Electronic Supporting Information

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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General. All reactions involving the use of substances sensitive to atmospheric oxygen were conducted in an inert nitrogen atmosphere. The anhydrous solvents were used according to the methodologies reported in the literature (Handbook of Preparative Inorganic Chemistry).
NMR spectra of the EPS solutions (EPS-13a and EPS-SC5II) were recorded at 25°C using a Bruker AV 600 spectrometer (Bruker, Germany) equipped with a TXI *z*-gradient probe operating at a frequency of 600.10 MHz for ^{1}H nucleus.
Absorption spectra were measured on a Shimadzu UV-3600 spectrophotometer double-beam UV–VIS–NIR spectrometer and baseline corrected. Quartz cuvettes with path length of 10.0 mm were used for the recording of UV-Vis absorption spectra. ICP-MS data were recorded with BRUKER aurora M90 ICP-MS (MA, USA).
The enantiomeric excesses and the molar conversions of the reactions were monitored by HPLC analysis with Merck-Hitachi L-7100 equipped with Detector UV6000LP and chiral column (Lux Cellulose 4).
For dynamic light scattering (DLS), the measurements were conducted on a Delsa Nano C Particle Analyzer (Beckman Coulter, operative wavelength 655 nm).
FTIR spectra were collected by using a Perkin Elmer (MA, USA) FTIR Spectrometer "Spectrum One" in a spectral region between 4000 and 450 cm^{-1} and analyzed by transmittance technique with 32 scansions and 4 cm^{-1} resolution.
Scanning electron microscopy (SEM) images were recorded with SEM-EDS JSM-IT500 LV of JEOL Spa (Italia) with an acceleration voltage of 20 kV.
Elemental analyses were performed using a Perkin Elmer SeriesII/CHNS/O 2400 Analyzer. The SEM-EDX measurements were carried out on all samples by means of a Hitachi TM-1000 scanning electron microscope equipped with an energy dispersive X-ray spectrometer (Oxford Instruments SwiftED).
Identification of environmental isolates. Strains were identified according to 16S rRNA nucleotide sequence analysis.

Strain	Species
SC5II	*Serratia plymuthica*
As3-5a	*Serratia plymuthica*
SC23	*Serratia plymuthica*
SC3I(2)	*Serratia plymuthica*
SC37	*Serratia plymuthica*
SC26	*Rhodococcus qingshengii*

Culture conditions. Bacterial strains were routinely maintained on LB medium slants (18 g/L agar, 5 g/L yeast extract, 10 g/L tryptone, 5 g/L NaCl, pH = 7.0). The strains, grown on LB medium slants for 48 h at 28 °C, were inoculated in a 500 mL Erlenmeyer flask containing 100 mL of the same liquid medium and incubated on a reciprocal shaker (150 rpm) for 48 h at 28 °C. In the case of liquid-liquid growth of the microorganisms, a 24 h pre-inoculation was carried out (20 mL liquid medium in 100 mL Erlenmeyer flask) and later they were inoculated (0.5 vol/vol) in 50 mL liquid medium in a 500 mL Erlenmeyer flask. Bacterial strains were incubated on a reciprocal shaker (150 rpm) for 48 h at 28 °C before testing.

Cell growth evaluation. Cell growth was evaluated by UV/Vis analysis, using optical density as a parameter, and by evaluation of cell's dry weight. The test sample were analyzed using a spectrophotometer at 600 nm. For the evaluation of dry weight, 20 mL of broth culture were centrifuged (6000 rpm for 15 min), the pellet was washed twice with milliQ water and re-suspended in milliQ water. The cell suspension was placed in an oven for 12 h at 104 °C.

Adsorption procedure. Cells obtained by centrifugation (6000 rpm for 15 min) of the culture broth (20 mL) were washed twice with millQ water. At the pellet, 6 mL of the solution containing 3 mg/mL of $Cu(OAc)_2 \cdot H_2O$ was added and the suspension was put on disk rotator (60 rpm for 30 min). For Cu(I), 2 eq. of ascorbic acid were added to the methanolic suspension. After the copper cells absorption, the suspension was centrifuged (6000 rpm for 15 min) and the supernatant analyzed by UV-Vis spectroscopy.

Cu(II) adsorption determination. After Cu(II) cells adsorption, 1 mL of supernatant containing free Cu(II) was put in a 5 mL graduated flask and afterward 2 eq. of 1,10-phenanthroline (PN)was added followed by 1 eq. of ascorbic acid to reduce in situ Cu(II) to Cu(I). The methanol was added up to 5 mL and the sample was diluted 1 to 10 and analyzed by UV-Vis spectroscopy. By calibration line the free Cu(II) was evaluated and the adsorbed Cu(II) was calculated by difference between added Cu(II) and free Cu(II).

UV-Vis spectroscopy analysis: calibration line: 3 mg/mL of $Cu(OAc)_2 \cdot H_2O$ was dissolved in 50 mL of methanol and the solution was used to obtain 5 solutions at different concentrations of Cu(II): 0.0205, 0.0154, 0.013, 0.0054, 0.0026 mg/mL. To the solutions 1 eq. of 1,10-phenanthroline (PN) and 2 eq. of ascorbic acid were added one by one. The so formed red Cu(I) complex gives an absorption maximum between 430-445 nm. The data were used to establish the correlation of absorbance and unabsorbed copper concentration.

ICP-MS spectroscopy analysis: calibration line: The 5 solutions used for UV-Vis spectroscopy were diluted with methanol by a factor of 250 and 500, in accordance with the sensibility of the instrument.

