

Exploring ZnO microcrystals through experimental design: from solar photocatalysis to antibacterial activity

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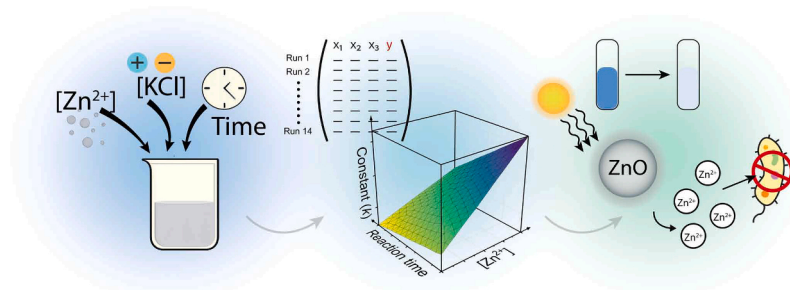
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HIGHLIGHTS

- DoE enables simultaneous optimization of ZnO yield and photocatalysis in one run.
- RSM and PCA reveal precursor, time and KCl effects on ZnO microcrystals.
- DoE-optimized ZnO shows improved colloidal stability and antibacterial action.
- Antibacterial activity of optimized ZnO is validated through Zn^{2+} release analysis.

GRAPHICAL ABSTRACT



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ABSTRACT

The concern on emerging pollutants and antibiotic resistance is pushing scientists to advance materials-based approaches by expanding their wet chemical synthetic parameter space. Herein, we leverage Design of Experiments (DoE) approach to optimize the synthetic yield and photocatalysis performances of wet chemistry prepared ZnO microcrystals. By varying three independent parameters (precursor salt concentration, reaction time and KCl concentration), we correlated synthetic yield and photocatalytic activity via Response Surface Methodology and Principal Component Analysis. ZnO microcrystals were incorporated into cellulose-based supports, facilitating their sustained release in solution. Photocatalytic efficiency was assessed on methylene blue degradation under simulated solar irradiation, and the role of photogenerated reactive radical species was elucidated. The antibacterial properties of the ZnO were investigated, underscoring the importance of their colloidal stability and finding that the amount of the released zinc ions was a critical factor in triggering their antibacterial activity, highlighting the multifunctional potential of these microstructures.

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1. Introduction

The worldwide pollutants emergency caused by anthropic activities demands a rational response driven by sustainable materials-based depuration technologies [1]. Similarly, antibiotics misuse has resulted in the threat of antibiotic-resistant bacterial strains, challenging the conventional treatments against bacterial infections [2]. In this context, materials science research can offer a joint effort to provide viable solutions to address these emerging concerns. Indeed, approaches based on adsorption and/or photocatalysis have been employed to facilitate pollutants degradation [3,4]. These methods rely on the interaction between the pollutant and the degrading material, with the efficiency of the process being enhanced by an increase in the exposed surface area of the latter [5,6]. Due to their nanoscale size, high surface area, and quantum size effects, nanoparticle-based approaches have been widely shown to yield efficient photocatalytic and antibacterial systems [7,8]. In fact, nanoparticles can promote photocatalytic processes through different mechanisms, including both the action of photogenerated charge carriers and, in some cases, the formation of Reactive Oxygen Species (ROS), which can induce damage to DNA and proteins, as well as inhibiting the growth of bacteria, fungi and viruses [9]. Depending on the material and reaction environment, the relative contribution of these pathways may vary, and direct oxidation mediated by photogenerated holes can represent a dominant degradation route. For instance, Ferrara et al. demonstrated that titanium dioxide nanoparticles, as photocatalysts, induce protein unfolding and oxidation [10]. However, nanoparticles might also induce unfavorable interactions with cellular membranes, inducing potential harmful cytotoxicity effects [11]. To this end, different strategies have been demonstrated to stabilize nanoparticles, such as the use of biosurfactants for capping [12] or employing structural/compositional paradigms [13].

This complex scenario underscores the urgent need for efficient strategies to tailor materials with multiple functionalities. However, considering the intricate relationship between structural features and functional properties, the conventional materials synthesis approaches based on the “One Factor at a Time” might be insufficient. Instead, a strategy that simultaneously varies multiple variables at once is needed to assess their combined effects [14]. To this aim, multiparameter approaches based on Design of Experiments (DoE) [15] or machine learning [16] accelerate materials discovery, ultimately enabling the tailoring of their functionality as needed. In particular, DoE is a statistical approach, that allows for studying systematically the effect of synthesis conditions on the expected observables. The outcomes of this process are then analyzed using the Response Surface Methodology (RSM), which provides a multifactor model to describe the relationship between the factors and the specific responses [17].

Accordingly, an ideal system for exploring the synthetic space in a reduced number of experiments through DoE could be Zinc Oxide (ZnO), a wide bandgap n-type semiconductor, known for its versatile electrical, photocatalytic and antimicrobial properties [18–20]. ZnO morphology can be easily tuned by varying synthesis conditions, both in the vapor [21] and aqueous phases [22] resulting in the formation of nanoparticles, nanocrystals, nanowires, nanoflakes and other multiscale structures. These structural variations also influence the material's stability and dispersibility in aqueous environments, which in turn can affect its photocatalytic or antimicrobial properties, highlighting that surface area alone is not sufficient to fully describe the functional performance of ZnO-based systems. In particular, ZnO microcrystals, unlike nanoparticles [23], exhibit higher stability in aqueous environments, given their higher crystallinity and lower surface energy, making them resistant to surface degradation processes, which is a key aspect for sustained photocatalytic operation under irradiation and for practical reuse of the material. However, their reduced dispersibility can limit their antimicrobial activity, compared to the more easily dispersed ZnO nanoparticles, suggesting that an optimal balance between surface-related effects, crystallinity, and stability must be considered

when targeting multifunctional photocatalytic and antibacterial applications.

Accordingly, DoE can be leveraged for exploring ZnO synthesis space in a programmed number of experiments, to better correlate structural features with functional properties and potentially improving the colloidal properties of ZnO microcrystals. However, most of the reports that leverage DoE or machine learning approaches are focused on optimizing the synthesis of nanosized systems such as nanoparticles [24], [25], [26] or nanowires [27]. More specifically, DoE has also guided the synthesis of ZnO nanoparticles used for the photocatalytic degradation of different dyes, such as Orange G [17] tartrazine [28], rhodamine B [29], or recently ciprofloxacin [30] showing a synergic antibacterial and photocatalytic activity [31].

In this scenario, to the best of our knowledge, no studies have analyzed ZnO nano/microstructures based on DoE, focusing on the synergic correlation between photocatalytic and antibacterial activities. The rationale for this investigation stems from the observation that, similarly to photocatalysis or photovoltaics, the antibacterial activity of ZnO is significantly influenced by its morphology, as shown in studies on ZnO nanoparticles [32]. This evidence highlights the need for further optimization based on a rational experimental strategy to enhance its performance. With the aim of rationally exploring such route, this study employs a DoE-based approach for exploring the different photocatalytic responses of ZnO microcrystals synthesized via a simple wet chemistry approach, based on a previously optimized ZnO synthesis tailored for printed circuit boards [22]. In this framework, the combination of rational synthesis strategies with comprehensive physicochemical characterization plays a pivotal role in understanding how synthetic parameters determine the structural, morphological, optical, and surface features of a material, and how these, in turn, dictate its functional behavior. For instance, Babayevska et al. showed that ZnO nanoparticles and nanorods with high specific surface area substantially exhibit higher antibacterial efficacy due to enhanced ROS production and ion release [33]. Detailed analyses of crystal structure, morphology, surface area, and optical response provide the basis for correlating synthesis conditions with the resulting performance, ultimately enabling the modeling and prediction of the material's properties.

More specifically, in this study, hybrid ZnO/cellulose supported films were used to study solar-driven photocatalysis on the model methylene blue dye, which was photodegraded by photogenerated charge carriers and ROS. This hybrid system allowed us to explore the integration of ZnO onto cellulose-based matrices, which were found to enhance performance by enabling a sustained release of Zn^{2+} ions into solution. Similar strategies employing functionalized cellulose derived from agri-food waste have been reported, for example, grafted citrus peel cellulose has been successfully modified to enhance hydrophobicity and adsorption properties for water purification applications [34]. More generally, hybrid or encapsulated systems have been widely explored as a strategy to improve the stability, availability, or functional performance of active components in diverse applications [35–37]. Furthermore, the antibacterial activity of the selected ZnO materials was evaluated using standardized tests on two reference bacterial strains [38] (*Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922) and the correlation with zinc ions release was investigated.

2. Experimental section

2.1. ZnO microcrystals synthesis and ZnO/cellulose supported films preparation

The following reagents were purchased from Sigma Aldrich and used without further purification: zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6 \text{H}_2\text{O}$, 98 %), potassium chloride (KCl, 99.0–100.5 %), ethanolamine (≥ 99.0 %, $M_w = 61.08$ g/mol), polyethylenimine (PEI) ($M_w \sim 800$ g/mol, $M_n \sim 600$), hexamethylenetetramine HMTA ($\text{C}_6\text{H}_{12}\text{N}_4$, ≥ 99.0 %, $M_w = 140.19$ g/mol), methylene blue (methylthionium chloride, certified by

the Biological Stain Commission), ethyl cellulose (viscosity 22 cp, 5 % in toluene/ethanol 80:20), glycerol (ReagentPlus, $\geq 99.0\%$ GC), and 1,4-benzoquinone (98 %). The reagent ammonium hydroxide (NH_4OH , 28 %, FW = 35.05 g/mol) was purchased from Alfa Aesar. Ethanol (absolute anhydrous, 99.9 % v/v) was obtained from Carlo Erba, and silver nitrate (AgNO_3 , 99.8–100.5 %, AnalaR NORMAPUR® Reag. Ph. Eur., ACS) from Avantor.

For the synthesis of ZnO microcrystals, independent variables such as zinc nitrate hexahydrate concentration, KCl concentration and reaction time were defined, to investigate their influence. The experiments were designed to systematically collect data on how these factors influenced the response. The employed DoE consisted in a two- or three-level factorial design. In a two-level design, the levels were defined as "high" and "low", represented by +1 and −1 respectively. This experimental scheme was structured as a matrix that, once populated with response data, enabled the generation of response surfaces [15,39,40].

The effect of three main variables was investigated, namely zinc nitrate hexahydrate concentration, KCl concentration and the reaction time. The reaction temperature was deliberately fixed at 85 °C, within the well-established optimal range of 80–95 °C for wet-chemical synthesis at ambient pressure. This choice is based on previous studies on high-quality ZnO synthesis [22,41–43], where this temperature range consistently yielded highly crystalline ZnO. Lower temperatures (< 80 °C) lead to simultaneous $\text{Zn}(\text{OH})_2$ formation, whereas higher temperatures (> 100 °C) require autoclave conditions. Post-synthesis annealing (> 200 °C) further improves crystallinity, eliminating the need to vary the synthesis temperature in this study.

