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IN SITU X-RAY ABSORPTION STUDY OF COPPER-SURFACE COMPLEXES: MICROBIAL INFLUENCED OXIDATION OF METALLIC COPPER

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Extended X-ray absorption fine structure (EXAFS) measurements of solid and colloidal $Cu^{\pm 0}$, Cu-oxides and hydroxides have been performed as model compounds, involved in microbially influenced corrosion.

Normally metals dissolved in natural waters due to corrosion processes, and often become sorbed onto various solid materials, e.g. clay minerals, oxides or surfaces covered with exopolymeric materials of organic origin produced by microorganisms [1-3]. In the last years it has been observed that copper pipes in large offices, buildings and hospitals deteriorate under the influence of microorganisms, e.g. *Pseudomonas fluorescence, Ps. aerugenosa, Nocardia, Azotobacter vinelandii, Xanthomonas campestris*, and other uncharacterized strains including fungi and diatoms, known to be present in surface waters. The mechanisms of these corrosion processes are still unknown. The biomineralization of copper ions as well as of metallic copper clusters from the solid Cu-materials imposes considerable problems to drinking water supplies due to the release of copper ions into the environment. One important aspect of this phenomenon is the partitioning of ions at the interfaces between solids, e.g. copper metal, and liquids, e.g. water and ions in the presence of an exopolymeric biofilm of microbial origin. This biofilm is mainly composed of polysaccharides of anionic nature, highly crosslinked of molecular weight of approximately of 1.9 to 2.5 million Dalton, containing equally spaced peptides with a constant ratio of serine to threonine, chemically very reactive histidines and a few tyrosines [4]. The biofilm is insoluble in water also when attached to the solid copper surface. This interaction between the biofilm and the copper surfaces is poorly understood at the atomic or molecular level. Therefore detailed information on the Cu-surfaces and complexes at the molecular level is warranted for a quantitative description of these interfacial processes, including the biomineralization of copper by microorganisms [3].

Among the appropriate physical and spectroscopic techniques that are commonly used to study interfaces X-ray absorption spectroscopy has been applied to determine fine structural features e.g., number of nearest neighbour atoms including its identity, interatomic distances in the aqueous environment when coming into contact with the solid Cu-surfaces. Thus X-ray absorption spectroscopy (XAS) unlike Mössbauer spectroscopy or photoelectron spectroscopy produces relatively accurate measures of the stereochemistry of the surrounding ligands. This investigation of in-situ XAS of copper-metal and copper complexes generated by microbial influenced corrosion processes, as well as on Cu-metal-like-thin-films at the solid or hydrated biofilm interface, reveals the usefulness of this technique. Particularly, by identifying the type of either atoms from solution or solid, including the number of atoms in the first, second and greater coordination shells around the sorbed Cu-atom including its bonding environment reveals valuable information on the sorption and biomineralization mechanism of metallic copper or their oxides, respectively.

We obtained XAS, in particular X-ray absorption fine structure (EXAFS) spectra, at an in-house EXAFS system, which basically consists of a high-power X-ray generator (Rigaku, RU-200), a goniometer with a flat Ge (220) perfect crystal monochromator, appropriate slits, an automatic sample positioning system, and a germanium solid state detector. The X-ray source was a Ag target and operated

at 20 kV and 200 mA. The resolution of the spectrometer close to the CuK absorption edge was 2.5 hwhm [5, 6]. The EXAFS spectra were recorded as the dependence of photon energy (E) to the ratio to the transmitted photon numbers (I) to the incident beam intensity, I(0). The ratios are converted to the absorbance (μt) and plotted against the magnitude of the photon electron momentum (k), as: $\mu(k)t=\ln(I_0/I)$, with $k=2\pi[2m(E-E_0)]^{1/2}/h$ and t is the sample thickness. Data analysis including the standard procedures to analyse and validate the spectra are essentially those as described in reference 7. Curve fitting analysis was performed according to Cramer [8]. XAS and EXAFS spectra were recorded in-situ of Cu products obtained in laboratory test systems, from field incidents in a County Hospital, and from reconstitution experiments [9, 10].

