

# ***Escherichia coli* removal during domestic wastewater treatment in outdoor high rate algae ponds: long-term performance and mechanistic implications**

Paul Chambonniere, John Bronlund and Benoit Guieysse

## **ABSTRACT**

*Escherichia coli* (*E. coli*) first-order decay rates ranging from 3.34 to 11.9 d<sup>-1</sup> (25–75% data range, *N* = 128) were recorded in two outdoor pilot-scale (0.88 m<sup>3</sup>) high rate algal ponds (HRAPs) continuously fed primary domestic wastewater over two years (influent *E. coli* cell count of 4.74·10<sup>6</sup> ± 3.37·10<sup>6</sup> MPN·100 mL<sup>-1</sup>, *N* = 142). The resulting removal performance was relatively constant throughout the year (log<sub>10</sub>-removal averaging 1.77 ± 0.54, *N* = 128), apart from a significant performance drop during a cold rainy period. *E. coli* removal performance was not strongly correlated to any of the meteorological or operational parameters recorded (e.g. sunlight intensity, pH, temperature). Hourly monitoring of *E. coli* cell count evidenced that *E. coli* removal, pH, dissolved oxygen (DO) and pond temperature peaked in the late afternoon of sunny summer days. Such improved daytime removal was, however, not evidenced in spring, even under sunny conditions causing milder increases in pH, DO and temperature. Overall, the data confirm the potential of HRAPs to support efficient *E. coli* removal during secondary domestic wastewater treatment and suggests *E. coli* decay was mainly caused by dark mechanisms episodically enhanced by indirect sunlight-mediated mechanisms and/or high pH toxicity.

**Key words** | disinfection, *Escherichia coli*, high rate algal pond, inactivation mechanisms, wastewater treatment

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## **INTRODUCTION**

Like maturation ponds (MP), high rate algal ponds (HRAPs) are shallow ponds (0.1–0.5 m for HRAPs and 1.0–1.5 m for MP) operated at short to medium hydraulic retention time (1–14 d in HRAPs and 3–15 d in MP) (Norvill *et al.* 2016). This combination of design and operation drives photosynthesis, which contributes to high pH (>10) and dissolved oxygen (DO) concentration (up to 30 mg·L<sup>-1</sup>) being episodically experienced in both systems (Curtis *et al.* 1992; Norvill *et al.* 2016). HRAPs and MPs primarily differ from each other due to the presence of broth mixing in HRAP, creating a linear flow to prevent settling (Norvill *et al.* 2016).

Although MPs are well known for providing efficient disinfection during agricultural or domestic wastewater treatment, with log<sub>10</sub> reduction of common wastewater indicators such as *Escherichia coli* (*E. coli*) being frequently reported above 2 (Buchanan 2014; Papadopoulos *et al.* 2014; Dahl *et al.* 2017), little consideration has been given to this essential

wastewater treatment service in HRAPs. This could be a missed opportunity due to the similarities in design and characteristics between both systems, and particularly in light of pathogen decay mechanisms identified to be significant in MPs. Davies-Colley *et al.* (1999) hypothesized that three sunlight-mediated mechanisms were the main causes for faecal indicator decay in MPs, including (1) direct absorption of solar UV-B by DNA resulting in DNA damage, (2) photo-oxidation of key cellular biomolecules by reactive oxygen species (ROS) generated by endogenous photosensitizers (i.e. photosensitizers existing within the organism) reacting with UV light and (3) photo-oxidation of key cellular biomolecules by ROS generated by exogenous photosensitizers. Both endogenous and exogenous photo-oxidation mechanisms were previously shown to be probably enhanced by high DO concentration, and exogenous photo-oxidation was also favoured by high pH conditions (Curtis

*et al.* 1992; Benchokroun *et al.* 2003). Several dark mechanisms have also been reported to mediate pathogen decay in algal ponds (e.g. heat inactivation, sedimentation, DO toxicity, pH toxicity, predation, starvation/competition with biomass, algal toxins; Dias *et al.* 2017) but there has been limited research on these mechanisms. Of note, Parhad & Rao (1974) noticed an increase of *E. coli* decay for pH above 9.4 in algal cultures grown in sterilized wastewater, and reproduced these results when adding different alkalis in cultures containing only *E. coli*, proving pH to be responsible for the decay observed. Because it combines high sunlight penetration and sustained photosynthetic activity during the day, resulting in high pH and DO levels, HRAP are expected to provide the conditions for enhanced pathogen removal during domestic wastewater treatment.

In this context, the present study was undertaken to quantify pathogen removal performance in two outdoor pilot HRAPs continuously fed real wastewater (primary effluent) over two years. *E. coli* was targeted because it is a well-established indicator of faecal contamination and disinfection of water (Edberg *et al.* 2000). The study of *E. coli* removal was supplemented by the monitoring of wastewater compounds, wastewater treatment performances, environmental parameters (e.g. temperature, pH) and weather parameters to verify long-term treatment efficiency and identify mechanisms of *E. coli* decay.