Having selected the variables of interest and their corresponding values, an experimental matrix was constructed (Table 1), comprising a total of fourteen distinct experiments along with three replicates of the run nine, which corresponds to a control experiment following the conditions of our previous work [44]. Further control experiments resulting in the synthesis of ZnO nanowires obtained from printed circuit boards [22] were also performed as a reference. However, these experimental conditions lead to extremely low synthesis yield, given the low supersaturation. In this matrix, each row represents a single experiment and its related experimental conditions, while the columns are dedicated to the representation of the variables considered in the study. Specifically, the rows of the matrix correspond to each individual experiment, specifying the combinations of variables employed in each case. The experimental conditions are indicated for each row, reflecting the different levels of the variables, namely the zinc salt concentration,

Table 1

Experimental matrix selected for the study based on a central composite design. The variables studied were Zn^{2+} concentration (7.5–30 mM), KCl concentration (1–50 mM), and synthesis time (12–48 h). Coded values (−1, 0, 1) represent low, central, and high levels. Three replicates were conducted at the control point (15 mM Zn^{2+} , 5 mM KCl, 24 h) and at the design's central point (19 mM Zn^{2+} , 25.5 mM KCl, 30 h) to assess reproducibility.

Run	X_1 [Zn^{2+}] coded values	X_2 [KCl] coded values	X_3 Time coded values	[Zn^{2+}] (mM) real values	[KCl] (mM) real values	Synthesis time real values (hours)
1	−1	−1	−1	7.5	1	12
2	1	−1	−1	30	1	12
3	1	1	−1	30	50	12
4	−1	1	−1	7.5	50	12
5	−1	−1	1	7.5	1	48
6	1	−1	1	30	1	48
7	1	1	1	30	50	48
8	−1	1	1	7.5	50	48
9	−0.33	−0.84	−0.33	15	5	24
10	−0.33	−0.84	−0.33	15	5	24
11	−0.33	−0.84	−0.33	15	5	24
12	0	0	0	19	25.5	30
13	0	0	0	19	25.5	30
14	0	0	0	19	25.5	30

KCl concentration and the reaction time.

The experimental values were fitted to a second order polynomial model and yielded the model of MB degradation efficiency (Y) as a function of Zn concentration (X_1), KCl concentration (X_2) and reaction time (X_3). The model adopted for data analysis is a linear regression model with second-order interactions.

$$Y = b_0 + \sum_{i=1}^k b_i X_i + \sum_{1 \leq i < j}^k b_{ij} X_i X_j + \sum_{i=1}^k b_{ii} X_i^2 + \varepsilon$$

The equation contains a constant value denoted by b_0 , and the coefficients for the linear (b_i), quadratic (b_{ii}), and cross product (b_{ij}) terms and ε stands for the residual. The coefficient b_{ij} stands for the degree of the impact of the interaction between the i and j factors. Note that given the limited number of experiments, it is not possible to introduce the quadratic terms X_i^2 . The software CAT (Chemometric Agile Tool) was employed to construct the chemometric model [45].

As shown in Table 1, the synthesis was carried out at four different selected times (ranging from 12 to 48 h) at a constant temperature of 85 °C. At the end of each period, ZnO microcrystals precipitated at the bottom of the container. These were then collected and dried in an oven at 60 °C, followed by calcination in a muffle at 200 °C for two hours. The concentrations of the solution used for the synthesis were chosen based on preliminary trials, our previous work [22], and solubility constraints, ensuring sufficient supersaturation for ZnO nucleation while avoiding uncontrolled precipitation: $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ at four different concentrations (7.5 mM, 15 mM, 19 mM and 30 mM), KCl at four different concentrations (1 mM, 5 mM, 25.5 mM and 50 mM), 7.5 mM HMTA, 0.10 M NH_4OH , 2 mM PEI, 15 mM EA. These ranges were specifically chosen to explore the effects of precursor supersaturation and KCl-mediated electrostatic stabilization on the size and morphology, crystallinity, synthetic yield, and facet exposure of the ZnO microcrystals. Varying the Zn^{2+} concentration within this range allows assessment of its influence on crystal nucleation and growth, while the KCl concentration range enables the study of stabilization of high-energy (002) surfaces and its impact on the relative growth of (100) and (101) planes [46–48]. Finally, replicates at the control and central points were performed to ensure reproducibility of the experimental design and allow reliable assessment of response variability. All aqueous solutions were prepared using ultrapure water (18.2 M $\Omega\text{-cm}$, Direct-Q® 3 UV Water Purification Systems). ZnO microcrystals were cast onto preformed ethyl cellulose films to prepare the hybrid ZnO/cellulose supported films. The ethyl cellulose films were obtained by dissolving ethyl cellulose in ethanol, followed by drying and stabilization. ZnO microcrystals were then deposited onto the films and dried to obtain the final hybrid materials (see Section S1 and Figure S1 for full experimental details).

2.2. Principal component analysis and clustering

To assess the relationship between the photodegradation rate constant and the synthesis yield of the nine ZnO samples, Principal Component Analysis (PCA) was performed using the software CAT, obtaining the first and second principal component scores (PC1 and PC2). For the clustering analysis, the Euclidean distance method was applied using Origin 2025 v. 10.2.0.188 software to evaluate similarity between samples. Hierarchical clustering was conducted to group the samples based on their proximity in the PCA space. A 95 % confidence level was established to determine the significance of the cluster separations. The final cluster centers were identified, and a distance matrix was computed to quantify the spatial separation between the three primary clusters.

2.3. Morphological and spectroscopic characterization of ZnO microcrystals

The simulated irradiation was obtained by using a solar simulator,

model 10500 ABET Low Cost (Abet Technologies, USA), equipped with a xenon arc lamp (150 W power) and an AM1.5 G filter. The distance between the sample and the solar simulator was calibrated with a commercial KG5-filtered Si calibration cell (model 15151, Abet Technologies, USA) to obtain an incident light with a power of $100 \text{ mW} \cdot \text{cm}^{-2}$, corresponding to 1 sun. The ζ -potential experiments were performed using Zetasizer Nano-ZS (Malvern Instruments) under isothermal conditions at 25°C . The measurements were carried out using a disposable folded capillary cell on aqueous dispersions of ZnO crystals at a concentration of 0.5 mg mL^{-1} . The pH was fixed at 6. UV-Vis diffuse reflectance spectra were recorded by using a UV-Vis-NIR V-770 Jasco spectrophotometer equipped with a 60 mm integrating sphere (ISN-923). ZnO powder was placed in a solid sample holder, and measurements were performed in the range 200–800 nm by setting a scan speed of 100 nm/min and a bandwidth of 2 nm. The photocatalyst band gap was calculated using the Kubelka-Munk method [44]. X-ray diffraction (XRD) data were acquired by a Rigaku Smartlab SE XRD multipurpose diffractometer, using a Cu $K\alpha$ radiation source ($\lambda = 0.154 \text{ nm}$), run at 40 kV and 50 mA.

Raman spectra of the ZnO samples were acquired using a LabRam HR-Evolution spectrometer (HORIBA France SAS, Lyon, France) in back-scattering configuration, integrated with a confocal microscope equipped with $50 \times$ long working distance objective, and a $200 \mu\text{m}$ pinhole aperture. Excitation was provided by a 532 nm laser source using non-destructive laser power conditions, and spectral dispersion was achieved with a grating of 600 lines per millimeter.

The morphology of the ZnO samples was examined using a TESCAN MIRA 3 field emission scanning electron microscope (FE-SEM, TESCAN, Brno, Czech Republic) equipped with an energy-dispersive X-ray (EDX) spectrometer (OXFORD XPLORE 30). The FE-SEM was operated at electron energy of 30 keV and a beam current of 1 nA. The working distance was set to 8 mm during image acquisitions, while an analytical distance of 15 mm was used for EDX analysis.

The surface area and pore size distribution of ZnO were assessed using a Quantachrome Autosorb iQ mp 8619 instrument, with data analysis performed using the ASiQwin software, and nitrogen adsorption at 77 K. Prior to analysis, the sample was degassed in two steps at 80°C and 120°C . Subsequently, the degassed sample was weighted, and adsorption analysis was carried out at 77 K employing nitrogen as the probe gas [49]. The specific surface area was calculated according to the Brunauer-Emmett-Teller (BET) theory [50]. Full details of the degassing procedure, calculations, and equations are provided in [Supplementary Information](#), Section S2.

2.4. Photocatalytic characterization of ZnO structures

The reagent Methylene Blue (MB) was employed for the photocatalysis experiments and used without further purification. The hybrid films were first incubated with MB solution (3 mL, $25 \mu\text{M}$) in the dark to allow adsorption equilibrium to be reached for 1 h, and the initial absorbance was recorded as control condition [44]. Irradiation was then applied, and the degradation of MB was monitored by measuring the absorbance at regular time intervals (20 min) over the course of the experiment up to 120 min. Magnetic stirring was used to ensure proper dispersion of ZnO in the solution. Control experiments were performed under identical conditions but without light exposure. Moreover, similar photocatalytic experiments were also performed using Methyl Orange solutions (MO) as a different dye.

The trapping experiments were carried out in the presence of specific scavenger species and subsequently performed under the same conditions as the standard photodegradation tests, as elsewhere reported [44]. Stock solutions of glycerol, ethanol, benzoquinone, and silver nitrate were freshly prepared in ultrapure water and then added to the 3 mL of MB solution so that their final concentrations were 1 mM for glycerol, 1 mM for ethanol, 0.2 mM for benzoquinone, and 1 mM for silver nitrate.

2.5. Antibacterial assays

The antibacterial activity was evaluated on Runs 3, 8, and 9 among the synthesized samples to examine whether structural variations affected antibacterial behavior. Antibacterial activity was quantified by measuring the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) against *S. aureus* ATCC 29213 (Gram-positive) and *E. coli* ATCC 25922 (Gram-negative), used as reference strains for antimicrobial susceptibility testing [38]. MIC and MBC determinations were performed to evaluate the antibacterial activity of the synthesized ZnO samples, following standardized broth macrodilution procedures. Additional methodological and interpretative details are reported in the [Supporting Information](#) (Section S3).

Bacterial suspensions were prepared from exponentially growing cultures and diluted to achieve a final bacterial concentration of $5 \times 10^5 \text{ CFU/mL}$. ZnO was dissolved in sterile H_2O and solubilized by adding 3 % v/v acetic acid to obtain a stock solution at a concentration of 10 g/L. Serial dilutions of ZnO were prepared from 1024 to 32 mg/L in CAMHB (BD, Becton, Dickinson and Company, NJ, USA), with the final tube serving as positive control [51].

