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Removal of *E. coli* and enterococci in maturation pond and kinetic modelling under sunlight conditions

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ABSTRACT

Bacteria, including pathogenic microorganisms are very sensitive to sunlight (solar ultraviolet radiation (UV)). Light stimulates algal photosynthesis; algae consume rapidly carbon dioxide, release oxygen and increase the pH of the water. Under high pH conditions, UV radiations are responsible for the elimination of pathogens. The aim of this study is to improve the modelling for a better design of disinfection in maturation ponds (MPs) and to identify the key parameters influencing the process. This paper addresses *Escherichia coli* and enterococci disinfection in a full-scale MP under Tunisian conditions. The evolution of wastewater temperature, dissolved oxygen and pH were continuously recorded using probes. The initial bacterial wastewater concentration before disinfection testing lies in the range 10^6–10^7 and 10^4–10^6 CFU/100 ml, *E. coli and* enterococci, respectively. Kinetic coefficients are determined on the basis of the first-order kinetic. Significant relationships between variables were determined by multiple regressions using Statistica Software. Two equations are suggested to calculate the kinetic coefficient *K*. Significant correlations between kinetic disinfection coefficients and pH, DO, T and UV intensity were observed. The kinetic coefficient (*K*) values for *E. coli* and enterococci are closely dependent on physicochemical parameters. Light has a main role in the disinfection process in MP. It has a synergistic effect with pH, DO and T in the pond.

Keywords: Maturation ponds; Kinetics; Disinfection; *E. coli*; Enterococci; UV

1. Introduction

Wastewater reuse is becoming increasingly important in developing countries which are characterized by the scarcity of water resources. One of the critical factors involved in wastewater reuse implementation is the provision of treated wastewater in conformity with reuse standards. Wastewater Stabilization Ponds (WWSP) are one of the most appropriate extensive wastewater treatment methods to reduce pathogens [1]. This technology has been widely used over the last few years as an alternative to conventional sanitation systems of small communities, due to their low energy and maintenance costs [2]. Previous studies have focused on the ability of these systems to reduce microorganisms from wastewater, especially indicator

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microorganisms like the faecal coliform and streptococci groups of bacteria [3].

Many authors have developed different empirical disinfection models in WWSP and especially in the maturation ponds (MPs). According to the review of the models conducted by [4], there are huge differences between the models describing this process. It is also proved that the improvement of pond design for better disinfection is acutely needed. The effectiveness of properly designed wastewater ponds in removing pathogens has been acknowledged by a number of key commentators. Ouali et al. [5] note that well-designed MPs are extremely efficient in the removal of *Escherichia coli* and enterococci. Several parameters governing the removal process of bacteria have been studied and identified as key parameters of disinfection. The design of MPs is based on pathogen removal; usually bacterial decay [6,7]. Whatever is the mechanism of bacterial inactivation, the kinetic disinfection follows first-order kinetics [8,9].

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

where N_0 and N_t: number of bacteria at the inlet and outlet of the pond; K: kinetic coefficient (h^{-1}).

Many studies have been conducted to identify the main factors involved in bacterial reduction, including the exposure to sun or ultraviolet radiations (UVs) [4], temperature [8,10], hydraulic retention time [3], the depth of water in the pond, number of ponds and the length/breadth ratio [7,11,12]. Reinoso et al. [13] confirmed that temperature, hydraulic retention time, solar irradiation and physicochemical characteristics should be taken into consideration when microorganisms decay models are assumed.

The survival of faecal indicator bacteria in aquatic environments is strongly influenced by abiotic (sunlight and temperature) and biotic (predation and competition) factors. The biological tolerance of *E. coli* to physicochemical factors has been especially well-studied, albeit mostly in the laboratory [14]. Of these factors, incoming solar radiation (sunstroke) is the most potent in the inactivation or killing of faecal indicators [15,16] and pathogenic bacteria [17]. Liu et al. [18] also mentioned that sunlight is a major contributor to the inactivation of bacteria at the surface, but the formulation based on sunlight, temperature and sedimentation is preferred over the first-order inactivation.

