

## 1. BACKGROUND

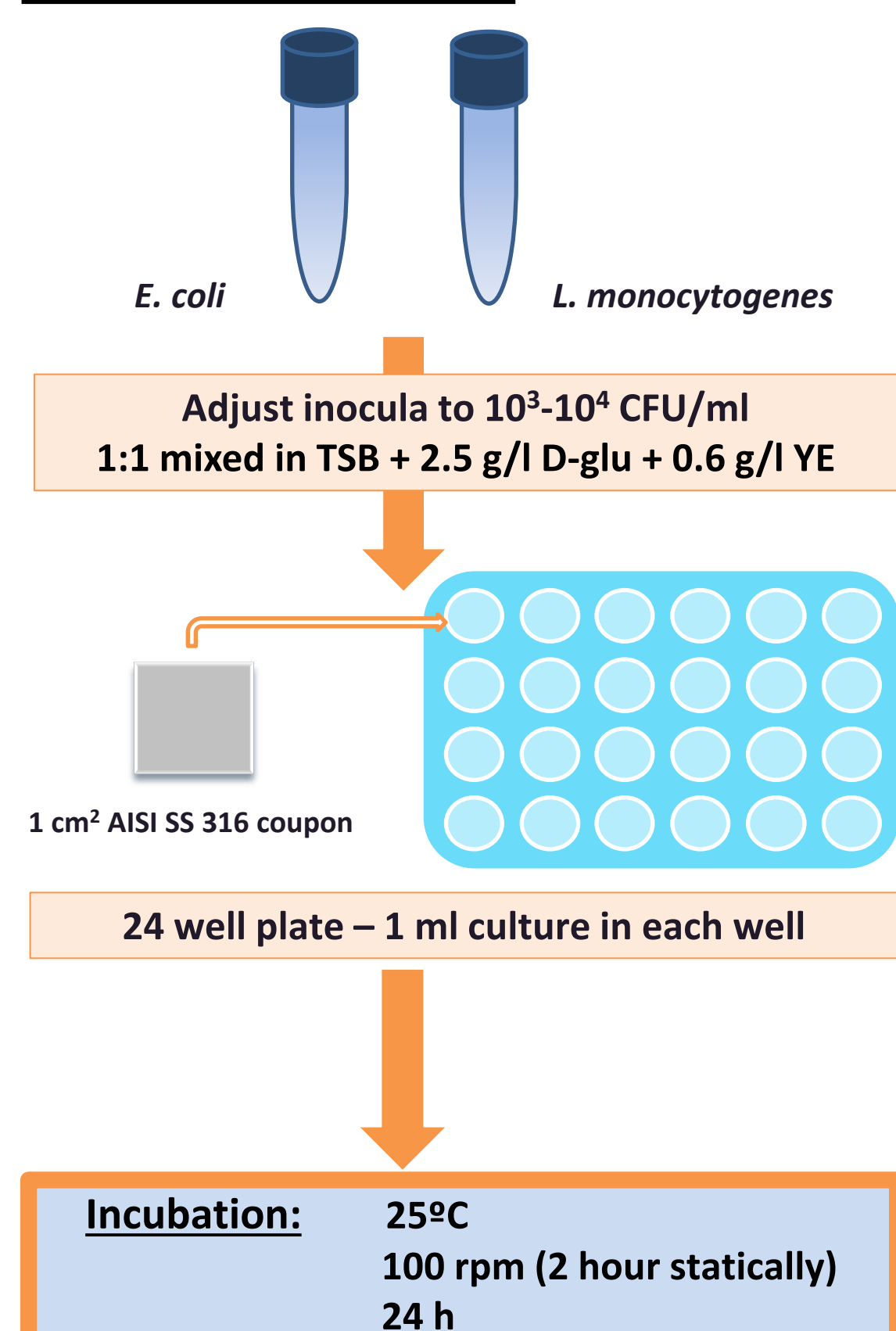
*Listeria monocytogenes* is considered one of the main food-borne pathogens in food industry presenting an increasing incidence tendency over the last years [1]. Its ability to form biofilms associated with other bacteria developing higher tolerance to antimicrobial treatments [2] is nowadays one of the main issues regarding food safety.