Figure 1 shows the Fourier-transforms $\phi(R)$ vs. R of the EXFAS spectra of a Cu-foil, the same material and purity used for the copper pipes installed in the County Hospital, of the crystalline and noncrystalline materials in the pits which are mainly Cu_2O, the black material covering the surfaces of the pipe, mostly CuO according to the EXAFS, including the EXAF spectrum of the reconstituted biofilm. A phase shift has not been taken into account. It is obvious that the main portion of the EXAF-spectrum obtained from the materials cut off the pits is Cu_2O. Similarly, the EXAF-spectra of the black layers on the biofilm is due to CuO. Surprisingly in both spectra, there is no interference of the biofilm on the Cu_2O or CuO-spectra to be detected which is also seen in the Fourier transform of the spectra. Wet and reconstituted biofilms with copper salts of oxidation states +1 and +2 show a different Fourier-transform of EXAFS as in Fig. 1. Figure 2 shows a Fourier transform of these EXAFS, whereas Fig. 2 depicts an EXAFS of colloidal metal like $Cu^{\pm 0}$ in the presence of imidazole at pH 8.0 in 0.001 M TRIS-buffer. Note, no structurally characterized discrete Cu^{O} complexes are known. However, they do develop at inverted micellar interfaces, it seems to be an intermediate in the corrosion process. The Fourier transform of the EXAFS of Fig. 2, which correspond to a certain kind of "radial distribution function" around the Cu-atom including correction for phase shift, reveals two peaks; one at 1.97 Å. and one at 3.05 Å. The former peak can well be attributed to $[Cu(H_2O)_6]^{2+}$ since it is very similar to the EXAFS-data obtained for $Cu(NO_3)\cdot 3H_2O$ in solution containing $[Cu(H_2O)_6^{+}]$ ions. The atoms flatly scattered in a distance from the Cu-atom due to

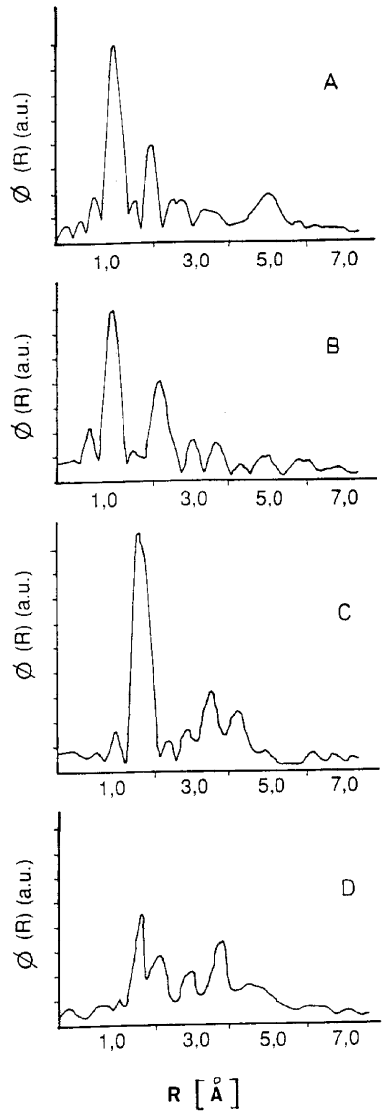

Fig. 1 Fourier transforms of the EXAFS spectra of colloidal $Cu^{\pm 0}$ (a), the mechanically removed biofilm with the associated Cu-oxides and hydroxides (b); the "reconstituted" biofilm with copper salts due to corrosion (c), and the Fourier transform of the EXAFS spectrum of colloidal $Cu^{\pm 0}$ with adsorbed imidazole on the Cu-surface (d).

disorder or vibration, and are ignored in the "radial distribution function". The value of 1.97 Å is very similar to that reported in the solid state and the determined value in solution for $[Cu(H_2O)_6]^{2+}$. The second peak at about 3.05 Å has no counterpart in distances of Cu-O, Cu-Cu, Cu-N, or Cu_2O (Fig. 1), which are 1.92 Å, 2.58 Å and 2.25 Å (peptide), respectively. However, by dehydrating the specimen the EXAFS change (Fig. 2) having main peaks at 1.92 Å, 2.28 Å, 2.58 Å and 4.85 Å corresponding to the Cu-O, Cu-N (peptide), Cu-Cu, CuO and/or Cu-Cu-

