A bentonite/ZIF-8 derived ZnO photocatalyst for the effective elimination of endocrine disruptors mixture under simulated solar light: estrogenicity mitigation

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S1. Gas chromatography with mass detection analysis

The concentration of BPA, PPB, and EE2 was monitored by GC/MS analysis following the procedure described previously [1]. One milliliter of the sample from the *batch* reactor was filtered through a 0.45 μm diameter membrane (Phenex). Then, 3 mL of NaCl (6 mol L^{-1}, Sigma Aldrich, ≥99%) and one milliliter of chloroform (TEDIA, HPLC/GC grade) were added, and the mixture was vortexed for one min. After 30 min of phase separation, 200 μL of the organic phase was placed in an amber vial and dried under a N_2 stream. The trimethylsilylation derivatization of the dried extract was performed with 150 μL of BSTFA – TMCS (1%) (Sigma Aldrich) and 50 μL of pyridine (Sigma Aldrich, ≥99%) as a catalyst for 30 min at room temperature [2]After this, the derivatized sample was diluted to 1 mL with acetone (J.T. Baker, HPLC/GC grade).

The derivatized samples were analyzed using an 8890 gas chromatography system coupled to a 5977B mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The analytes were separated using an HP-5MS capillary column (30 m x 0.25 mm, 0.25 μm film thickness). High-purity helium (99.999%) was used as the carrier gas at a flow rate of 1 mL min^{-1}. The injector was set to 300 °C in *pulsed splitless* mode, and the injection volume was 5 μL. The temperature program of the column oven was set to 100 °C for 2 min; from 100 to 150 °C at 20 °C min^{-1}, maintained at 150 °C for 2 min; a new ramp from 150 to 280 °C at a rate of 25 °C min^{-1}, and held at 280 °C for 5 min. The ion source and quadrupole temperatures were maintained at 230 and 150 °C, respectively. Mass spectra were obtained by electron impact (70 eV). Quantitative analysis was performed using SIM mode, while qualitative study was conducted to identify reaction intermediates in SCAN mode. Calibration curves were constructed using a dilution of standards, ranging from 50 to 1000 ng L^{-1}. Standard sample and procedure blanks were also monitored to detect signals corresponding to the analyzed compounds.

S2. In vitro YES assay - Estrogenic activity evaluation

The solutions for the YES assay were prepared according to the protocol described by Routledge and Sumpter (1996) [3]. The YES (Yeast Estrogen Screen) assay was performed in 96-well microplates, using serial dilutions of aqueous samples in ultrapure water. E2 was used as a positive control to construct the standard curve, covering a concentration range of 2724 to 1.33 ng L^{-1}, while ultrapure water was used as a negative control. In each well of the assay microplate, 10 μL of the sample and 190 μL of culture medium containing yeast and CPRG (chlorophenol red-β-D-galactopyranoside) were added. After a 72-h incubation period at 30 °C, absorbances were measured at 575 and 620 nm wavelengths using the VersaMax microplate reader (Molecular Devices).

Dose-response curves for the positive control (E2) were fitted to a symmetric logistic function using the Origin 6.0 software package (OriginLab, USA). From these curves, the mean EC_{50} value of E2 was determined. Estradiol equivalents (E2-Eq) in the samples were calculated by interpolating sample data into the E2 standard curve. The method's detection limit (LOD) was 25.7 ± 2 ng L^{-1}. To minimize the effects of turbidity on the measured estrogenic response, absorbances were corrected according to Equation E1, considering the negative control:

$$Abs_{Corrected} = Abs_{575\ (Sample)} - \left(Abs_{620\ (Sample)} - Abs_{620\ (Negative\ control)}\right) \quad \text{(E1)}$$

Yeast growth inhibition, potentially caused by sample cytotoxicity, was evaluated through absorbance control at 620 nm, as described by Equation E2, proposed by Frische et al. (2009) [4]No dilution showing cytotoxicity (Equation 2) was used for the E2-EQ calculation, as it would have led to an underestimated estrogenicity value.

$$Citotoxicity = 1 - \left(\frac{Abs_{620\ sample}}{Abs_{620\ NC}}\right) \quad \text{(E2)}$$

The corrected absorbance and concentration data were plotted, and the resulting sigmoidal curves were fitted to a symmetric logistic function, described by Equation E3:

$$y = \frac{A_1 - A_2}{1 + \left(x/x_0\right)^p} + A_2 \quad \text{(E3)}$$

Where: A1 and A2 represent the maximum and minimum β-galactosidase induction values in corrected absorbances; x_0 is the median effective concentration (EC_{50}) of E2, expressed in ng L^{-1}; p is the sigmoidal curve slope; (x,y) are the coordinates corresponding to the sample concentration and its corrected absorbance response.

