

# Mechanisms implied in *Escherichia coli* removal during wastewater treatment

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## Background

Wastewater treatment reduces environmental contamination:

- removing gross solids and mitigating its polluting effect
- reducing the number of indicator organisms and pathogens

Recycling of sludge as an organic fertilizer is environment friendly but:

- some pathogens can be present (viruses, bacteria and protozoa) (8)
- these microorganisms could be concentrated in sludge

## Materials and methods

**Biological model:** *Escherichia coli* ABCgfp, isolated from wastewater (Crispiana WWTP) and modified to express GFP protein. This strain was indistinguishable from the parental non-tagged strain with respect to growth and behavior in sterilized wastewater (6).

**Wastewater samples;** from the Crispiana wastewater treatment plant (WWTP) (5).

**Laboratory-scale activated-sludge unit (ASU)** fed with influent and sludge from Crispiana WWTP. Similar working conditions for Crispiana WWTP and ASU.

## Aim of the work

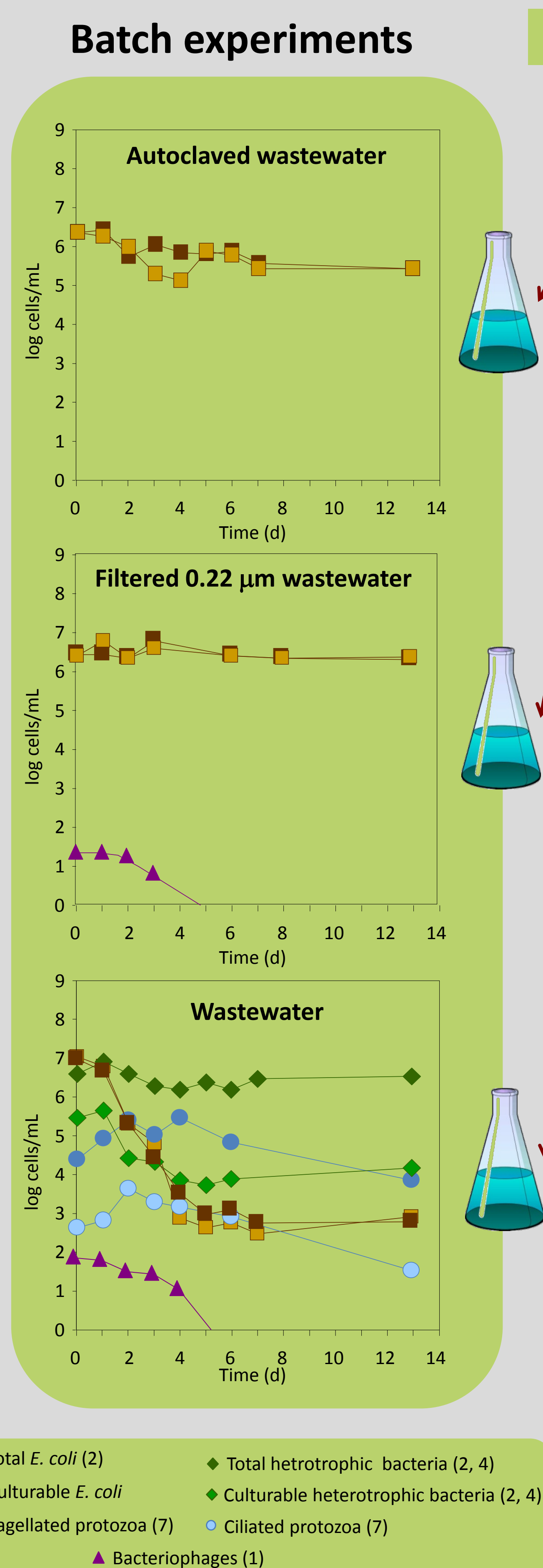
To take an approach to the main mechanisms involved in the reduction of pathogenic microorganisms during activated sludge wastewater treatment.

## Results and Discussion

In **absence of microbial population**, the exposure of tagged *E. coli* to wastewater did not affect the culturability, activity and integrity of the cells. Entry into the viable but nonculturable state (3) could not be induced during wastewater treatment.