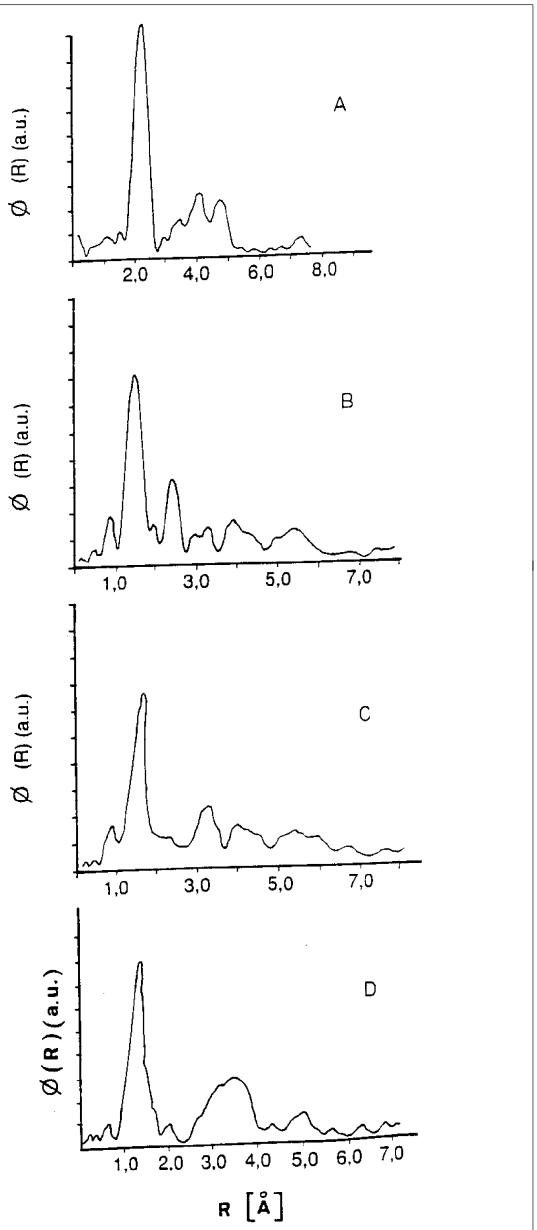

Fig. 2 Fourier transforms of the EXAFS spectra of colloidal $Cu^{\pm 0}$ (a), the mechanically removed biofilm with the associated Cu-oxides and hydroxides (b); the "reconstituted" biofilm with copper salts due to corrosion (c), and the Fourier transfrom of the EXAFS spectrum of colloidal $Cu^{\pm 0}$ woth adsorbed imidazole on the Cu-surface (d).

distances, respectively. The process of dehydration appears to be irreversible since it was not possible to retain the EXAFS of Fig. 1 upon dehydration.

EXAFS of colloidal $Cu^{\pm 0}$ and imidazole (Fig. 2d) are compared with the spectra of a thin Cu foil, CuO and Cu_2O, respectively. Although the main peak intensity is lower for the system Cu/imidazole, pH 8.0 (Fig. 3), when compared to the metal, the position of the peak coincides well with that of the Cu-foil. However, the radial distribution function is very different from that of the Cu-foil. Peaks were discovered at 2.05 Å, 2.58 Å and at 3.62 Å, respectively. The peak at 3.67 Å in the EXAFS is not seen in any of the other EXAFS obtained (Fig. 1).