MIC and MBC were determined according to EUCAST and CLSI guidelines (EUCAST 2022; CLSI 2022). After 24 h and 48 h of incubation at 37°C , MIC was assessed by observing the turbidity of the tubes. While MBC was determined by transferring 100 μL of aliquots from the corresponding one- and two-fold dilution MIC tubes, seeded on Mueller-Hinton agar (Oxoid, Thermo Scientific, Basingstoke, United Kingdom), and incubated overnight. For both *S. aureus* and *E. coli*, the tests were repeated under both static and dynamic conditions (500 rpm) to evaluate any differences in efficacy between the two modalities. The standard deviation in MIC testing is typically ± 1 fold dilution for broth macrodilution methods [52]. Additional experimental details are reported in the [Supporting Information](#) (Section S3).

2.6. Electrochemical analysis of Zinc ions release

The electrochemical characterization was performed using Anodic Stripping Voltammetry (ASV) to detect zinc ions released from ZnO under dark conditions. Measurements were carried out with a potentiostat/galvanostat (Metrohm Autolab PGSTAT 128 N, Utrecht, Netherlands) connected to a three-electrodes serigraphic-printed sensor (ItalSens-Au sensor, Houten, Netherlands). The analytical protocol follows a previously reported method [53], with the following experimental parameters. Briefly, a reduction potential of -0.9 V was applied for 90 s without stirring to pre-concentrate Zn^{2+} ions (step potential: 4 mV; amplitude: 50 mV, frequency: 50 Hz) using 0.1 M Britton-Robinson buffer (pH 7.0) as the background electrolyte. The buffer was prepared from acetic acid (Sigma Aldrich, ReagentPlus $\geq 99\%$), boric acid (Sigma Aldrich, BioReagent $\geq 99.5\%$) and orthophosphoric acid (Sigma Aldrich, 85 wt% in H_2O) and the pH was adjusted to 7.0 using 0.1 M NaOH (Merck) and measured with a pH meter (Eutech Instruments Europe B.V.). The samples were selected from the antibacterial assay conducted according to the protocol outlined in paragraph 2.5, utilizing the ZnO powder from Run 3. For zinc ion analysis, 1 mL samples were collected 24 h after the MIC point tubes and from a lower concentration of the MIC point for each bacterial strain, as well as from the corresponding control tubes containing ZnO without bacteria. To ensure data normalization, additional samples were also taken from the positive (bacteria and CAMHB) and negative control tubes (CAMHB alone). Since the average bacterial cell sizes of the strains used are in the range of $0.5\text{--}2 \mu\text{m}$ and Run 3 particle size distribution in the range of $0.5\text{--}3 \mu\text{m}$ (Fig. 4), the samples were filtered using $0.2 \mu\text{m}$ filters in order to analyze the dissociated Zn^{2+} ions present in the aqueous solution without interference from cells or microcrystals. For each measurement, 10 μL of sample was diluted into 490 μL of 0.1 M Britton-Robinson buffer, resulting in a 1:50 dilution (v/v).

3. Results and discussion

3.1. Chemometric analysis of ZnO microcrystals synthesis

The synthesis approach employed in this work is based on a previously optimized work for ZnO nanowires synthesis on electronic interfaces [22]. Notably, the ZnO microcrystals obtained by this method are obtained at moderate supersaturation, to favor the formation of ZnO at the copper PCB interface rather than in the solution phase, leading to extremely low yield (4 mg of ZnO produced from a typical experiment). The synthesis yield is an important parameter to consider in synthetic design because it directly impacts the overall efficiency of the process, as it reflects the amount of product produced relative to the initial precursor. High synthesis yields reduce waste, making the process more sustainable, and allow for higher product quantities per synthesis batch, which is crucial for optimization [54]. While the photodegradation rate constant (k) gives insight into the performance of the photocatalyst, synthesis yield provides a complementary perspective on the effective process.

To solve this issue, a DoE was initially employed to optimize both the synthetic yield and the photocatalytic activity. The present study aims to provide a practical demonstration of the photocatalytic activity of ZnO/cellulose composites, using methylene blue (MB) as a model compound to study the light-induced formation of charge carriers and ROS from ZnO microcrystals in each of fourteen experiments. The photocatalysis experiments were conducted randomly to minimize the potential biases and ensure the reliability of the results. To this aim, pseudo-first-order kinetic was employed to determine an apparent rate constant (k) using the linear regression model $\ln(C/C_0) = kt$, where C is the MB concentration after a given time exposure to 1 sun irradiation, and C_0 is the concentration after adsorption/desorption equilibrium [55–57]. The synthesis yield of ZnO microcrystals and the apparent rate constant (k) are reported in Table 2.

It is well known that the mechanism of ZnO photocatalysis includes the absorption of photons with energy equal or higher than the band gap, resulting in the generation of excited electrons and holes in the conduction and in the valence bands, respectively [58,59]. The excited electrons reduce molecular oxygen, producing reactive species such as superoxide radicals ($O_2^{\bullet-}$) and hydroxyl radicals ($\bullet OH$), which can lead even to complete mineralization of MB [44]. MB can also directly absorb on the photocatalyst surface and undergo degradation through a direct photocatalytic pathway, independent of ROS mediation [60].

Control experiments performed under dark conditions showed an almost negligible degradation of the dye, confirming that the

photodegradation process is primarily driven by the ZnO photocatalytic activity. The apparent photodegradation kinetic constants obtained are in good agreement with those reported in the literature (Table S1).

Moreover, to further support the versatility of the ZnO microcrystals as photocatalysts, tests were also performed using an anionic dye, Methyl Orange (MO). These tests confirmed effective photocatalytic degradation, demonstrating that the composites can act on molecules with different charges (Figure S2). The use of MO, together with MB allows us to demonstrate the general photocatalytic capability of the ZnO/cellulose composites toward organic molecules with different physicochemical properties. In this context, the present results should be regarded as a proof of concept, while future investigations will be specifically devoted to the degradation of environmentally relevant emerging organic contaminants.

The correlation between the synthetic yield and the photocatalytic performance suggests that the efficiency of ZnO is influenced not only by the material's structural properties but also by the synthesis conditions. To further investigate this relationship, a second-order polynomial model was employed to describe the MB degradation efficiency (Y) as a function of Zn concentration (X_1), KCl concentration (X_2), and reaction time (X_3). The coefficients obtained from the model are reported in Fig. 1A and in Table S2. This regression analysis was used to identify the dominant effects and interaction trends within the experimental domain investigated, while quadratic terms, describing curvature effects, were not included as the aim was to compare discrete synthesis conditions and identify the most performant ones.

The results indicate that both reaction time and the Zn concentration significantly ($p < 0.01$) influence the photocatalytic activity of the system. The positive coefficient associated with X_1 indicates that increasing the zinc precursor concentration enhances the photocatalytic response, likely by increasing the amount of Zn^{2+} ions in solution, which likely promotes nucleation and crystal growth. This results in the formation of a larger number of microcrystals, enhancing the overall availability of active sites. Conversely, the negative coefficient associated with X_3 indicates that prolonged reaction times negatively affect the response, as they allow crystals to grow larger, reducing the surface area. Shorter reaction times limit crystal growth, leading to smaller microcrystals with higher specific surface area. This increased surface area is directly associated with a greater number of active sites for photocatalytic reactions, thereby improving the interaction between the catalyst and the target molecules [46,61,62]. In contrast, the interaction terms involving KCl show limited statistical relevance, suggesting that synergistic effects between salt concentration and the other variables are weak within the investigated experimental space. Finally, response surfaces were generated, which are three-dimensional color-scaled graphical representations that illustrate how the response varies as two factors change simultaneously. Fig. 1B shows the response surface as a function of reaction time and zinc concentration. This representation, in agreement with the coefficient plots, emphasizes that reducing reaction time and increasing zinc concentration are key parameters for maximizing efficiency.

Both the coefficient plot and the response surface provide valuable insights into the effects of experimental variables on the photodegradation rate constant. However, these analyses can also be extended by considering synthesis yield as an additional response variable. In this context, the relationship between synthesis yield and photocatalytic activity was evaluated simultaneously through a multivariate analysis was performed using Principal Component Analysis (PCA) [63,64]. PCA allows for accounting for the relationships between these effects, providing insight into which of the 14 experimental runs exhibit optimal characteristics. The two selected parameters allowed for the extraction of the first and second principal component scores (PC1 and PC2), which are presented in the biplot of Fig. 2A. The score plot illustrates the distribution of samples revealing the separation of them into three primary clusters. These clusters were identified by applying the K-Means algorithm on the PCA-reduced data [65] (Fig. 2B), thereby enabling the

Table 2

Synthetic yield and apparent photodegradation kinetic constants (min^{-1}) for each run. The synthetic yield represents the molar percentage of the final ZnO product, calculated relative to the initial zinc precursor. The apparent photodegradation kinetic constants were obtained from the linear fit of the MB photodegradation experiments under solar simulator light exposure.

Run	Synthesis Yield	Apparent photodegradation kinetic constant (min^{-1})
1	11.7 %	-0.0179 $\pm$ 0.0005
2	75.7 %	-0.065 $\pm$ 0.005
3	76.4 %	-0.067 $\pm$ 0.006
4	25.8 %	-0.0177 $\pm$ 0.0004
5	22.1 %	-0.0096 $\pm$ 0.0003
6	77.5 %	-0.0289 $\pm$ 0.001
7	73.1 %	-0.0276 $\pm$ 0.0006
8	24.5 %	-0.0072 $\pm$ 0.0003
9	60.5 %	-0.0321 $\pm$ 0.0008
10	63.2 %	-0.0313 $\pm$ 0.0007
11	61.8 %	-0.0285 $\pm$ 0.0005
12	58.2 %	-0.026 $\pm$ 0.006
13	55.4 %	-0.0269 $\pm$ 0.001
14	56.5 %	-0.027 $\pm$ 0.003

