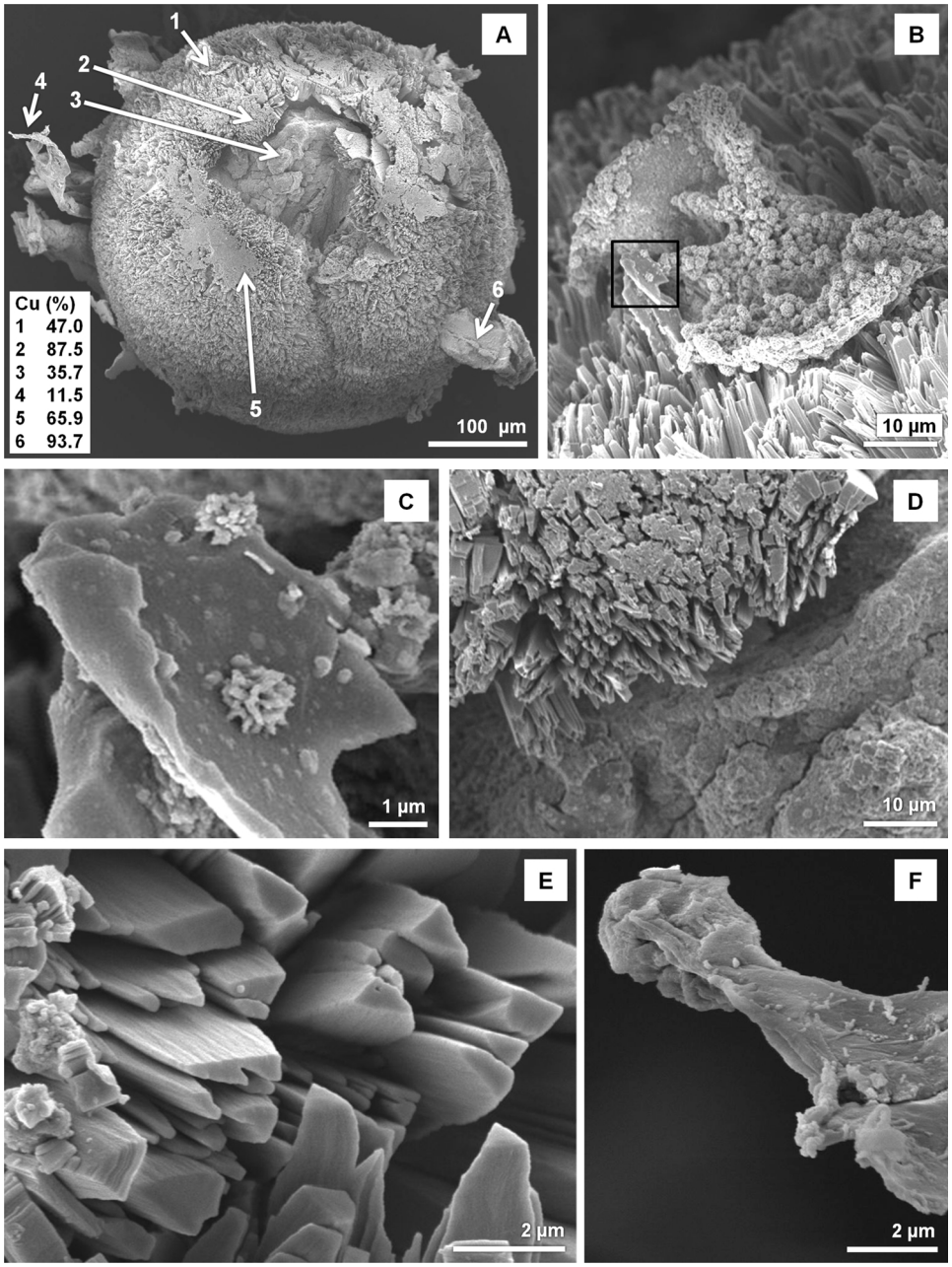

Fig. 3. Crystal arrangements observed during the sphere's formation. (A) Whole BS in incomplete formation stage. Table inside show the mean of copper concentration in six area of BS. (B) Detail of area 1 show structures like druse attached to the membrane. Rectangle enclose druse. (C) Detail of druse attached to membrane. (D) Detail of area 2 e 3 show crystals formed by strates and amorphous material, respectively. (E) Details of crystals formed by strata. (F) Detail of membrane observed in the area 4, without druse attached.

acyl chain with 5 to 19 carbons, i.e. arabinoic, tetradecanoic, hexadecenetrile or octadecanoic acid (SS1).