The EXAFS of the mechanically detached biofilm show qualitatively CuO and Cu_2O only, which are not correlated to the biofilm in structural terms. For future experiments it may be useful to study the dehydration process of the biofilm in the presence of the Cu-salts or corrosion products in order to gain more insight into the complex nature Of $Cu^{2+}L^{(2\text{-}6)}$ (L=Ligand) with residues at or in the biofilm. However, on dehydration of the biofilm $[Cu(H_2O)_6]^{2+}$ forms CuO or Cu_2O, respectively, the peak at 1.86 Å remaining unchanged in position, but the second peak shifted from 2.85 Å to 2.60 Å before phase shift correction. It is known that Cu^{2+}-ions are easily polymerized in aqueous alkaline solutions with Cu-OH-Cu linkages and the air calcination leads to formation of CuO. The EXAFS parameters were fitted to the CuO structure (crystallite) determined by X-ray diffraction: the first and second neighbor shell distances, R_{CuO}=1.95 Å and $R_{Cu\text{-}Cu}$=2.85 Å and the coordination numbers $N_{Cu\text{-}O}$=4 and $N_{Cu\text{-}Cu}$=8, respectively. Analyzing our spectra we found a neighbour shell distance of $R_{Cu\text{-}O}$=2.09 Å and a coordination number $N_{Cu\text{-}O}$=2.8 for the first peak and $R_{Cu\text{-}Cu}$=3.15 Å and $N_{Cu\text{-}Cu}$=2.1 for the second, respectively. These studies show that the copper-oxide species existing in the dehydrated biofilm is different from CuO crystallite in the numbers of the first and second neighbours of each copper atom. These findings suggest that non-crystalline and small copper oxide aggregates are formed in this dehydrates biofilm.

The EXAFS result for the reduced form of the Cu as colloidal solution in the presence of imidazole ($R_{Cu\text{-}Cu}$=2.85 Å, N=8) is very similar to that of Cu-foil, since the Cu-Cu distance is the same as that for the copper metal as shown in Fig. 3. The Fourier transform of the EXAFS spectrum, however, reveals that the Cu metal peaks are lower than those for the Cu-foil. However, the distance of 3.01 Å precludes this possibility of direct Cu-Cu bonding. It was possible to assign the peaks in the EXAFS spectrum unequivocally since curve fitting procedures applied to known distances, specially for Cu-imidazole were applied. However, for a more accurate representation of ligands coordinated to the metal sites, it is necessary