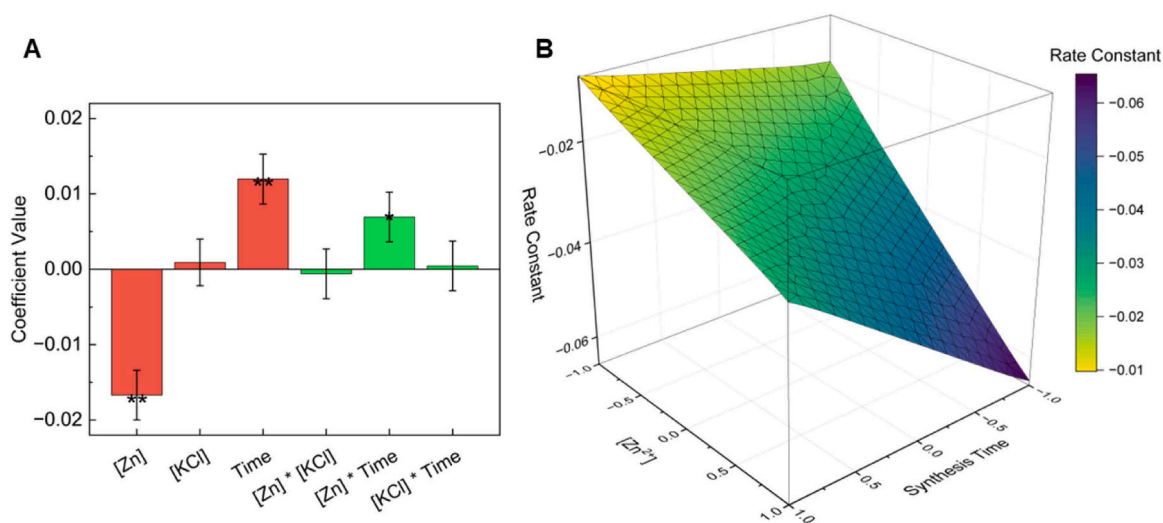


Fig. 1. Coefficient plot showing the influence of each linear term ([Zn], [KCl], Time) and their interaction terms ([Zn]*[KCl], [Zn]*Time, [KCl]*Time) on the response. The error bars represent the confidence intervals provided by the CAT software (A). 3D response surface plot illustrating the effect of reaction time (x-axis) and precursor Zn concentration ([Zn], y-axis) on the photodegradation rate constant (z-axis). Both reaction time and [Zn] vary within the interval from -1 – 1 , as defined by the experimental matrix. The photodegradation rate constant is represented by the color scale on the side of the graph. The third variable [KCl] is held constant at level 0 (B).

grouping of samples and a rational selection of representative systems for further investigation.

The spatial separation between clusters is evident, in particular, Cluster 3 is the most separated from both Cluster 1 and Cluster 2, indicating a distinct grouping in terms of the evaluated parameters. Cluster 2 represents samples with intermediate performance, while Cluster 1 groups samples that are more closely distributed, suggesting a higher degree of similarity among them. The inter-cluster distances, ranging from 2.16 to 4.07, highlight a clear differentiation based on both photocatalytic efficiency and synthesis yield, as will be discussed in detail in the following section. On this basis, samples 1, 3, 8, and 9 were selected as representative systems for further investigation. In particular, two samples were chosen from Cluster 1 to account for the internal variability of this group, while one representative sample was selected from each of the other clusters.

3.2. Surface and bulk features of ZnO microcrystals determining the photocatalytic activity

The optical response of ZnO microcrystals plays a key role in

determining their photocatalytic activity. In this context, diffuse reflectance spectroscopy was performed to evaluate the optical properties of the materials obtained under different synthetic conditions. Across the three analyzed runs, the diffuse reflectance (Figure S3B) exhibit a similar trend, displaying low reflectance in the UV region followed by a steep increase in the visible range above 400 nm. This similarity suggests that the differences in synthesis conditions have not significantly altered the overall optical response of the materials. This analysis allows for the calculation of the optical band gap using the Kubelka-Munk method. In particular, in this approach, the diffuse reflectance is converted into the function $[F(R)h\nu]^n$, which is then plotted as a function of photon energy ($h\nu$). The optical band gap is obtained by extrapolating the linear portion of the plot to the energy axis, where the function $F(R)h\nu$ equals zero, revealing an estimated band gap of approximately 3.24 eV, corresponding to 380 nm, for the three different samples, in line with other studies [66–68]. The consistency of this value indicates that the bulk electronic structure remains unchanged despite the morphological variations induced by different synthetic approaches. Therefore, the observed variations in photocatalytic performance cannot be ascribed to changes in the band gap, but

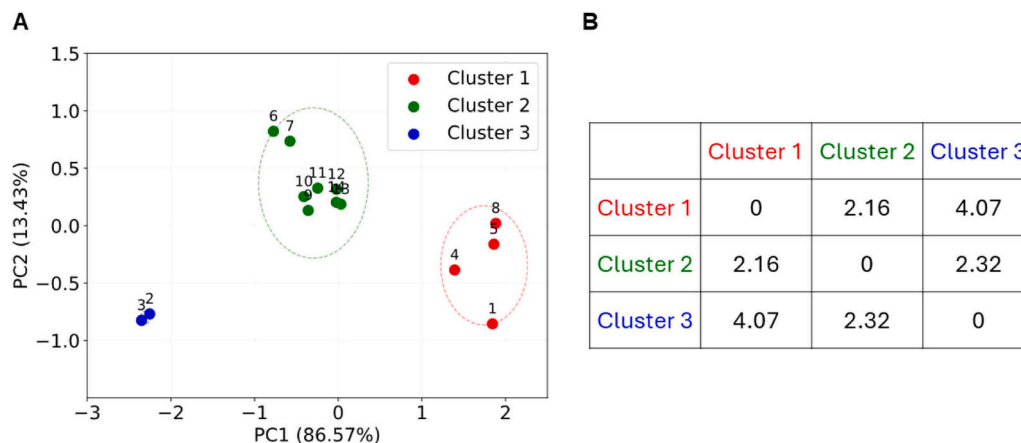


Fig. 2. Score plot showing the distribution of the 14 runs based on the first and second principal components (PC1 and PC2). The samples are grouped into three distinct clusters, which reflect their relative performance in terms of photocatalytic efficiency and yield (A). Matrix of Euclidean distances between the final cluster centers, highlighting the spatial separation between the three identified clusters (B).

are instead attributed to differences in surface and interfacial properties, as widely reported for ZnO-based photocatalysts [69–71]

The calculation of optical band gap values for all the selected samples represents a limited characterization for the description of the photocatalytic activity of the ZnO particles herein studied. Therefore, a comprehensive multi-technique analysis of ZnO microcrystals was carried out in order to characterize the surface properties of the several ZnO samples synthesized, as well as their bulk structure and chemical composition. This approach did allow to rationalize the photocatalytic behaviour of ZnO samples observed in the experiments of MB photocatalytic degradation by evaluating both ZnO surface properties and bulk characteristics. The surface analyses included ζ potential measurements to assess surface charge and aqueous colloids stability, scanning electron microscopy (SEM) for morphological characterization and average diameter estimation, and X-ray diffraction (XRD) for bulk crystalline parameter analysis. Additionally, diffuse reflectance measurements are employed to derive the band gap energy, while Fourier-transform infrared (FTIR) and Raman spectroscopies used to identify functional groups and chemical bonds.

The ζ potential is a key parameter for evaluating the surface charge of the particles, which determines the colloidal stability of the suspensions, as a consequence of the electrostatic repulsion between particles in solution [61,62,72]. Higher ζ potential values indicate greater repulsion, reducing the likelihood of aggregation and leading to more stable dispersions, which can directly influence photocatalytic performance through improved particles dispersion in suspension. This measurement is particularly relevant for assessing the particles charge and the dispersion quality of ZnO microcrystals, which directly influences their photocatalytic performance. However, for Runs 1, 4, 5, the crystals did not disperse adequately, resulting in a non-homogeneous suspension that prevented ζ potential measurement. These observations agree with their low synthesis yields, which can be directly linked to their poor dispersibility and overall instability in suspension. (Table 2). All the measurable samples showed positive ζ potential values (ranging from +9.6 to +29 mV) in agreement with their surface chemical composition and specific ionization equilibria of the functional groups. For pH values lower than the ZnO isoelectric point (pH = 8.7), the surface functional groups of the microcrystals are protonated as $\text{Zn}(\text{OH}_2)^+$ [73]. Accordingly, the measurements, performed at pH = 6, revealed that ZnO microcrystals are positively charged (Fig. 3).

Among the measurable samples, Run 3 exhibited the highest ζ potential, suggesting superior colloidal stability, confirming that the material obtained from this experiment has a more uniform dispersion and higher stability than the others. Both Runs 6 and 7 resulted in a good colloidal stability, while Run 8 and 9 had significantly lower values, with run 8 showing the lowest ζ potential. These results confirm that higher ζ potential values correlate with improved dispersion stability and highlighted a relevant correlation between the ζ potential values and the observed photocatalytic performance of the samples. The ZnO samples herein investigated exhibit a similar optical band gap (~ 3.24 eV), indicating unchanged bulk electronic structure and the absence of significant doping or electronically active defects, even though some pronounced differences in photocatalytic activity were observed. This experimental observation can be rationalized by considering the dominant role of surface and interfacial properties on the photocatalytic process. In this sense, ζ potential measurements revealed a fundamental correlation of the ZnO crystals surface charge with the photocatalytic performances. Samples with higher ζ potential values, indicative of a more highly polarized surface, systematically show larger absolute k_{app} values, reflecting better photocatalytic performance. This correlation can be rationalized by considering that a highly polarized surface enhances charge separation and interfacial charge transfer, acting as local electric fields that reduce electron-hole recombination [69,70]. This prolongs carrier lifetimes and promotes the formation of ROS, such as $\bullet\text{OH}$ and $\text{O}_2\bullet^-$, which are primarily responsible for MB degradation. In addition, at pH 7, both ZnO and MB

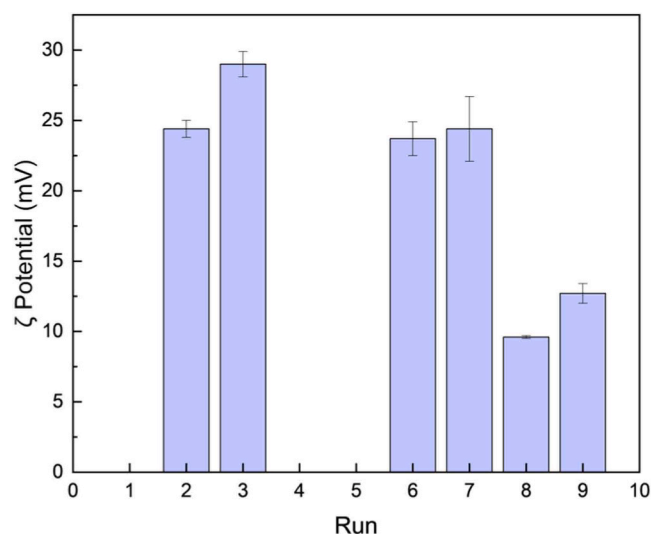


Fig. 3. ζ potential values (mV) for the different runs. Bars represent the mean $\pm$ standard deviation for each sample. Measurements could not be obtained for Runs 1, 4, and 5.

are positively charged, so direct electrostatic adsorption is unlikely. This further information suggests that an indirect degradation mechanism mediated by ROS primarily occurs, rather than direct dye adsorption.

