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## Short Communication

## Novel application of HKUST-1 metal–organic framework as antifungal: Biological tests and physicochemical characterizations

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## ABSTRACT

The HKUST-1 metal–organic framework (MOF) was successfully applied as a biocidal material against representative yeast and mold. The synthesized MOF showed a strong antifungal effect against *Saccharomyces cerevisiae* for which the growth was completely inhibited, whereas the growth of *Geotrichum candidum* was reduced from 6.16 to 1.29 CFU mL<sup>−1</sup>. The antifungal action was related to copper ions released into the culture medium due to a progressive degradation of the crystalline structure of the material that involved the formation of surface extra-framework Cu(I). This study shows the potential of using copper-based metal–organic frameworks for a controlled release of biologically active copper ions.

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## 1. Introduction

Cu compounds in solution such as CuSO<sub>4</sub> and Cu(OH)<sub>2</sub> are traditionally used for the control of contamination by microorganisms, but they should be added in high levels. In the search for alternatives, numerous studies for dosing Cu have been published, e.g. the use of Cu-exchanged zeolites to gradually supply Cu(II) ions [1,2] or the use of dispersed Cu(0) and CuO nanoparticles [3,4]. This latter approach has the disadvantage of particles aggregation. Moreover, inorganic–organic materials as polymer–metal complexes based on Cu(II) have also been applied for a more flexible dosification of ions [5–7]. A relatively new family of these hybrid materials are metal–organic frameworks (MOFs). MOFs have become of interest for a wide range of applications [8], but the unusual features of these materials suggest that they could be used in other areas so far unexplored. MOFs are being actively explored for biomedicine as imaging agents and as delivery vehicles for therapeutic agents [9]. Moreover, the search for new materials that allow a controlled release of ions with antimicrobial activity is an area in which MOFs could also be very promising. In this sense, it has been recently reported that a silver-based MOF presents good bactericidal action due to the release of ions of its own structure [10]. Most recently, a cobalt-based metal–organic framework also showed a high activity by itself against *Escherichia coli* [11]. HKUST-1 has an open pore system with accessible binuclear copper centers in which the Cu ions are interconnected in the three-dimensional

net. Then these atoms could be disconnected and the MOF act as a source of biologically active ions.

In this study, we first show that HKUST-1 has a strong antifungal activity against a prototypical yeast and mold which are commonly found in the industries of food processing. Then, based on characterizations, a possible action mechanism of the material is proposed.