Catalyst preparation. After absorption of Cu(II) as (L-Cu(II)), cells were washed two times with methanol and were used for the catalytic reaction in such an amount so that 3 mg of Cu(II) are present in the reaction system. In the case of the Cu(I)-catalyzed (L-Cu(I)) reaction, ascorbic acid (1.1 eq.) was added soon after the second wash.

General procedure for catalyzed addition of Bis(pinacolate)diboron on α,β-unsatured substrates. 0,1 eq. of substrate (S1-S6), 1.2 eq. of Bis(pinacolate)diboron ($B_2(pin)_2$) were placed in a round bottom flask and the catalyst (L-Cu(II) or L-Cu(I)) was transferred into the reaction system with 5 mL of Et_2O. The reaction mixture was magnetically stirred at room temperature. After 15 h the suspension was centrifuged (6000 rpm, 10 min) and the organic phase was concentrated under vacuum. To the residue dissolved with 4 mL of a THF/H_2O mixture (1.5:1), an excess of $NaBO_3.H_2O$ was added and the resulting mixture was stirred for 2 h at room temperature.
The mixture was then extracted with EtOAc (5 mL x 2 times), the collected organic phases dried over anhydrous Na_2SO_4 and the solvent evaporated. The sample was analyzed by ^{1}H-NMR and HPLC analyses.[1-4]
HPLC condition for *3-(4-chlorophenyl)-3-hydroxy-1-phenylpropan-1-one*: *S*-isomer: 5.7 min, *R*-isomer: 8.5 min.

EPS extraction. 1.1 g of microbial biomass was transferred into a balloon flask containing 30 mL of demineralized water and the mixture heated under reflux for 40 min. The sample was centrifuged at 5000 rpm at 20 ° C for 10 minutes, the supernatant was collected, and the pellet was discarded. The solvent was

concentrated in vacuo up to about 3 mL; then 9 mL of ethanol was added and left overnight at 4 ° C to promote the EPS precipitation. The day after, the solution was centrifuged at 5000 rpm, at 20 °C for 20 minutes; the supernatant (composed of ethanol and water) was discarded, whereas the EPS was washed with 10 mL of ethanol and dried in vacuo for several hours. From 1.1 g of EPS microbial biomass 49.6 mg of EPS were obtained as a white solid. (4.3 % yield)

Nuclear Magnetic Resonance analysis. 6-7 mg of EPS were dissolved in 550 µL of 99.96% D_2O, the pH was adjusted at 5.1 by a diluted solution of NaOD. The EPS samples were centrifuged for 2 minutes, and the supernatants were put in a 5 mm NMR tubes. The ^1H NMR spectra were acquired with 256 NS, 16 K points in resolution and calibrated on the residual water signal set at 4.78 ppm. Stock solution (0.15 mmol/ mL) of Cu(II) was prepared by dissolving $CuCl_2$ in D_2O, pH 5.0. NMR titrations were performed by adding increasing amounts of metal to the EPS samples from 0 to 2.4 mM.

DLS analysis: the DLS measurements were performed on dispersions of the particles in EtOH (ca. 0.1 mg/mL).

FT-IR analysis. The analysis was realized after lyophilization of the samples.

SEM analysis: Before being inserted into the instrumentation, part of the samples were fixed on aluminum stubs (ø 12.5 mm; 3.2 x 8 mm pin) and dried: by means of a pipette a drop of the material was made to fall on a coated stub from carbon-based adhesive scotch tape, on which a slide has in turn been glued.
The material was analyzed after metalization in gold by means of a SCANCOAT SIX SPUTTER COATER by the company EDWARDS (1996).
The photos (in SE) were taken under high vacuum (HV) conditions, using an acceleration voltage of 20 kV, a load current of 2.52 / 2.53 µA and a beam current (PC) of 40 µA.
The other values in the report are mostly variable depending on the analysis conditions and the height of the sample. In the case of WD, the working distance tends to vary between 10.7 and 10.9 mm between the analyzed samples.

EDX analysis. The analysis was realized after lyophilization of the samples

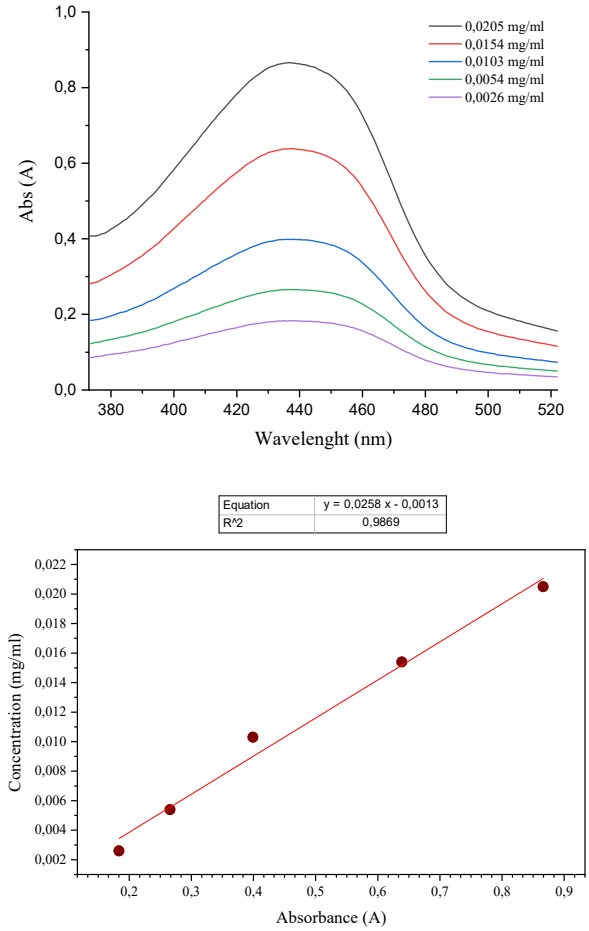


Figure S1. UV-Vis of 1,10-phenanthroline (PN) with Cu(I) and the corresponding calibration line.

