

# Active packaging approach: impact of zinc oxide nanoparticles shape and size in antimicrobial activity

Ana Rita Mendes<sup>1</sup>, Andreia Leite<sup>2</sup>, Francisco A.G. Soares Silva<sup>1,3</sup>, Carlos Granadeiro<sup>2</sup>, Eulália Pereira<sup>2</sup>, Paula Teixeira<sup>1</sup>, Fátima Poças<sup>1,3,\*</sup>

<sup>1</sup> Universidade Católica Portuguesa, CBQF—Centro de Biotecnologia e Química Fina—Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal;

<sup>2</sup> REQUIMTE/LAQV & Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal;

<sup>3</sup> CINATE, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal;

\*Corresponding author. Email: fpocas@ucp.pt

## Abstract

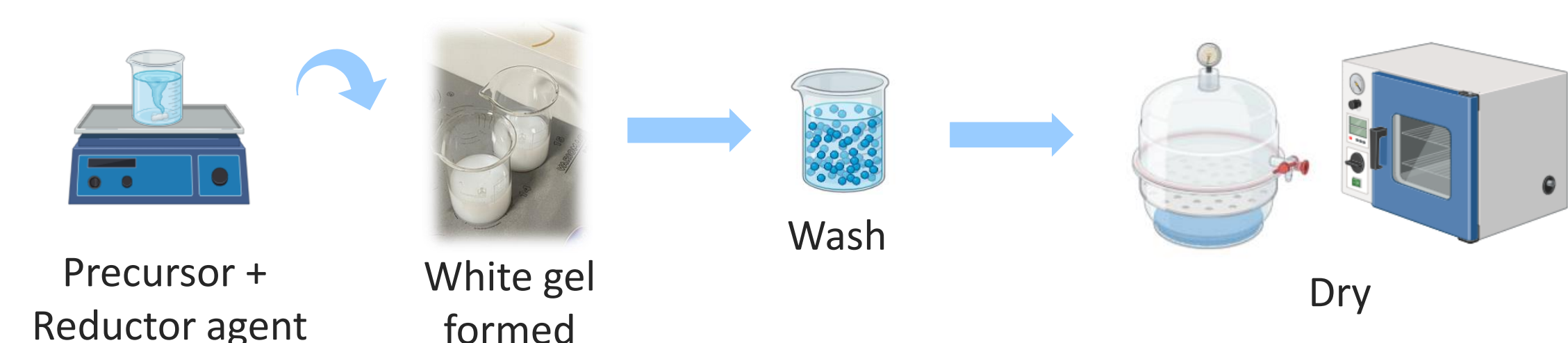
Nanotechnology has been used in food packaging to improve material properties such as barrier to gases, thermal and light stability, and mechanical strength, offering active and intelligent functionalities that assure protection and preservation, together with biobased materials due to requirements for using less plastic packaging.

Zinc oxide nanoparticles (ZnO NPs) has received a positive safety evaluation from European Food Safety Authority (EFSA) for packaging applications as transparent UV absorbers based on absence of significant migration in particulate form. It is also considered Generally Recognized As Safe (GRAS) by the FDA. ZnO NPs are also known to have good antimicrobial properties and therefore are suitable to be applied as active compounds. Despite the abundant literature addressing the use of ZnO NPs as antimicrobial component in packaging materials, the effect of particles size and morphology on the activity against different microorganisms is still poorly studied.

In this work the impact of different shapes (spherical, sheet and flower) and sizes of nanoparticles was studied in their antimicrobial activity against *Escherichia coli* at 4 and 22 °C. ZnO NPs characterization was performed to assess their structural and physicochemical properties using scanning electron microscopy (SEM), X-ray powder diffraction (XRD), ultraviolet–visible spectroscopy (UV-VIS) and nitrogen adsorption–desorption for specific surface area determination. A further preliminary study was performed to characterize the antimicrobial activity of in situ ZnO NPs in bacterial nanocellulose (BNC) against *E. coli* at 4 and 22 °C.

