Removal kinetic of *Escherichia coli* and enterococci in a laboratory pilot scale wastewater maturation pond

A. Ouali, H. Jupsin, A. Ghrabi and J. L. Vasel

ABSTRACT

During the last 15 years several authors studied the disinfection in waste stabilisation pond (WSP) and several empirical models were developed. There are huge differences between the models describing this process and there is really a need to improve the design of ponds for better disinfection. This paper addresses the *Escherichia coli* and enterococci disinfection in a laboratory pilot scale maturation pond (1.5 l) with light intensity (0, 12 and 25 W/m^2) under controlled pH, temperature and dissolved oxygen (DO) conditions. The aim of this study is to improve modelling for a better design of disinfection in maturation ponds (MP) and to identify the key parameters influencing the process. It was found that kinetic coefficients *K* values for *E. coli* and enterococci are closely dependent on physicochemical parameters. *K* values increase with increasing pH, *I*, *T* and DO. *E. coli* disinfection depends closely on the pH and the DO and increases strongly when the pH is above 8.5. The enterococci disinfection depends essentially on DO. Two equations are suggested to calculate the kinetic coefficient *K* related to the environmental average state variables.

Key words | disinfection, *E. coli*, enterococci, kinetic, maturation pond, UV

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INTRODUCTION

The waste stabilisation pond (WSP) and maturation pond (MP) could provide an interesting solution for disinfection of treated wastewater and reuse. The advantage of these systems is their ability to produce a good microbiological quality suitable for reuse without additional disinfection (Pearson *et al.* 2009). *Escherichia coli* and faecal coliforms have been widely used as biological indicators for monitoring wastewater treatment system (Davies *et al.* 2009).

Several authors have studied disinfection in WSPs and some empirical models have been developed. According to Andrianarison *et al.* (2010) there are huge differences between the models describing this process and the pond design for better wastewater disinfection. The design of maturation ponds is based on pathogen removal; usually bacterial decay (Bracho *et al.* 2006). Whatever the mechanism of bacterial inactivation, the kinetic disinfection follows first-order kinetics (Bastos *et al.* 2011).

Since Marais's equation, various studies have been conducted to understand the driving processes in WSPs. The effect of light, especially UVB and UVA, has been demonstrated by many authors (Nelson *et al.* 2009). More generally, studies demonstrated that disinfection depends on light intensity (Andrianarison *et al.* 2010), water depth (Maïga *et al.* 2009), algal biomass, light penetration, and the pond's hydrodynamics (Badrot-Nico *et al.* 2009; Ouali *et al.* 2012). Usually the main factors driving disinfection in ponds are, in decreasing order, light intensity, pH, dissolved oxygen (DO) (Curtis *et al.* 1992; Davies Colley *et al.* 1997), and temperature (Pearson *et al.* 1987).

Several mechanisms which explain the effect of the light on the disinfection in WSP and MP, are proposed in the literature (Davies-Colley *et al.* 1997, 1999; Nelson *et al.* 2009).

The aim of this study is to identify the key parameters influencing the disinfection process in maturation ponds to improve modelling for better design of disinfection in maturation ponds.

METHODS

This paper presents the *E. coli* and enterococci disinfection studies conducted in laboratory pilot scale maturation ponds with UVA and UVB and visible light (0, 12 and 25 W/m^2) under controlled pH, *T*, and DO conditions. The mixed batch reactor (Figure 1) consists of a 1.5 l tank exposed to radiation from two terrarium lamps (terrarium F30watt/6500 K) commercialised as 'UV lights'.

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The reactor was fed by treated domestic wastewater from the activated sludge wastewater treatment plant (WWTP) of Arlon city (Belgium). The wastewater sample collected was stored in sterile bottles and was directly analysed in the laboratory.

Wastewater (2 cm deep) was maintained homogeneously in the reactor by seven magnetic stirrers (100 rpm). Temperature within the reactor was controlled by rapid pumping of water from a temperature-controlled water bath (hot or cold water) through stainless steel tubing to keep it constant during the experiment. The wastewater temperature, DO, and pH levels inside the reactor were continuously logged using specific probes (tinitag, oxymeter WTW197i, pHmeter WTW197i). To avoid the usual studies on WSP disinfection where the disinfection effect is a combination of true disinfection and hydrodynamics of the reactor, we worked with a completely mixed reactor working in batch mode (no inlet – no outlet). Moreover we adopted a very small depth (2 cm) to guarantee that the light variation in the depth will be small and linear.

The initial bacterial wastewater concentration before disinfection testing was in the range 10^6–10^7 *E. coli*/100 ml and 10^4–10^6 enterococci/100 ml. Samples were collected every 12 h during 24 h. They were analysed immediately. The technique used to quantify *E. coli* and enterococci in the laboratory was the membrane filtration method using Chromocult Coliform-Agar (Merck, Germany) and Chromocult Enterococci-Agar (Merck) as culture medium.

