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Hybrid Catalysts from Copper Biosorbing Bacterial Strains and Their Recycling for Catalytic Application in the Asymmetric Addition Reaction of $B_2(pin)_2$ on α,β-Unsaturated Chalcones

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Abstract: The recycling of heavy metal contaminants from wastewater as a source of valuable products perfectly fits with the principles of a Circular Economy system in view of restoring pollutants back into the system endowed with new social and economic benefits. Heavy metals are often present in such a low concentration that it makes the removal efficiency difficult to realize through the conventional physicochemical methods with high selectivity. Biosorption, conversely, by EPSs (extracellular polymeric substances) produced by several bacterial cells' strains, is gaining a great deal of attention as an economic, efficient and sustainable depolluting process of wastewater from metal cations such as copper. Metal coordination to EPS components was thus deeply investigated by ^{1}H NMR titration experiments. The 1,10–Phenanthroline–copper complex was exploited for quantifying the ability of different strains to sequester copper by a practical UV-Vis spectrophotometric method. The obtained data distinguished *Serratia plymuthica* strain SC5II as the bacterial strain displaying copper-adsorbing properties higher than any other, with *Stenotrophomonas* sp. strain 13a resulting in the worst one. Different analytical techniques, i.e., Dynamic Light Scattering (DLS), FT-IR analysis and SEM spectroscopy were thus employed to rationalize these results. Finally, the obtained copper chelates were successfully employed as hybrid catalysts in the asymmetric boron addition to α,β-unsaturated chalcones for the synthesis of valuable pharmaceutical intermediates, thus placing waste management in a new circular perspective.

Keywords: bacterial EPSs; heavy metal recycling; copper recovery; wastewater depollution

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1. Introduction

The concept of a Circular Economy was created to avoid the permanent dissipation of resources and therefore to allow their circularity within the system. It therefore represents a way to reuse materials in subsequent production cycles, thus reducing waste. Currently, pollution due to heavy metals is due to the large quantities released into the environment by anthropogenic activities, such as the mining, refining and galvanic industries [1]. Industrial wastewater can cause severe water and soil pollution, and the main source of detectable human exposure to heavy metals is the consumption of contaminated drinking water, and the resulting health problems can include neuronal and kidney damage, cardiovascular disease and risk of cancer and diabetes [2].

Typical contaminants contained in galvanic wastewater can be classified into four categories: metals, the main ones being copper and nickel, and to a lesser extent, zinc, chromium, aluminum, cadmium and iron; anions, including chlorides, fluorides, borates,

nitrites, nitrates, phosphates and sulphates; cyanides and organic compounds, including surfactants, oils, greases and solvents. It is well-known that, unlike organic contaminants, heavy metals are not biodegradable and result in accumulation in living organisms, leading to toxicity [3]. To limit the release of heavy metals into the environment, national regulatory bodies impose effective wastewater treatments. Unfortunately, conventional methods developed to remove them from wastewater, i.e., precipitation, coagulation, complexation, solvent extraction and ion exchange techniques, become inefficient or very expensive when contaminants are present in low concentrations [4].

The biosorption method represents one of the most promising alternative techniques, thanks to the low costs, the short times and the possibility of recovering and reusing the bioadsorbed metals. This method is based on the exploitation of bacterial cells, resistant to heavy metals, to adsorb them inside or to bind them externally through the cell wall or extracellular structures (sheaths, capsules, lipopolysaccharides, etc.). The few available studies in the literature showed the effectiveness of this process and the possibility of recovering bioadsorbed metals with a high degree of purity [3]. In fact, the biosorption consists in a passive uptake of toxic agents by inactive or non-living cells. It is made possible by a series of metabolism-independent processes occurring at the cell wall level [5]. An example is provided by El-Sheekh and collaborators [6], who used colonies of cyanobacteria (*Nostoc muscorum* and *Anabaena subcylindrica*) to remove heavy metals from wastewater. In addition, no less important are the extracellular polymeric substances (EPSs), which are also able to bind metal cations with high affinity [7,8].

Carboxyl groups, phosphonates, amines and hydroxyl groups are among the different functional groups present on the bacterial components. Thanks to their negative charge and abundant availability, it is believed that the carboxyl groups actively participate in metal-binding. Among the various factors able to modulate the biosorption of metal cations, there are certainly the chemical characteristics of the EPS and the chemical-physical properties of the metal as well, which lead to a differentiation of the interactions between the metal and the other components in solution. In particular, EPSs constitute a layer of extracellular polymeric substances that can be both bound to the cell surface (EPS-bound) and free in solution (EPS-unbound) [9]. EPSs are heterogeneous mixtures of molecules, composed mostly of polysaccharides and proteins and to a lesser extent of nucleic acids and lipids. EPSs contain various weak acid functions (carboxylic, phosphoryl, amide, amino and hydroxyl groups) which ionize in response to changes in pH or ionic strength of the surrounding environment. In addition to the metal–proton exchange, there are other mechanisms involved in the adsorption of metals by EPSs, such as electrostatic interactions, cation exchange and precipitation. Some studies indicated the alcoholic, carboxylic, amino and phosphate groups as responsible for the metal-binding by EPSs [10]. Furthermore, previous studies have shown that the EPS molecules have great binding capacities towards metals, even stronger than any other known biosorbent. This behavior could be ascribed to the establishment of a network of multiple complexes with metal ions and, thanks to their high concentration in binding sites, EPSs can play a pivotal role in metal adsorption, with particular relevance to the treatment of contaminated wastewater [11,12].