## 2. OBJECTIVE

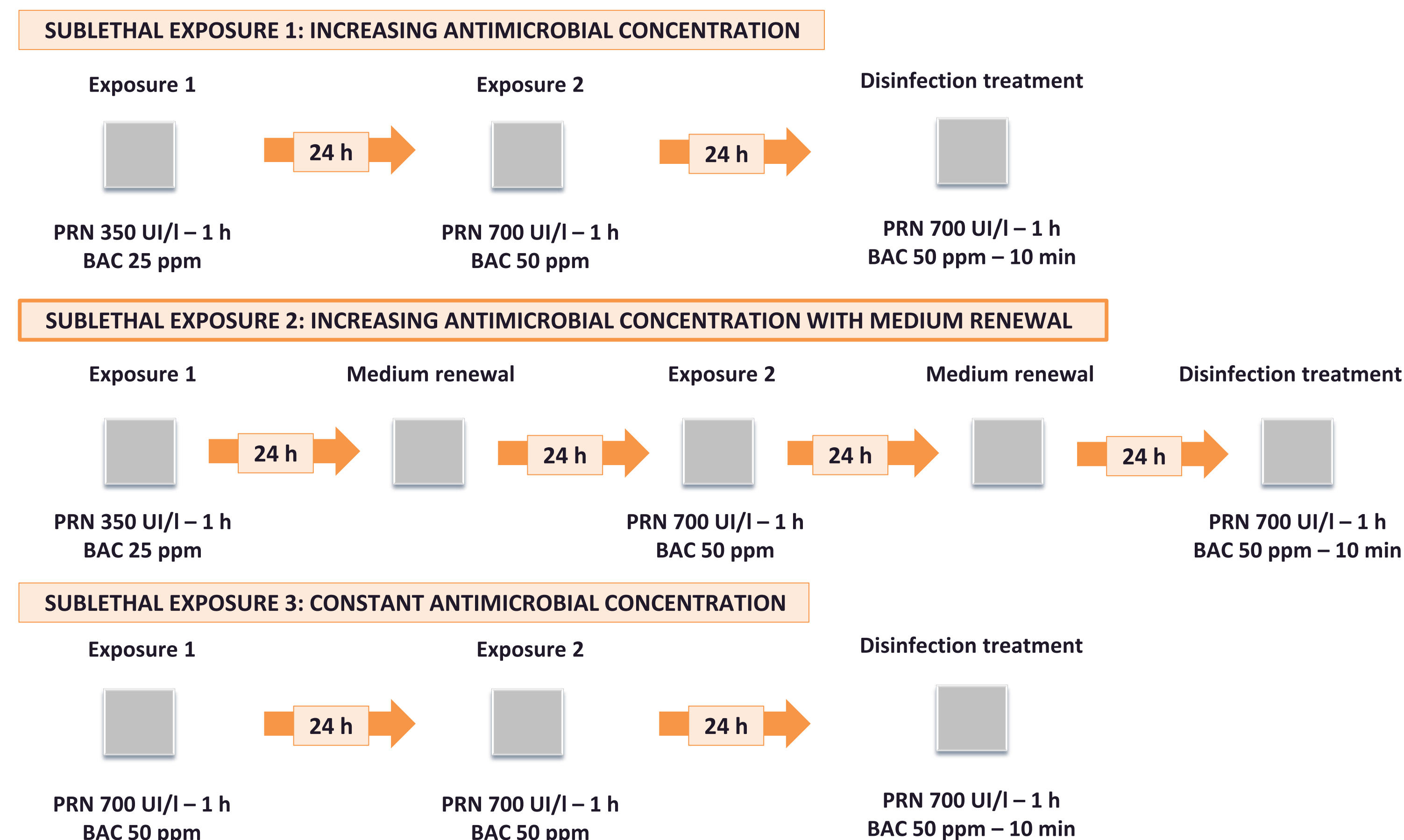
To assess the capacity of a *L. monocytogenes* and *E. coli* MIXED-SPECIES BIOFILM isolated from food industry [3] grown on a stainless steel (SS) to develop TOLERANCE to SUBLETHAL CONCENTRATIONS of a PRONASE (PRN) and BENZALKONIUM CHLORIDE (BAC) sequential treatment.

