Use of a predictive model to calculate *E. coli* survival in waste waters after secondary and tertiary treatments

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Abstract

The decay of *E. coli* in waste waters at mesophilic temperatures has been attributed to a complex mixture of environmental factors that include nutrient availability, the presence of microbial competitors or toxic compounds, the chemical properties of the waste water and the present microbiota. Consequently, the kinetics of the decay observed in different studies varies strongly with the conditions of both the strains and the effluent water used in each of them. To fit these data, several mathematical models have been tried, from log linear to sigmoid equations, but each of them fits only one type of behaviour. In this work we have used a model that is based on the assumption that the bacterial population is heterogeneous and the individual survival times follow a Weibull frequency distribution:

$$N= N_0 * EXP(-B*t^n)$$

The model equation has been fitted to data obtained in our laboratory with different effluents. The value of the exponent, n, determines the shape of the curve. With values above one it produces curves with downward concavity, typical of clean waters, tertiary effluents or regenerated waters. The values below one (producing upward concavity) appear in non-treated waters with high BOD or secondary effluents.

Keywords: self-regeneration, E. coli, Weibull equation, secondary effluent, tertiary effluent, inactivation kinetic.

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1 Introduction

Self-regeneration of contaminated waters is a biological process based on a selective inactivation of the pathogenic bacteria present in the waters, without any external human participation. This process is known from the early days of Microbiology. It was described, more than 100 years ago by E. H. Hankin, a medical doctor working in India. He described that *Vibrio cholerae* died three hours after its inoculation in Ganges waters, whereas it maintained its viability for more than 48 hours in distilled water. He suggested that this antibacterial activity, then of unknown origin, was responsible for limiting the spread of cholera (Hankin [1]). While Hankin did not study this phenomenon further, his work was nonetheless recognized a generation later as being among of the first observations of bacteriophage activity when Félix d'Herelle witnessed it at the Pasteur Institute. From that time, some other mechanisms have been related with the decay of *E. coli* in waste waters at mesophilic temperatures. In our days, the process is envisaged as a complex mixture of environmental factors which, beside bacteriophage activity, include nutrient availability, presence of microbial competitors or toxic compounds, the chemical properties of the waste water and the present microbiota. Crane and Moore [2], in a review on the mathematical models available to describe the die-off kinetics, published a list of more than 20 different factors affecting the process. The kinetics of the decay observed in different studies varied strongly with the conditions of both the strains and the effluent water used in each of them and it was necessary to use different mathematical models to describe the different curve shapes. However in studies in which the waters had similar origin and composition, the bacterial *E. coli* inactivation kinetics showed also similar kinetics (González [3]). These results supported the hypothesis that the observed kinetics depends on the physical, chemical and biological characteristics of the water. With this hypothesis, we decide to develop this research with the following objectives: 1) To develop and validate a general mathematical model able to describe the different inactivation kinetics observed in several secondary and tertiary effluents. This model should be as mechanistic as possible, that is, its structure should be based on and its parameters related with the biological mechanisms underlying the process. 2) To quantify the self-regeneration capacity of the effluents with different experimental treatments by the use of the mathematical model and to relate the changes in the parametric values of the model with the treatment performed. 3) To quantify and analyze the effect of industrial tertiary treatments on the self-regeneration capacity.

2 Results and discussion

2.1 Development and validation of a mathematical model describing the inactivation kinetics of *E. coli* in water

The biological basis of the model is the assumption that microbial inactivation is the result of the lack of the cell capacity to cope, after some time, with the

structural and biochemical problems imposed by any type of stress. It is assumed also that the microbial population is heterogeneous, meaning that each cell takes its own time to lose its viability. If the population is big enough, in such a way that the times of death distribution can be described by a continuous function, the inactivation curve can be described by a statistical distribution. We have selected the Weibull distribution, extensively studied and applied to the bacterial thermal inactivation by Peleg [4]:

$$N/N_0 = exp(-Bt^n)$$

And taking logarithms

$$logN = logN_0 - bt^n$$

in which b=B/ln10. This equation is very simple, having only two parameters, b, which is called location coefficient, and n, called the shape coefficient because its value determines the shape of the inactivation curve. This curve shows an upwards concavity when n has values below 1 and downwards concavity when is bigger than 1. When n is equal to 1, the Weibull equation is identical to the negative exponential equation describing first order kinetics, the most popular model to describe bacterial death. The negative exponential equation can be then considered as a special case of the Weibull more general equation. The values of these coefficients are logically related with the underlying statistical distribution and can be related with the individual sensibility of the cells to the inactivation factor. When n is greater than 1, this means that many of the cells are very sensible to the factor. Most of them will die quickly, in short times, and the few more resistant cells will accumulate and will die slowly, producing the tail in the inactivation curve. A value of n greater than 1 implies a different inactivation mechanism to which many cells would be resistant. However, an accumulation of injures produced by that mechanism, would eventually kill the cell. For this reason, in this case the kinetics shows no apparent cell death at the beginning, because the cells are able to cope with the small amount of injures produced at short times, but the death rate increases with time, when most of the cells are already injured and just one more stress injury will produce death.