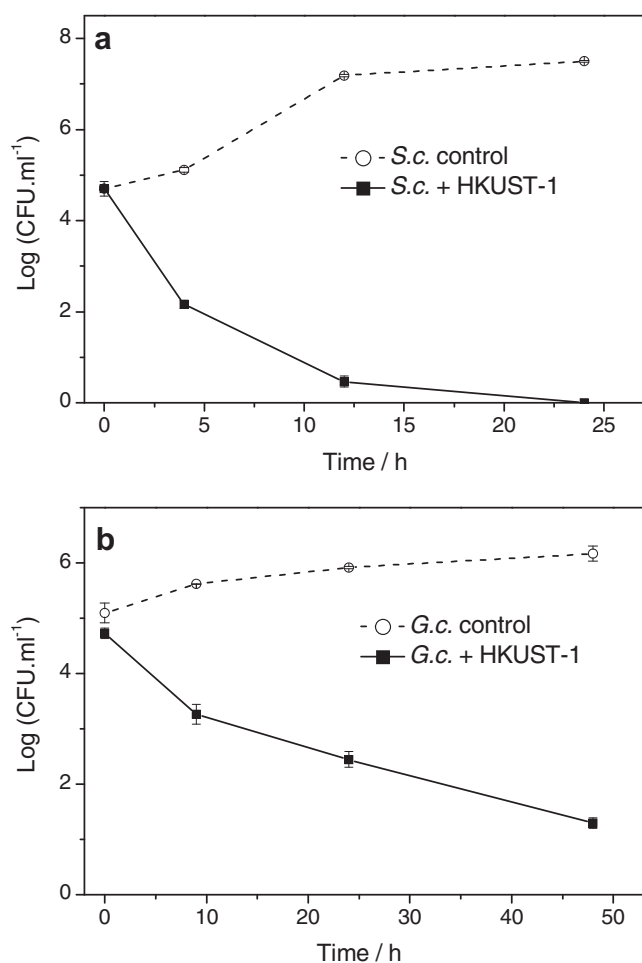
## 2. Experimental

The synthesis of HKUST-1 was carried out following a reported procedure [12] with slight variations. Basically, 0.875 g of Cu(NO<sub>3</sub>)<sub>2</sub>·3H<sub>2</sub>O (Sigma–Aldrich, 99%) were dissolved in 12 mL of distilled water and separately, 0.42 g of trimesic acid (Sigma–Aldrich 99%) were dissolved in 12 mL of ethanol (Cicarelli). Subsequently, both solutions were mixed, kept under stirring for 60 min and then placed in a Teflon-lined autoclave at 120 °C for 16 h. Finally, turquoise crystals were recovered and dried at 120 °C for 10 h.

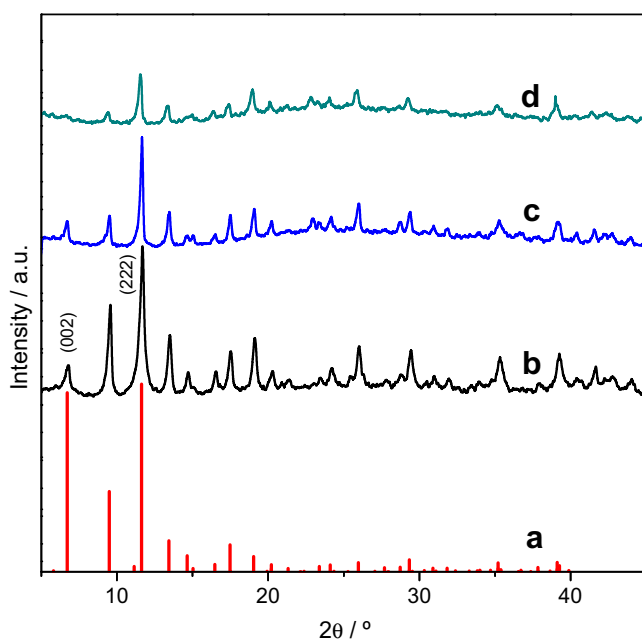
The microorganisms selected for the microbiological assays were the unicellular eukaryote *Saccharomyces cerevisiae* (LMFIQ444) and the coenocytic eukaryote *Geotrichum candidum* (LMFIQ269); they were isolated from spoiled food products and the strains were stored in the culture collection at the Universidad Nacional del Litoral. The tests were performed under fermentative conditions adding 9 mL of culture medium, 10 mg of HKUST-1 and 1 mL of freshly prepared inoculum suspension, and subsequently incubated in malt extract broth (MEB) – malt extract 2.0%; peptone bacteriological 0.1%, Glucose 2%, w/v in distilled water- (Biokar

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**Fig. 1.** Viable colony counting at different times with and without the addition of HKUST-1 for: (a) *S. cerevisiae*; (b) *G. candidum*.