The morphology of the ZnO microcrystals can add further information to elucidate the photocatalytic behaviour of the different ZnO microcrystals herein reported. Selected experiments, namely Run 1, Run 3, Run 8 and Run 9 (control experiment) were characterized by SEM and EDX (Figure S4). Importantly, Run 9 represents the control experiment, whereas Run 1 and Run 8 resulted in the worst yield and photocatalytic activity, respectively. The results of the analysis highlighted the distinct morphological characteristics and particle size distributions of the synthesized samples, as shown in Fig. 4. These morphology variations across the samples arise from differences in synthetic conditions, reflecting the interplay of precursor availability, ionic strength, and reaction duration. EDX analysis confirmed, as expected, the presence of zinc and oxygen (Figure S4).

In the case of Run 1, the sample obtained with low zinc and KCl concentrations and short reaction time, star-like, branched structures were observed (Fig. 4A). Such irregular structures reflect a broad particle size distribution (Fig. 4E), as the lower zinc concentration limits the availability of precursors, likely reducing nucleation density and creating conditions where fewer particles grow larger. Simultaneously, the low ionic strength due to reduced KCl concentration minimizes the electrostatic stabilization of growing particles, promoting directional growth and the development of anisotropic features, such as branches or star-like shapes. Furthermore, the short reaction time further inhibits particle coalescence or ripening, leading to heterogeneous particle sizes and the observed irregular morphology. Run 3, synthesized with high zinc precursor and KCl concentrations and a short reaction time, exhibits the most uniform morphology among the samples, characterized by compact and well-dispersed particles (Fig. 4B), and the size distribution is shifted toward smaller diameters, predominantly in the range of 1–2 μm (Fig. 4F). The high zinc concentration promotes rapid nucleation, resulting in a high density of growth sites that limit excessive crystal growth. The increased ionic strength, due to the higher KCl concentration, plays a crucial role in stabilizing these particles during their growth phase, preventing aggregation and enabling a more uniform size and shape. A correlation can therefore be observed between the morphological characteristics and the ζ potential of Run 3 ($+29 \pm 0.9$ mV) and can be attributed to the higher density of protonated functional groups ($\text{Zn}(\text{OH}_2)^+$) on the surface of smaller particles. The shorter reaction time

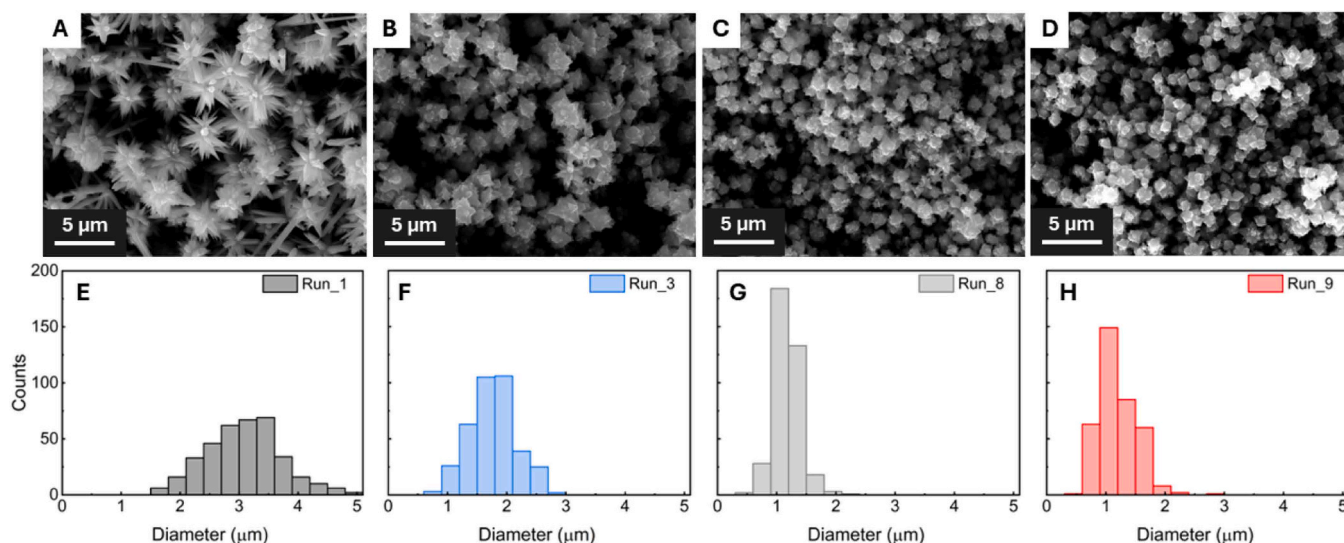


Fig. 4. Representative SEM images of ZnO microcrystals obtained from Run 1 (A), Run 3 (B), Run 8 (C), and Run 9 (D); scale bar 5 μm . Relative particle size distributions corresponding to Run 1 (E), Run 3 (F), Run 8 (G), and Run 9 (H).

ensures that growth is primarily kinetically controlled, limiting further particle enlargement or the development of irregular structures. This combination of factors yields particles with a consistent morphology and size, indicative of a synthesis process dominated by controlled nucleation and stabilized growth.

Run 8, which was synthesized under low zinc precursor concentration, high KCl concentration, and a prolonged reaction time (48 h), presents compact but densely aggregated particles (Fig. 4C). Although the corresponding distribution is narrow and centered around 1 μm (Fig. 4G), the micrograph reveals significant aggregation, with individual particles forming tightly packed clusters. This can be attributed to the low zinc precursor concentration, which likely limits the number of nuclei formed, while the high KCl concentration promotes rapid and anisotropic crystal growth. Over the extended reaction time, the limited availability of zinc and high ionic strength drive particle aggregation, as individual particles collide and fuse into larger clusters. This results in a morphology dominated by densely packed aggregates, despite the small individual particle size. In the case of Run 9, synthesized under intermediate zinc precursor and KCl concentrations with a reaction time of 24 h, it is possible to obtain compact, hexagonal-shaped particles with minimal aggregation (Fig. 4D). Their size distribution is comparatively narrow and centered around 1 μm (Fig. 4H), suggesting that under these conditions the interplay of precursor concentration, ionic strength, and synthesis time supports both shape regularity and dispersion stability.

In summary, higher zinc precursor concentrations (e.g., Run 3) result in smaller, more uniform particles due to rapid nucleation, while lower concentrations (Runs 1 and 8) lead to larger particles or aggregates. These differences in particle size and clustering directly affect the surface area and active sites available for photocatalysis, leading to improved photocatalytic performance in Run 3, while larger or less stable particles with lower ζ potential tend to cluster, resulting in minor photocatalytic activity. KCl concentration stabilizes ions in solution and influences directional growth. ZnO crystals from Runs 9 and 8 have narrow size distributions around 1 μm , while those from Run 3 are skewed toward 1–2 μm , and Run 1 shows the broadest distribution, up to 5 μm . Higher KCl concentrations (Runs 3 and 8) accelerate growth but can cause aggregation with extended reaction times. Lastly, reaction time is a critical factor in determining both particle growth and aggregation: shorter reaction times, as in Run 1 and 3, leads the formation of discrete particles, whereas longer reaction times, like those in Run 8, allow particles to interact and aggregate.

X-ray diffraction (XRD) analysis was conducted to investigate the

bulk crystallographic structure, phase purity, and lattice parameters of the ZnO microcrystals produced under the above selected conditions (Run 1, Run 3, Run 8 and Run 9). The obtained results are shown in Fig. 5A.

The XRD pattern revealed the characteristic diffraction peaks of ZnO at 31.9° , 34.1° , 36.4° , 47.9° , 56.6° , 61.6° , 66.1° , 67.3° , 68.9° , 72.2° and 76.6° , corresponding to the crystallographic planes (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202). These peaks align with the hexagonal wurtzite structure of ZnO, as indicated by ICDD card no. 36–1451, with no additional impure crystalline phases, thereby confirming the purity of the material [74]. This high purity is essential for ensuring the desired properties, such as photocatalytic activity, and suggests that the synthesis method effectively promoted the formation of a single-phase material. The similarity of the diffraction patterns indicates that differences in photocatalytic performance are not associated with changes in crystal phase or band structure. The lattice parameters of the ZnO structure, measured at room temperature, were found to be $a = 3.25 \text{ \AA}$ for the in-plane (horizontal) parameter and $c = 5.20 \text{ \AA}$ for the out-of-plane (vertical) parameter, values that are characteristic of the ideal wurtzite structure [75].

The texture coefficient analysis revealed that the highest texture coefficient (TC) for the (101) plane was observed for the optimal Run 3 with a value of 0.442, while Run 8 showed the highest TC for the plane (002). These observations suggest a correlation between the crystallographic orientation induced by the synthesis conditions and the resulting photocatalytic performance. However, it should be noted that photocatalytic activity in ZnO is governed by the interplay of multiple structural parameters, including crystallite size, defect density, surface states, and preferential crystallographic orientation. In particular, higher activity observed for samples enriched in the (101) plane due to its high atomic density and the presence of defect-rich surfaces [76], and lower activity for samples with a predominance of the (002) plane. Such differences are further reflected in the surface charge behavior, as evidenced by the ζ potential measurements. For instance, Run 3, which exhibited the highest photocatalytic activity, showed greater stability of the ZnO in solution, likely due to the dominant (101) plane. On the other hand, Run 8, which showed lower photocatalytic activity, demonstrated reduced stability, consistent with the presence of the (002) plane. The ζ potential data further support this interpretation. We detected that Run 3 possesses a ζ potential value three times larger as compared with Run 8. Overall, these results indicate that the observed trends in photocatalytic performance likely originate from a combination of

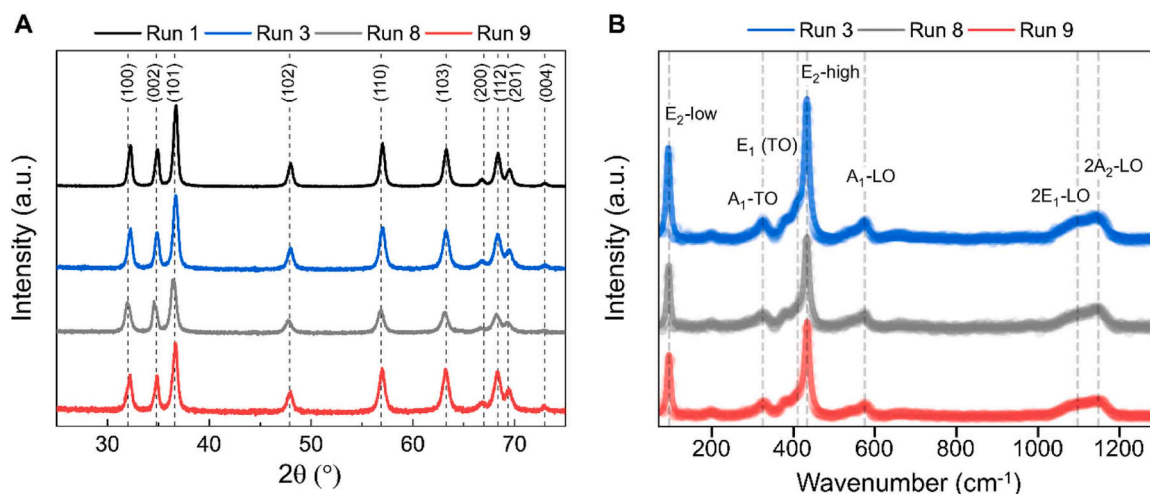


Fig. 5. XRD patterns of ZnO samples from Run 1 (black), Run 3 (blue), Run 8 (grey), and Run 9 (red). The diffraction peaks, highlighted by dashed vertical lines and labelled at the top of the figure, correspond to the characteristic reflections of the crystalline phases present in the ZnO samples (A). Representative Raman spectra (dots) of ZnO samples from Run 3 (blue), Run 8 (grey), and Run 9 (red) and their relative fitted curve (continuous lines), showing the characteristic vibrational modes of the wurtzite ZnO crystal structure, highlighted by labels and dashed vertical lines. (B).

preferential crystallographic orientation, defect-related surface properties, and associated surface charge effects, rather than from crystallographic orientation alone. Therefore, we can conclude that the better photocatalytic performances of Run 3 agrees with the higher surface density charge due to $\text{Zn}(\text{OH}_2)^+$ groups.