3.3. Fourier transform infrared spectroscopy (FTIR)

The FTIR analysis showed differences between spectra of copper-free lipopeptides (CuFLp) fraction (control) and blue spheres (BS) (Fig. 6).Spectral shift between BS and CuFLp were observed in four bands in the 900-700 cm^{-1} region. In the same way, the bands 3449.8 and 2850.9 cm^{-1} shifted to 3446.0 and 2880.8 cm^{-1}, respectively and the bands 2968.6, 2918.4, 1630.9, 1467.9, 1251.9 and 1069.6 cm^{-1} present in the control were not observed in the BS spectrum. The bands 3570.4, 2360, 2331.1, 1581.7, 1384.0 and 1357.0 cm^{-1} present in BS were not observed in the control fraction. In the BS spectrum, four bands associated to nitro group were observed at 2331.1 cm^{-1} (C≡N), 1581.7 cm^{-1} (N═N), 1384.0 cm^{-1} (N–O) and 1357.0 cm^{-1}(C–N). Differently, in control fraction only two nitro group were observed at 1467.9 cm^{-1}(C═N) and 1251.9 cm^{-1} (C–N). The bands 1630.9 cm^{-1} (C═C) and 1467.9 cm^{-1} (N═N) observed in the control, were not present in the BS.

4. Discussion

Metal recovery from e-waste bioleaching liquor can be an important source of valuable metals. However, specific metal recovery from polymetallic solutions can be difficult and expensive to perform. Thus, biosorption has been considered as an alternative process due to low operating costs, low use of chemicals and fewer residues [30].