## 3. EXPERIMENTAL DESIGN

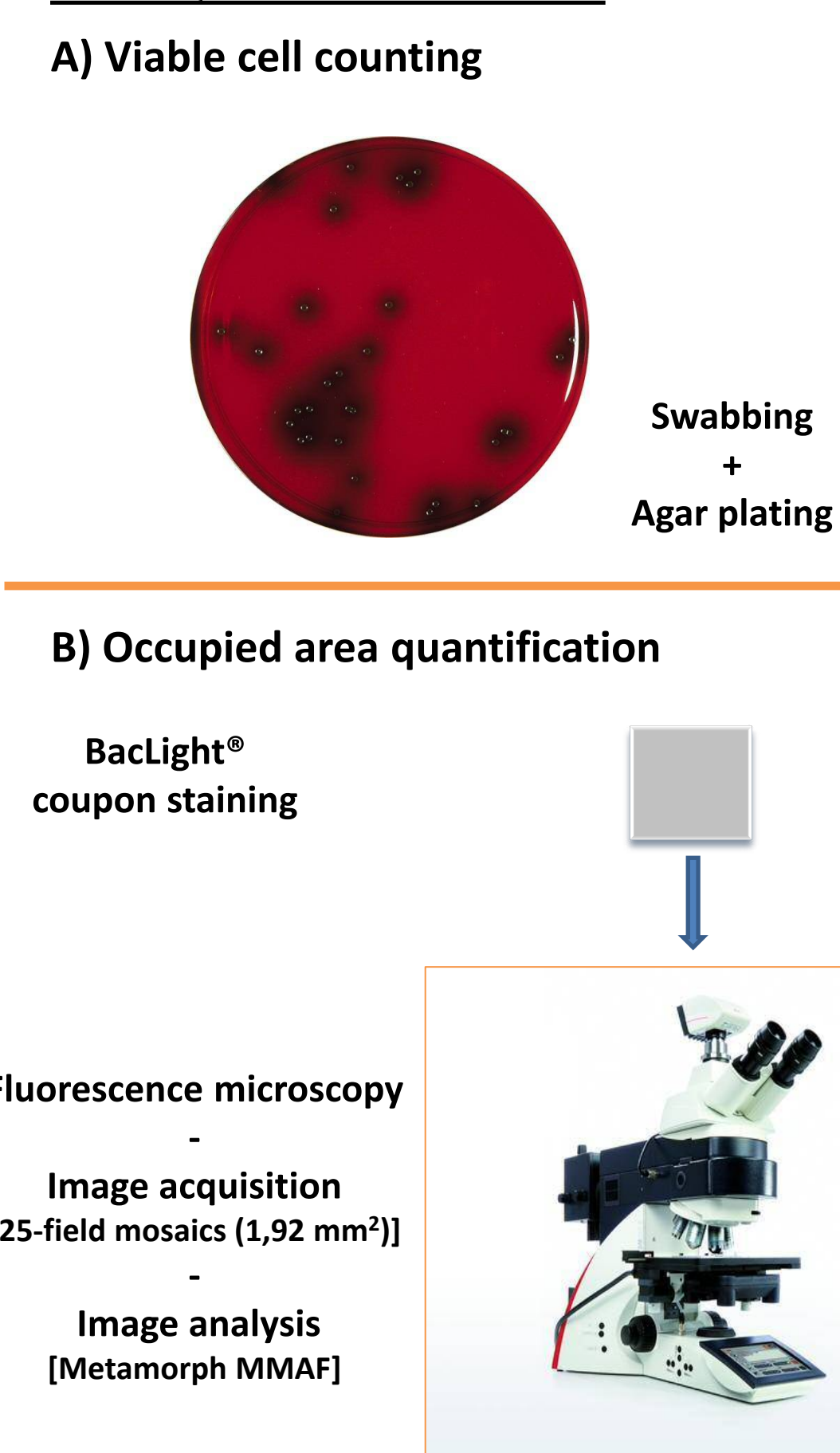
### 3.1. BIOFILM SETUP



### 3.2. EXPOSURE TO TREATMENTS