By exploiting the ability of bacterial cells to adsorb copper thanks to the presence of EPSs on their surface, we studied the possibility of converting the biomass obtained (intended as cell–metal complex) into organo–metallic complexes with catalytic properties. Among the transition metal complexes, copper-based derivatives have attracted a great deal of attention thanks to their earth-abundance, non-toxic properties and mild Lewis-acidity properties. Thanks to the low redox potentials of the different oxidation state, copper complexes can undergo oxidative addition/reduction elimination steps more easily, thus increasing the reactivity in many different catalyzed organic transformations comprising the synthesis of valuable heterocyclic scaffolds [13], such as Suzuki−Miyaura-Type Cross-Couplings [14]. C-H activation reactions are among the others [15]. An additional advantage offered by copper catalysis relies on the possibility to use open-flask conditions in aqueous medium, thus avoiding the use of organic co-solvents and paving the way for a

sustainable catalysis [16,17]. The use of this alternative and innovative strategy would give a second life to what has always been considered "waste" and therefore no longer useful. The so-formed hybrid catalysts are thus recycled in a representative copper catalyzed reaction, i.e., the asymmetric addition reaction of $B_2(pin)_2$ on α,β-unsaturated chalcones, which are pharmaceutically related intermediates [18–21]. Chalcones are naturally synthesized in plants as secondary metabolites within the flavonoids' biosynthetic pathway, and their open-chain flavonoid structure features two aromatic rings joined by a three-carbon α,β-unsaturated chain. The possibility to introduce substituents with different electronic and steric properties on both the phenyl rings along with the presence the unsaturated Michael acceptor function make the chalcone skeleton a privileged scaffold in medicinal chemistry, able to interact with different thiol-containing biomolecules [22,23]. Many studies reported the use of chalcones as anticancer agents with novel targets and mechanisms of action [24]. Chalcones have indeed a poor probability to directly interact with DNA, thus reducing the risk of mutagenicity and leading to potential cytotoxic compounds with reduced side-effects [25]. These intriguing structural features have sparked intense research in the field of pharmacologically active chalcones, leading to isolation of derivatives endowed with antioxidant and antitubercular activity [26], antimicrobial and anti-viral agents [27] and anti-diabetic properties [28] with the addition of chalcone derivatives employed in the chemistry of materials [29]. However, the promiscuous target profile exhibited by the chalcone scaffold, metochalcone (choleretic drug), hesperidin methyl chalcone (vascular protective) and sofalcone (antiulcer and mucoprotective) have also been approved for clinical applications [30].

2. Results and Discussion

An initial screening of EPS-producing strains was carried out on environmental isolates present at the Agricultural Environmental Lab (DeFENS). Strains were grown on LB medium and tested for their ability to remove copper supplied as 200 mg/L $CuCl_2$ solution. This concentration was chosen in order to simulate typical copper concentration in electroplating industry effluents, ranging between 100 and 500 mg/L. Cell biomass was washed with phosphate buffer (1 M KP buffer, pH 7.2) and resuspended in bi-distilled water to obtain OD_{600} = 2. The suspension (4 mL) was deposited onto a 0.2 µm cellulose acetate filter (Millipore, USA). $CuCl_2$ solution was allowed to pass the activated filter by vacuum pump. Copper concentration in the flow-through was determined by ICP-MS analysis according to Cavalca et al [31]. The quantification of the biomass-adsorbed Cu(II) was obtained by the difference of initially supplied Cu(II) with respect to the Cu(II) flown through the filter. Specific Cu(II) adsorption (mg/g d.w.) was calculated by dividing the adsorbed Cu(II) by biomass dry weight. The ability to remove copper was present to different extents in the analyzed strains (Figure 1). The best-performing strains belonged to the *Serratia plymuthica* species. Particularly, *Serratia plymuthica* strain SC5II removed 80 ppm Cu(II) corresponding to the maximum copper specific removal of 21.6 mg/g. *Stenotrophomonas* sp. strain 13a was the less efficient one, displaying removal of 31 ppm Cu(II) corresponding to a Cu(II)-specific removal of 11 mg/g. No changes in Cu(II) concentration occurred in abiotic controls. *Stenotrophomonas* strains able to adsorb Cu(II) have been isolated from vineyard soils [32]. *Serratia marcescens* strains have been considered in the past for their ability to remove toxic metals such as Pb, Cd and Cr from natural water [33]. One strain has been recently tested for its ability to remove Ni, Co, Zn and Co from mono- and bi-metallic solutions [34].

The tested strain was able to absorb Cu(II) 8.6 mg/g_{cells}. Considering the higher biosorption ability of the *Serratia plymuthica* strains tested in the present study, future analyses will be performed in order to envisage their use in Cu(II) biorecovery and bioremediation of heavy-metal-contaminated industrial wastewaters. Operational conditions should be optimized since the left-over copper of about 120 mg/L determined in the present study far exceeds the legal limits of 1 mg/L (D.Lgs. 152, 2006).