Moreover, the analysis of lattice parameters provided further insights, as shown in the [Supporting Information \(Figure S5\)](#). The lattice parameter “*a*” showed changes across the different experiments, suggesting a possible alteration of the lattice structure, likely due to the variations in synthesis conditions. Conversely, the “*c*” parameter exhibits greater consistency across the runs, with the exception of Run 8, where an increase is observed, suggesting a reduced expansion of the lattice structure. Given the intermediate properties exhibited by Run 1, which did not significantly distinguish it from the others, this condition was excluded from further analysis. To streamline the subsequent spectroscopic investigations and focus on the most representative samples, the characterization was continued with Runs 3, 8, and 9, to investigate potential differences among the ZnO microcrystals.

The Raman spectra collected in back scattering configuration, reveal features characteristic of the wurtzite crystal structure for sample in powder form [77–79], confirming the high structural order of the material. The observed Raman bands correspond to the vibrational modes expected for ZnO, which, according to literature [77,80] comprises six optical phonon modes, three of which are Raman-active. Some of these correspond to longitudinal optical modes (LO) and trasversal mode (TO). For a clear classification of the peaks collected, the Raman spectra of Runs 3, 8, and 9 were fitted using Lorentzian line shapes. The fitting procedure and the resulting spectral components are reported in the [Supporting Information \(Figure S6\)](#), while the extracted peak positions and full width at half maximum (FWHM) values are shown in [Figure S7](#). The first order peaks collected are located at approximately $\sim 95 \text{ cm}^{-1}$ (E_2 -low), $\sim 382 \text{ cm}^{-1}$ (A_1 -TO), $\sim 410 \text{ cm}^{-1}$ (E_1 -TO), $\sim 434 \text{ cm}^{-1}$ (E_2 -high) and $\sim 574 \text{ cm}^{-1}$ (A_1 -LO). The E_2 modes correspond to nonpolar vibrations confined within the basal (*a*–*b*) plane: E_2 -low involves mainly Zn atom displacements, while E_2 -high arises from the oxygen sublattice. In addition, A_1 -TO and A_1 -LO mode represents a polar vibration along the *c*-axis (out-of-plane), associated with the oscillation of atoms along the polar direction of the wurtzite lattice, instead E_1 -TO mode represents a polar vibration perpendicular to the *c*-axis [77,81]. More details about second-order peaks and their classification are reported in [Supporting Information in Table S3](#).

The E_2 -low, E_2 -high, and A_1 -LO modes of all samples are

systematically red-shifted by approximately 4 cm^{-1} with respect to bulk ZnO values. Since this behavior is observed for all runs, it is attributed to a general relaxation of internal lattice strain or to growth-induced structural distortions rather than to run-dependent effects [82]. This result is consistent with the similar average lattice parameters obtained from XRD analysis.

Differences among the three runs mainly emerge from the analysis of the peak widths. The E_2 (high) mode exhibits slightly narrower line-widths for Runs 3 and 8 compared to Run 9, although the difference remains limited ($13.7 \pm 0.5 \text{ cm}^{-1}$, $13.8 \pm 0.5 \text{ cm}^{-1}$ and $10.4 \pm 0.5 \text{ cm}^{-1}$ respectively), indicating that variations in bulk crystalline order are modest and consistent with the XRD results. A more pronounced run-dependent behavior is observed for the A_1 (LO) mode, whose linewidth is significantly narrower for Run 8 ($18 \pm 2 \text{ cm}^{-1}$) compared to Runs 3 and 9, which both show broader values ($24 \pm 2 \text{ cm}^{-1}$). Since LO phonon modes are particularly sensitive to lattice disorder and oxygen-related defects, this behavior reflects a reduced heterogeneity of the local vibrational environment for Run 8 [83,84]. Complementary evidence of structural quality and chemical interactions was obtained from ATR-FTIR spectroscopy ([Figure S3A](#)). The ATR-FTIR spectra of the three Runs display a prominent peak near 500 cm^{-1} , characteristic of the Zn–O stretching vibration in the wurtzite lattice. In addition, weaker absorptions bands at 380 and 415 cm^{-1} can be attributed to the Zn–O–Zn bond stretching vibrations, consistent with the transverse optical modes expected for the wurtzite phase at $\omega_{\text{T//}} = 377 \text{ cm}^{-1}$ and $\omega_{\text{T⊥}} = 406 \text{ cm}^{-1}$ [85–87].

Building on these findings, and to gain further insight into the structural features governing photocatalytic performance, particular attention was directed toward the investigation of the textural properties of the material.

To this end, the specific surface area of the best performing sample, Run 3, was determined by nitrogen adsorption measurements. [Fig. 6](#) presents the N_2 adsorption–desorption isotherms, which, according to the IUPAC classification, correspond to a type IVa isotherm with an H3-type hysteresis loop [88]. This profile is characteristic of mesoporous solids formed by aggregates of plate-like particles, in which slit-shaped voids and interstitial pores lead to capillary condensation in the relative pressure range (0.4–0.9) [89]. In agreement with the SEM data, Run 3 arises from voids between aggregated particles, rather than from pores within the individual particles. Moreover, the absence of a plateau at low relative pressures suggests negligible microporosity, whereas the steep rise in nitrogen uptake close to $P/P_0 = 1$ reveals the presence of

interparticle voids, which can be directly correlated with the interstitial spaces between the well-dispersed particles observed in the SEM micrographs. The specific surface area, calculated by the multipoint BET method, was found to be $7.3 \text{ m}^2/\text{g}$, which lies within the range commonly reported for microcrystalline ZnO materials showing good photocatalytic performances, with values depending on synthesis conditions and aggregation state [90–93]. Although this surface area is lower than that typically reported for highly nanostructured ZnO, the observed photocatalytic activity indicates that, in the present microcrystalline system, photocatalytic efficiency cannot be ascribed to surface area alone. Instead, the photocatalytic performance likely results from the combined contribution of surface area and high crystallinity, favorable surface charge, controlled morphology, and reduced recombination losses. The pore size distribution, obtained from the desorption branch by applying the BJH model, reveals a predominant mesoporosity with a main peak centered at approximately 3–5 nm. At larger pore diameters, the differential pore volume (dV/dD) gradually decreases, indicating a progressively lower contribution of wider pores to the total pore volume [94–96].

3.3. Investigation of radical species photogenerated and photocatalyst reusability

To clarify the contribution of different ROS to the photocatalytic process, specific scavengers were employed and the resulting effects on degradation efficiency were analyzed. The selectivity of these scavengers makes it possible to correlate the observed changes with the predominant active species. In Fig. 7A the scavengers employed in this study are reported, each selectively interacting with the different radical species photogenerated upon illumination of ZnO with a solar simulator. When photons with energy exceeding the semiconductor band gap are absorbed, an electron is excited from the valence band (VB) to the conduction band (CB), resulting in the formation of an electron–hole pair (e^-/h^+). Glycerol is widely reported in the literature as an efficient scavenger of photogenerated holes (h^+) [97,98], whereas AgNO_3 acts as an electron scavenger [44]. Once the e^-/h^+ pairs are generated, they interact with molecular species at the interface; in particular, aqueous species and dissolved oxygen are converted into hydroxyl radicals ($\bullet\text{OH}$), which are trapped in the presence of ethanol [99], and into highly reactive singlet oxygen ($\text{O}_2\bullet$) and superoxide-derived species, which are in turn scavenged by benzoquinone (BQ) [100,101].

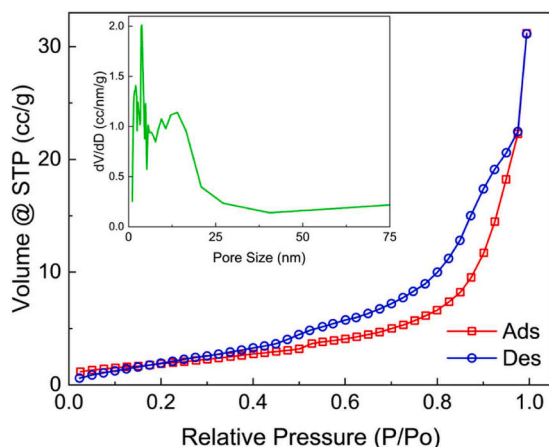


Fig. 6. BET nitrogen adsorption–desorption isotherms of Run 3, obtained at 77 K. The adsorbed nitrogen volume (cc/g at STP) is plotted as a function of the relative pressure (P/P_0). The specific surface area was determined by the Brunauer–Emmett–Teller (BET) method from the linear region of the adsorption isotherm. The inset graph reports the pore size distribution calculated from the desorption branch using the Barrett–Joyner–Halenda (BJH) model, expressed as differential pore volume (dV/dD , cc/nm^3) versus pore diameter (nm).

The results of the study shown in Fig. 7B, highlight that glycerol exhibited the most pronounced decrease in efficiency, limiting the degradation to approximately 88 %. Ethanol and BQ, produced comparatively milder effects, with efficiencies of 97 % and 94 %, respectively. Experiments carried out in the presence of silver nitrate showed an increase in photodegradation efficiency, thus precluding a direct comparison in terms of trapping activity. On the basis of literature data, this effect may be tentatively rationalized by the ability of Ag^+ ions to accept photogenerated electrons from ZnO, thereby reducing electron–hole recombination and indirectly increasing the availability of photogenerated holes and hydroxyl radicals [102–104]. Nevertheless, further dedicated studies would be required to conclusively elucidate this behavior. Altogether, these findings strongly suggest that photogenerated holes represent the key reactive species driving the observed photodegradation, as their removal by glycerol resulted in the most significant loss of activity, similarly to what has been reported in the literature for other comparable photocatalysts [97,98,105], thus providing further insights into the efficiency of the process.