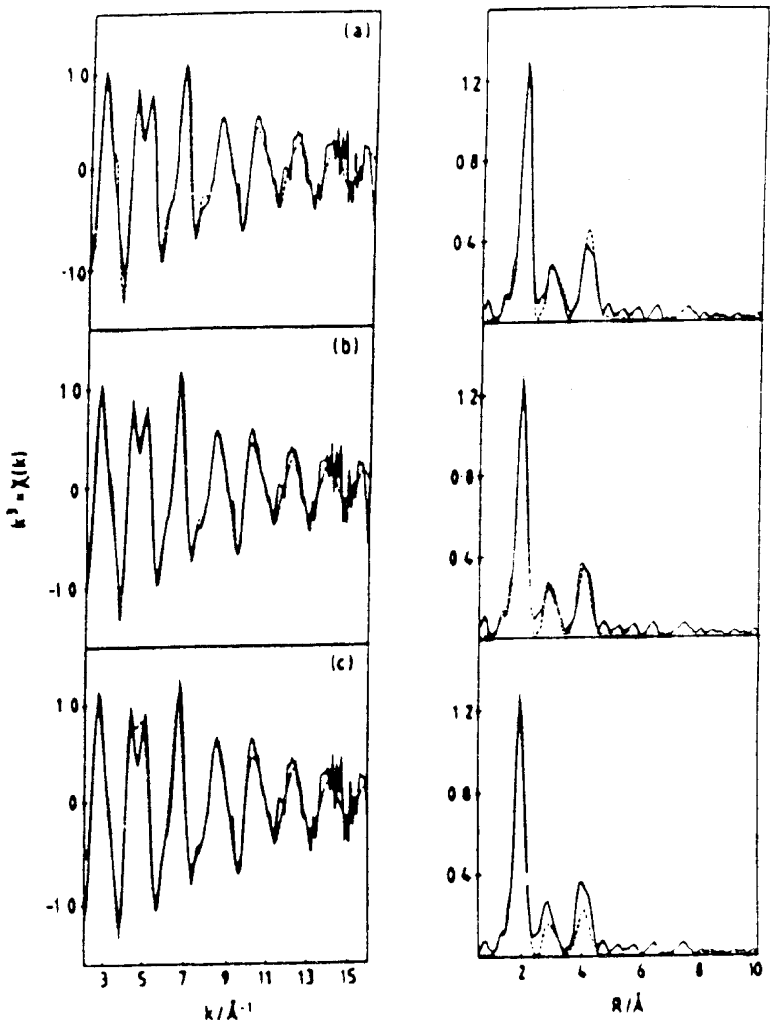

Fig. 3 Left: The EXAFS functions generated by Fourier-back-transformation of the second peak of the radial structure function for Cu_2O-imidazol (solid line) is shown, comapred with least sqaures fits (dashed lines) with imidazol nitrogens (1,3) and Cu_2O in the second coordination sphere.

Right: Fourier transforms of the EXAFS spectra of $Cu_2O \cdot xH_2O$ and imidazol (a); $Cu_2O \cdot xH_2O$ and L-(+)-histidine (b); and (c) Cu2O and undecylimidazol at pH 7.9 (20°C).

to have reasonable curve fitting in order to obtain nearest neighbours, distances and coordination in solution. Qualitatively it can be said, in light of the XPS-results obtained, the broadened peak at 3.5-3.6 Å is probably due to backscattering from N, and C. of the imidazoles, which are the third shell of atoms in the imidazole group. The peak at 2.08-2.11 Å can be assigned to a distance between Cu-N_3 of the imidazole system according to crystallographic analysis from $Cu(IM)_4(ClO_4)_2$ salts. At the present time it is not possible to distinguish Cu^{1+}-N_3-structures from Cu^{2+}-N_3-structures unequivocally. However, upon oxygenation of the system Cu±/imidazole at pH 7.9 (20°C) the EXAFS-spectrum as well as the XPS-spectrum changed considerably as seen in Fig. 2: the large and broad peak at approximately 3.51 Å appeared probably from backscattering by a copper atom separated from another copper atom (absorbing) by 3.51 Å, the distance of 2.02 Å appears to be the distance of Cu^{2+}-N_3, and the distance of 1.93 Å seems to be the distance of CuO found in the other EXAFS spectra and in the dehydrated state of the reconstituted biofilm (Figs. 1, 2). The Cu-Cu-distance of 2.85 Å as seen in the Cu-foil is surprising. Apparently upon oxygenation the Cu has been oxidized completely to a Cu^{2+}-species, coordinated by imidazole. Another Cu-

O distance appeared at 1.93 Å which is different from the Cu_2O and CuO-distances known from crystalline structures. Furthermore, whether a dioxygen complex as observed in other systems is involved in this structure has to await further studies of this heterogeneous system. In addition recent X-ray photon spectroscopy (XPS) and Raman scattering results reveal binding energy values for Cu in both adsorbate and adsorbent of 368.3 eV.

Furthermore, through a careful comparison with all the spectra obtained including from Cu-, Cu_2O, CuO and Cu_xO_y standards, and in complex with imidazol or undecylimidazol, it seems clear that there are two distinct features in this spectrum from the "Cu-monolayer". They correspond to the positive oscillations in the real part of the Fourier transform occurring at 1.05 Å and 2.15 Å. This has to be due to oxygen neighbours of the liquid like metal film atoms of Cu in the monolayer. Careful fits to the experimental data are consistent with approximately 4.0 oxygen atoms at appr. 1.95 Å. The peak at 2.15 Å could be assigned to oxygen atoms at approximately 2.55 Å or Cu atoms at 2.60 Å. This needs more clarification. However, the surprising finding from the spectrum of the Cu monolayer is that the peak in the Cu-O nearest neighbor distribution is prominent, sharply defined which suggests strong interactions of Cu-atoms in the monolayer. Taken all together, it suggests that oxygen or even chloride play an important role in the formation of the Cu/Cu_xO_y monolayer at the interface, which can be shown nicely by EXAFS spectra obtained by reconstitution experiments [11].