Kinetic coefficients were determined on the basis of first-order kinetics

$$N_t = N_0 e^{-kt} \quad (1)$$

This is the integration of a first-order kinetic in a batch mode reactor, where N_0 and N_t are the number of bacteria at the beginning and time t, respectively, k is the kinetic coefficient (h^{-1}).

The K coefficient (base 10) is the slope of the fitted linear regression of log N vs time. The r^2 values of the semi-log regressions corresponding to the various tests were close thus identical weights were chosen for the multilinear regressions.

Significant relationships between variables were determined by multiple regression analysis using Statistica® Software. Table 1 presents the experimental conditions conducted on *E. coli* and enterococci.

RESULTS AND DISCUSSION

Inactivation without light

The first experiment tests were conducted in dark conditions. The disinfection coefficients calculated by Chick's law ranged between 0.021 h^{-1} and 0.046 h^{-1} and 0.025 h^{-1} and 0.046 h^{-1} for *E. coli* and enterococci, respectively. These coefficients are similar to those reported in the literature. Maïga *et al.* (2009) found similar K values in dark conditions: 0.045 h^{-1} for *E. coli* and 0.047 h^{-1} for enterococci. However, Noble *et al.* (2004) reported lower K values of 0.029 and 0.020 h^{-1}, for *E. coli* and enterococci, respectively. In addition, in night experiments, Craggs *et al.* (2004) reported a mean K value of 0.020 h^{-1} for *E. coli* in high-rate algal pond operating at temperature lower than 20 °C.

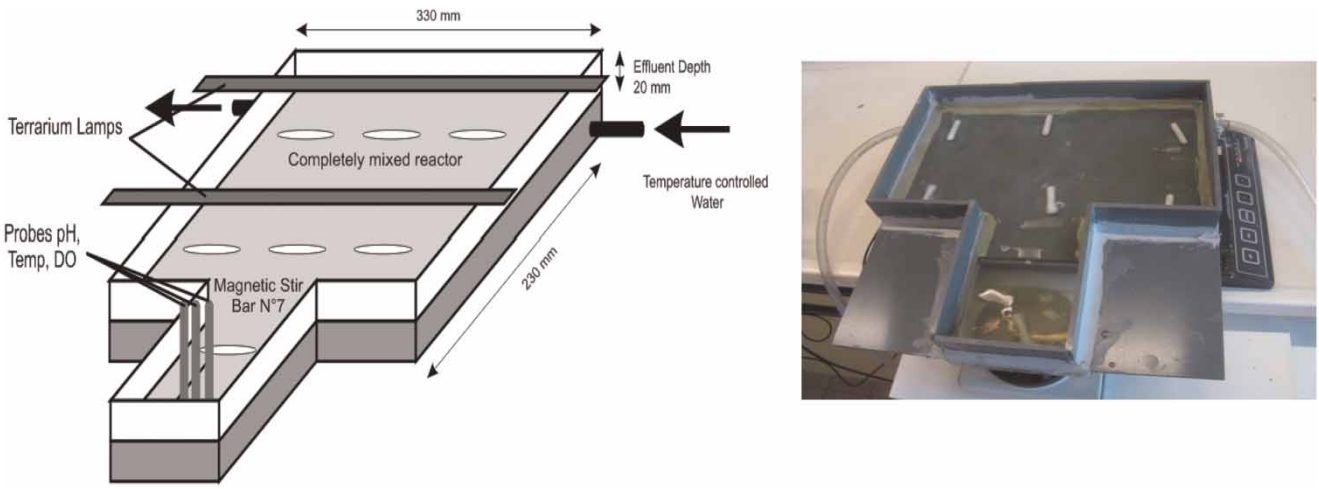

Figure 1 | Layout and photograph of laboratory pilot plant reactor.

Table 1 | Range of experimental conditions for *E. coli* and enterococci

Parameters	Range *E. coli*	Range enterococci
K_{meas} (h^{-1})	(0.04–0.43)	(0.04–0.34)
Irradiation (W/m^2)	0–25	0–25
pH	5.16–12.07	5.16–12.07
DO (mg/l)	1.2–8.19	1.2–8.19
T (°C)	14–28.9	14–28.9

Inactivation by light

In order to show how the impact of light is affected by conditions in the pond, the following model equation has been created to calculate the removal rate for different light intensities, pH, DO concentrations and water temperature for *E. coli* and enterococci. However, it should be noted that Curtis *et al.* did not take temperature into account in their experiments (Curtis *et al.* 1992). They worked in a 30–40 °C range but results were not significantly different.

$$K = (K_0 + K_{pH} \cdot pH + K_{DO} \cdot DO + K_I \cdot I) \cdot \theta^{(T-20)} \quad (2)$$

Figures 2 and 3 illustrate the relationship between the calculated kinetics (K_{cal}) from the first-order equation and the measured (K_{meas}). There are significant correlations between Curtis-type models and our experimental results ($R^2 = 0.66$ and $R^2 = 0.609$ for *E coli* and enterococci, respectively).