The experiments to study the inactivation kinetics included a previous inoculation of the effluent with a lab strain of *E. coli* to reach a population of about 10^7 CFU/ml. It was incubated in the dark at 28ºC, to minimize the effect of these environmental factors [3, 5]. One ml samples were incorporated in Chromocult agar plates. The studied effluents were provided by waste water treatment plants from Madrid and Cantabria regions (Spain) in autumn and spring (Mad 1 and 2) and winter (Can1). Results of several experiment of this type are shown in Fig. 1, where the decay of the endogenous *E. coli* populations of the effluents along time is compared with that of the inoculated effluents. The continuous curves are the result of the fitting of the Weibull model to the experimental data. It can be observed that the model describe with reasonable accuracy the experimental results in all the cases. We can conclude that both the experimental design employed to measure the inactivation kinetics and the mathematical model used to describe quantitatively the process, seems to be scientifically valid.

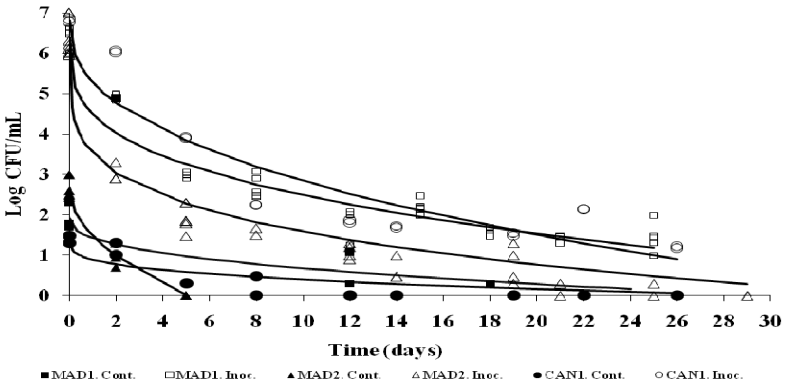

Figure 1: Inactivation kinetics of secondary (Control) and inoculated (Inoc.) effluents. Continuous lines represent the Weibull fit. Effluents came from waste water treatment plants from Madrid (MAD) and Cantabria (CAN).

2.2 Effect of the removal of the endogenous microbiota and thermosensitive molecules from the effluent on its self-regeneration capacity

To test our hypothesis relating the type of kinetics observed with the predominant factor responsible of *E. coli* inactivation we decided to eliminate sequentially these factors. To remove the endogenous microbiota the effluent were centrifuged (3500 rpm, 10 minutes) and filtered (Millipore, 0,22 μm) the effluents (labelled, CF). To inactivate the thermosensitive molecules with toxic potential, the filtered effluent was heated in a boiling bath at 100ºC for 5 minutes (labelled, CFT). These treated effluents, and those non-treated as controls, were inoculated and incubated as described above, to measure their inactivation kinetics. The results are shown in Figs. 2 and 3. We can conclude that the removal of the inactivation factors decreases, as could be expected, the self-depuration capacity. Moreover, the shapes of the curves, that change from clearly upwards concavity in the control effluents to downwards concavity in the effluent without microbiota neither toxins (CFT) support our hypothesis relating the shape of the curves with the predominant mechanism responsible for the inactivation.

Our results support a view of the self-regeneration process in which protozoa predation, nutrient competition and, in general, interactions with the present microbiota, would be the predominant group of inactivation factors in the self-regeneration of the effluents. This type of factors, to which most of the cells would be sensitive, would produce a concave kinetics, with n values above 1. The tail of survivals could be explained by the accumulation of more resistant cells and/or to a decreased accessibility due to the low population density. The inactivation that is maintained in the effluents without microbiota or toxic compounds (see Fig. 2) has to be due to another mechanism that displays another kinetic.

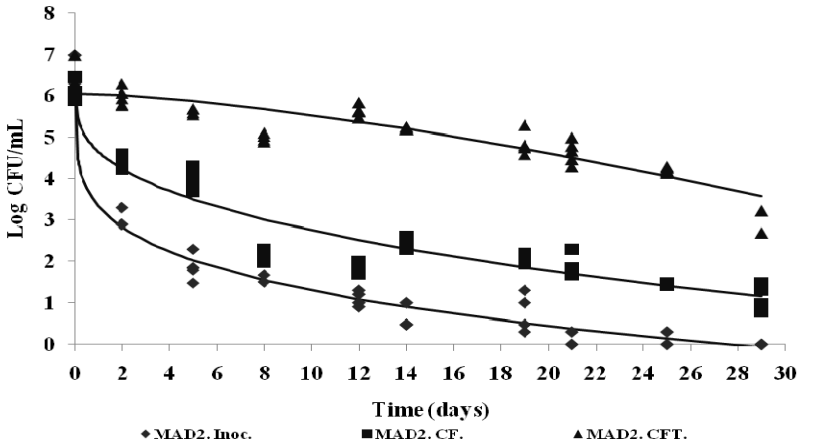

Figure 2: Effect of removal of inactivating factors on the inactivation kinetics of *E. coli* inoculated in a secondary effluent from Madrid (MAD2). Effluent without any treatment (Inoc.). Effluent centrifuged and filtered (CF). Effluent centrifuged, filtered and heat treated (CFT). Continuous lines represent the Weibull fit.