UV (ppm)	ICP (ppm)
20,5	19,645
15,4	13,68167
10,3	8,586667
5,4	5,915833
2,6	2,929167

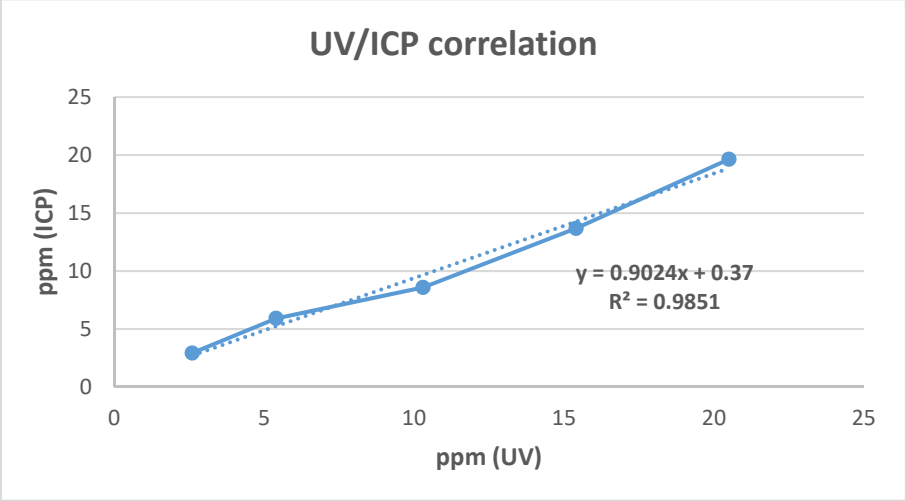


Figure S2. Correlation curve between UV and ICP data for determination of copper concentration.

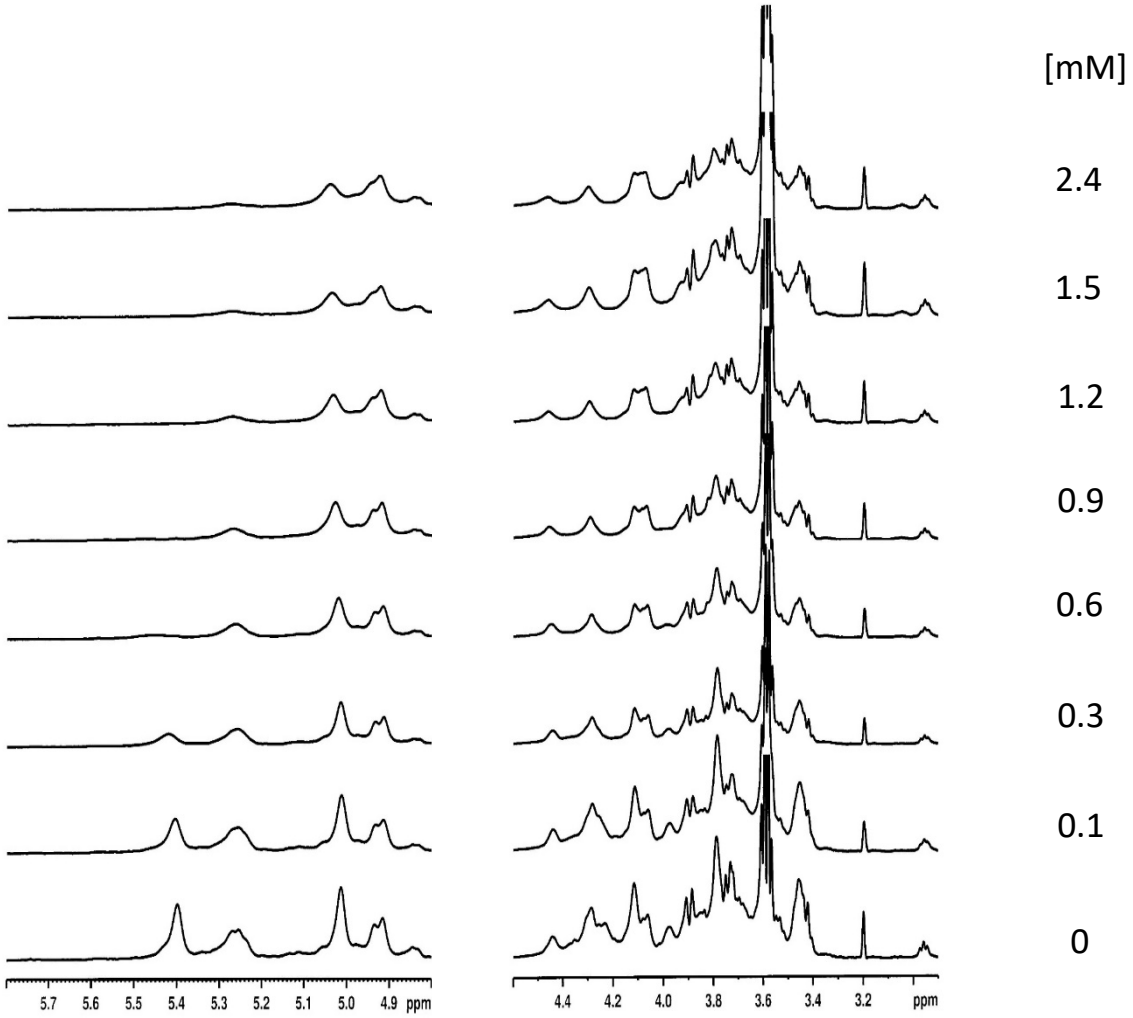


Figure S3. ^{1}H NMR spectra of EPS-SC5II in presence of various Cu(II) concentrations, pH 5.1, T = 25 °C.