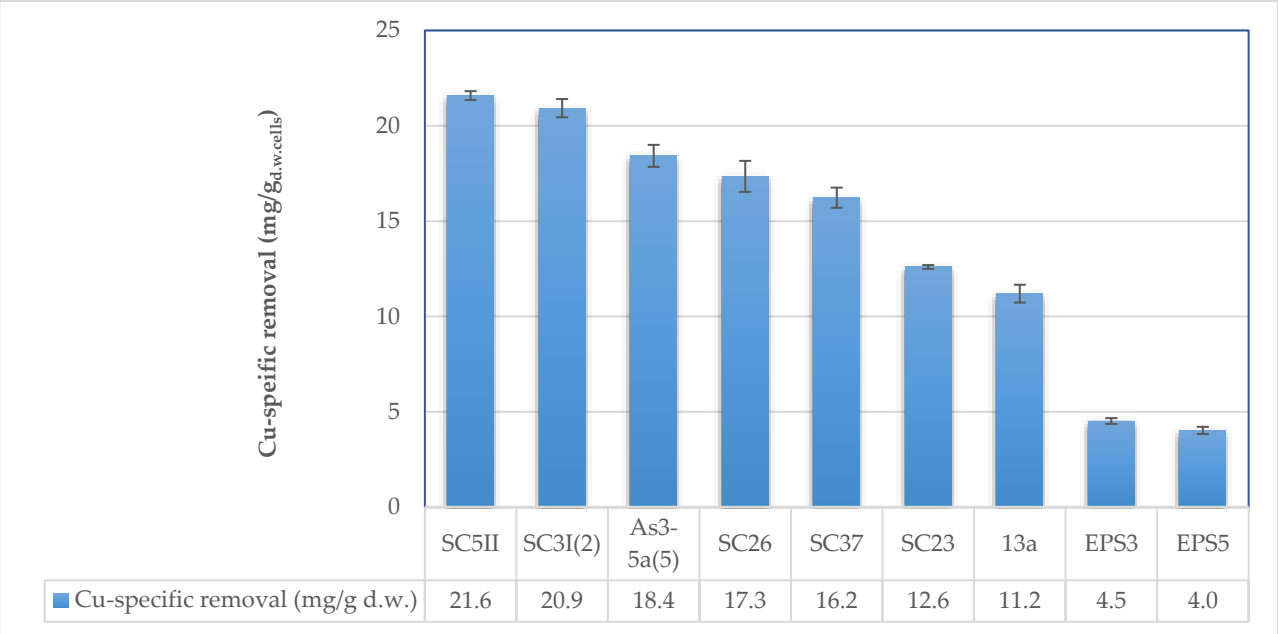


Figure 1. Cu-specific removal (mg/g d.w.) by bacterial isolates in filter-adsorbed biomass trials.

A common approach for the quantification of the different ability of microorganisms to adsorb copper relies on the use of ICP-MS. Although this analytical technique offers evident advantages in terms of precision and accuracy, it also has limitations, being time-consuming and expensive. In order to overcome these drawbacks, the first objective was to develop an easy and fast analytical method that would allow us to obtain reliable results in the normal practice of research laboratory analysis. It was therefore decided to use UV-Vis spectrophotometry to determine the concentration of copper in our unknown samples.

Therefore, 1,10-phenanthroline (1,10-PN) was chosen as the optimal ligand capable of coordinating copper in its monovalent form as Cu(I) [35,36]. In this regard, a Cu(II) reduction method in situ has been developed, using $Cu(OAc)_2 \cdot H_2O$ as the starting salt and ascorbic acid in a stoichiometric ratio of 1:1.2 as a reducing agent. The solvent chosen was MeOH, as it is miscible in water and able to meet the required characteristics mentioned above (See SI, Figure S1). In particular, instead of using the cellulose filter method, we chose to disperse the biomass and the copper solution in a vial with the aim to facilitate the use of the catalyst for the subsequent catalytic reactions. Once the optimal analysis conditions had been set up, a comparison study was carried out between the two analytical methods used (ICP-MS and UV-Vis analysis) in order to evaluate the reproducibility of the two methods by analyzing the same solutions at a known concentration with both analytical techniques (See SI, Figure S2). The correction coefficient ($CC_{UV/ICP}$) between the two methods was then determined and found to be equal to 0.978 ± 0.01, calculated as an average value. Considering the replicability of the results with the absorption in the bacteria, the UV-Vis method was applied for all the further analyses.

Considering the intrinsic variability dictated by the biological world, experiments were performed to determine the best conditions for cell adsorption, using strain 13a as reference, and for the subsequent standardization of the method to obtain results that are as reproducible as possible. The growth and adsorption conditions of the strains were then varied according to the type of inoculum (solid–liquid (SL) and liquid–liquid (LL)) inoculum, both using fresh cells and cells stored in the refrigerator for a maximum time of 72 h). The incubation times were varied (48 h and 72 h) and we also decided to evaluate the concentration of the copper salt and the volume of the aqueous copper solution employed for adsorption. The greatest reproducibility of results was obtained using cells grown for 48 h (S-L). Cells from 20 mL of broth culture were used for copper adsorption by adding

6 mL of a $Cu(OAc)_2 \cdot H_2O$. Different copper concentration was evaluated in a range of 1–6 mg/mL, with 3 mg/mL proving to be the optimal absorption condition.

Based on the obtained data, the strains were classified in terms of mg of adsorbed Cu(II) per grams of dry weight cells as reported in Figure 2.

