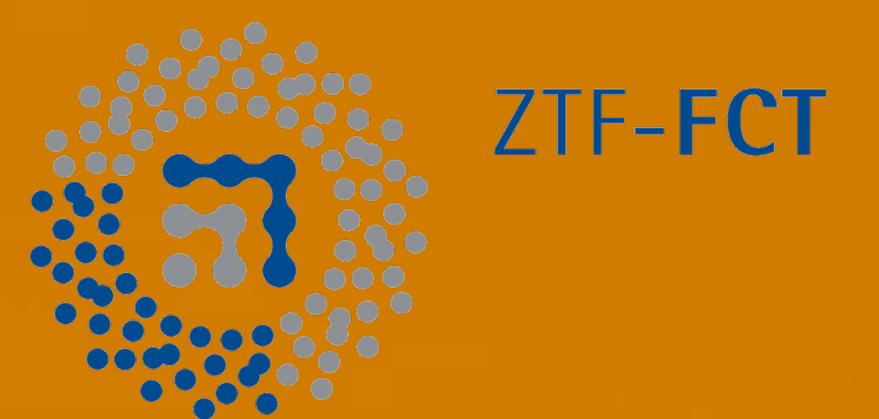


# Fate of *Escherichia coli* during an activated sludge wastewater treatment



Orruño M., Arana I., Garaizabal I., Muela A., and Barcina I.

Dpto. Inmunología, Microbiología y Parasitología. Facultad de Ciencia y Tecnología. Universidad del País Vasco. Apdo.644. E-48080. Bilbao. Spain



Email: maite.orruno@ehu.es, ines.arana@ehu.es, isabel.barcina@ehu.es

Activated sludge process is the most widespread solution for the treatment of wastewater and one of the key components of a wastewater treatment plant (WWTP).

Wastewater → reduces BOD  
Suspended solids and nutrients  
Bacterial concentration

The gene codifying the fluorescent protein (GFP, DsRed) used as a marker for tracking and visualizing bacteria in environmental samples allows the study of the behaviour of indicator bacteria in different aquatic systems (1, 2)

## MATERIAL AND METHODS

- **Bacterial Strains**  
Lab Strains: *E. coli* MC1061 pEGFP luc (3)  
*E. coli* DH5 $\alpha$  pGEN222 (V.de Lorenzo)  
*E. coli* MC1061 DsRed (4)  
Wild Strain: *E. coli* ABCgfp (our lab)
- **Preparation of inocula:** Overnight cultures in LB
- **Bacterial counts:** Total number of bacteria: stained (5) or non-stained samples  
Culturable bacteria (CFU) on YE Agar with or without marker antibiotics. Observation under UV lamp
- **Experiments:** batch culture and pilot WWTP

## AIM OF THE WORK

To study the fate of *E. coli* during wastewater treatment by using fluorescent strains.