## MATERIALS AND METHODS

### HRAPs set-up and operation

Two pilot-scale HRAPs (0.86 m<sup>3</sup> working volume, 3.42 m<sup>2</sup> of illuminated surface area, 0.25 m working depth) were set up at the wastewater treatment plant of Palmerston North, New Zealand (Latitude: 40° 23' 7" S; Longitude: 175° 34' 47" E). These ponds were made of a concrete trough equipped with a central concrete wall to create a raceway path. Mixing was provided by a paddlewheel operated at approximately 10 rpm, generating an average linear fluid velocity of 23 cm·s<sup>-1</sup> (within the typical range of 15–30 cm·s<sup>-1</sup> reported by Craggs *et al.* 2014). Wastewater was pumped from the outlet channel of the primary settler of Palmerston North domestic wastewater treatment plant and continuously supplied to the ponds to reach a median hydraulic retention time (HRT) of 10.3 d, within the typical range of 1–14 d reported by Norvill *et al.* (2016). The first HRAP (HRAP-A) was seeded prior to the start of this study using an algal/bacterial broth obtained from an MP located in Rongotea,

New Zealand (Latitude: 40° 17' 40.6" S; Longitude: 175° 24' 47.8" E, previously described in Hom-Diaz *et al.* 2017). The second HRAP (HRAP-B) was seeded on 7 July 2016, using half of the volume from HRAP-A, and both ponds were gradually filled with wastewater on the same day. Data were therefore collected over 22 months for HRAP-A and 10 months for HRAP-B. The ponds were visited twice per week for maintenance, onsite monitoring and sampling (see SI-A in the Supplementary information for details).

### Sampling and analysis

During each visit, 250 mL grab samples were withdrawn from each HRAP and influent wastewater was sampled by letting the inlet tubing directly fill the sampling bottle. The wastewater sample was collected prior to cleaning the inlet tubing to prevent sample contamination with biofilm. Pond broth and wastewater aliquots of 1.5 mL were also saved in Eppendorf tubes for *E. coli* counting. All samples were immediately stored in the fridge upon arrival at the laboratory. Total suspended solids (TSS) concentration, colorimetric analyses and bacterial cell counting were completed within 4 h of sampling. For other analysis, both filtered and unfiltered samples were stored at -20 °C and thawed on the day of analysis. On specific days, a refrigerated autosampler (ISCO 6712FR, Teledyne ISCO, USA) was used to grab 200 mL samples every hour from 9 am. These samples were withdrawn downstream from the paddlewheel approximately 5 cm below the water surface. All samples were stored at 4 °C in the refrigerated chamber of the autosampler until collection the next day. Samples were then processed as described above.

TSS concentration was quantified via dry weights measurements following the standard method 2540.D (Eaton *et al.* 1998) using GF/C grade fibreglass filters (General Electric, USA). The filtrate was used to quantify the concentrations of chloride, nitrite, nitrate, sulphate and phosphate using the ion chromatography system described by Hom-Diaz *et al.* (2017), as well as the concentration of ammonium using a colorimeter (Orion AQUAfast AQ3700 from Thermofisher, USA or DR3900 from Hach, USA) and tests from the corresponding manufacturers. The concentrations of chemical oxygen demand (COD) and its soluble fraction (sCOD) were also measured with the same colorimeters and corresponding tests. The concentrations of total and dissolved organic carbon, total carbon, and total and dissolved nitrogen were quantified in raw samples and filtrates using a Shimadzu TOC-L analyzer (Shimadzu,

Japan) equipped with an autosampler and TBM-L unit from the same manufacturer.

The total cell counts of *E. coli* were quantified using the IDEXX Quantitray Colilert-18 method, following the manufacturer procedure. Wastewater influent and HRAP samples were diluted 20,000 and 1,000 times, respectively, in distilled water, using glassware cleaned with detergent and thoroughly rinsed with distilled water prior each measurement.

## Data analysis

Assuming the HRAPs were at pseudo-steady state and well mixed (the validity of this hypothesis is further discussed in SI-B), the *E. coli* decay rate  $k$  ( $\text{d}^{-1}$ ) was computed as (Craggs et al. 2004):

$$k = \frac{1}{HRT} \cdot \left( \frac{C_{IN}}{C_{OUT}} - 1 \right)$$

where  $HRT$  is the hydraulic retention time (d),  $C_{IN}$  is the *E. coli* cell count of the influent (most probably number (MPN)·100 mL<sup>-1</sup>) and  $C_{OUT}$  is the *E. coli* cell count of the effluent (MPN·100 mL<sup>-1</sup>). Measurements made at days when severe issues occurred were excluded for analysis (see SI-A). Although it may not always provide a fully accurate mechanistic representation of the disinfection processes occurring in HRAP system, first-order decay rates are commonly used to quantify performance of algae-based wastewater treatment (Maynard et al. 1999; Von Sperling 2005).

Meteorological data (shown in SI-C) were obtained from the National Institute of Water and Atmospheric Research Ltd (NIWA) database (Palmerston North, location agent number 21963).