Moreover, the ZnO–cellulose hybrid photocatalyst exhibits good stability and sustained photocatalytic efficiency over multiple cycles. As shown in Supplementary Figure S8, the temporal evolution of C/C_0 during four consecutive cycles remains comparable, despite moderate variations in the apparent kinetic constant (-0.047 min^{-1} in the first cycle, -0.043 min^{-1} in the second, -0.031 min^{-1} in the third, and -0.039 min^{-1} in the fourth). In all cases, complete photodegradation is achieved. XRD patterns collected before and after dispersion in water (Figure S9) show very slight differences of the ZnO diffraction peaks, with no appreciable changes in peak positions. These variations are within the experimental uncertainty and indicate that the overall crystal structure of ZnO is preserved. Consistently, the photocatalytic performance remains stable upon repeated use, supporting the reusability of the material.

3.4. Antibacterial activity of ZnO microcrystals

ZnO has garnered significant attention for its antibacterial properties, which are attributed to its ability to disrupt bacterial cell structures and functions [32]. The antibacterial activity of ZnO is primarily driven by the generation of ROS and zinc ion release under light exposure or in dark conditions, which leads to oxidative stress, damage to cell membranes, and ultimately bacterial cell death [106,107]. Additionally, the release of zinc ions further contributes to its antimicrobial effects by interfering with cellular processes [107]. In this context, the determination of the Minimum Inhibitory Concentration (MIC) under standardized conditions is a critical step in assessing the efficacy of antimicrobial agents. By analyzing MIC results for ZnO in Run 3, Run 8, and Run 9 samples, it was possible to identify the lowest concentration of ZnO microcrystals capable of inhibiting the visible growth of a microorganism. Interestingly, similar approaches have been explored in the development of alternative dosage forms incorporating conventional antibiotics, such as polymeric microparticles, hydrogels, and nanofiber systems [108–110]. Table 3 shows the MIC values obtained after 24 and 48 h of exposure, revealing significant differences among the samples, reflecting the distinct effectiveness against *S. aureus* and *E. coli* under both static and dynamic conditions.

Run 3 exhibits good antibacterial activity, particularly against *S. aureus*, with MIC values of 128 mg/L under static conditions at 24 h, which increase to 256 mg/L at 48 h and remain constant under dynamic conditions. In Run 8, the values for *S. aureus* show similar values, with a greater fluctuation between static and dynamic conditions. This variability could indicate that incubation time and movement may influence its behaviour more than in Run 3. Run 9, on the other hand, demonstrates higher MIC values, especially under static conditions and with prolonged exposure time, in accordance with other studies [111].

This behaviour suggests that Run 3 possesses enhanced initial efficacy, potentially due to an optimal ZnO particle morphology or an

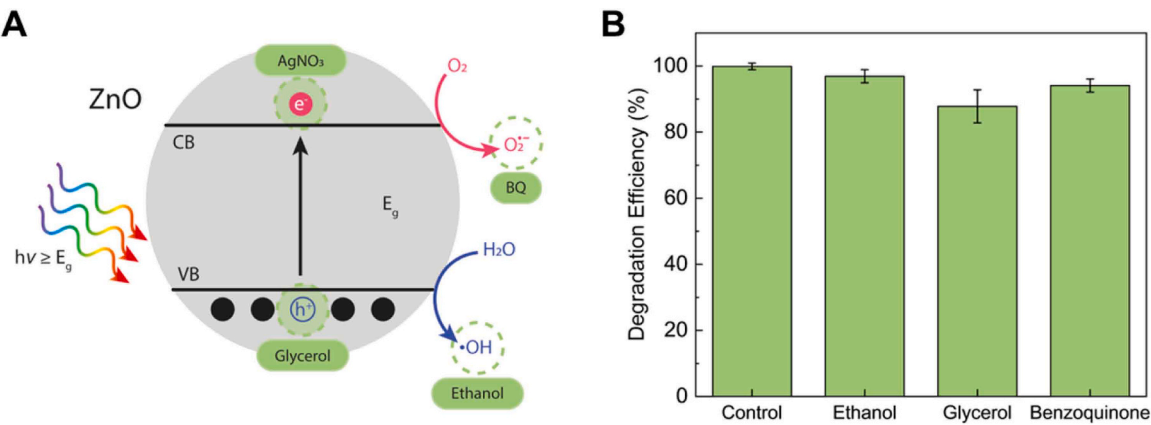


Fig. 7. Schematic representation of the photocatalytic mechanism at the ZnO interface, illustrating the generation of electron–hole pairs upon solar light irradiation ($h\nu \geq E_g$) and the subsequent formation of reactive oxygen species. The role of scavengers is highlighted: glycerol as h^+ scavenger, ethanol as $\cdot OH$ scavenger, benzoquinone (BQ) as superoxide/singlet oxygen scavenger, and silver nitrate ($AgNO_3$) as electron scavenger (A). Photodegradation efficiency of ZnO after 2 h irradiation in the absence (control) and presence of different scavengers (B).

Table 3
Minimum Inhibitory Concentration (MIC) expressed as mg/L of ZnO samples From Run 3, Run 8, and Run 9 under static and dynamic conditions for *S. aureus* and *E. coli*. The MIC was determined after 24 h and 48 h of incubation. The values are reported as mg/L of the ZnO suspension.

MIC values (mg/L)								
	<i>S. aureus</i>					<i>E. coli</i>		
	Static 24 h	Static 48 h	Dynamic 24 h	Dynamic 48 h		Static 24 h	Static 48 h	Dynamic 24 h
Run 3	128	256	256	256	Static 24 h	256	256	128
Run 8	128	256	128	256	Static 48 h	128	256	256
Run 9	512	512	256	512	Dynamic 24 h	512	512	256

increased release of bactericidal species (Zn^{2+} or ROS). Additionally, the Minimum Bactericidal Concentration (MBC) analysis further confirms the MIC trends as reported in Table 4.

The high MBC value raises the possibility that further investigations could explore whether these effects might be improved through photocatalytic action activated by light, to enhance the bactericidal activity. However, in the dynamic conditions, the data for *S. aureus* show a range of values, with some instances indicating a high bactericidal concentration at the 48-hour time point, which could reflect the influence of the dynamic environment on the bacteria’s susceptibility to the treatment. For *E. coli*, the MBC values are more consistent, though still exhibit considerable variation. In the static conditions, higher concentrations are often required for bacterial eradication, especially in the 48-hour static conditions. In contrast, the dynamic conditions show more varied responses, with some runs showing a decrease in the required concentration over time, suggesting the dynamic environment might alter how *E. coli* responds to the treatment. These differences between *S. aureus* and *E. coli* may be attributed to the distinct structural and compositional characteristics of their cell walls. *E. coli*, a Gram-negative bacterium, has a thin peptidoglycan layer and an outer membrane, characterized by the presence of a liposaccharides layer, that carries negative charges due to dissociated carboxyl groups. ZnO microcrystals might electrostatically interact, causing greater damage to the membrane and requiring lower concentrations for inhibition. In contrast,

S. aureus, a Gram-positive bacterium, has a thicker peptidoglycan layer, which provides more resistance to ZnO [112,113].

For each experimental condition tested, the MBC/MIC ratio was calculated, which establishes whether a substance has bactericidal (≤ 4) or bacteriostatic (>4) characteristics. The obtained ratios are reported in Table 5.

Under most tested conditions and incubation times, samples from the different Runs showed bactericidal properties; however, the transition between bacteriostatic and bactericidal behavior was found to depend on both incubation time and experimental setup (static vs dynamic), suggesting that multiple factors contribute to the antibacterial outcome. To contextualize these findings, it is useful to consider the antibacterial mechanisms of ZnO-based materials reported in the literature. Indeed, the antibacterial activity of ZnO-based materials arises from multiple, synergistic mechanisms involving both chemical and photochemical pathways (summarized in Table S4). Under dark conditions, the primary contribution arises from the release of Zn^{2+} ions, which penetrate the bacterial cell, disrupting membrane and intracellular components. In addition, surface-related effects, including mechanical interactions and surface oxygen species, may contribute to membrane damage and leakage [106]. Even in the absence of irradiation, a light-independent oxidative stress degree has been reported, due to the basal generation of ROS [115]. Noteworthy, several studies further demonstrate the antibacterial performance is significantly amplified under light exposure

Table 4
Minimum Bactericidal Concentration (MBC) expressed as mg/L of ZnO samples from Run 3, Run 8, and Run 9 under static and dynamic conditions for *S. aureus* and *E. coli*. The MIC was determined after 24 h and 48 h of incubation. The values are reported as mg/L of the ZnO suspension.

MBC values (mg/L)								
	<i>S. aureus</i>					<i>E. coli</i>		
	Static 24 h	Static 48 h	Dynamic 24 h	Dynamic 48 h		Static 24 h	Static 48 h	Dynamic 24 h
Run 3	> 1024	> 1024	1024	512	Static 24 h	512	1024	1024
Run 8	> 1024	> 1024	1024	> 1024	Static 48 h	512	> 1024	512
Run 9	> 1024	1024	> 1024	> 1024	Dynamic 24 h	1024	1024	1024

Table 5

The relationship between MIC and MBC (MBC/MIC ratio) provides the basis for distinguishing bactericidal (≤ 4) from bacteriostatic (> 4) antimicrobial activity in vitro [114].

MBC/MIC ratio								
	<i>S. aureus</i>				<i>E. coli</i>			
	Static 24 h	Static 48 h	Dynamic 24 h	Dynamic 48 h	Static 24 h	Static 48 h	Dynamic 24 h	Dynamic 48 h
Run 3	> 4	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	> 4	≤ 4
Run 8	> 4	≤ 4	> 4	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4
Run 9	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4

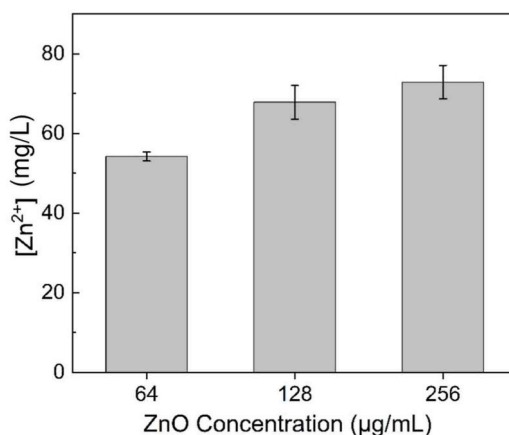


Fig. 8. Bar graph showing the average concentration of Zn^{2+} ions (mg/L) released at increasing Run 3 ZnO suspensions concentration (64, 128 and 256 $\mu\text{g/mL}$) in CAMHB culture, measured by ASV. Error bars represent the standard deviation of three independent measurements, indicating variability in ion release across samples.

through photocatalytic activity, leading to increasing ROS production, particularly H_2O_2 , and consequently higher bacterial killing rate compared to dark conditions [107]. These two diverse mechanisms highlight that while a consistent baseline activity is dependent to Zn^{2+} release, the irradiation-dependent photochemical response provides a potent augment to the material responsible for the increasing of the antibacterial activity.