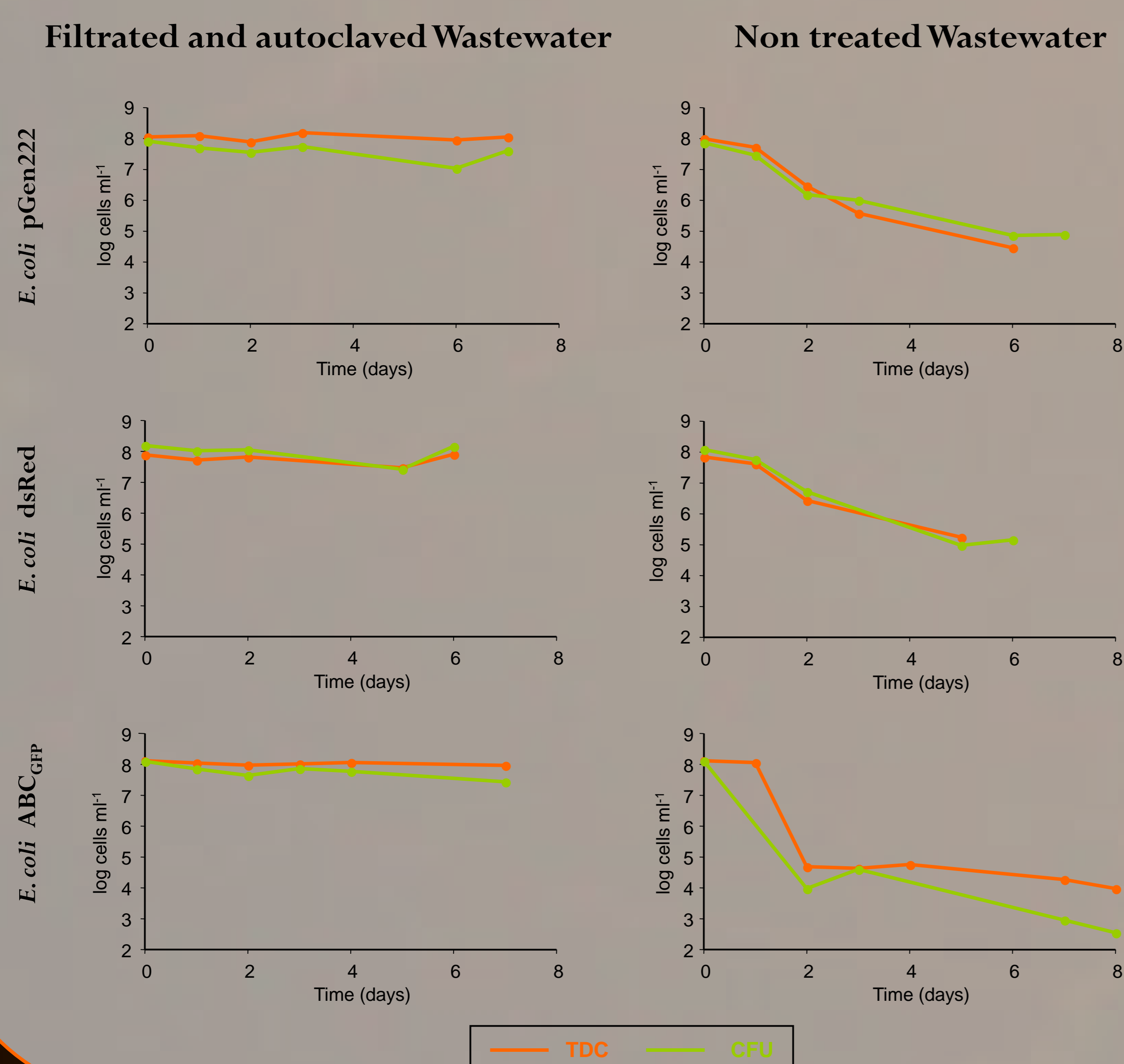


| Parameter                            | Efficiency     |             |
|--------------------------------------|----------------|-------------|
|                                      | Crispiana WWTP | Pilot plant |
| BOD <sub>5</sub>                     | 91,30          | 98,99       |
| SS                                   | 82,90          | 98,15       |
| TB ml <sup>-1</sup>                  | 94,64          | 97,51       |
| EC 100 ml <sup>-1</sup>              | 98,14          | 99,75       |
| EF 100 ml <sup>-1</sup>              | 97,96          | 99,85       |
| HBC <sub>37°C</sub> ml <sup>-1</sup> | 98,74          | 99,79       |
| HBC <sub>20°C</sub> ml <sup>-1</sup> | 98,99          | 99,85       |