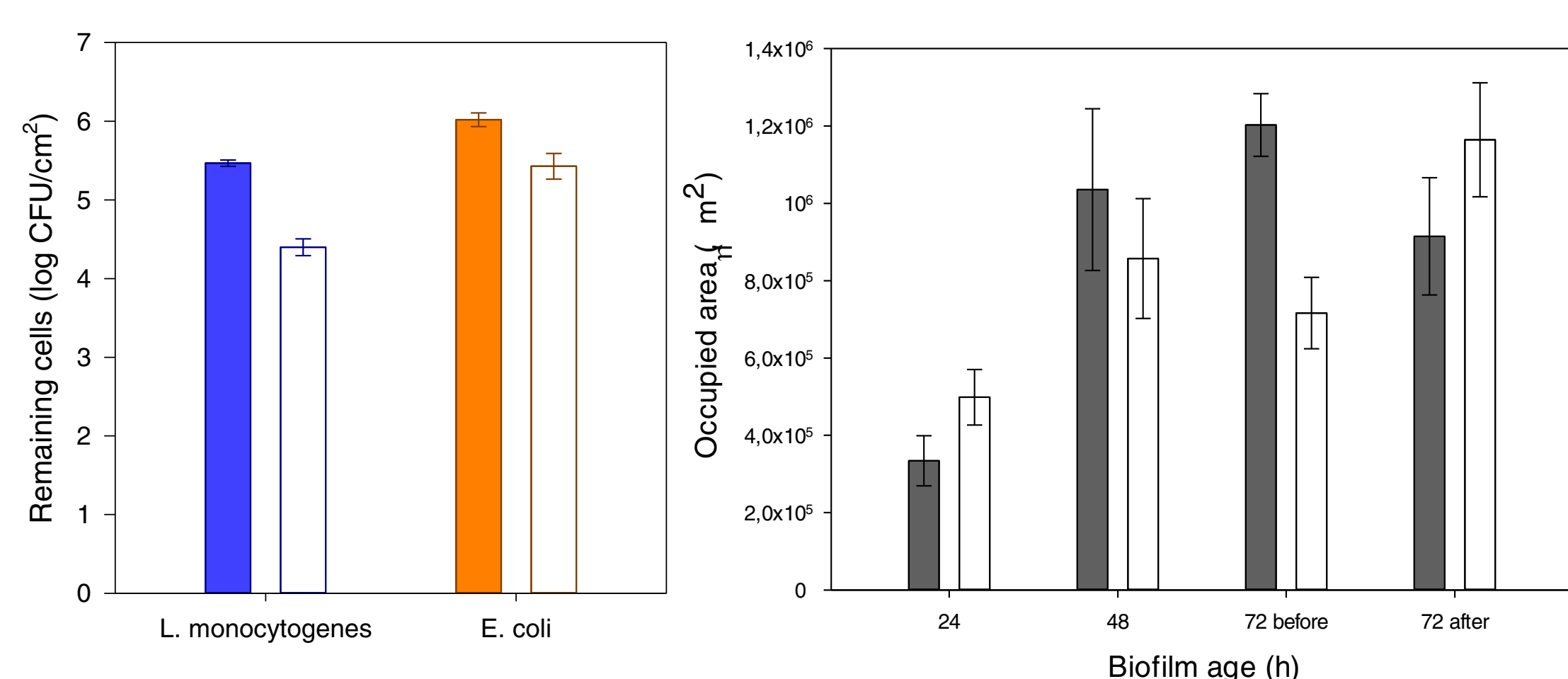


### 3.3. QUANTIFICATION

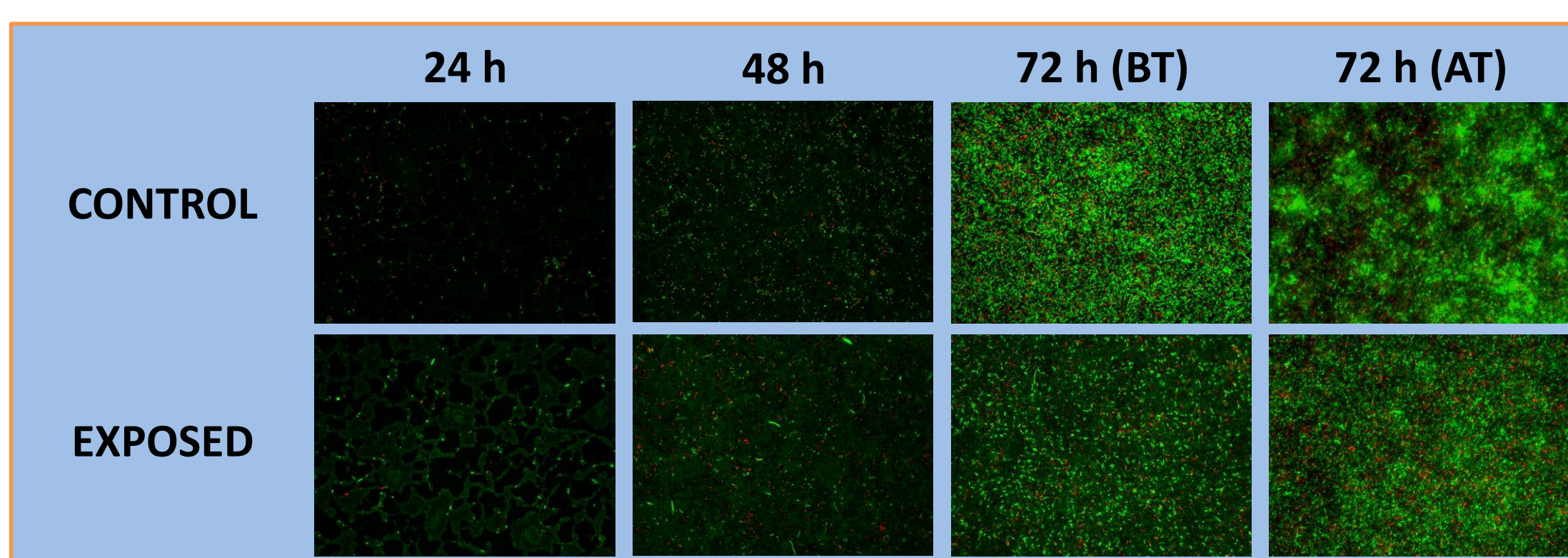


## 4. RESULTS

### 1) INCREASING CONCENTRATIONS