Sunlight acts on the interaction with other factors including dissolved oxygen (DO) and pH [19,20] and the presence of light absorbing constituents which may function as photosensitizers. These parameters, determinant in the sunlight inactivation of bacteria, are submitted to important diurnal variations [21]. Therefore, it is important to investigate the behaviour of faecal indicators in MP in order to predict their efficiency in countries where sunlight is available. Also, knowledge on pathogen removal efficiency could provide the basis for improvement in the design, operation and maintenance of WWSP.

Davies-Colley et al. [20,22] suggested three mechanisms to explain the disinfection effect of light:

- The direct effect of UVB [23,20,22,24];
- The effect of UVB and visible light. These are combined with external photosensitizers [19], but they are dependent on external DO, pH [19,20] and the wavelength [19,20];
- And finally, the effect of UVB combined with internal photosensitizers, but it is dependent on external DO.

According to Bosshard et al. [25] solar UVA light is the agent that inactivates bacteria during the treatment.

This study was conducted in Tunisia, using pilot-scale experiments under full-scale conditions to determine the effect of solar radiation in association with potential environmental factors on the survival of *E. coli* and enterococci, which are of public health importance as indicators of faecal pollution in water. The objectives were to improve the modelling for a better design of disinfection in MP and to study the effect of the physicochemical parameters (pH, DO, temperature (T) and light intensity (I)) influencing the disinfection of *E. coli* and enterococci.

2. Methods

Thirteen separate short-term (6 h) experiments on sunlight exposure of a domestic treated wastewater from the Korba WWTP (activated sludge) were carried out during the period between April and October using pilot-scale experiments (Figs. 1 and 2). The aim of these experiments was to investigate the effects of the variables (DO, pH, T, light intensity) on sunlight inactivation of *E. coli* and enterococci. During the experiments, solar radiation was monitored on site with a Skye SKS 1110 pyranometer connected to a data logger (Agilent 34970A data logger) recording 10 min averages. A Reactor completely covered with two layers of aluminium foil was used for dark controls.

The wastewater (2 cm deep) was maintained homogeneous in the reactor by seven magnetic stirrers (100 rpm). Temperature within each reactor was controlled by rapid pumping of water from a temperature-controlled water bath (20°C) through rubbery tubing (Fig. 2). DO, pH and temperature were

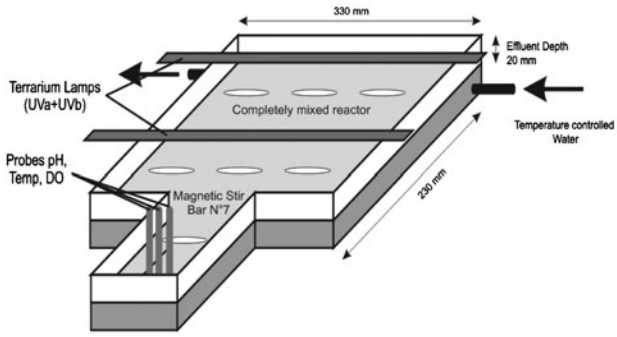

Fig. 1. Schematic diagram of reactor (pond microcosm).

Fig. 2. Photograph of reactor.

continuously logged using probes (tinitag, oxymeter WTW197i and pHmeter WTW197i). The initial concentration of faecal indicators was known and was sufficiently high for the study of inactivation kinetics. The initial bacterial wastewater concentration before disinfection testing was in the range of 10^6–10^7 and 10^4–10^6 CFU/100 ml for *E. coli* and enterococci, respectively. Samples were collected every 2 h during 6 h. They were analyzed 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 Entecocci-Agar (Merck) as culture medium. Details are given in Table 1.

3. Results

The importance of sunlight as an inactivating factor has been demonstrated in very different surface water environments, including seawater [26] and WWSP [19].

Kinetic coefficients were determined on the basis of first-order kinetics. Significant relationships between variables were determined by multiple regression analysis using Statistica Software. Table 2 presents the experimental conditions conducted on *E. coli* and enterococci. Fig. 3 shows an example of a test [pH = 10, T = 23°C, DO = 9 mg/l and light intensity = 2,466 W/m^2]. After 6 h, the number of *E. coli* and enterococci decreases by about 4 log units.

In order to show how the impact of light is affected by other factors in the MP under Tunisian conditions, the following model equation was developed to calculate the removal rate for different light intensities, pH, DO concentrations and water temperature for *E. coli* and enterococci. Then the results were adjusted according to Curtis model.