In general, the decreases in the *E. coli* and enterococci concentrations during the experiment were between 2 and 3 log units. The kinetic coefficient values (K_{meas}) of all experiments corresponded to the values obtained from the curves of the numbers of *E. coli* and enterococci. A significant relationship ($n = 22$, $p < 0.00003$ for *E. coli* and $p < 0.00005$ for enterococci) was identified between (K_{meas}) and pH, DO, I, and T. This relationship explains 66 and 60.94% of the K variance of *E. coli* and enterococci, respectively (Table 2).

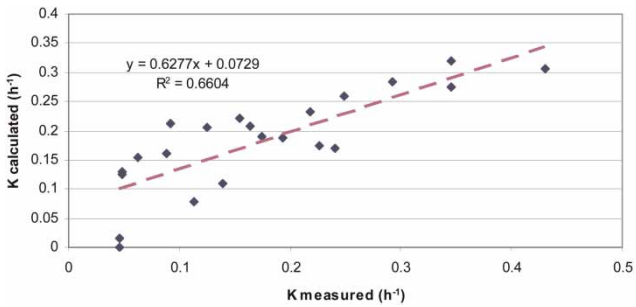

Figure 2 | Relationship between the kinetic coefficients (K_{cal}) and (K_{meas}) for *E. coli*.

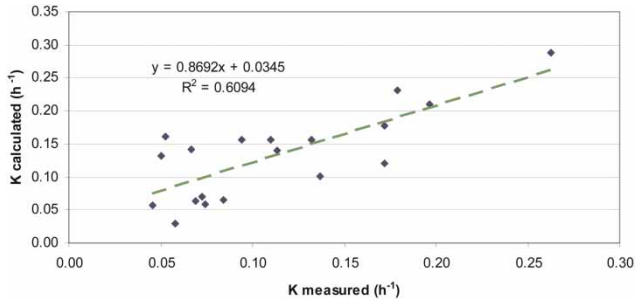

Figure 3 | Relationship between the kinetic coefficients (K_{cal}) and (K_{meas}) for enterococci.

The coefficients obtained for *E. coli* and enterococci are relatively different. The fitted parameters of the K values confirm this difference. By comparing the precision on the pH and DO measurements, it seems that the removal mechanisms of *E. coli* and enterococci by the light are influenced differently by the physicochemical parameters.

The decay rate in illuminated conditions ranged from 0.046 to 0.43 h^{-1} for *E. coli* and from 0.046 to 0.346 h^{-1} for enterococci. The difference between the kinetic coefficients obtained in dark and illuminated conditions shows that sunlight is a major factor (operating alone or synergistically) in the inactivation of bacteria in maturation ponds.

Effects of variables on *E. coli* and enterococci under light

The present study is significant because it was conducted taking into account the combined effect of all damaging factors and their interaction generating changes in the density of *E. coli* and enterococci to improve modelling for better design of disinfection in maturation ponds.

Five separate experiments on the influence of temperature under the same conditions of pH, DO concentrations and light intensity, show clearly that temperature is an important factor in the disinfection process of *E. coli* and enterococci. The kinetic coefficient increased from 0.07 to 0.34 h^{-1} and from 0.04 to 0.31 h^{-1} respectively for *E. coli* and enterococci when the water temperature increased from 13 to 28 °C.

Table 2 | Fitted parameters of the $K = (K_0 + K_{pH}.pH + K_{DO}.DO + K_I.I).\ \theta^{(T-20)}$

Parameters	*E. coli*	Enterococci
K_0	-0.23 ± 0.358	0.022 ± 0.053
K_{pH}	0.037 ± 0.029	0.011 ± 0.031
K_{DO}	0.008 ± 0.03	0.02 ± 0.025
K_I	0.006 ± 0.004	0.005 ± 0.003
θ	1.066 ± 0.065	1.072 ± 0.066

Our findings are consistent with those of Pearson *et al.* (1987). They found reduced faecal coliform counts over vertical transects within WSPs only where pH > 9.3 and temperature was high.