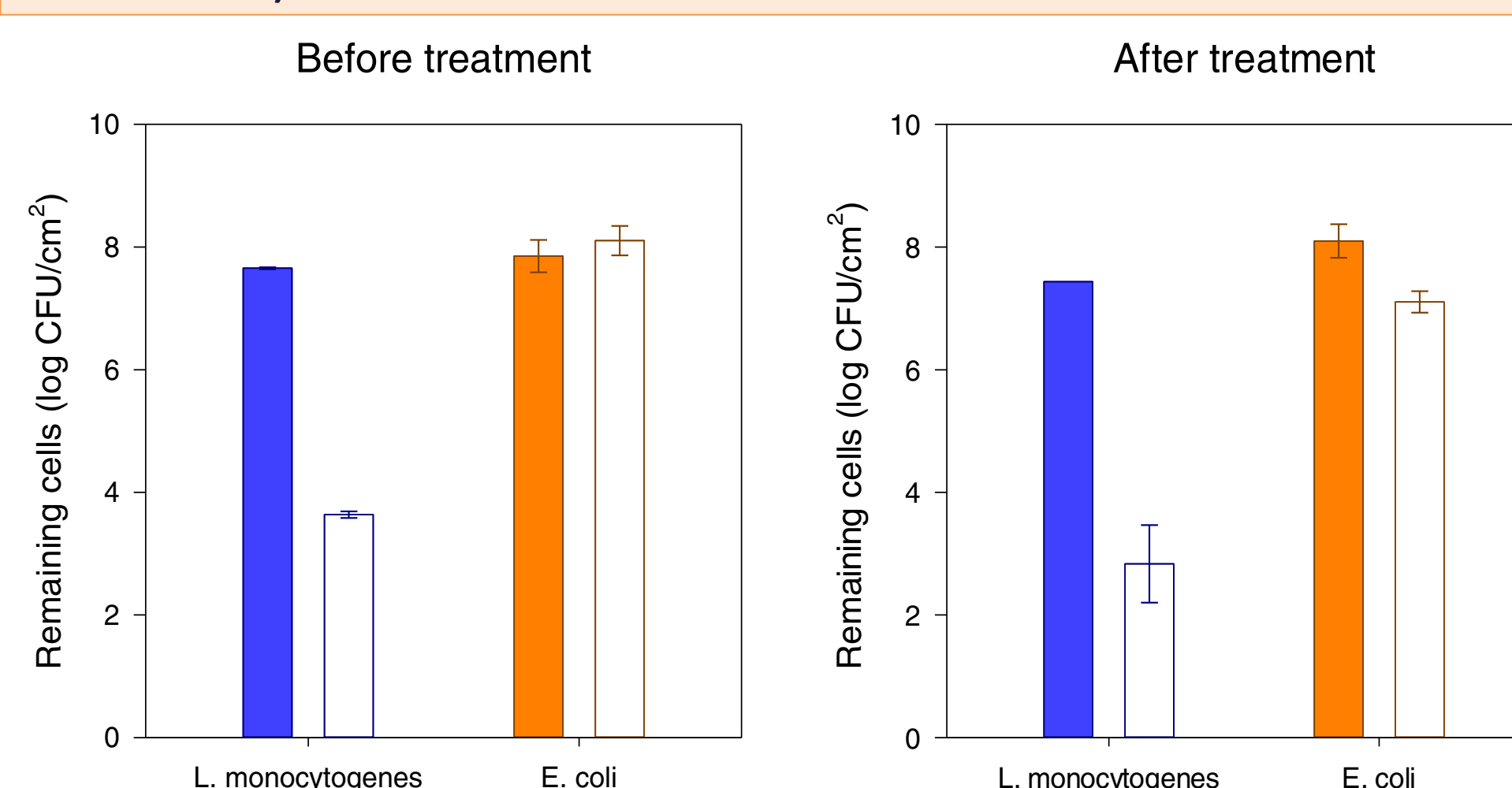


- ❖ EXPOSED BIOFILMS (void bars) presented LOWER CELL COUNT compared with control (filled bars).
- ❖ LOWER OCCUPIED AREA VALUES at 72 h BEFORE TREATMENT in EXPOSED SAMPLES (void bars). However, NO DIFFERENCES were appreciated AFTER TREATMENT.