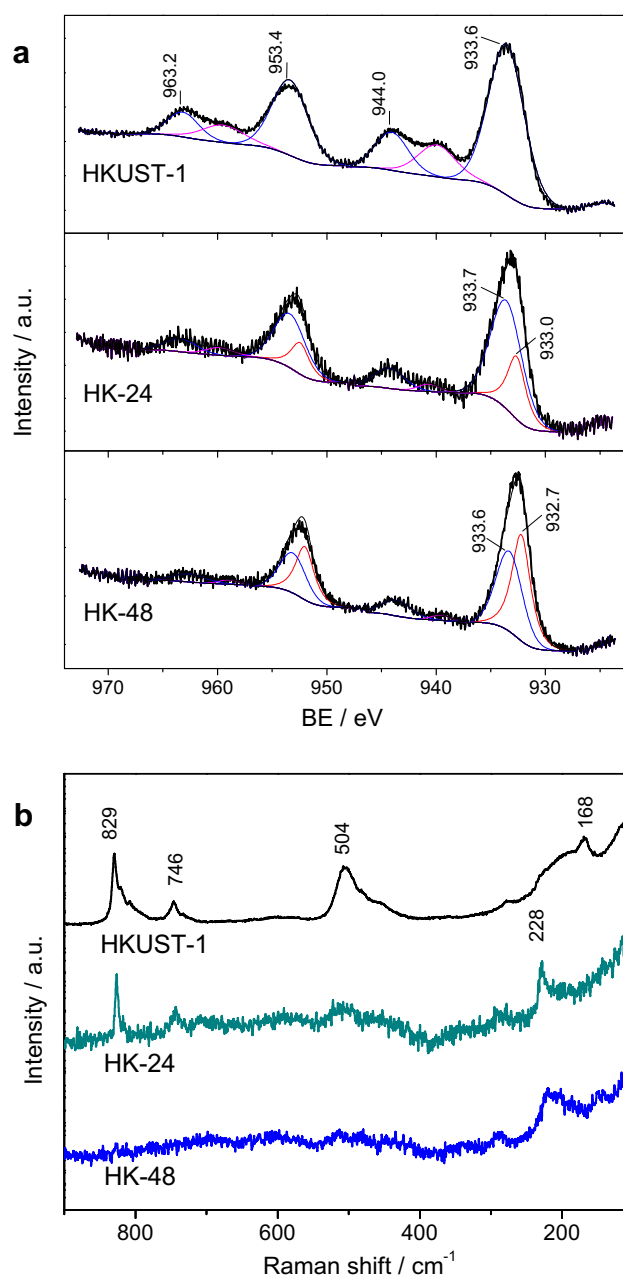


**Fig. 2.** X-ray diffraction: (a) simulated pattern using Diamond software with CCDC archive for HKUST-1; (b) as-synthesized HKUST-1; (c) HKUST-1 after 24 h in MEB; (d) HKUST-1 after 48 h in MEB.

**Table 1**  
Results from XRD, AAS and XPS data.

Sample	Crystallinity (%)	[Cu] in sol. (ppm)	Cu2p <sub>3/2</sub> /Cu2p <sub>1/2</sub> sat.	Surface Cu(II)/Cu(I)
HKUST-1	100	–	4.2	∞
HK-24	70	183	9.1	3.7
HK-48	48	184	7.7	2.9

Diagnostics, Beauvais, France) at 25 °C for 48 h. In parallel, the respective control growths were conducted. Each of the tests was performed in quadruplicate. After incubation, counts using decimal dilutions in MEB-agar (1.2% w/v) were conducted.



**Fig. 3.** Spectroscopic analyses of fresh and used HKUST-1 samples: (a) processed XPS spectra in Cu<sub>2p</sub> region; (b) LRS spectra.

Characterization through X-ray diffraction (XRD) was performed with a Shimadzu XD-D1 instrument in a Bragg–Brentano configuration ( $\text{CuK}\alpha$  radiation,  $\lambda = 1.5418 \text{ \AA}$ , 30 kV, 40 mA,  $2^\circ \text{ min}^{-1}$ , 100 mg sample). Crystallinity of used solids was calculated considering the main peaks and taking 100% for the as-synthesized MOF. Atomic absorption spectroscopy (AAS) was performed on filtrates (Sartorius  $0.45 \mu\text{m}$  membrane filters) with a Perkin Elmer 3110 flame emission instrument (324.8 nm). X-ray photoelectronic spectroscopy (XPS) analyses were carried out in a SPECS system (Mg  $\text{K}\alpha$  X-ray source, 200 W and 12 kV; C 1s peak at 284.8 eV as internal reference). The data processing and peaks deconvolution were performed using the Casa XPS software (splitting of the Cu  $2p_{3/2}$  and Cu  $2p_{1/2}$  signals was 19.8 eV; peaks were fitted by a Gaussian–Lorentzian component wave-form (GL = 30 for Cu(I) and GL = 80 for Cu(II)). Laser Raman spectroscopy (LRS) was conducted with a Horiba-Jobin–Yvon coupled to an Olympus confocal microscope (30 mW, 532 nm). Scanning electron microscopy (SEM) was performed with a JEOL JSM-35C instrument (20 kV; Au coating). Electron probe micro analyses (EPMA) were performed with a dispersive instrument coupled to the SEM (graphite coating).