Finally, the E2-Eq was calculated as the lowest xx eliciting a detectable agonist response divided by the sample's final enrichment factor in the assay.

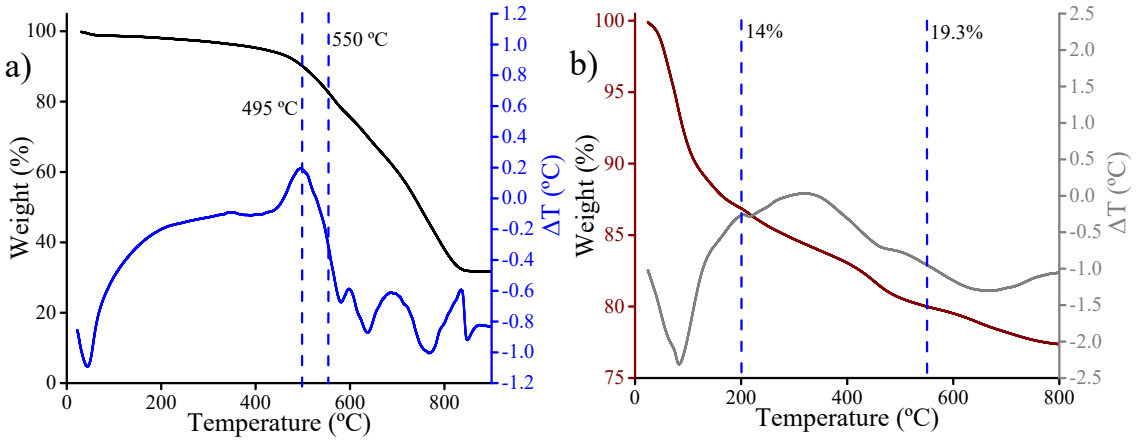
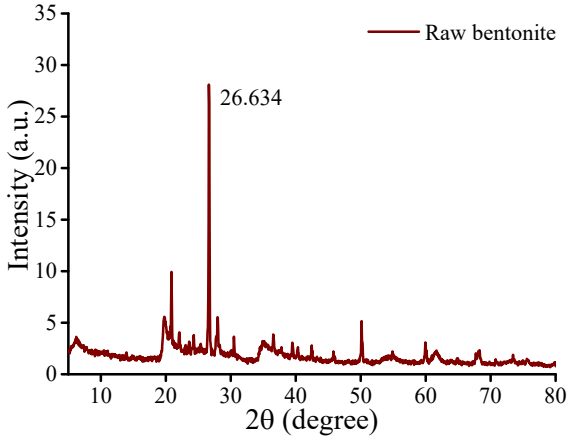


Figure 1S. Thermogravimetric and DTA analysis of a) ZIF-8 and b) raw bentonite.



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95 **Figure 2S.** a) XRD analysis of natural bentonite used to prepare the materials.