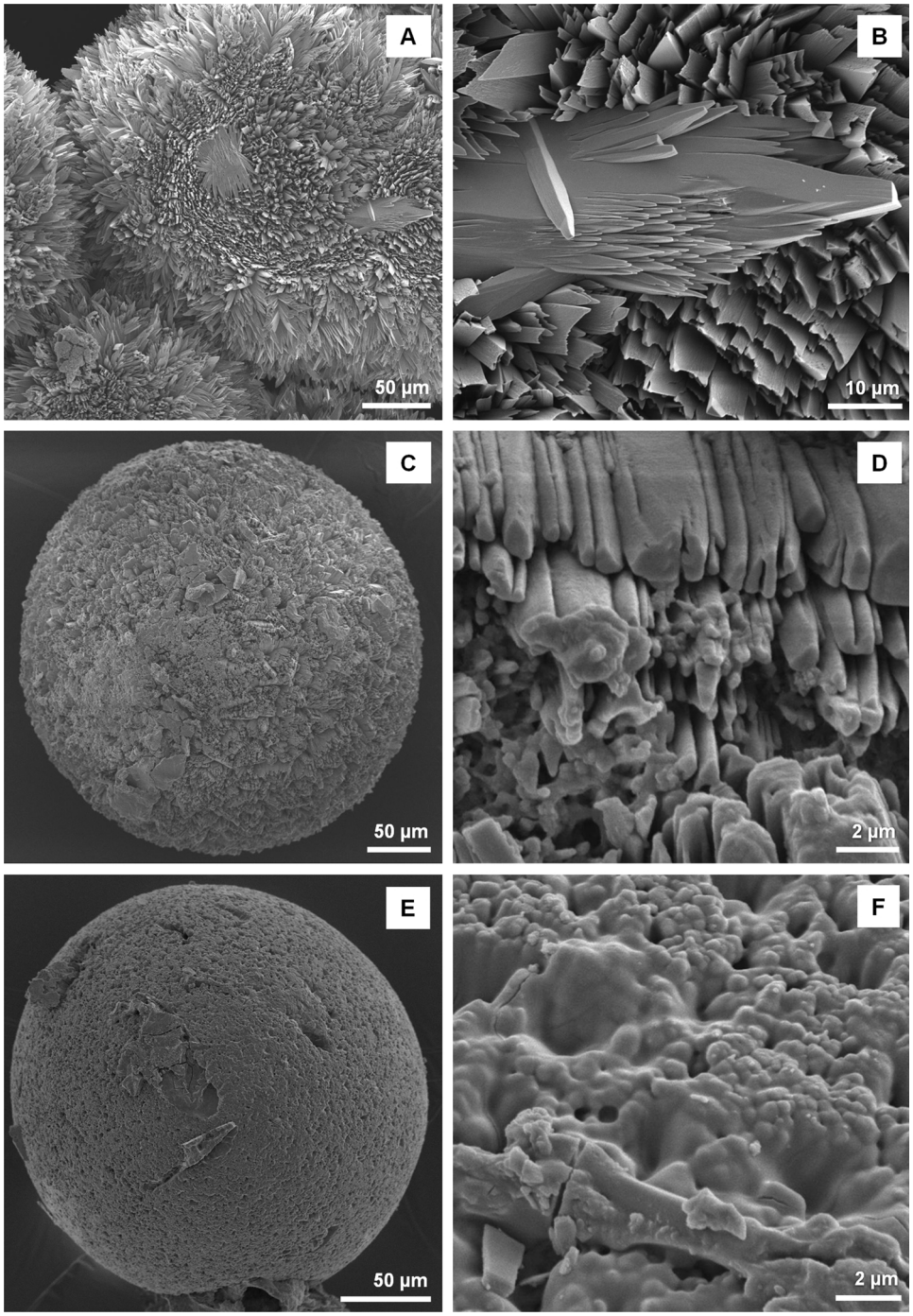

Fig. 4. Different formation stage of spheres. (A) First formation stage showed a crystalline formation like "sunflower". (B) Detail of crystals in the surface of sphere. (C) second formation stage show crystals covered by amorphous material. (D) Detail of amorphous material at the top of crystals. (E) Final formation stage showed a compact sphere. (F) Detail of amorphous material covering the crystals.

Considering this, our work has shown an alternative and innovative biosorption process, by which copper-lipopeptides are isolated from a bacterial culture, composing blue spheres of organic copper crystal in ethanol medium.

The blue spheres, initially characterized by SEM-EDS, showed an elemental composition based of copper, carbon and nitrogen, copper being the most abundant element. This composition suggests that CuCN could be involved in the initial stage of BS construction. The copper cyanide (CuCN) structures synthesized in laboratory are usually described as highly disordered polymorphic structures, producing polycrystallines samples which are morphologically dependent on the Cu/CN ratio [25]. CuCN as pure compound showed an intense band at 2170 cm^{-1}, which can be correlated to the C≡N group [23,21], which were observed at 2331.1 cm^{-1} in the BS FTIR spectrum. This shift on frequency could be explained by the large degree of interchain disorder observed in the crystal structure [23] or by the presence of other groups that compose the BS crystals. For example, the N═N group that had shown the most intense frequency in BS spectrum (1582.7 cm^{-1}). Considering that C≡N, N═N and N−O were not observed in CuFLp, it is possible that macrocyclic peptides were broken and re-organized into new structures defined by the interaction between copper and lipopeptides. In addition, FTIR spectrum of CuFLp showed bands which