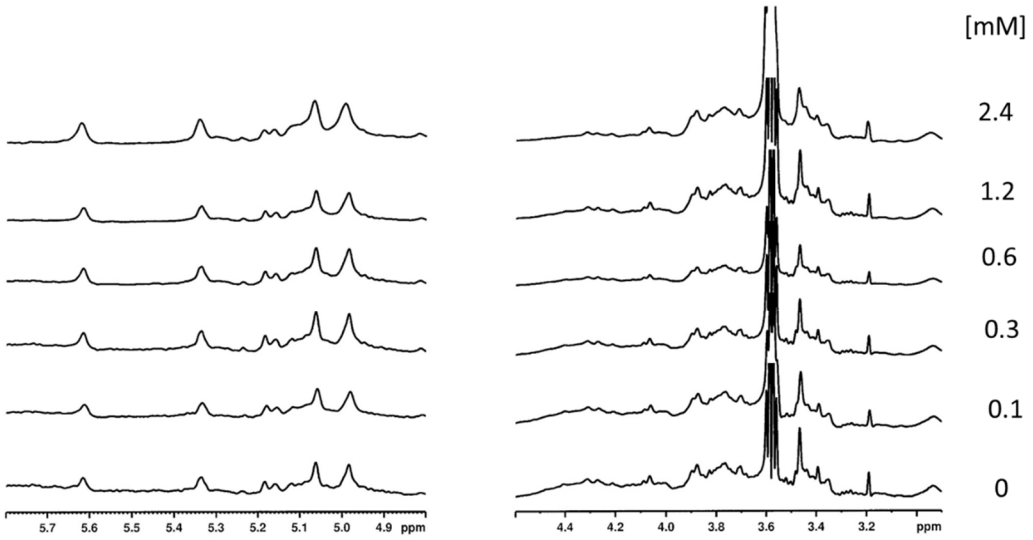


Figure S4. ^{1}H NMR spectra of EPS-13a in presence of various Cu^{2+} concentrations, pH 5.1, T=25°C.

Table S1. DLS analysis of 13a and SC5II with or without coordinated copper.

Bacteria	Sample	Size (nm)	Z Potential (mV)
13a	Only cells	845.30 ± 38.61	-35.15 ± 0.52
13a	Cells + Cu(II)	739.80 ± 12.89	-17.02 ± 0.54
13a	Cells + Cu(I)	781.40 ± 22.,40	-24.26 ± 0.34
SC5II	Only cells	3008.00 ± 86.70	-29.83 ± 1.06
SC5II	Cells+ Cu(II)	2789.00 ± 174.30	-16.24 ± 0.77
SC5II	Cells+ Cu(I)	2458.00 ± 293.00	-18.17 ± 0.57

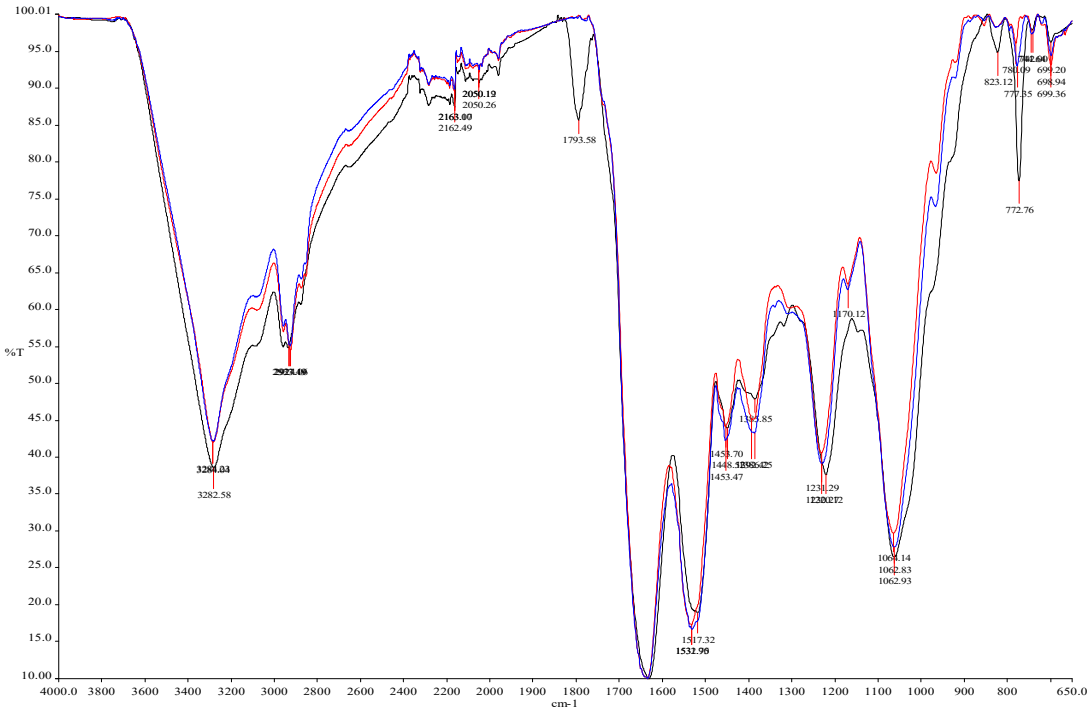


Figure S5. FT-IR of 13a (line red), 13a-Cu(II) (line black) and 13a-Cu(I) (line blue).