### 3. Results and discussion

#### 3.1. Microorganisms growth assays

Fig. 1 shows the growth curves for the microorganisms in the culture media added with HKUST-1. The control growth curves are also depicted. The curves using trimesic acid (not shown) were similar to the control curves. It should be remarked that in all experiments there were no significant differences in the initial condition concentration. A remarkable biocidal activity of the MOF was

noticed for both microorganisms, *S. cerevisiae* being the most sensitive and achieving a fast reduction in the viable counts (Fig. 1a). After 24 h of incubation a total inhibition of the growth was observed while for the control the colony reached  $7.5 \pm 0.2 \log \text{CFU mL}^{-1}$ . For *G. candidum* there was also a strong inhibition effect which increased with incubation time. A reduction from  $6.2 \pm 0.1$  to  $1.3 \pm 0.1 \log \text{CFU mL}^{-1}$  was observed in the microorganism development after 48 h. The marked decreasing logarithmic profile of the growth curves obtained with the addition of HKUST-1 both for *S. cerevisiae* and *G. candidum* indicates a strong inhibitory effect caused by the MOF. In order to try to establish the mode of antifungal action, physicochemical studies were conducted.

#### 3.2. Physicochemical studies

Fig. 2a shows the XRD pattern of HKUST-1 simulated from crystallographic data (CCDC112954) and it can be observed that the as-synthesized HKUST-1 (Fig. 2b) presented all the indexed diffraction signals for this material [12]. The lower relative intensity of the (002) reflection with respect to that of (222) compared with the simulated pattern is due to a partial hydration HKUST-1 [13]. Moreover, the BET surface area and the micropore volume (from  $\text{N}_2$  adsorption isotherms) were  $608 \text{ m}^2 \text{ g}^{-1}$  and  $0.253 \text{ cm}^3 \text{ g}^{-1}$ , respectively, indicating a correct solvent evacuation that leaves the MOF porosity available. Meanwhile, the XRD patterns of the MOF upon 24 and 48 h in MEB (Fig. 2c and d) showed a decrease in the intensity of all signals which increased with contact time, indicating a gradual loss of the MOF crystallinity (Table 1). It should be noticed that none of the analyzed samples presented obvious signals from bulk CuO or  $\text{Cu}_2\text{O}$ , which could be possible by-products of the MOF degradation. On the other hand, the AAS