## Materials and Methods

### Synthesis of ZnO NPs: Sol-gel method

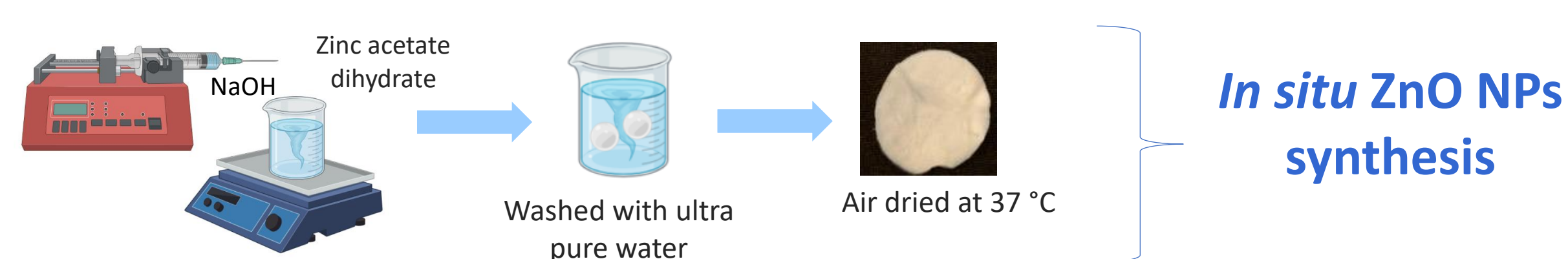


### ZnO NPs characterization:

- Scanning electron microscopy (SEM) → Size and morphology
- Powder X-ray diffractometer (XRD) → Purity and crystallinity
- Nitrogen isotherms (BET) → Specific surface area
- UV-visible spectroscopy (UV-VIS) → UV absorption

### Production of films in bacterial nanocellulose and *in situ* ZnO NPs:

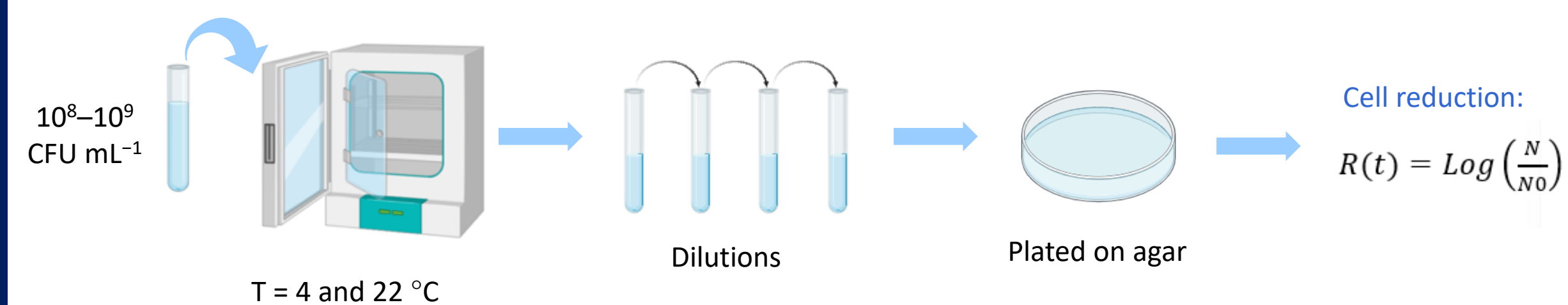
- *Komagataeibacter xylinus* + Hestrin-Schramm medium: 15 days fermentation