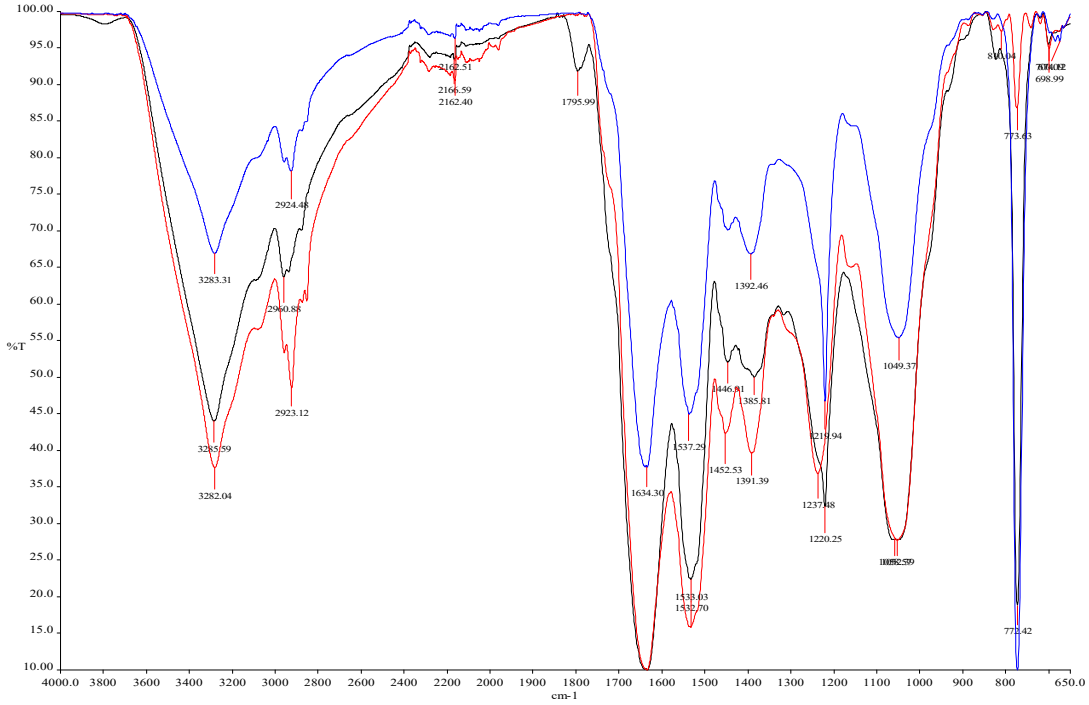


Figure S6. FT-IR of SC5II (line red), SC5II-Cu(II) (line black) and SC5II-Cu(I) (line blue).

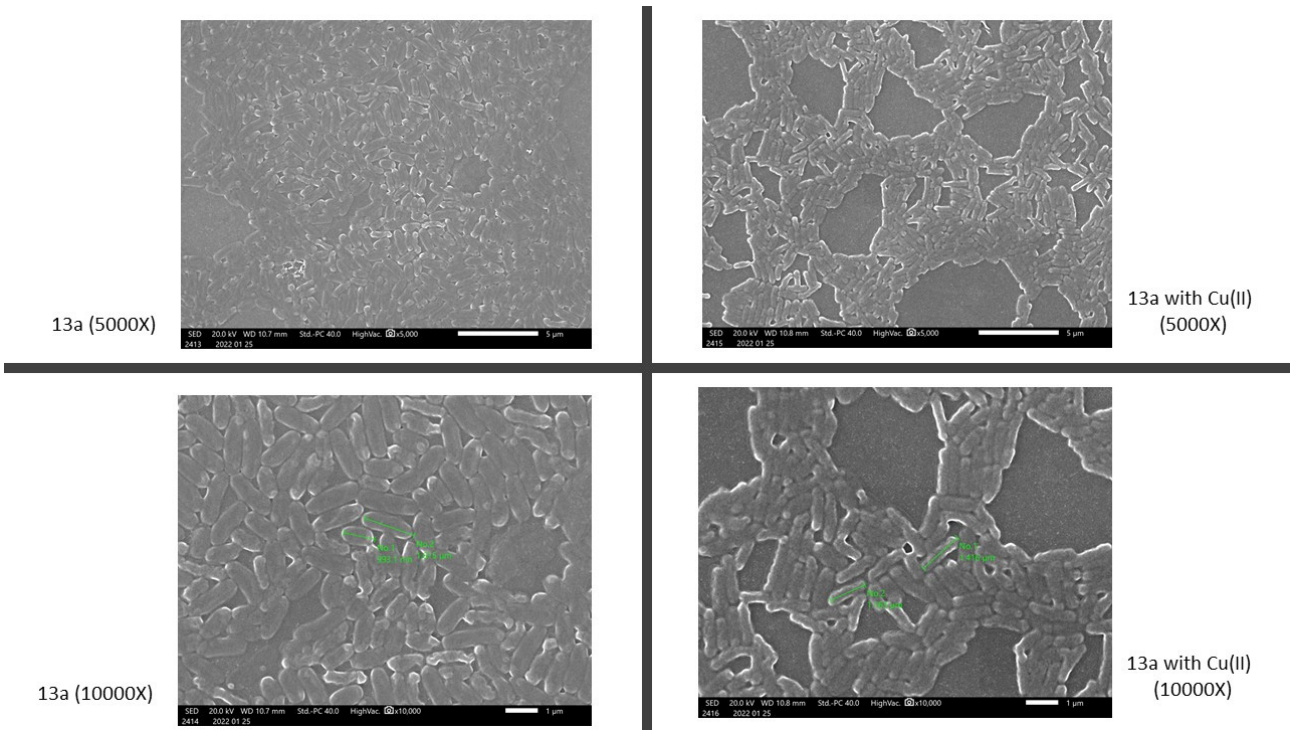


Figure S7. SEM analysis of 13a with or without Cu(II) (5000× and 10,000×).