However, it should be noted that Curtis did not take into account the temperature factor. The temperature interacts with other factors influencing the disinfection process in MPs.

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

Figs. 4 and 5 illustrate the relationship between the calculated kinetics (K_{cal}) from the first-order equation

Table 2
Range of experimental conditions for *E. coli* and enterococci

Parameters	*E. coli*	Enterococci
K_{meas} (h^{-1})	(0.047–1.81)	(0.058–1.66)
Irradiation (Wm^{-2})	1313.55–3082.06	
pH	8–10	
DO (mg/l)	7.28–7.97	
T (°C)	26–31.6	

Table 1
Parameters, sampling frequency and analytical methods

Parameters	Unit	Method	Frequency
pH	–	pH meter WTW 197i	Continuous
(DO)	mg/l	Oxymeter WTW197i	Continuous
T	°C	Probe (tinitag)	Continuous
I, light intensity	W/m^2	Skye SKS 1110 pyranometer Agilent 34970 A data logger	10 min
E. coli	UFC/100 ml	Chromocult Coliform Agar, (incubation: 36°C, 24 h) membrane filtration 0,45 µm	0, 2, 4, and 6 h
Enterococci	UFC/100 ml	Chromocult Entero agar, (incubation: 36°C,48 h) membrane filtration 0,45 µm	0, 2, 4, and 6 h

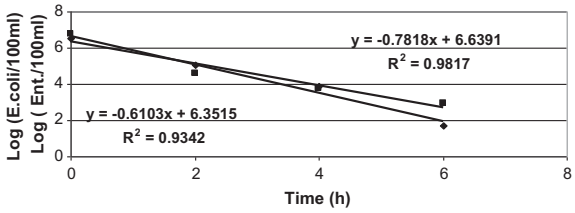

Fig. 3. Sample analysis.

and the measured (K_{meas}). There are significant correlations between Curtis-type models and the experimental results.

The decreases in the *E. coli* and enterococci concentrations during the experiment were between 1.5 and 5 log units. The kinetic coefficient values (K_{meas}) of all the experiments corresponded to the values obtained from the curves of the numbers of *E. coli* and enterococci. A significant relationship (n = 13, $p < 0.00001$ for *E. coli* and $p < 0.00001$ for enterococci) was identified between (K_{meas}) and pH, DO, I and T. This relationship explains 95.9 and 66% of the K variance of *E. coli* and enterococci, respectively (Table 3).

The *E. coli* and enterococci coefficients are relatively different. The fitted parameters of the kinetic coefficient confirm this difference. The removal mechanisms of *E. coli* and enterococci by sunlight seem influenced differently by the physicochemical parameters. The removal process of these bacteria responds differently to the variations of the key parameters.

According to Bolton et al. [27], whatever the sunlight wavelength regions, the effect of DO and pH on inactivation rates was dependent on the bacteria. Bolton et al. [28] considered that the inactivation of *E. coli* in the WWSP is due to sunlight interacting with photosensitizers, high pH, DO, predation and sedimentation in the presence of the suspended solids.

The disinfection coefficients calculated by Chick's law ($dC/dt = Kt$) are 0.047 and 0.058 h h^{-1} for *E. coli* and enterococci, respectively, in dark conditions. These coefficients are similar to those reported in the literature. Maïga et al. [21] found similar K values

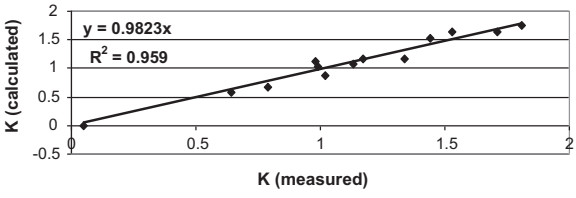

Fig. 4. Relationship between the kinetic coefficients (K_{cal}) and (K_{meas}) for *E. coli*.

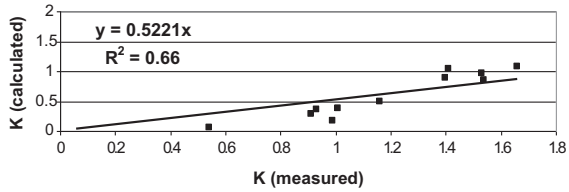

Fig. 5. Relationship between the kinetic coefficients (K_{cal}) and (K_{meas}) for enterococci.