*In situ* ZnO NPs synthesis

### Antimicrobial activity: Viable cell count assay

Pathogenic bacteria: *E. coli*

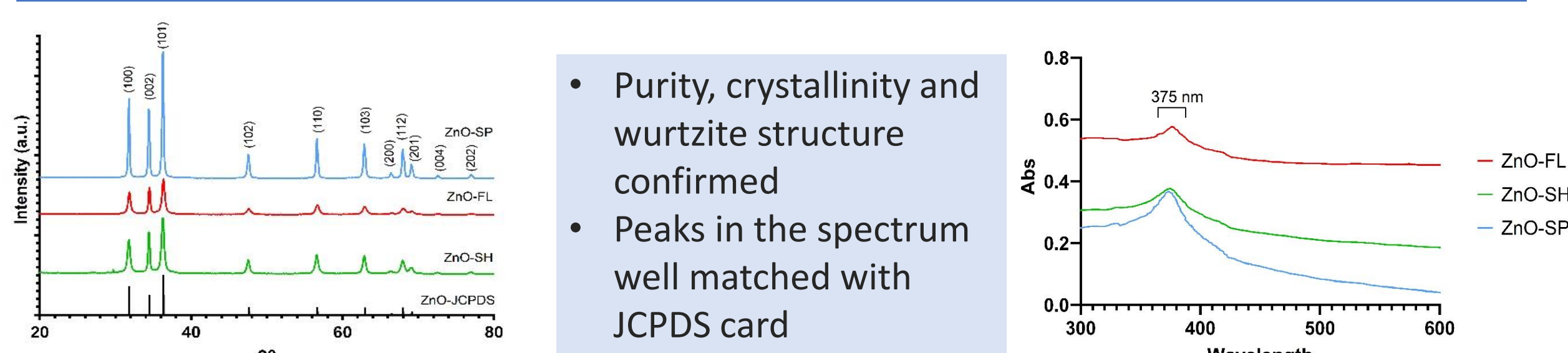


## Results

### ZnO NPs characterization:



Parameter	Spherical	Flower	Sheet
Average size (nm)	31 ± 6	249 ± 74	243 ± 96
Specific surface area (m <sup>2</sup> g <sup>-1</sup> )	13.4	5.3	18.5



### Antimicrobial activity: ZnO NPs

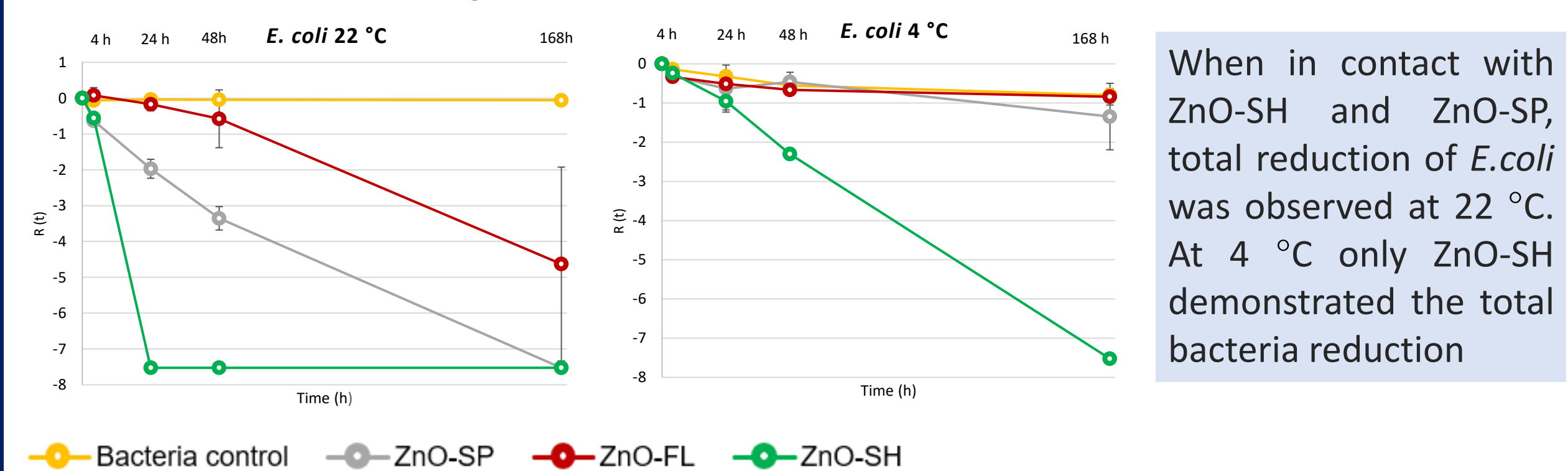


Figure 1 - Antibacterial activity of ZnO-SP, ZnO-FL and ZnO-SH for *E. coli* at 22 and 4 °C.