In the present study antibacterial assay were performed without photoactivation; therefore, the observed bactericidal behavior is most reasonably attributed to Zn^{2+} -mediated effects and surface-associated interactions. This interpretation is further supported by the slightly acidic aqueous environment introduced by acetic acid, which favors partial ZnO dissolution and enhances Zn^{2+} availability in solution [116].

According to Sirelkhatim et al. ZnO particles interact with bacterial cell surfaces and induce membrane disruption, a mechanism that is widely recognized as a primary contributor to their antibacterial activity [117]. Electrostatic interactions between positively charged ZnO particles and negatively charged bacterial envelopes promote strong adhesion at the cell exterior. In this framework, the release of zinc ions from the ZnO particle surface is correlated with the generation of ROS, leading to oxidative damage of the bacterial membrane and associated cellular components.

On the basis of these considerations, the antibacterial activity of ZnO observed under the present experimental conditions is likely attributed to the release of zinc ions, as ZnO suspensions were prepared in an aqueous solution containing 3 % acetic acid to improve dispersibility.

Among the tested samples, Run 3, which showed the most pronounced antibacterial effect, was selected for further investigation of zinc ion release by ASV. To estimate the concentration of Zn^{2+} ions present before bacterial incubation, a control sample referred to as the zero point was analyzed. This sample was prepared with the same ZnO of the test samples but was not incubated with bacteria. As shown in

Fig. 8, the concentration of released Zn^{2+} ions follows a logarithmic increase with respect to ZnO content. Conversely, the degree of ionization, defined as the percentage of free ionized zinc to theoretical amount present in CAMHB, decreases linearly as ZnO concentration rises. Specifically, ionization decreases from approximately 85 % at 64 mg/L ZnO to about 53 % at 128 mg/L and further down to 28 % at 256 mg/L. This behavior is consistent with a concentration-dependent dissolution equilibrium of ZnO in aqueous solution, in agreement with literature data on zinc solubility [107,118,119].

To further clarify the interaction between Zn^{2+} ions and bacterial cells, the concentration of released Zn^{2+} ions in CAMHB culture medium was investigated after 24 h of incubation with *E. coli* and *S. aureus*. These values were compared with Zn^{2+} ion concentration released in the absence of bacteria to determine the percentage of the Zn^{2+} ions remaining in the culture medium at concentrations lower or equal to the MIC point. Intriguingly, as reported in Fig. 9 and Table S5, the concentration of Zn^{2+} ion concentration decreases by 74 % in samples where visible bacterial growth occurred, whereas at the MIC point, showing no visible bacterial growth, the concentration of Zn^{2+} ions decreases only by 29 %.

This differential reduction suggests that bacterial cells actively interact with Zn^{2+} ions, potentially through a biological uptake, likely due to internalization or surface binding by bacterial cells [120,121], thereby reducing the pool of free zinc ions in the medium. Such uptake of zinc ions could be a crucial component of the antibacterial mechanism, since intracellular accumulation of Zn^{2+} is known to impair key cellular processes and homeostasis [107,122]. Therefore, the depletion of free Zn^{2+} in the presence of bacteria not only supports the hypothesis of Zn^{2+} -mediated toxicity but also provides indirect evidence of its uptake by microbial cells, which could underlie the observed bacteriostatic or bactericidal effects. The concentrations of released Zn^{2+} ions measured by ASV under various experimental conditions suggested that

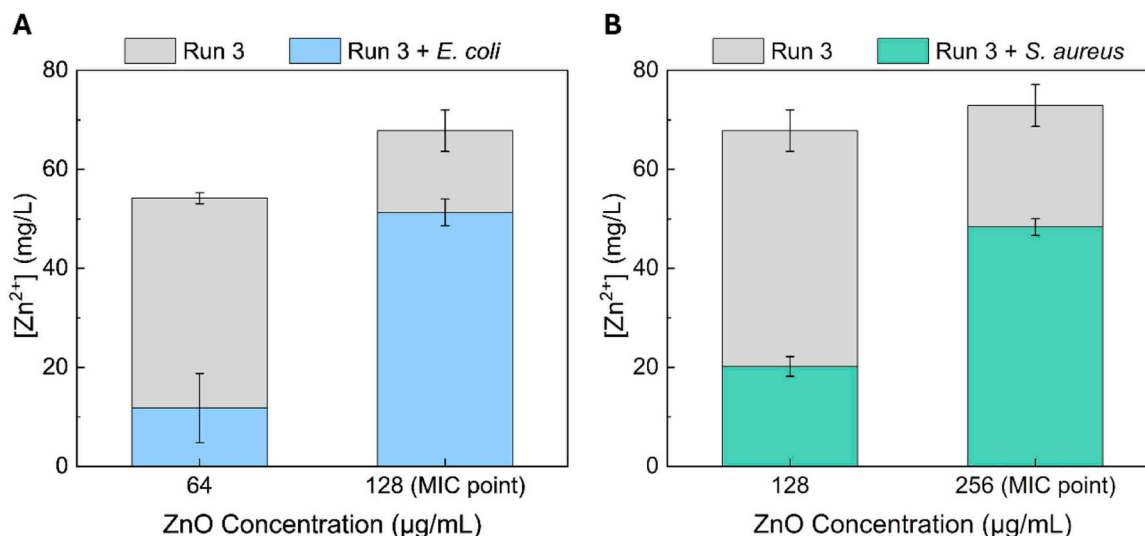


Fig. 9. ASV quantification of Zn^{2+} ion concentration (mg/L) release in CAMHB culture medium containing (A) *E. coli* and (B) *S. aureus*. “Run 3 + bacteria strain” samples report the concentration of Zn^{2+} ions in CAMHB culture medium at two levels: one corresponding to the MIC point (no visible bacterial growth) and one where visible bacterial growth occurs. “Run 3” samples serve as positive controls, measuring Zn^{2+} ions released from ZnO microcrystals in CAMHB culture medium in the absence of bacteria. Data represent average values with error bars indicating deviation from three independent measurements.

the antibacterial activity of ZnO is closely associated with its ability to release Zn^{2+} ions under the tested conditions.

4. Conclusions

In this work, it has been demonstrated that the combined investigation of synthetic yield, photocatalytic and antibacterial properties of ZnO microcrystals, achieved through a rational experimental design approach, enables the rational design of advanced materials that can be supported on cellulose. A two levels central composite design, based on a previously optimized protocol, revealed that optimizing the synthesis parameters of ZnO microcrystals not only leads to a higher synthetic yield but also enhances both photocatalytic efficiency and the overall functional performance of the material. The convergence of these optimal conditions underscores how a fine-tuned balance between precursor concentration and reaction time directly governs the structural and surface properties responsible for the improved activity. These findings were further supported by PCA analysis, which identified three different clusters of synthetic conditions (optimal, suboptimal and control). Comprehensive characterization by SEM, XRD, Raman, FT-IR, and diffuse reflectance confirmed that the optimal synthetic conditions promote the exposure of polar crystallographic planes in the ZnO microcrystals, ultimately improving their colloidal stability. BET surface area analysis additionally confirmed the high specific surface area of these mesoporous materials, making them highly suitable for photocatalytic applications. Trapping experiments provided insight into the mechanisms underlying the generation and efficiency of electron-hole pairs. Moreover, the materials showed reusability, retaining their functional activities.

These properties were completed by a promising antibacterial performance against *S. aureus* and *E. coli* strains, which was slightly improved with respect to the control and suboptimal conditions. The mechanisms responsible for this antibacterial behavior were further investigated through quantitative voltammetric analysis of Zn^{2+} ion release under different experimental conditions.

The results demonstrate that ZnO releases bioavailable zinc ions in sufficient quantities to exert a biological effect. These observations provide further insight into the contribution of ion-mediated toxicity and reinforce the hypothesis that zinc ion release is a central, though not

exclusive, mechanism of antibacterial action. Future investigations will focus on elucidating the influence of ZnO microcrystal size and morphology on their antibacterial efficacy, aiming to optimize their activity.

These results open the way to a data-centric perspective for material design. The systematic collection of experimental data, coupled with the identification of clear quantitative relationships between synthesis variables and performance metrics, provides a robust foundation for future machine learning applications. In the framework of *materiomics*, the information obtained here can be exploited to train predictive algorithms capable of guiding the synthesis of next-generation photocatalysts and antibacterial materials with tailored properties [123–125].

CRediT authorship contribution statement

Giorgia Puleo: Writing – original draft, Validation, Formal analysis, Data curation. **Maria Maggiore:** Writing – original draft, Validation, Formal analysis, Data curation. **Ester Rosa:** Validation, Formal analysis. **Claudia Pellerito:** Validation, Formal analysis. **Giuseppe Cavallaro:** Writing – review & editing, Writing – original draft, Data curation. **Pier Gianni Medaglia:** Validation, Resources, Formal analysis. **Emanuele Sangiorgi:** Writing – review & editing, Validation, Formal analysis. **Paola Conti:** Validation, Resources, Formal analysis. **Fortuna Sebastiano:** Validation, Formal analysis. **Vittorio Ferrara:** Validation, Resources, Formal analysis. **Simonpietro Agnello:** Writing – review & editing, Validation, Formal analysis. **Bruno Pignataro:** Writing – review & editing, Writing – original draft. **Floriana Campanile:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Giuseppe Arrabito:** Writing – original draft, Supervision, Project administration, Funding acquisition.

Ethical approval and consent to participate

This study did not involve human or animal participants.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used OpenAI's GPT-4

model, in order to improve text clarity and grammar checks. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Supplementary material

The following files are available free of charge. The [Supporting Information](#) includes the experimental procedure for the preparation of ZnO/ethyl cellulose hybrid films, surface area and pore size analysis of ZnO microcrystals, a literature comparison of ZnO-cellulose-based photocatalysts, coefficients and statistical analysis from the DoE study, UV-vis absorption spectra of methylene blue during photocatalytic tests, ATR FT-IR and diffuse reflectance analyses, EDX spectra, XRD-derived lattice parameters, Raman spectra with peak fitting, FWHM analysis and second-order Raman mode assignments, photocatalytic cycling stability tests, and zinc ion release measurements in bacterial and control samples.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.colsurfa.2026.139958](https://doi.org/10.1016/j.colsurfa.2026.139958).

Data availability

Data will be made available on request.

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