**Bacteriophages** did not affect the survival of tagged *E. coli* in wastewater.

**Complex interactions** between microbial populations and *E. coli* occur in presence of wastewater microbiota. Wastewater bacteria maintained their population density, while *E. coli* ABCgfp, behaving as predation non-escaping prey, was predated (2). Predation by **protozoa** is an important removal mechanism of bacteria in activated sludge.



*E. coli* ABCgfp

ASU experiments

60-70% of introduced *E. coli* cells remained in the ASU system, and a very few cells were recovered in effluents, so **predation** could be responsible of the removal of resting fraction.

Almost 95% of remaining *E. coli* cells was present in flocs and sludge; what points out:

- the importance of **adhesion** to solid fractions to eliminate cells
- the protective role of adhesion to flocs and sludge to face up to predation by protozoa.

| Time (h)                                      |                                      | Influent inoculated in continuous with gfp-tagged <i>E. coli</i> |        |        |        |        |        | Influent non-inoculated |        |        |        |
|---|--------------------------------------|--|--------|--------|--------|--------|--------|-------------------------|--------|--------|--------|
|   |                                      | 0.25   | 0.5    | 1      | 2      | 3      | 4      | 2                       | 4      | 24     | 48     |
| Aeration tank                                 | <b>Aqueous fraction</b>              | 2.23   | 2.98   | 3.97   | 17.4   | 10.1   | 15.8   | 1.79                    | 1.59   | ND     | ND     |
|   | (10 <sup>3</sup> <i>E. coli</i> /mL) | (0.46)   | (0.35) | (0.56) | (1.07) | (1.11) | (0.98) | (0.45)                  | (0.38) |        |        |
| Secondary clarifier                           | <b>Flocs</b>                         | 0.35   | 1.05   | 2.92   | 24.8   | 25.7   | 47.3   | 2.69                    | 3.38   | 1.06   | 0.12   |
|   | (10 <sup>7</sup> <i>E. coli</i> /g)  | (0.05)   | (0.11) | (0.21) | (1.35) | (1.48) | (2.35) | (0.43)                  | (0.31) | (0.85) | (0.02) |
| Effluent (10 <sup>3</sup> <i>E. coli</i> /mL) | <b>Aqueous fraction</b>              | 0.99   | 0.99   | 1.98   | 21.8   | 17.8   | 18.6   | 4.47                    | 1.69   | 0.33   | ND     |
|   | (10 <sup>3</sup> <i>E. coli</i> /mL) | (0.54)   | (0.43) | (0.75) | (1.78) | (1.12) | (0.98) | (0.45)                  | (0.65) | (0.05) |        |
| Effluent (10 <sup>7</sup> <i>E. coli</i> /g)  | <b>Flocs</b>                         | 0.22   | 0.99   | 1.10   | 33.4   | 39.8   | 42.0   | 2.66                    | 4.42   | 0.66   | 0.05   |
|   | (10 <sup>7</sup> <i>E. coli</i> /g)  | (0.05)   | (0.43) | (0.65) | (5.89) | (3.72) | (3.72) | (0.41)                  | (0.54) | (0.43) | (0.01) |
| Effluent (10 <sup>3</sup> <i>E. coli</i> /mL) |                                      | ND   | ND     | 1.06   | 4.10   | 5.23   | 5.10   | 1.49                    | 1.88   | 0.33   | ND     |
|   |                                      |  |        | (0.32) | (0.43) | (0.44) | (0.43) | (0.32)                  | (0.55) | (0.21) |        |

Study of the residence time of cells in the system by stopping *E. coli* inoculation:

- cells disappeared faster from aqueous fractions (aeration tank, secondary clarifier and effluent) than from solid fraction
- elimination of cells could be attributed to the washing with new influent, to the sludge removal and/or to the cell death, mainly by predation.

## Conclusions

Due to the quantitative importance of cell adhesion to sludge with respect to cell removal via predation (real elimination), the recycling of sludge can suppose a sanitary and ecological risk.

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## Acknowledgments

- Authors thank to AQUALIA Gestión Integral del Agua S.A.-Lagunketa (UTE Crispiana) and Aguas Municipales de Vitoria-Gasteiz S.A. (AMVISA) for their collaboration.
- This study was funded to the research projects CTM2006-09532/TECNO from the Science and Technology Ministry of Spain, IT376-10 from Basque Government, EHU08/56 from the Basque Country University and Basque Government Predoctoral Grant BF109.103 to I. Garaizabal