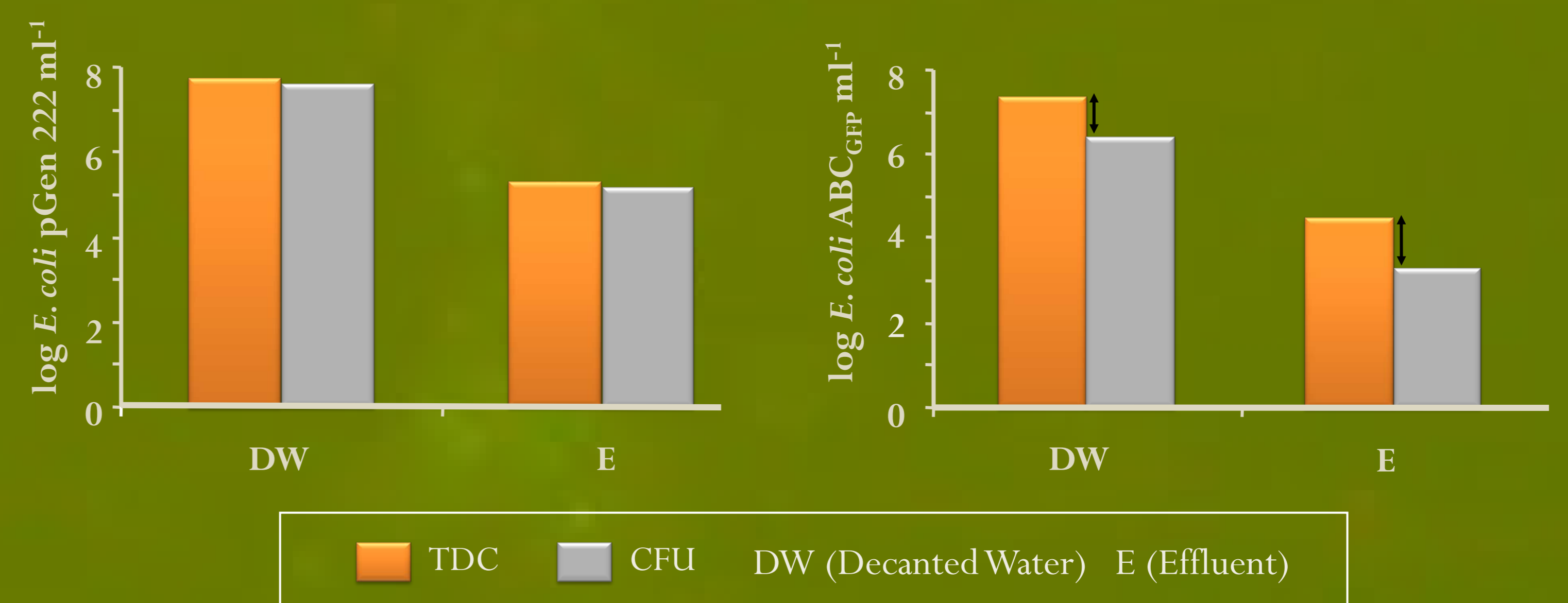
## RESULTS

### SURVIVAL ASSAYS. BATCH CULTURES



After a week of permanence of *E. coli* strains in wastewater, there was no variation in their fluorescence. The expression of fluorescent proteins was maintained. In batch culture assays, in absence as well as in presence of natural microbiota of wastewater, it was not detected formation of viable but nonculturable cells (VBNC, active cells but unable to grow on appropriate culture media). Decreases in *E. coli* population were only detected in presence of microbial populations. In pilot plant assays, the strains introduced in the pilot WWTP suffered a deep fall in both total and CFU counts as consequence of treatment (> 99%) and they did not adopt the VBNC state. These results can be attributed to predation by protozoa, to transfer to sludge or to lysis processes.

### SURVIVAL ASSAYS. PILOT PLANT



### Bibliography

1. Arana I, Irizar A, Seco C, Muela A, Fernandez-Astorga A & Barcina I (2003) Microb Ecol 45:29-38.
2. Cho JC & Kim SJ (1999) J Microbiol Methods 36:227-235.
3. Lehtinen J, Nuutila J & Lilius EM (2004) Cytometry 60:165-172.
4. Hakkila K, Maksimow M, Rosengren A, Karp M & Virta M (2003) J Microbiol Methods 54:75-79.
5. Hobbie JE, Daley RJ & Jasper S (1977) Appl Environ Microbiol 33:1225-1228.

© 2009 Maite Orruño et al.

## CONCLUSIONS

Wastewater treatment provokes a great decrease of *E. coli* populations. There is no formation of VBNC cells during treatment.

### Aknowledges

This study was funded by the research projects CTM2006-09532/TECNO from the Science and Technology Ministry of Spain and EHU 08/56 from the Basque Country University.