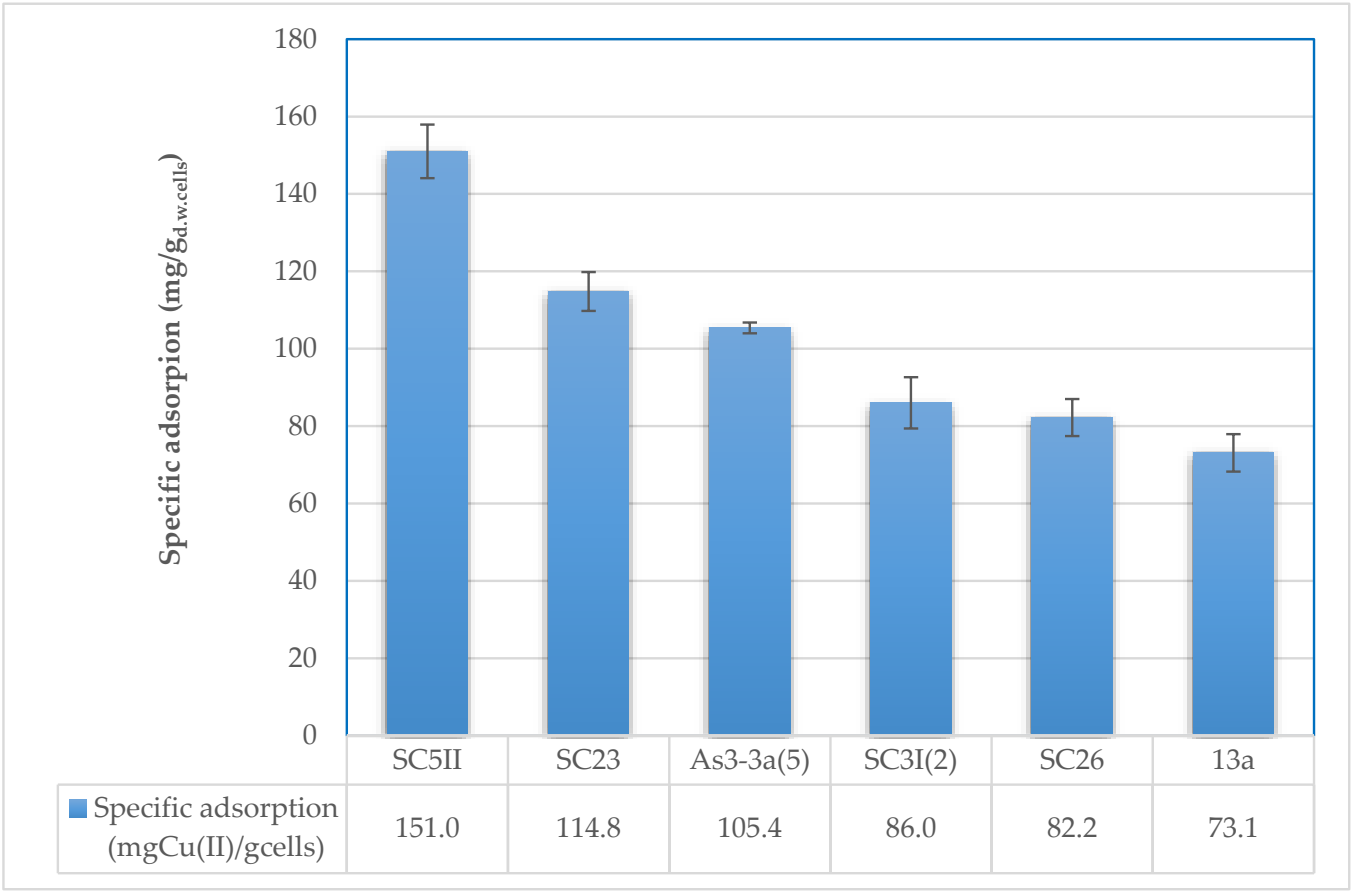


Figure 2. Specific adsorption (mgCu(II)/$g_{d \cdot w \cdot cells}$) using vials.

Starting from the obtained results, we chose to proceed in the evaluation of the behavior of the two endpoints of the list, SC5II as the strain endowed with the best adsorbing properties and strain 13a as the worst one. This choice stems from the hypothesis that the difference in terms of adsorption was related to the different type or different distribution of EPSs on the surface of the bacteria.

To investigate the interaction of EPS with Cu(II), ^{1}H NMR titration experiments were carried out and the spectra in the presence of an increasing amount of Cu(II) were evaluated. Cu(II) is a paramagnetic ion that affects the chemical shift of protons that are close to the Cu(II) binding sites.

For the carbohydrate components of EPS samples, the ^{1}H proton signals with chemical shifts ranging from 5.8 and 4.8 ppm were attributed to α anomeric protons of the sugar units, whereas those between 4.6 and 4.2 ppm were related to the β-type configuration. In the spectrum of EPS-SC5II, four intense signals in the anomeric region at 5.40, 5.27, 5.03 and 4.95 ppm were observed as well as signals between 4.20 and 4.60 ppm (See SI, Figure S3). The spectrum of EPS-13a showed intense signals at 5.62, 5.34, 5.07 and 4.95 ppm as well. Moreover, other minor broad anomeric signals at 5.19, 5.16 and 5.12 ppm were detectable. This could indicate a different monosaccharide composition and/or increased conformational mobility in comparison with EPS-SC5II. ^{1}H signals between 4.0 and 2.9 ppm were attributed to pyranose saccharide protons and characterized, for all EPS samples, by the extent of overlapping chemical shifts, as expected for a complex polysaccharide with high

molecular weight. Signals between 2.3 and 1.2 ppm are attributed to methylated groups of 6-deoxy and acetyl sugars. In any case, the spectra showed that the carbohydrates were co-extracted with lipids and/or proteins, as demonstrated from the signals at 3.0–0.5 ppm.