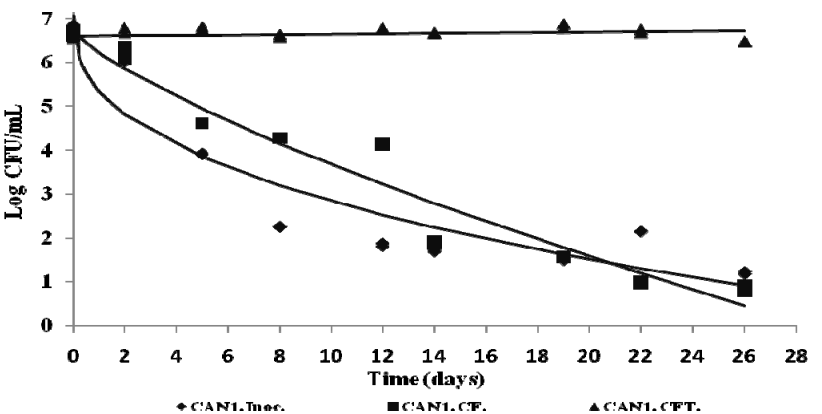

Figure 3: Effect of experimental treatments (centrifugation and filtration –CF–, centrifugation, filtration and heat treatment –CFT–) on the kinetics of inactivation of *E. coli*. The same secondary effluent from Cantabria, inoculated (CAN1. Inoc.), was used as control. The continuous line represents the Weibull fit.

Our hypothesis is that this mechanism is the natural mortality of the cells, due to an accumulation of metabolic failures, and, for this reason would show a convex kinetics. This hypothesis is supported comparing the results of Madrid (Fig. 2 and Cantabria (Fig. 3). In the case of Cantabria all the self regeneration capacity has been eliminated by the treatment, whereas in the Madrid effluent, with the same removal treatment, some inactivation capacity remains. This difference is in accordance with the hypothesis that the underlying inactivation mechanism is the natural cell death, which would imply a minimum of metabolic activity that the Madrid effluent would permit and that of Cantabria would not. The hypothesis about this minimum amount of metabolism that bacteria would need to develop and complete a death process is strongly support by published data showing that in environments without any energy source, as sterile distilled water, many species are maintained viable for years [6].

2.3 Effect of a tertiary treatment (microfiltration) on the self-regeneration capacity of a secondary effluent

The results described above pointed that tertiary treatment, by removing inactivation factors of the secondary effluent, would produce a decrease in its self-regeneration capacity. We have made a series of experiments to quantify this effect. Fig 4 shows one example with a microfiltration treatment of a Cantabria effluent.

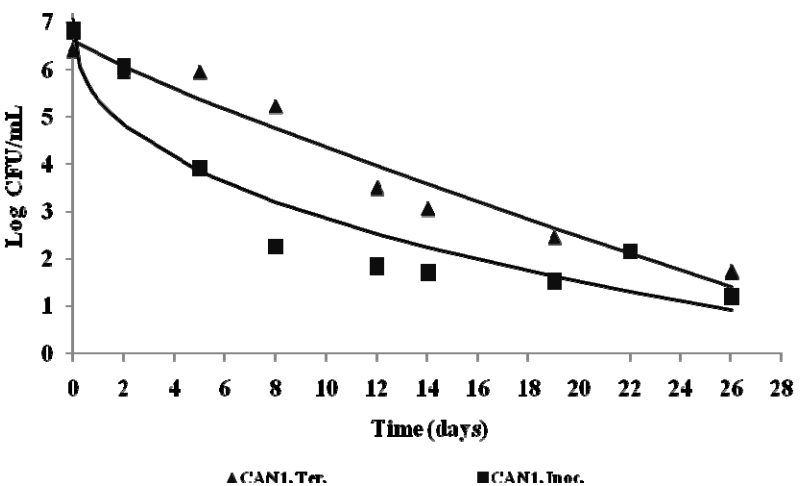

Figure 4: Effect of tertiary treatment (microfiltration) performed in Cantabria (CAN1) on the same secondary effluent of Figure 3. The experimental treatment is equivalent to centrifugation and filtration (CF). The continuous line represents the Weibull fit.

As it was expected, the treatment decreased the self-regeneration capacity with a pattern very similar to that observed with the equivalent laboratory treatment (centrifugation/filtration). This confirmation of the predicted effect is relevant because, although in a first approach, all the treatments that remove microorganisms from water may seem positive, they have the negative consequence of decreasing its capacity of self-regeneration. The more clean the water (in microbiological terms) the more defenceless will be in relation with a new contamination. This a danger that will have to be considered in a risk analysis of water re-use, especially in some cases as the refilling of aquifers in places in which faecal recontaminations would occurs.

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