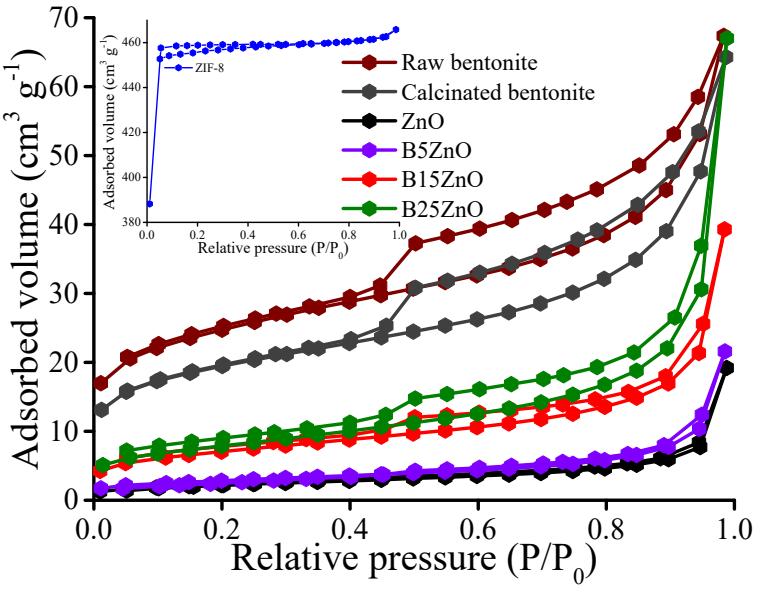


Figure 3S. N_2 adsorption-desorption isotherms of the prepared materials: in the graphical insert, the isotherm of ZIF-8.

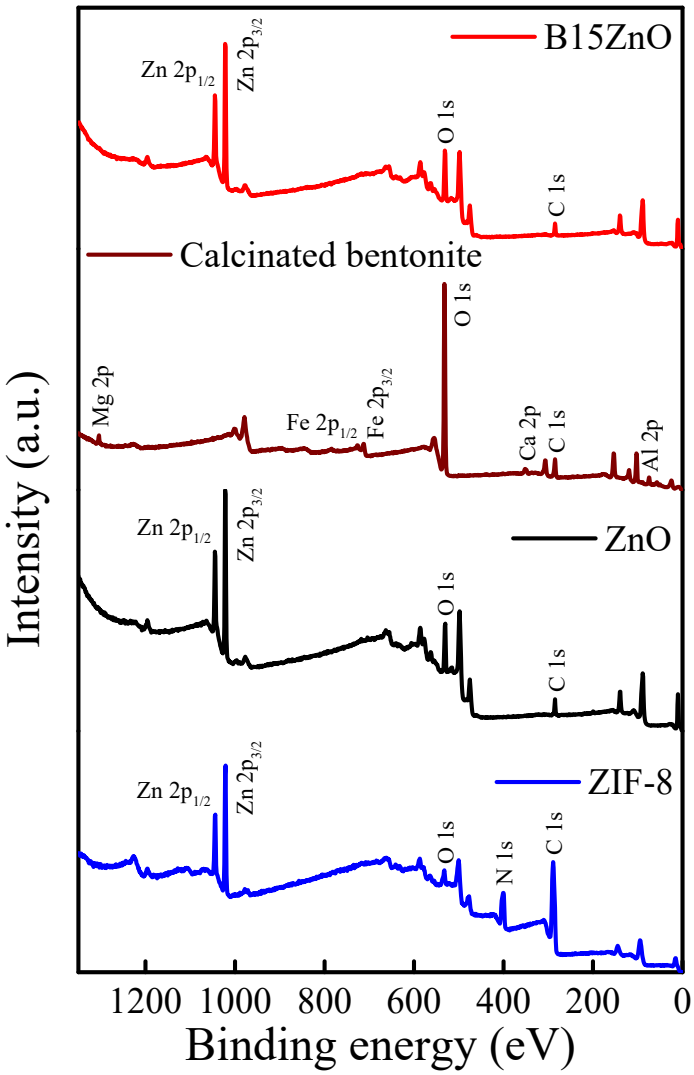


Figure 4S. Full scan XPS spectra of the ZIF-8, ZIF-8-derived ZnO, calcinated bentonite and B15ZnO composite.