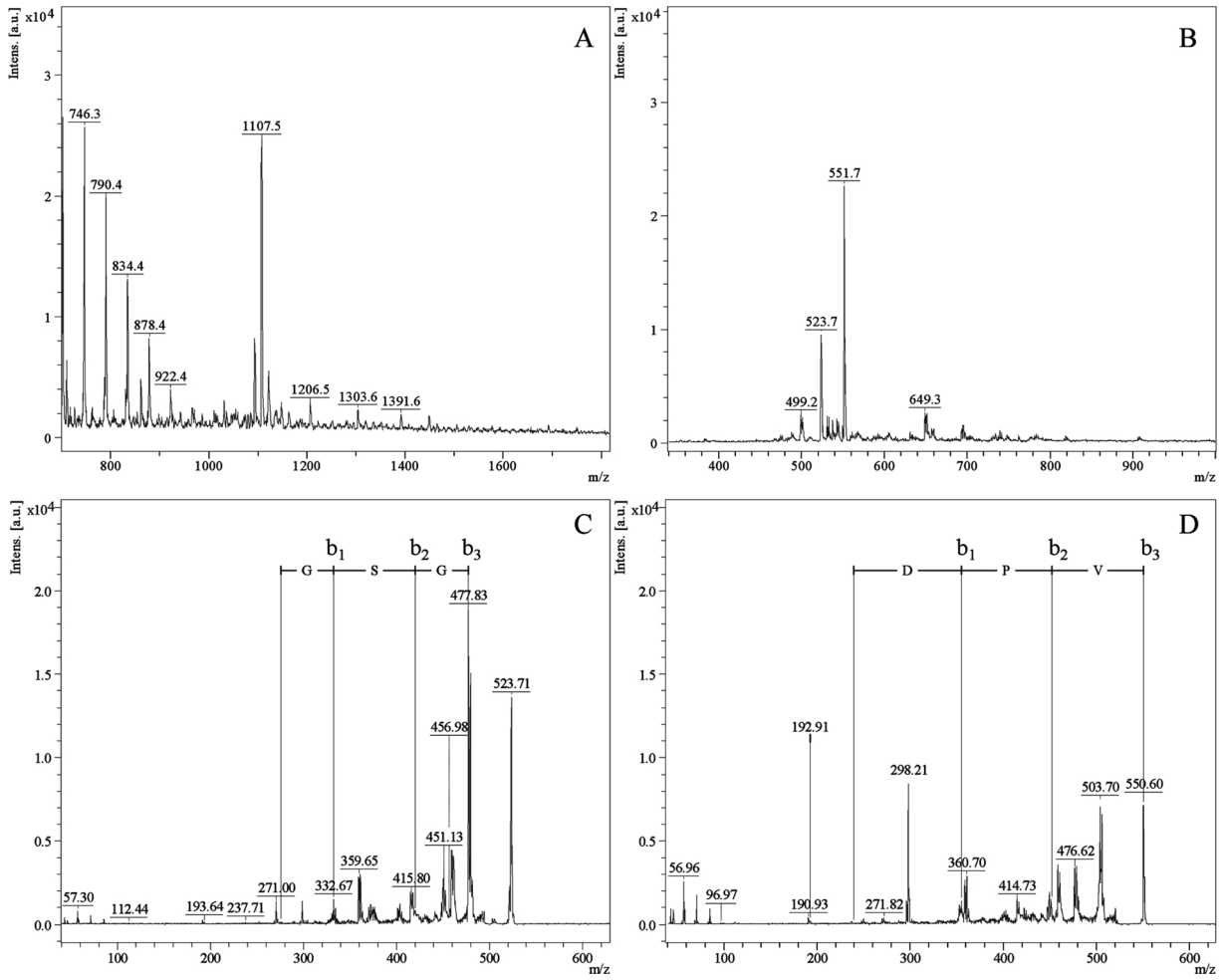

Fig. 5. MALDI-TOF-MS/MS analysis of blue spheres. (A) Spectrum of Cu-lipopeptide fraction immediately after separation of free-cells bioleaching liquor. (B) Spectrum of Cu-lipopeptide fraction after 3 months at 8 °C. (C and D) show the mass spectrum fragmentation of precursor ions *m/z* 523.7 and 550.6, respectively. The ions b_1, b_2 and b_3 from precursor ion *m/z* 523.7 are *m/z* 275.688, 332.372 and 420.196; and from precursor ion *m/z* 550.6 are *m/z* 238.956, 354.884 and 451.568, respectively.

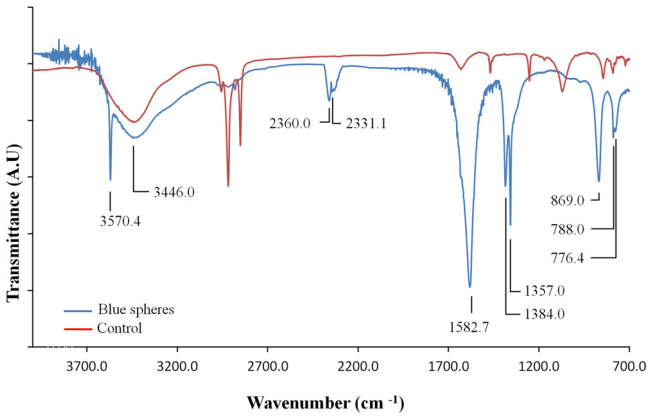

Fig. 6. FTIR spectrum of blue spheres produced by Cu-lipopeptides fraction and lipopeptides copper free fraction (CuFLp) of control culture. Blue line represent FTIR spectrum of BS structures and red line represent spectrum of CuFLp fraction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

indicate surfactant compound features produced by *Bacillus subtilis*, conserving the C−O, C−N and C═N in all variant [31,32].

To confirm these results, mass spectrometric analysis showed that after the formation of BS, degraded peptide macrocycles and fatty acyl chain (5–19 C) were observed. The data suggest that amino acid of the CuLp contributed to the BS formation. In this sense, presence of hexadecenetrile in the BS surface, identified by GCMS, indicate that the C≡N group from amino acid was formed before separating from the lipid chain.

Studies in supramolecular self-assembly of surfactants, especially amino acid-based, have shown that the addition of macrocycles induce micellar aggregation, and increasing its content induce tubular structure and vesicles formation [8,30]. Thus, headgroups of amino acid-based surfactant and macrocycles combined show the ability to complex cation, maintaining its alkyl chain unaffected and electrostatically driving the formation of spherical structure, as an inverse vesicle [33]. Biosurfactants produced by *Bacillus* are especially interesting for heptapeptide cycles linked β-hydroxy fatty acid with variable chain lengths [13] These anionic lipopeptides are known as selective cation carriers through lipid membranes and participate in supramolecular structures as micelles [16], but were never reported as part of crystalline supramolecular structures.