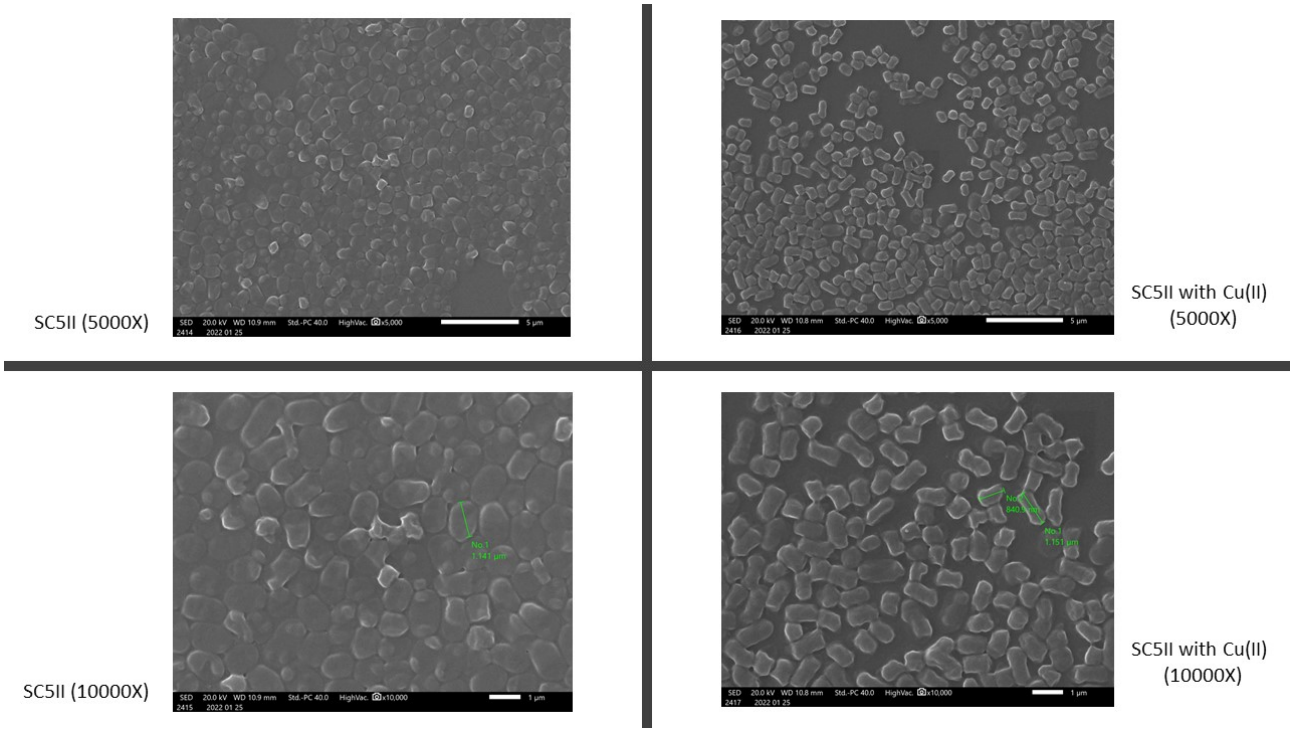


Figure S8. SEM analysis of SC5II with or without Cu(II) (5000× and 10,000×).

Table S2. Elemental analysis of strains 13a and SC5II with or without Cu(II) or Cu(I) coordinated.

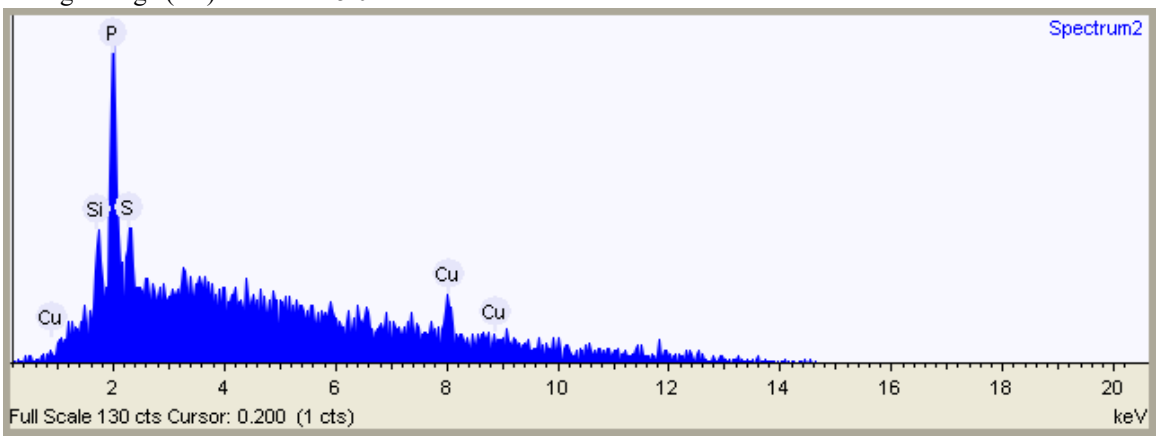
Sample	C %	H %	N %	Total % in C, H, N
13a	48.52	7.32	12.83	68.67
13a with Cu(II)	47.42	6.78	12.55	64.75
13a with Cu(I)	45.04	6.45	11.83	63.32
SC5II	46.59	7.07	10.82	64.48
SC5II with Cu(II)	44.77	6.65	10.49	61.91
SC5II with Cu(I)	43.19	6.09	9.94	59.22