The addition of up to 2.4 mmol/L of Cu(II) to the EPS-13a solution did not affect the chemical shift or a line-broadening of the ^{1}H proton signals. Conversely, upon the addition of 0.3 mmol/L of Cu(II) to EPS-SC5II, the selected anomeric protons (5.40 and 5.27 ppm) shifted downfield and showed a remarkable line-broadening, due to the reduction of the electron density on the receptor EPSs, and gradually disappeared. No shifts were observed for other anomeric protons, but they became slightly broad. All other signals at the up-field region remained unaffected or exhibited insignificant variations. These findings supported the better interaction of Cu(II) with EPS-SC5II in comparison with EPS-13a as reported above.

From a structural point of view, the two strains were analyzed by other different analytical techniques, i.e., Dynamic Light Scattering (DLS), FT-IR analysis (see SI, Table S1 and Figures S4 and S5) and SEM spectroscopy (Figure 3 and see SI, Figures S6 and S7), both with and without the presence of the coordinated copper ion.

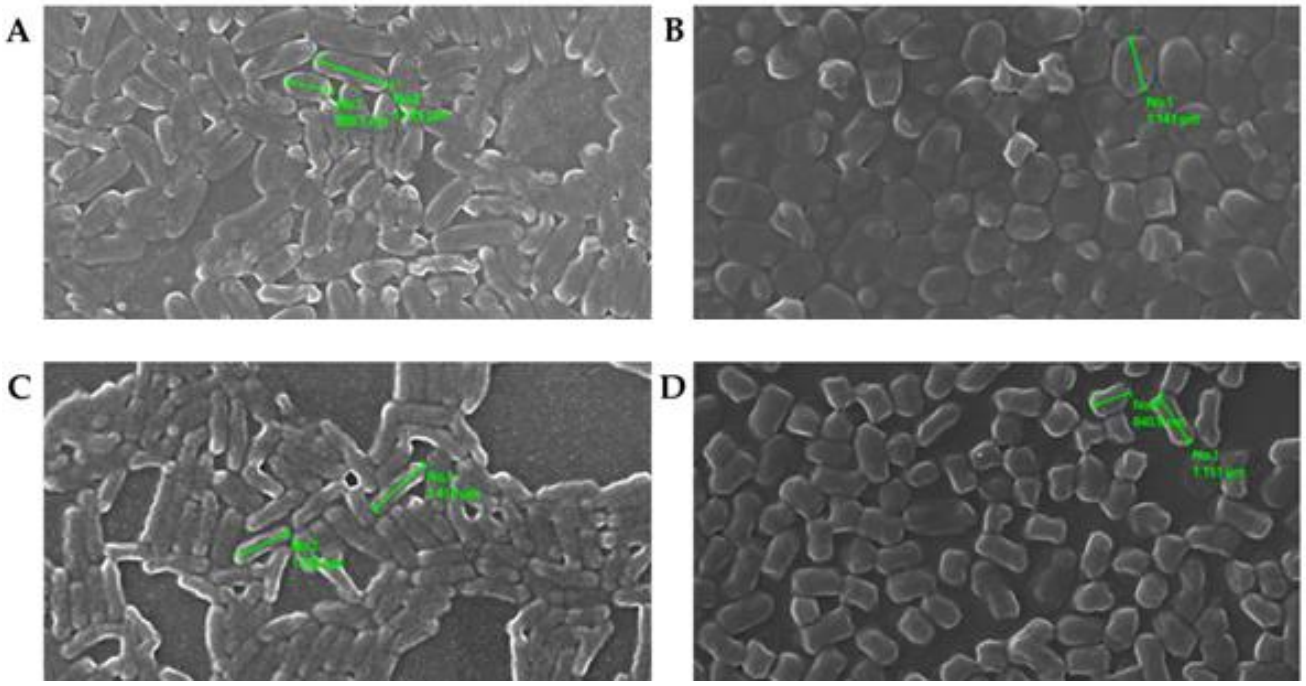
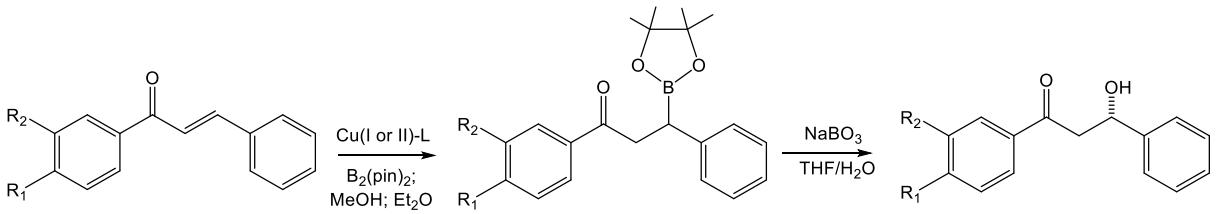


Figure 3. SEM analysis (10,000×) of 13a and SC5II before (**A**,**B**, respectively) and after absorption of copper(II) (**C**,**D**).