Furthermore, comparing the radial distribution functions of this system with model systems comprising of a peptide and $Cu^{+/}$Cu2+, or the with the peptide containing exopolymer it can be concluded that a certain number of Cu-solvate species are present, which are CuCl(H2O)n $a_{s\ the}$ main component for amino acid complexation, whereas sufficient Na+ (H2O)n ions $w_h$ich $_{incomplete}$ hydration shell are found in order to provide a strong source of water removal in the corrosion process in the presence of the biofilm as a exopolymer (H2O)nNa+ in $f_o$rm $_o$f a bicontinious phase [12].

Taking the chemical properties of imidazole and histidine, which has been found extensively in the biofilm, into consideration as well as the known structures of (imidazolato)-copper(I) as a polymer we can infer a following corrosion mechanism [13] (Fig. 4).

The first step of the corrosion process may be the formation of a cuprous complex by ligation of N_3 of imidazole with metallic copper. (N_3 is the pyridine nitrogen having a large electronegativity, N_1 equals a pyrrole nitrogen). In solutions at pH 7 or above the unprotonated imidazole functions as a ligand through the two unpaired electrons on N_3. In this complex (step 1) the Cu atom and the amino group become reactive. When the solution is exposed to oxygen or hydroperoxide which has been observed in the corrosion products as it is the case of the corrosion process in a County Hospital, the Cu is oxidized and imidazole will be deprotonated, resulting in the formation of (imidazolato)-copper(I) and H_2O (step 2). Step 3 shows the formation of an infinite polymer consisting of imidazolato-copper(I) which forms a thin layer on the surface of the Cu-metal in addition to the biofilm which has a thickness of at least 100-500 Å. This structure and the possible dioxygen complex

$$Cu^{\pm 0} + nL + \frac{1}{2}O_2 \rightleftharpoons [Cu(L)_n]^{1+} + H_2O$$

$$2[Cu(L)_n]^{+} + O_2 \rightleftharpoons [Cu^{2+} - (L)_n - Cu^{2+}]^{2+}$$

$$[Cu^{2+} - (L)_n - Cu^{2+}] + H_3O^{+}$$

$$\rightarrow [Cu^{2+} - (L)_n](OH)_2 + H_2O_2$$

$$\rightarrow 2H_2O + 2CuO$$

as mentioned above, is consistent with another proposed reaction scheme as shown below:

where L=imidazole containing ligand, e.g. glycoprotein. However, until the dioxygen complexes in this corrosion process has been characterized in more detail. The proposed reactions scheme is no more high hypothetical since Cu-dioxygen complexes of this kind are known since Wieland (1923) [14], and regained new interest very recently [15].

The imidazole-copper-complex is also consistent with molecular structures of Cu-undecylimidazole or Cu-dodecylimidazole complexes [16] in the presence Of Cu_2O (Fig. 3). The incorporation of O_2 or HO_2^- produced either by microorganisms in the biofilm or by catalytic amounts of colloidal copper nano-particles during the initial stage of oxidation is also observed at the interface between Cu_2O and Xanthan or alginate. The most plausible explanation which is consistent with the EXAFS-experiments involves the decomposition of $HO_{2\text{-}}$ to free radicals, accelerated by