Table 3
Parameters of equation (2) ($+/-\sigma$)

Parameters	*E. coli*	σ	Enterococci	σ
K_0	–2.46	±2.358	–2.75	±2.053
K_{pH}	0.260	±0.29	0.083	±0.031
K_{DO}	0.049	±0.03	0.206	±0.025
K_I	0.44	±0.46	0.36	±0.53
θ	1.01	±0.065	1.02	±0.066

without UV (dark conditions): 0.045 h^{-1} for *E. coli* and 0.047 h^{-1} for enterococci. However, Noble et al. [29] reported lower K values of 0.029 and 0.020 h^{-1}, respectively for *E. coli* and enterococci. In addition, in night experiments, Craggs et al. [30] 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. Dark inactivation of bacteria may be attributed to the activities of predatory and lytic organisms. Sinton et al. [31] confirmed that dark inactivation of bacteria may be attributed to inhibitory substances, predation (grazing by protozoa) and the lack of substrate after depletion.

The decay rate measured under light conditions ranged from 0.047 to 1.81 $h^{-1}$and from 0.058 to 1.66 h^{-1} for *E. coli* and enterococci, respectively (Table 2). The comparison between the kinetic coefficients obtained in dark and UV conditions shows that sunlight is a major factor in the inactivation of bacteria in the MP. Over comparatively short period of time (up to 6 h in our experiments), sunlight dominated the inactivation of faecal indicator organisms in MP effluent as shown by comparing inactivation kinetic coefficient for sun-exposed reactors at different intensities with those maintained in dark conditions (Fig. 6).

The results show that the removal of *E. coli* and enterococci by UV radiation is not a simple process. Many factors (light, high pH, high oxygen concentration and temperature) are required for light to have any effect. However, the action of sunlight can be modified by other factors, including DO, pH [20].

The inactivation of *E. coli* increased with increasing pH (Fig. 7). High kinetic coefficients (1.13 and 1.34 h^{-1}) have been observed in 11 tests conducted with

pH values between 8.19 and 8.26 at saturation DO and temperatures of 27˚C. Enterococci had apparently similar rates of inactivation at all pH values tested. The inactivation rate of *E. coli* increased when pH values are ≥ 9. *E. coli* was inactivated more rapidly when the pH exceeded 8.5. The most important kinetic coefficient (K = 1.81 h^{-1}) is obtained with pH 10 and DO concentration of 8.99 mg/l, but enterococci were not affected by pH; Enterococci can grow at pH 10.

These findings are consistent with numerous studies reviewed by [32] that reported a strong pH dependence of inactivation of faecal coliforms, notably the experimental studies of [19] and [20]. Under moderate pH conditions (7.0–8.5), only UVB caused inactivation of *E. coli*.

According to Davies-Colley et al. [20,23], *E. coli* are removed by an indirect mechanism of photo-oxidation by exogenous sensitizers (solid and dissolved humic substances) causing the destruction of cell membranes [22,33,34]. Exogenous photo sensitizers react with pH and DO to promote the photo inactivation process [27]. The pH effects may be due to the 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 or due to damage to host or cell attachment sites [25,35].

Sunlight inactivation of enterococci and *E. coli* increased with increasing levels of DO (Fig. 8). Under the same conditions of pH, temperature and light intensities, enterococci disinfection coefficient increases from 0.91 to 1.54 h^{-1} when the DO concentration increased from 7.28 to 9.97 mg/l. A DO dependence of sunlight inactivation strongly suggests that a photo-oxidative process is involved. Sunlight may damage DNA via reactions between iron and hydrogen and hydrogen peroxide. The results for *E. coli* are consistent with those of [19], who reported a strong influence of DO on the inactivation of faecal coliforms in WWSPs.

The strong relationship between the inactivation of *E. coli* and DO concentration would support an indirect photo-oxidation process by endogenous sensitizers. The inactivation of enterococci depends essentially on the DO concentrations. The disinfection coefficient of enterococci increased from 0.91 to 1.54 h^{-1} with increasing levels of DO (from 7.28l to 9.97 mg/l). These results are in agreement with those of [19,20,24,27,28]. Enterococci are eliminated by an indirect mechanism related to exogenous sensitizer (humic substances). These results demonstrate the importance of DO in sunlight-mediated inactivation of the *E. coli* and enterococci in MPs.