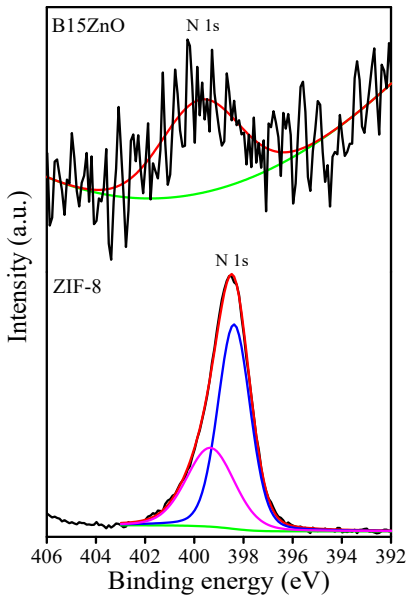
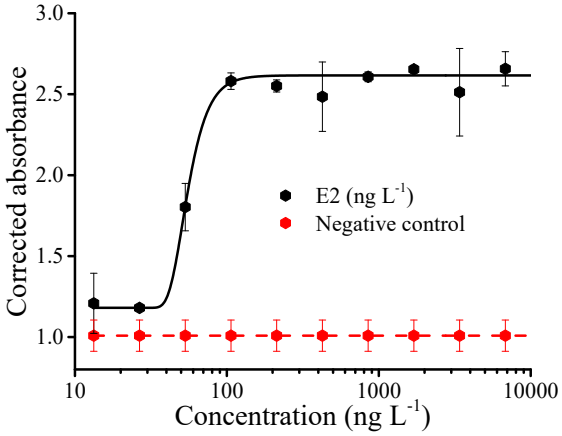


Figure 5S. High-resolution XPS spectra of a) N 1s in the ZIF-8 and B15ZnO.



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106 **Figure 6S.** a) Positive control (estradiol, E2), and negative control (Milli-Q water) curves for two replicates.

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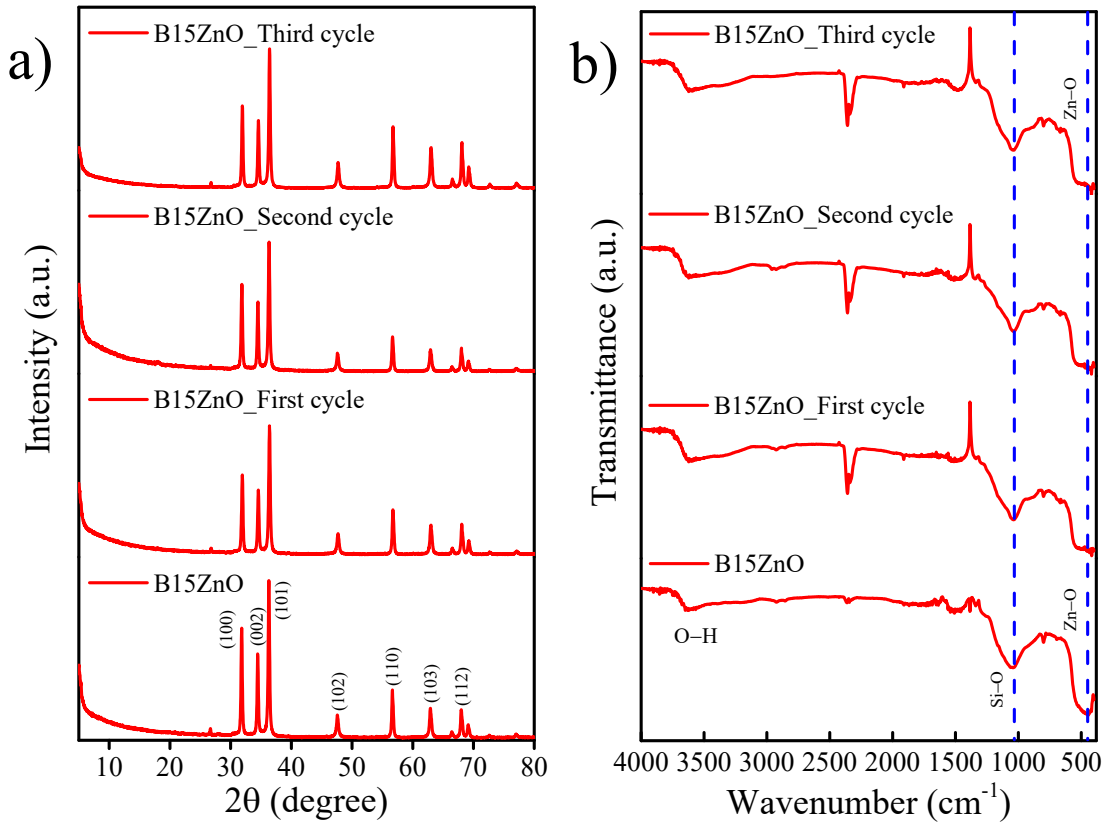

Figure 7S. a) XRD and b) FTIR of B15ZnO composite before and after each of the three reuse cycles.

References

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