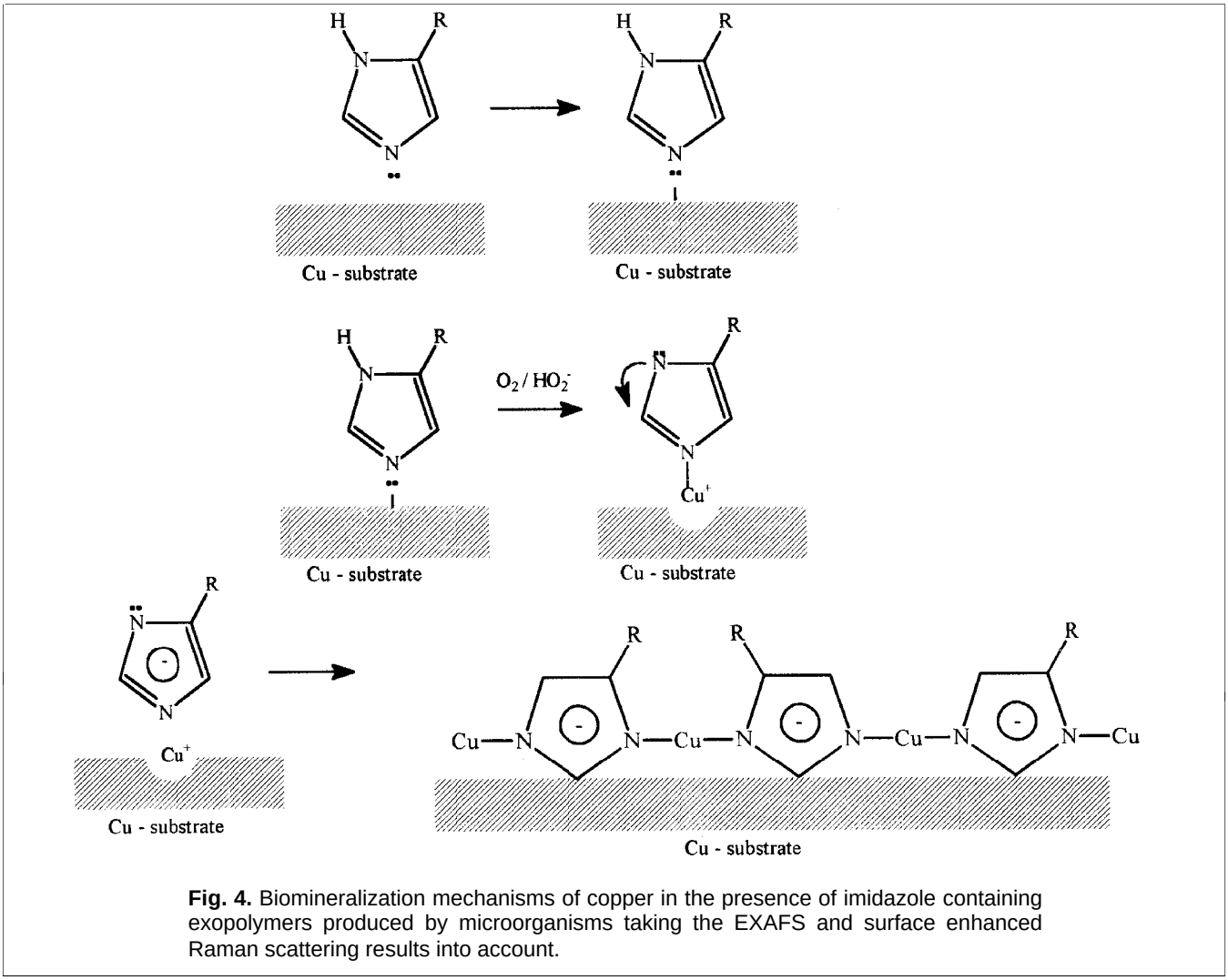

Fig. 4. Biomineralization mechanisms of copper in the presence of imidazole containing exopolymers produced by microorganisms taking the EXAFS and surface enhanced Raman scattering results into account.

biofilm formation and microorganisms [17]. The catalytic effects of powdered copper, Cu_2O and CuO, revealing the highest reactivities showing the EXAFS spectra as of Fig. 1 are obtained with cuprous oxide and a copper surface exposed to air at room temperature. These oxidation processes are carried out at the interface of the biofilm and the Cu or Cu_2O-Substrate, yielding nanoclusters of $Cu_2O \cdot xH_2O$ which are light sensitive and of fractal nature [18].

The EXAFS results clarify the interpretation of the effects of the biofilm on the interactions with the copper surface at low ionic strength revealing the formation of imidazole-Cu-complexes, hence dissolution of metallic copper. The anionic imidazole possesses two equivalent sites for coordination, so that each copper cation can coordinate two nitrogens of the imidazole. These polymeric Cu-complexes are water insoluble and form a thin layer on the surface of the copper metal within the biofilm which has in addition also cationic selective membrane mimetic properties [11].

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