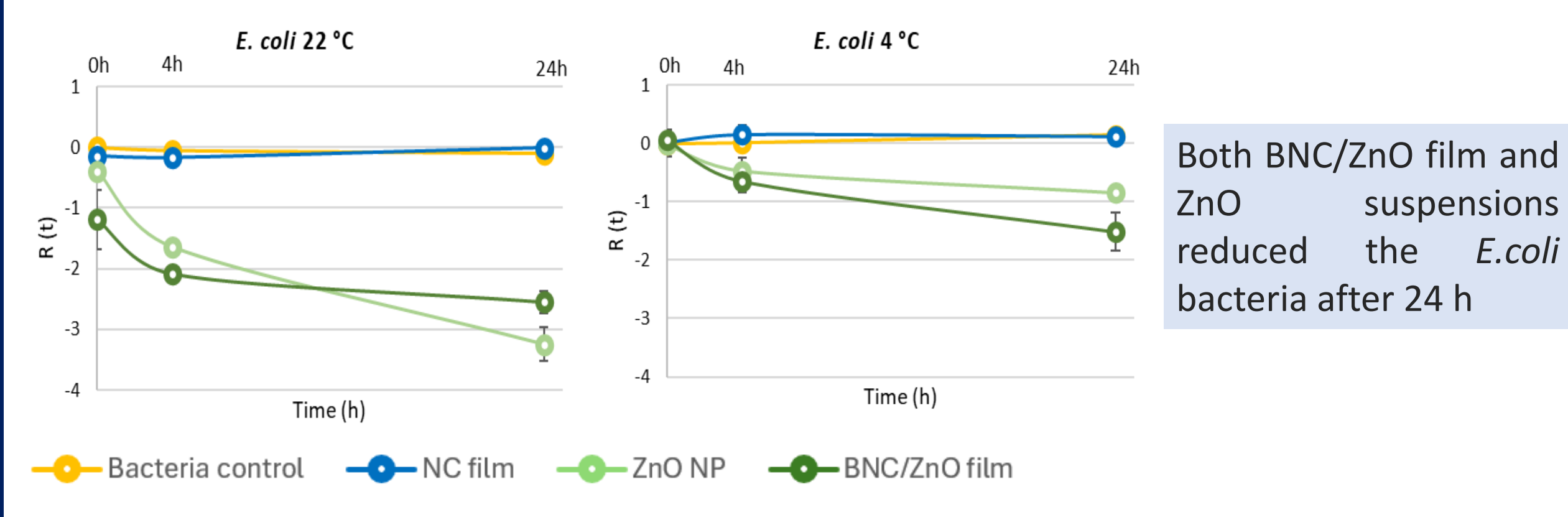


Figure 2 - Antibacterial activity of ZnO NPs and BNC/ZnO films for *E. coli* at 22 and 4 °C.

## Conclusions:

- Significant antimicrobial activity results were observed depending on shape, size and surface area of ZnO NPs.
- 22 °C studies revealed to have more efficient microbial reduction compared to 4 °C experiments.
- The incorporation of antimicrobial agents, such as ZnO NPs, in polymeric matrices, can inhibit the growth of microorganism, offering an active packaging for protection and preservation of the food.

## Acknowledgements:

- Financial support of the FCT through the grant given to Ana Rita Mendes (UI/BD/151387/2021).
- This work was supported by National Funds from FCT through project UIDB/50016/2020.