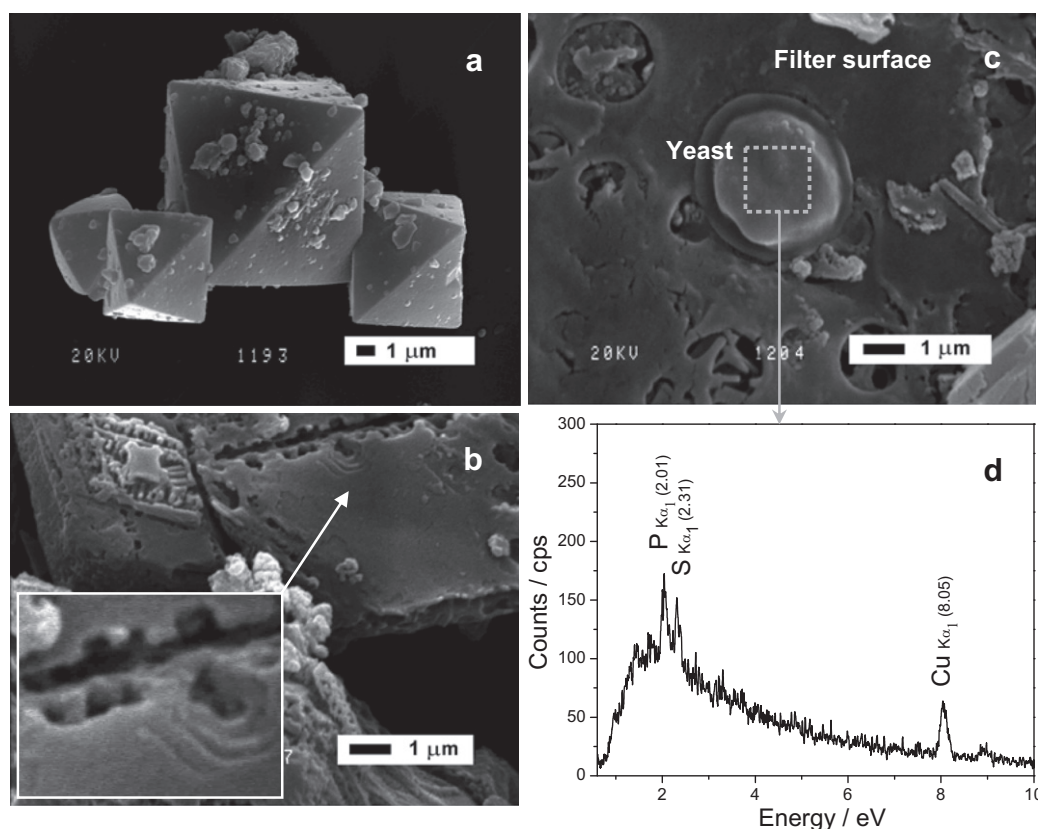


Fig. 4. SEM images of: (a) fresh HKUST-1 crystals; (b) HKUST-1 crystals recovered after 24 h of the growth assay; (c) specimen of *S. cerevisiae* after 24 h of the microbiological test with HKUST-1; (d) EPMA analysis in a small selected area on *S. cerevisiae* (indicated in Fig. 4c).

analyses of the filtrates of these samples indicated a leaching of Cu from the MOF which reached a concentration almost invariant after 24 h (Table 1). However, for a MOF sample maintained in water for 48 h, the amount of dissolved copper was of 39 ppm. The higher MOF degradation in MEB should be related to certain components of the culture medium that promoted the dissolution of Cu.

XPS spectra of a fresh HKUST-1 sample (Fig. 3a) presented two main signals from Cu 2p<sub>3/2</sub> and Cu 2p<sub>1/2</sub> levels with BE of 933.6 and 953.4 eV, respectively, which are characteristic of Cu(II). In addition, the presence of satellite peaks confirmed this species [14,15]. Whereas for the used samples (HK-24 and HK-48), the 2p signals became slender (i.e. the FWHM decreased) and the intensities of the satellite peaks were reduced. It is known that 2p signals of Cu(I) are narrower than those of Cu(II) and also that Cu(I) has no satellite peaks [15]. The higher ratios of the 2p<sub>3/2</sub> signal and their satellite peak for used samples (Table 1) coupled with the narrowing of their 2p signals indicates Cu(I) on the MOF surface. In fact, the bands found at BE near 933 and 952 eV are typical of Cu(I). Moreover, a higher enrichment of surface Cu(I) for the HK-48 sample was observed (Table 1). The presence of Cu(I) in the used samples was confirmed by LRS (Fig. 3b). Despite the high fluorescence due to organic residues, the typical Raman signal of a second-order overtone at 208 cm<sup>-1</sup> from Cu<sub>2</sub>O [16] was observed. In addition, a marked decrease MOF signals due to a loss of the structural integrity was noticed. The vibrational modes of Cu(II) in the MOF (504 cm<sup>-1</sup>; 200–170 cm<sup>-1</sup>) and from out-of-plane ring (C–H) bending (746 cm<sup>-1</sup>; 829 cm<sup>-1</sup>) [17], were reduced. The generation of such Cu(I) species was most likely caused by reducing components of the culture medium, such as sugars. In addition, the low partial pressure of O<sub>2</sub> under the test conditions could contribute to their stability. Then, this extra-framework copper in the amorphized structure of the MOF is available to be released to the culture medium, as determined by AAS.

The as-synthesized MOF crystals (Fig. 4a) showed a typical polyhedral morphology with a smooth surface [18]. Meanwhile, after being used for 24 h in the microbiological assays an eroded surface was observed, with craters that penetrated into the crystal (Fig. 4b). On the other hand, EPMA analysis in small selected areas of the microorganisms, as shown for *S. cerevisiae* (Fig. 4c), revealed the presence of Cu in the cell. Moreover, S and P from cellular components were also observed (Fig. 4d). It is known that Cu ions can bind to fungal cell walls, disrupting the transport of nutrients and inhibiting intracellular enzymes [19]. Moreover, fungal walls differ between them depending on the taxonomic group [20] that may explain the variable sensitivity to HKUST-1 for *S. cerevisiae* and *G. candidum*.

#### 4. Conclusions

It was shown that HKUST-1 is a material that exerts a strong inhibitory activity against *S. cerevisiae* and *G. candidum*. The anti-fungal action is due to the ability of the MOF to release Cu ions from its own structure, which slowly degrades producing extra-framework surface Cu(I). The possibility of regulation of connectivity and type of bonds in MOFs show the potential of using other copper-based MOFs for a controlled-release rate of active copper ions.

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