The differences between the two strains from a structural point of view, probably related not only to the different nature of the strain but also to the different amount of EPSs produced by 13a and SC5II strains as indicated by NMR spectroscopy, were confirmed by all the structural analyses, both FT-IR (changes in the anomeric region of the spectrum of SC5II at 772 cm^{-1} [37]; see SI Figure S5) and SEM (disaggregated structures in presence of coordinated copper always for SC5II, Figure 3D). The DLS analysis confirmed a slight decrease of dimension along with an increase in the zeta potential values due to the coordination of positively charged copper. A deeper investigation of the surface of the catalysts was realized by SEM-EDX analysis, confirming the presence of copper on the surface due to the adsorption properties of EPS by the selected strains. Moreover, SC5II showed a higher capability of adsorbing copper for both the oxidation state in comparison with 13a (32.6% with Cu(II) and 29.9% with Cu(I) vs. 13.3% with Cu(II) and 16.2% with Cu(I) for 13a, see SI Figures S9–S14).

In order to prove the validity of our approach in the circular management of metals, we have investigated the proposed copper-strain systems in catalysis. For evaluating the capability of the new hybrid catalysts obtained by the merging of the chelating properties of bacteria with the transition metals to be recycled, we decided to set up a catalytic reaction employing copper in both its oxidation states, Cu(I) and Cu(II), using a $B_2(pin)_2$ addition reaction on α,β-unsaturated substrates [38,39]. The selected substrates belong to the class of chalcone-derivatives, well-known intermediates of pharmaceutical interest [40–43] (Scheme 1).

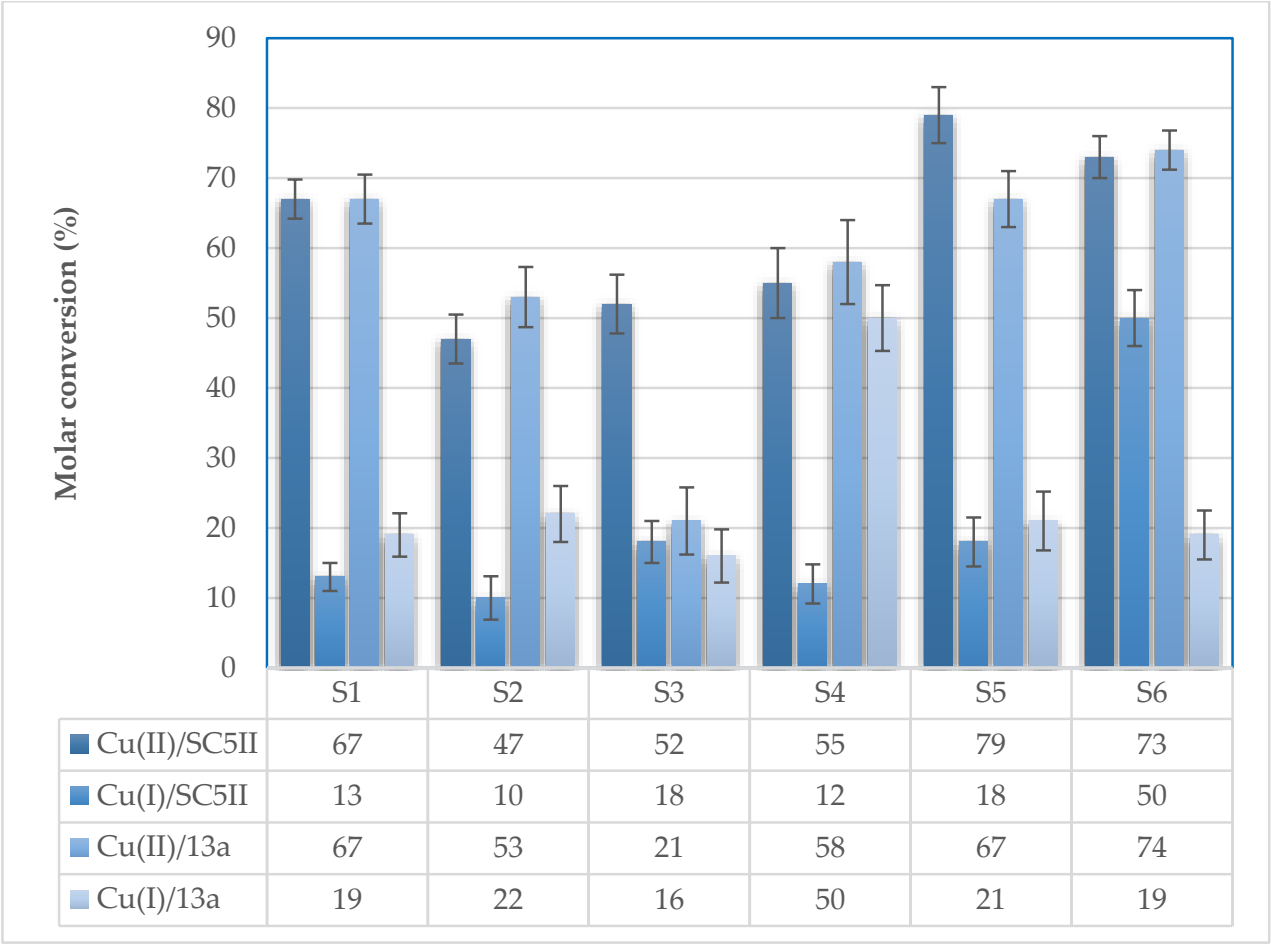


S1 R'= -Cl; R''=-H
S2 R'=-OCH_3; R''=-H
S3 R'=-CF_3; R''=-H
S4 R'=-H; R''=-Cl
S5 R'=-CH_3; R''=-H
S6 R'=-H; R''=-H

Scheme 1. Asymmetric addition of Bis(pinacolate)diboron on α,β-unsaturated substrates.