Six separate experiments on the influence of DO at pH around 7.5, showed the DO effect on light inactivation. Light inactivation of *E. coli* and enterococci increased with increasing levels of DO. The kinetic coefficient increased from 0.053 to 0.34 h^{-1} for *E. coli* and from 0.048 to 0.43 h^{-1} for enterococci when the DO concentrations increased from 1.2 to 8.19 mg/l under controlled conditions of pH, temperature and light intensity.

These results demonstrate that DO is an important factor influencing the disinfection process. The strong dependence of *E. coli* and enterococci inactivation and DO implies that photo-oxidation predominates.

These results match those found by Curtis *et al.* (1992). They found that fecal coliform survival was completely dependent on the presence of oxygen and decrease with increasing oxygen concentration.

The results of the study are also in agreement with those of Davies Colley *et al.* (1999). They found that the endogenous photo-inactivation of *E. coli* and enterococci was strongly dependent on DO.

Four separate experiments on the influence of pH at DO concentration around 4 mg/l showed the light inactivation of *E. coli* dependence on pH. The kinetic coefficient of *E. coli* increased from 0.046 to 0.49 h^{-1} when the pH increased from 5 to 12 under controlled conditions of DO concentration, water temperature and light intensity.

The results of this study are evidence that when the pH exceeds 8.5 the kinetic coefficient of *E. coli* increased. A synergistic increase in inactivation was observed when both pH and DO increased.

The results of the study are in agreement with those of Davies Colley *et al.* (1997, 1999).

They found that under moderate pH conditions *E. coli* was inactivated by endogenous sensitisers. When the pH was elevated above 8.5, *E. coli* was inactivated more rapidly by exogenous mechanism.

The linear relationship between the kinetic coefficient of *E. coli* and pH is in agreement with the results already found by previous research on WSPs (Curtis *et al.* 1992; Davies-Colley *et al.* 1997, 1999; Nelson *et al.* 2009) and has less effect on the elimination of enterococci (Davies-Colley *et al.* 1999; Nelson *et al.* 2009).

Three other separate experiments on the influence of pH on the enterococci disinfection at DO concentration around 4 mg/l showed that the kinetic coefficient of enterococci presents a very small variation (0.15 to 0.2 h^{-1}) when the pH increased from 5 to 12 under the same conditions of DO, water temperature and light intensity. The results of this study show that the inactivation of *E. coli* increased with increasing pH. In contrast, enterococci had similar rates at all pH. Our findings are in agreement with those of Davies Colley *et al.* (1999) and Nelson *et al.* (2009).

Nelson *et al.* (2009) found that the inactivation rate of *E. coli* was lower in pond water compared with DO and the inactivation rate increased dramatically with pH ($K = 0.3$ at pH 7; $K = 1.2$ at pH 10). In contrast, the inactivation rate of *E. coli* was higher in pond water than for DO, and was roughly similar between pH 7 and 10.

From these laboratory tests it was concluded that the investigated micro-organisms indicators respond differently to the pH, water temperature and DO levels investigated yielding two different regressions which could correspond to the two different mechanisms presented in the literature for those indicators.

Our findings are consistent with the results of past research on the importance of cell structure, conformation and genomic properties on organism sensitivity (Love *et al.* 2010). They demonstrate that different micro-organisms are affected differently by potential stressors (e.g. sunlight, pH, DO, etc.) due to variations in genome structure, length and sequence; cell membrane and conformation.

Bolton *et al.* (2011) found that the effect of DO on inactivation rates was organism dependent. Increasing DO had a positive effect on Enterococcus faecium. They found also that the effect of pH on photo-inactivation was variable, depending on the micro-organism and the other experimental conditions. Bosshard *et al.* (2010a, b) attribute the pH effects to conformational changes to the membrane of the bacteria. A damaged membrane will lead to inactivation due to physical breakdown which exposes nucleic acids to environmental stressors, damage to the respiratory chain in bacteria.

CONCLUSIONS

Rather significant correlations between the kinetic disinfection coefficient (K) and pH, DO, T, and light intensity were observed. The kinetic coefficient (K) values for *E. coli* and enterococci were found to be dependent on physicochemical parameters (pH, DO, water temperature (T °C), and light intensity (I)). K increases with increasing pH, I, and T.

The variation coefficients of the fitted parameters are still high and more experience is needed to improve the robustness of the model.

The pilot scale reactor has been designed to be used on the field with pond water (and algae) and direct sunlight. The light intensity and algal concentration have to be measured, but the principle of the measurement will remain the same. Other correlations taking the algal concentration into account could be generated. These laboratory results are now being validated by tests carried out directly under full-scale conditions.

As the kinetic model is fitted for a completely batch reactor with small depth, it is independent of the hydrodynamic.

This means that the model can be combined with other hydrodynamic models such as plug flow, dispersive plug flows or even computational fluid dynamics (CFD) models to simulate disinfection in real maturation ponds.

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