Spectrum details

Project	New project
Spectrum name **13a**	Spectrum2

Acquisition conditions

Acquisition time (s)	73.6
Process time	4
Accelerating voltage (kV)	15.0



Quantification Settings

Quantification method All elements (normalised)

Summary results

Element	Weight %
Silicon	14.7
Phosphorus	59.8
Sulfur	25.5
Copper	0.0

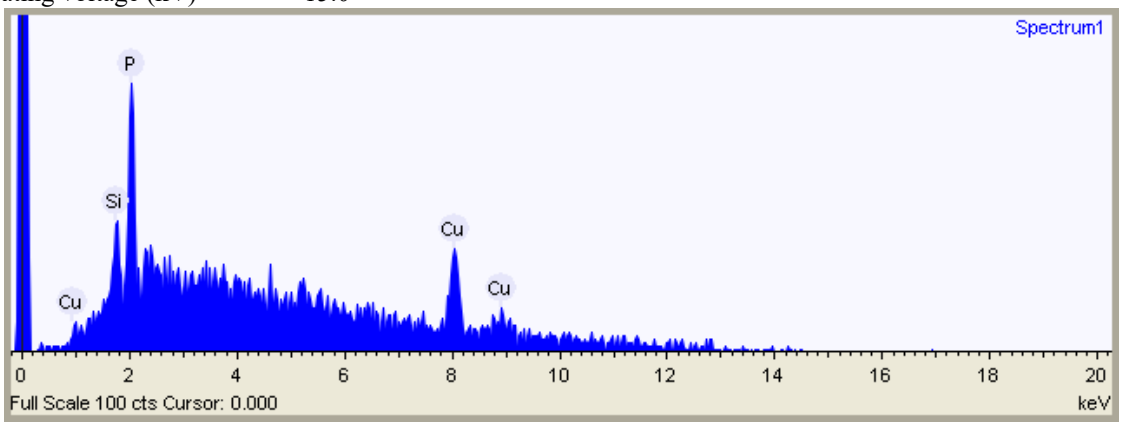
Figure S9. EDX analysis of 13a strain.

Spectrum details

Project	New project
Spectrum name **13a with Cu(II)**	Spectrum1

Acquisition conditions

Acquisition time (s)	51.0
Process time	4
Accelerating voltage (kV)	15.0



Summary results

Element	Weight %
Silicon	16.5
Phosphorus	70.1
Copper	13.3

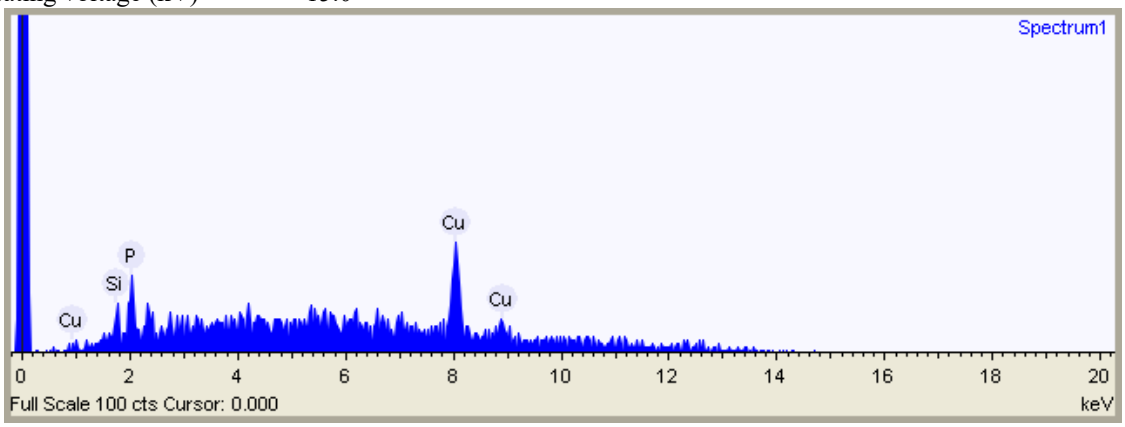
Figure S10. EDX analysis of 13a with adsorbed copper(II).

Spectrum details

Project	New project
Spectrum name **13a with Cu(I)**	Spectrum1

Acquisition conditions

Acquisition time (s)	46.1
Process time	4
Accelerating voltage (kV)	15.0



Summary results

Element	Weight %
Silicon	22.0
Phosphorus	61.8
Copper	16.2

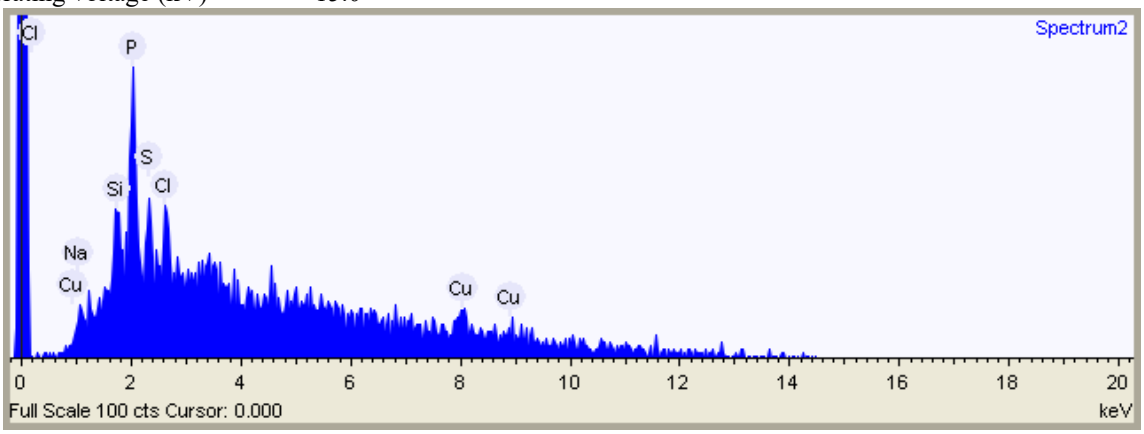
Figure S11. EDX analysis 13a with adsorbed copper(I).