Catalytic experiments were set up on the basis of the behavior of the standard substrate S1 [13]: diethyl ether as solvent, MeOH as protic co-solvent and a substrate ratio: catalyst = 20:1. Moreover, as a main advantage, the reaction proceeded even in air, avoiding the use of an inert atmosphere as is often required for metal-based catalysts. Initially, the selected copper salt ($Cu(OAc)_2 \cdot H_2O$), the two strains SC5II and 13a, the preformed complex (1,10-PN-Cu(I)) and the two hybrid complexes formed by SC5II and 13a after adsorption of either Cu(I) or Cu(II) ions, were used as catalysts to study the catalytic capacity of the different species in this type of reaction. It is worth noting that the bacteria cells, after growth, were repeatedly washed with the reaction solvent and centrifuged to minimize the amount of water present in the reaction mixture and thus make the reaction conditions comparable for all types of possible catalysts.

When the copper salt $Cu(OAc)_2 \cdot H_2O$ and the resulting complex (1,10-PN-Cu(I)) were used, under the tested conditions, the reaction proceeded with a conversion of 10–15%, whereas for both the strains, the reaction did not proceed at all in the absence of the metal ion, thus underlining that the bacteria themselves do not have any catalytic activity in this type of reaction. With regards to the experiment employing the two strains in the presence of the metal ion in its two oxidation states, i.e., as Cu(I) or Cu(II), better results were obtained in terms of conversion for both strains in the presence of copper in the oxidized form (67% with Cu(II) vs. 13–19% with Cu(I)). The reaction conditions were therefore furthermore varied, such as by the type of inoculum, the quantity of cells, the reaction temperature, the solvent used (for example a green solvent as 2-MeTHF) and the substrate/catalyst ratio. The reaction conditions considered optimal are the following: 30 °C in the presence of diethyl ether as solvent and a substrate/catalyst ratio of 10:1. In particular, for the latter condition, we decided to work always in presence of 3 mg of coordinated copper in order to standardize the amount of active catalyst in all the reactions set up, checking the absorbent capacity of the strains each time after each growth by UV-Vis analysis. We then extended the scope of the reaction to different substituted substrates (S2–S6), as shown in Scheme 1. From the reported results (Figure 4), it is possible to see how with both strains, under the tested conditions, complete conversion into the product was never achieved even after 15 h of reaction, even if in the case of S5 and S6 the product was isolated by an appreciable 79% and 73%, respectively.



	S1	S2	S3	S4	S5	S6
Cu(II)/SC5II	67	47	52	55	79	73
Cu(I)/SC5II	13	10	18	12	18	50
Cu(II)/13a	67	53	21	58	67	74
Cu(I)/13a	19	22	16	50	21	19

Figure 4. Catalytic evaluation of Cu(I) or Cu(II) hybrid catalysts on different substrates S1–S6.

Furthermore, for both strains, the reactions catalyzed by Cu(II) lead to higher conversion; regarding the reactions catalyzed by Cu(I), a different behavior was observed for the substrate S4 (*m*-Cl) employing the 13a strain and for the substrate S6 (*p*-H) with the SC5II strain, in which the conversions with Cu(I) were comparable to those in the presence of Cu(II).

The presence of a small amount of brick-red precipitate observed with the direct adsorption of Cu(I) had led us to hypothesize a possible preference of EPSs in favor of the oxidized rather than the reduced metal species. If the affinity was different, there could be a phenomenon of undermining in the case of Cu(I) adsorption, identifiable by the formation of the observed precipitate stemming from the exceeding copper fraction. Cu(I) adsorption and catalysis tests under an inert nitrogen atmosphere were then conducted in order to avoid the oxidation of Cu(I) by atmospheric oxygen.

As it can be seen from Table 1, the hypothesis is confirmed by the fact that for both strains, the conversions increase considerably, becoming comparable also for the substrate S1 to those obtained in the presence of Cu(II). The studied reaction could also lead to an enantiomerically enriched product. The enantiomeric excess sets at around 15% for all the substrates and for both the strains, even when working under an inert atmosphere, but it should be emphasized that, however, the fact that an enantiomeric excess is present, albeit minimal, makes us suppose that the induction of chirality depends on the structure of the EPSs and the type of coordination to the metal center occurring with the approaching of the substrate.

Table 1. Catalytic reactions under an inert atmosphere in comparison to an air one. The enantiomeric excess was evaluated by HPLC equipped with chiral column (Lux Cellulosa 4) [14].

Bacteria	Atmosphere	Molar Conversion (%)	e.e. (%)
13a	inert	47	12
13a	air	19	14
SC5II	inert	72	15
SC5II	air	13	18

Furthermore, consecutive catalytic cycles were carried out, always reusing the same cellular biomass, in order to determine for how many cycles the catalyst can be used, fulfilling our plan to develop a strategy matching the principles of a circular economy. The experiments were performed with strain 13a and substrate S1, using diethyl ether as the reaction solvent at 30 °C. The results obtained showed that the reaction displayed a positive outcome for eight consecutive catalytic cycles, although the conversion progressively decreased over time (Figure 5).