The presence of hexadecenetrile in the BS suggest that broken peptides from CuLp were responsible for building the sphere and fatty acid chains of different length (C_5–C_{19}) remain attached. This data was coherent with SEM-EDS results indicating that BS crystals were mainly constituted by CuCN. The FTIR results showed that the C≡N group present in BS was not observed in CuFLp, indicating a structural change.

Differently than the traditional acid bioleaching process, the copper was specifically removed by linking atoms to lipopeptide amino acids, resulting in copper-lipopeptides [26]. The GC–MS, SEM-EDS and FTIR data suggested that CuFLp peptide macrocycles were broken, releasing linked copper and amino acids and forming the CuCN structure.

Considering than lipopeptide Surfactin can show a β-sheet conformation at high micelle concentration [34], it is possible that the amino acids present in CuLp were responsible for inducing β-sheet formation to organize the initial membrane that constituted the BS. This membrane folded to form crystal layers that after fusion originated other crystalline structures. These were covered by fatty acid chains from the broken CuLp, acting as a cement that was able to compact the sphere. Similar crystal growth was described to other CuCN three-dimensional structures that exhibit considerable variability, despite the presence of common building blocks constructing networks of Cu_2X_2 and Cu_8CN_8 [22]. Considering the BS's elemental composition regarding Cu, C and N (Fig. 1B), it was possible to determine the molar composition as Cu_6CN_2, which showed an excess of copper compared to the expected Cu_2CN_2. This data suggests a cuprophilic interaction with other 4 copper atoms to form the CuCN network, indicating that N bonded to Cu and Cu bonded to Cu, forming a 3D structure similar to CuCN networks with diimines [35]. Due to the non-covalent bond assemblies, this versatility of structural motif accommodating has led to an increased interest in these macromolecules as supramolecular coordination biopolymers in the synthesis of new materials [36].

The supramolecular auto-assembly ability of lipopeptides isolated from *Bacillus* HyHel1 and its free-cell culture to form BS were tested using chemical e-waste leached as copper source. However, no structure was observed in this case (data not shown). This leads to the belief that the copper-lipopeptide's macrocycle peptide is broken to form CuCN unities and released as CuCN-C16 fatty acid, thus auto-assembling the BS.

Considering this, it would be possible to prepare functional materials controlling the crystal morphology by understanding the factors that influence polycrystalline BS formation from copper-lipopeptides (temperature, pH, solute concentration, binding interaction and other). This would allow the synthesis of complex structures in a simple and highly specific manner, using metal-lipopeptides as template.

The experiment was designed considering that lipopeptides produced by *B. subtilis* Hyhel1 would transport the leached cooper into the cell, in order, to produce copper nanoparticles that aggregated in cubic copper crystals [26]. However, we were surprised to find how different the behavior was with copper-lipopeptides isolated from bioleaching liquor, which produced polyhedric crystals similar to flower petals instead of the expected cubic crystals observed inside bacteria.

Although bioleaching reaction pathway is still not well known, lipopeptides which carry copper from e-waste into bacteria cells during bioleaching processes (in neutral pH environment) could be responsible [26]. In addition, even though lipopeptides transporting copper inside of bacteria are well known, it is not clear if these lipopeptides are the unique factor involved in the intracellular crystals production. For these reasons, future proteomic analysis of this process will most likely provide a deeper understanding of synthesis by lipopeptides and copper-crystal formation.

5. Conclusion

Copper-lipopeptides isolated from bioleaching liquor produced supramolecular structures by auto-assembling of CuCN blocks, which in turn were originated in the copper-amino acids complexes. The macrocycle of CuLp broke down into copper-amino acids and underwent structural modifications, until a C≡N group was produced and remained linked to copper. Thus, the CuCN block was originated from each amino acid and auto-assembled in a 3D polycrystalline structure, producing blue spheres of visible size. The lipopeptide produced by *B. subtilis* Hyhel1 had a different amino acid sequence and was responsible for producing the polycrystalline structure of blue sphere. Considering these findings, future efforts should induce bacteria to produce specific lipopeptides in high concentration in order to fabricate homogenic crystalline structures. In sum, copper recovery from a polymetallic solution was also achieved simultaneously to the production of supramolecular CuCN structures, which in turn may be used as template for superconductor or computing devices.

Competing financial interests

The authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2019.01.038.

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