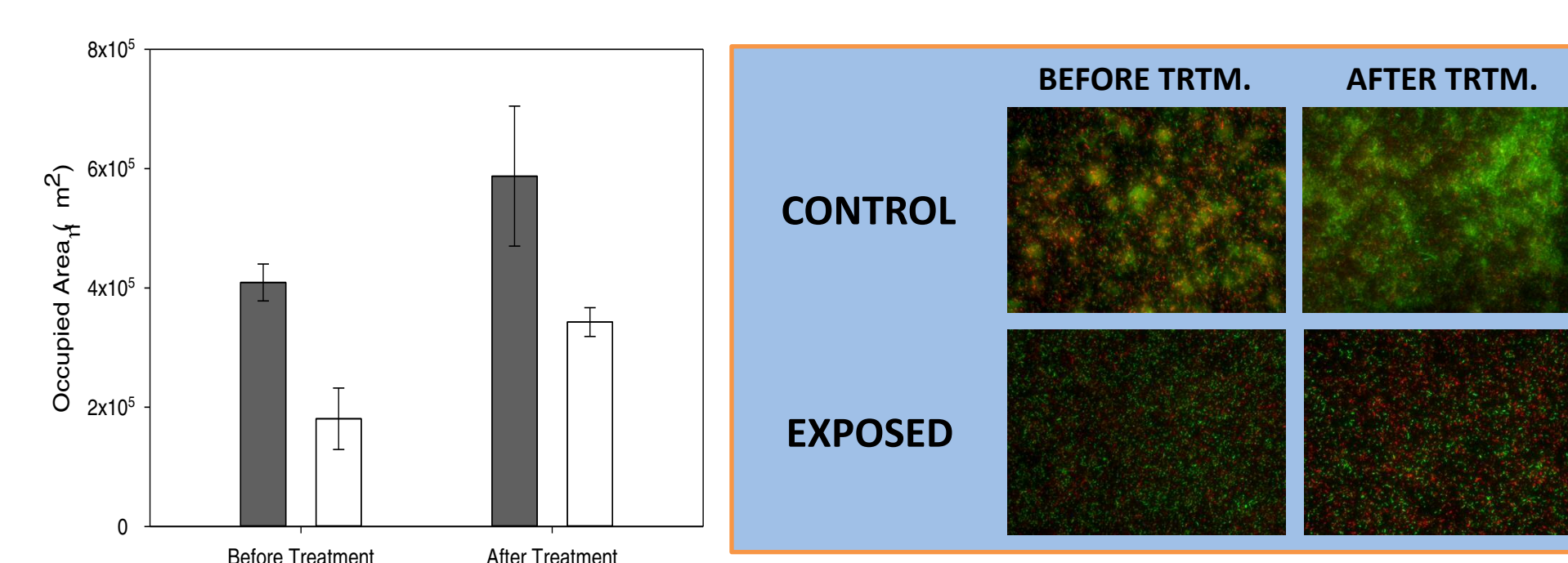


- ❖ EXPOSED SAMPLES PRESENTED AN ALTERED STRUCTURE if compared with control biofilms.
- ❖ Differences were more evident when samples before treatments (BF) and after treatments (AF) are compared where MATRIX STRUCTURE WAS LOST due to the proteolytic action of the pronase.