Spectruum details

Project	New project
Spectrum name **SC5II**	Spectrum2

Acquisition conditions

Acquisition time (s)	54.8
Process time	4
Accelerating voltage (kV)	15.0



Summary results

Element	Weight %
Sodium	14.0
Silicon	10.0
Phosphorus	37.8
Sulfur	18.9
Chlorine	17.0
Copper	2.2

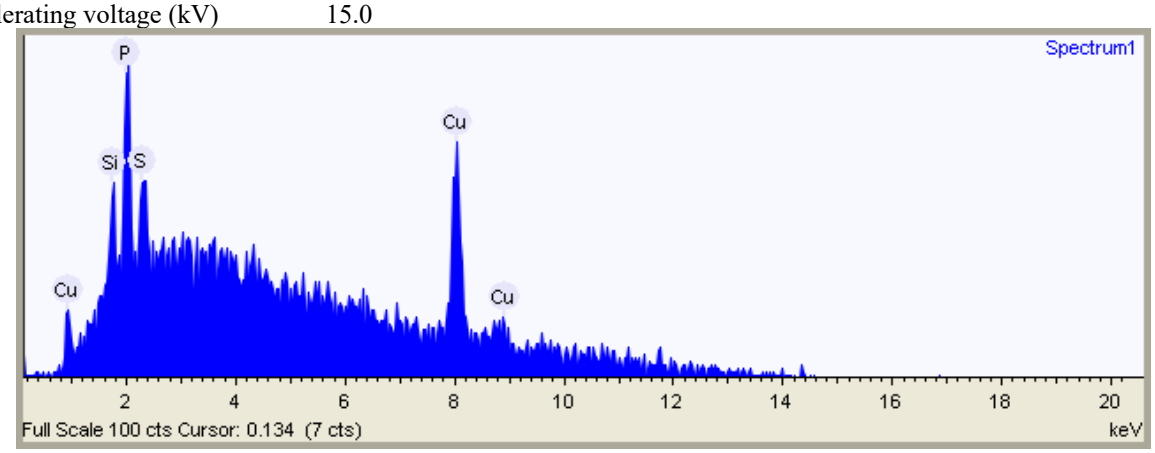
Figure S12. EDX analysis of SC5II strain.

Spectrum details

Project	New project
Spectrum name SC5II with Cu(II)	Spectrum1

Acquisition conditions

Acquisition time (s)	92.5
Process time	4
Accelerating voltage (kV)	15.0



Summary results

Element	Weight %
Silicon	11.9
Phosphorus	35.9
Sulfur	19.6
Copper	32.6

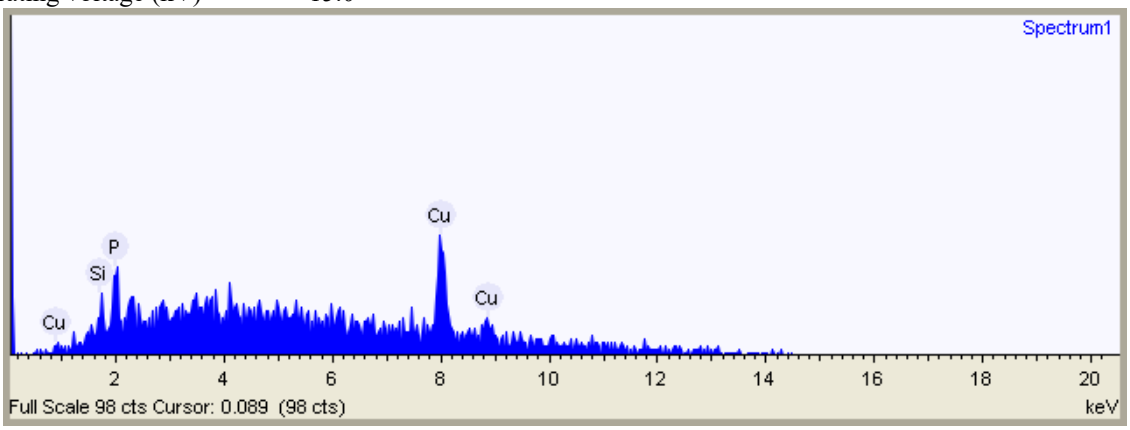
Figure S13. EDX analysis of SC5II with adsorbed copper(II).

Spectrum details

Project	New project
Spectrum name **SC5II with Cu(I)**	Spectrum1

Acquisition conditions

Acquisition time (s)	46.2
Process time	4
Accelerating voltage (kV)	15.0



Summary results

Element	Weight %
Silicon	19.7
Phosphorus	50.4
Copper	29.9

Figure S14. EDX analysis of SC5II with adsorbed copper (I).

References

1. R. Gandolfi, G. Facchetti, M. S. Christodoulou, M. Fusè, F. Meneghetti and I. Rimoldi, *ChemistryOpen*, 2018, **7**, 393-400.
2. L. Zhu, T. Kitanosono, P. Xu and S. Kobayashi, *Chem. Commun.*, 2015, **51**, 11685-11688.
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4. M. Heidlindemann, A. Berkessel and H. Gröger, *ChemCatChem*, 2017, **9**, 1383-1388.