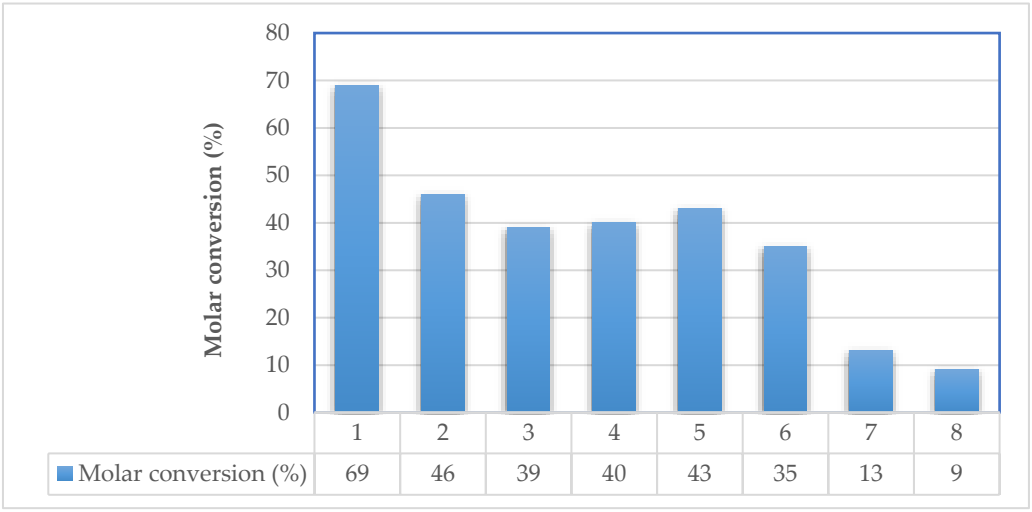


Figure 5. Repeated batch of catalytic reaction using Cu(II)-13a on S1.

These data showed that it is therefore possible to reuse the catalyst for several consecutive cycles, leaving open future possibilities for the development of a continuous process (Repeated Batch) at an industrial level.

3. Conclusions

Depollution of wastewater from heavy-metal pollutants and the possibility of converting them into valuable organometallic complexes useful for catalytic organic transformations perfectly matches the idea of a circular economy that should be restorative by design. In this context, EPS-mediated biosorption produced by *Serratia plymuthica* could be a valid tool for heavy-metal recovery. A preliminary screening of the selected bacterial strains was realized in order to quantify the differential ability towards copper chelation by the biomass. This was realized by the development of a UV-Vis spectrophotometric method, taking into account the formation of a copper-1,10-phenanthroline complex, which has been revealed to be comparable in terms of accuracy to the ICP-MS technique, but is more practical and less time-consuming. Different conditions for cell adsorption such as the type of inoculum, the incubation time and the copper concentration were evaluated, revealing SC5II as the strain characterized by the most adsorbing abilities (151.0 mg of Cu(II)/g cells), conversely to 13a, which was less effective in copper chelation (73.1 mg of Cu(II)/g cells). In order to shed light on the origin of this behavior, ^{1}H-NMR spectroscopy analyses were performed on both EPS samples. The addition of Cu(II) to the EPS-13a solution

did not affect ^{1}H proton signals. However, the addition of Cu(II) to EPS-SC5II shifted downfield and showed a remarkable line-broadening of the selected anomeric protons. These data confirmed the previous results and demonstrated a better interaction of Cu(II) with EPS-SC5II in comparison with EPS-13a. The resulting copper complexes were thus deeply investigated by DLS, SEM and FT-IR spectroscopies, confirming the difference in terms of behavior between the two strains.

Finally, we explored the possibility of recycling the recovered copper as a hybrid catalyst in the catalytic enantioselective β-borylation of α,β-unsaturated chalcones. Both oxidation states of copper were evaluated within this reaction, showing better results in terms of conversion in the presence of copper in its oxidized form for both strains (67% with Cu(II) vs. 13–19% with Cu(I)). Moreover, products were isolated in an enriched form (more or less 15% e.e.) underlying the structural impact of the supposed chiral EPS on the stereoselective outcome of the reaction. Surprisingly, the catalyst proved to be effective for up to eight consecutive cycles, thus paving the way for a future industrial application within the depollution process of galvanic wastewaters.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/catal12040433/s1, Figure S1: UV-Vis of 1,10-phenanthroline (PN) with Cu(I) and the corresponding calibration line; Figure S2: Correlation curve between UV and ICP data for determination of copper concentration; Figure S3: ^{1}H NMR spectra of EPS-SC5II in presence of various Cu(II) concentrations, pH 5.1, T = 25 °C; Figure S4: ^{1}H NMR spectra of EPS-13a in presence of various Cu^{2+} concentrations, pH 5.1, T = 25 °C; Figure S5: FT-IR of 13a (line red), 13a-Cu(II) (line black) and 13a-Cu(I) (line blue); Figure S6: FT-IR of SC5II (line red), SC5II-Cu(II) (line black) and SC5II-Cu(I) (line blue); Figure S7: SEM analysis of 13a with or without Cu(II) (5000× and 10,000×); Figure S8: SEM analysis of SC5II with or without Cu(II) (5000× and 10,000×); Figure S9: EDX analysis of 13a strain; Figure S10: EDX analysis of 13a with adsorbed copper(II); Figure S11: EDX analysis 13a with adsorbed copper(I); Figure S12: EDX analysis of SC5II strain; Figure S13: EDX analysis of SC5II with adsorbed copper(II); Figure S14: EDX analysis of SC5II with adsorbed copper (I); Table S1: DLS analysis of 13a and SC5II with or without coordinated copper; Table S2: Elemental analysis of strains 13a and SC5II with or without Cu(II) or Cu(I) coordinated. References [18,38,44,45] are cited in the Supplementary Materials.

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