### 2) INCREASING CONCENTRATIONS WITH MEDIUM RENEWAL

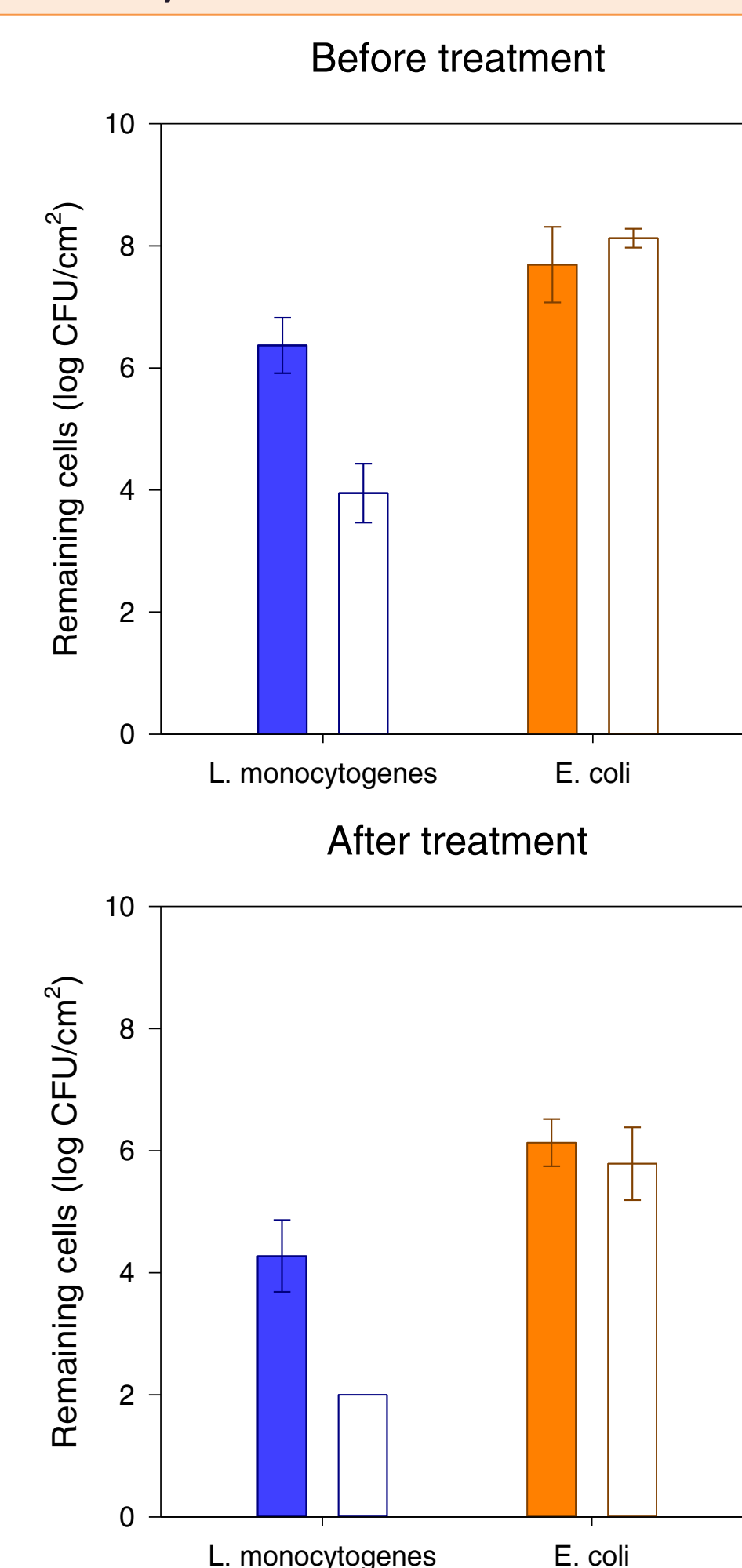


- ❖ After treatments NO SIGNIFICANT DIFFERENCES were observed in the cell count compared with samples before treatment, neither in exposed samples (void bars) nor in controls (filled bars).
- ❖ *L. monocytogenes* COUNTS WERE AFFECTED by the previous sublethal exposure.



- ❖ As in the first approach, BIOFILMS EXPOSED TO PRN-BAC PRESENTED AN ALTERED ARCHITECTURE where the cloudy-shape structure present in controls, was absent.
- ❖ A SIGNIFICANT REDUCTION OF OCCUPIED AREA was observed in exposed samples (void bars) compared with controls (filled bars).

### 3) CONSTANT CONCENTRATIONS



- ❖ Regardless of exposure a DECREASING OF  $\approx 2$  log CFU/cm<sup>2</sup> was observed in both species.
- ❖ *L. monocytogenes* in exposed samples (void bars) was MORE SENSITIVE compared with control (filled bars).
- ❖ NO DIFFERENCES were observed in *E. coli*.

## 5. CONCLUSIONS

1. NO TOLERANCE DEVELOPMENT WAS DETECTED in *Listeria monocytogenes* – *E. coli* mixed-species biofilm to the PRN – BAC treatment applied in the experimental conditions used.
2. PRN – BAC TREATMENTS WERE MORE EFFECTIVE IN YOUNG BIOFILMS, indicating that MATURENESS OF THE STRUCTURE PLAYS AN ESSENTIAL ROLE IN RESISTANCE to antimicrobial treatments.
3. Considering the effects on individual species, *L. MONOCYTOGENES* APPEARED TO BE MORE SENSITIVE to the treatment than *E. coli*. In this latter, viable cell numbers remained almost unaltered in all approaches followed.
4. The application of a PRN – BAC sublethal treatments, DRAMATICALLY AFFECTS THE MATRIX STRUCTURAL FEATURES of the biofilm, especially in prolonged exposures. These changes may be one of the major causes of the higher sensitivity to BAC.

## 6. REFERENCES

- [1] EFSA, *EFSA J.* 2015, vol. 13, no. 1, p. 3991, Jan. 2015.
- [2] E. Giaouris, et al. *Front. Microbiol.*, vol. 6, no. August, pp. 1–26, 2015.
- [3] P. Rodríguez-López, et al. *Int. J. Food Microbiol.*, vol. 206, pp. 84–95, 2015.

## 7. ACKNOWLEDGMENTS

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