Fig. 9 shows that inactivation of *E. coli* and enterococci were strongly dependent on the temperature. It seems clear that temperature is an important factor in the disinfection process of *E. coli* and enterococci. The kinetic coefficient increased from 0.79 to 1.71 h^{-1} and from 0.62 to 1.66 h^{-1}, respectively, for *E. coli* and enterococci when the water temperature increased from 26 to 31.6˚C. The temperature accelerates bacterial die-off presumably by increasing metabolic

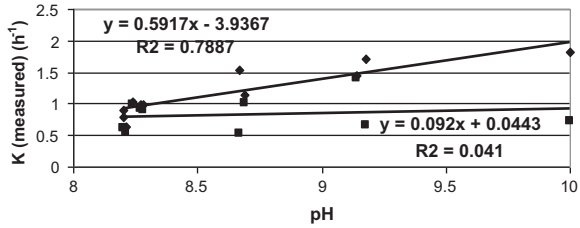

Fig. 7. pH dependence of sunlight inactivation of *E. coli* and enterococci.

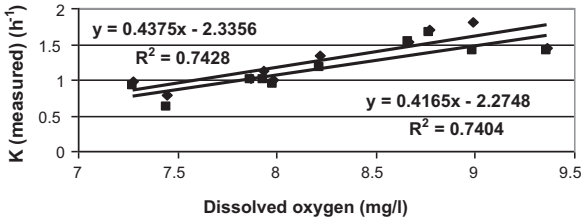

Fig. 8. DO dependence of sunlight inactivation of *E. coli* and enterococci.

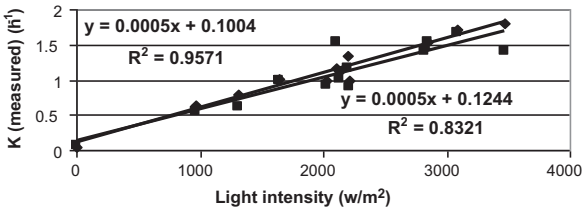

Fig. 6. Inactivation curves for *E. coli* and enterococci for various light intensities.

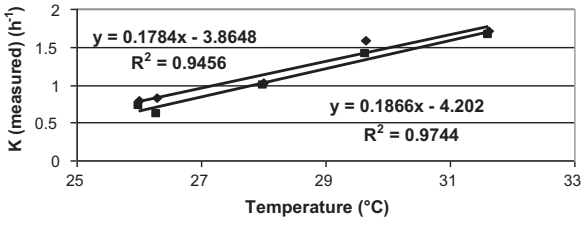

Fig. 9. Temperature dependence of sunlight inactivation of *E. coli* and enterococci.

activity, and thus susceptibility to toxic substances. It will also accelerate substrate utilization and thus the onset of starvation conditions.

Pearson et al. [36] confirm the elimination of faecal coliform in WWSPs only where pH exceeds 9.3 and the concentration of DO was high. These authors disregard light and emphasized pH and temperature as the main factors involved in pond disinfection. Saqqar and Pesscod [37] developed a model of faecal coliform removal from a statistical analysis of data for a large WWSP system. These authors chose to include temperature in their model rather than solar radiation with which it was strongly correlated.

4. Conclusion

A mathematical model of bacterial die-off in MPs was developed, by combining the environmental variables (pH, temperature, DO and light intensity) with perfectly mixed reactor for MPs. The model was tested against measured bacterial die-off in a laboratory scale MP, using secondary effluent and found to give a good account of *E. coli* and enterococci die-off in the laboratory pond. This model provides a useful tool for the design and performance prediction of MPs. Light has a main role in the disinfection process in MP. It has a synergistic effect with pH, DO and T in the ponds.

Significant correlations between the kinetic disinfection coefficient (K) and pH, DO, T and UV intensity were observed. The kinetic coefficient (K) values for *E. coli* and enterococci were found to be dependent on physicochemical parameters. K increases with increasing pH, I and T. *E. coli* is less resistant to the light radiation than enterococci. It is primarily inactivated by photo-oxidation damage which is closely dependent on the pH, DO and water temperature. Enterococci are inactivated by photo-oxidation damage depending essentially on DO and water temperature.

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