## Statistical analysis

Simple linear regressions were performed between *E. coli* decay rate and each monitored parameter using Matlab R2015a or R2018a (MathWorks, Natick, Massachusetts, USA) with built-in function *fitlm*. Regressions were deemed significant when the associated  $p$ -value (for the  $t$ -statistic of the hypothesis test where the corresponding coefficient is equal to zero or not) was below 0.05. The strength of the relationship between the tested parameters was then analyzed based on the coefficient of determination of the linear regression ( $R^2$ ).

$t$ -tests were performed to compare (1) the distribution of *E. coli* cell counts in the effluents of HRAP-A and HRAP-B

(*E. coli* cell counts were log10 transformed to respect the  $t$ -test hypothesis of normal distribution of the data, which was confirmed by Anderson–Darling tests at the 95% significance level), and (2) winter and summer *E. coli* cell counts in the influent (log10 transformed for the same reason). All tests were performed using Matlab R2015a or R2018a.

One-way analysis of variance (ANOVA) was used to evaluate the significance of the differences between monthly *E. coli* decay rates (performed using Matlab R2018a).

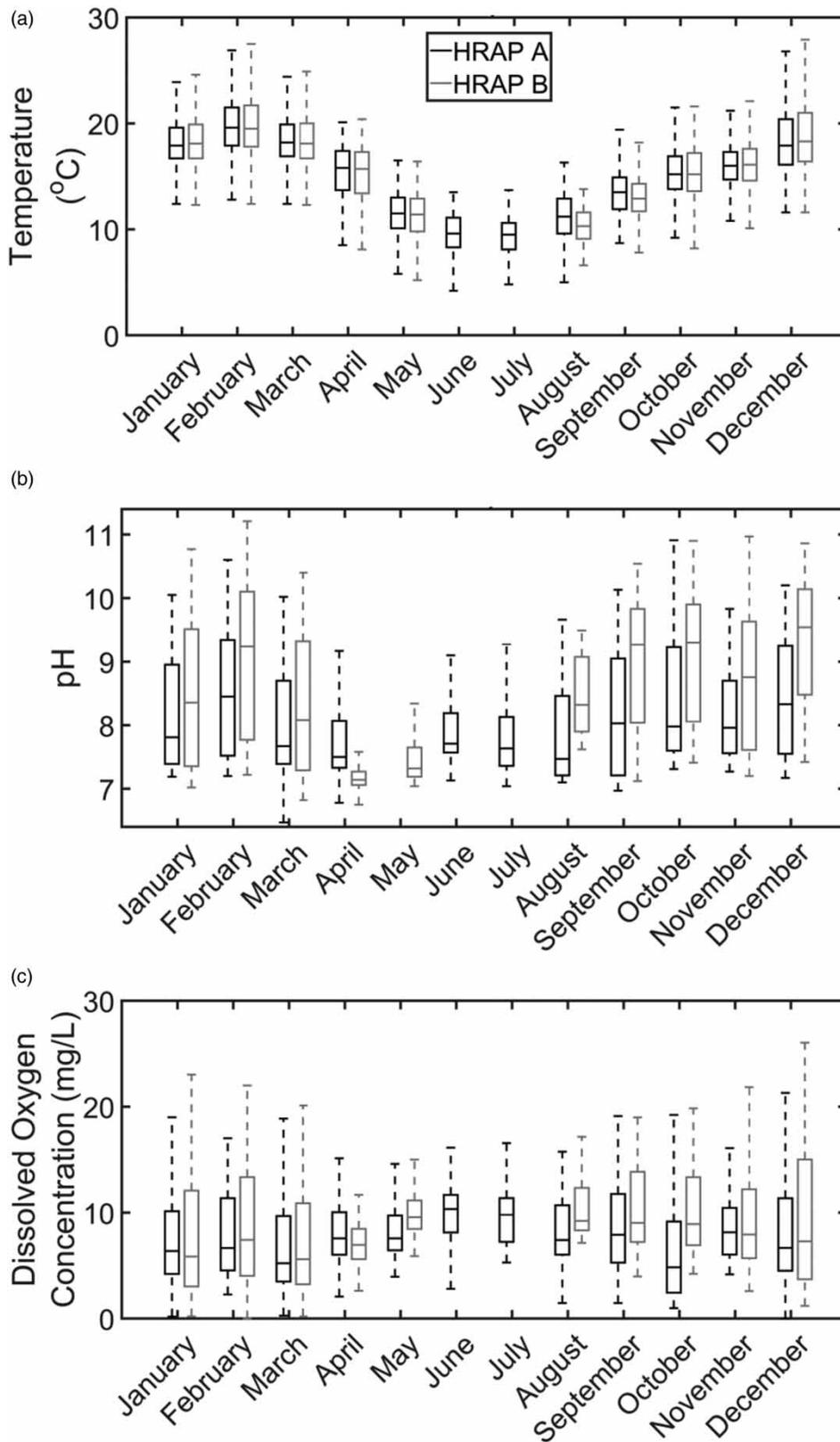
## RESULTS AND DISCUSSION

### General wastewater characteristics and treatment performance

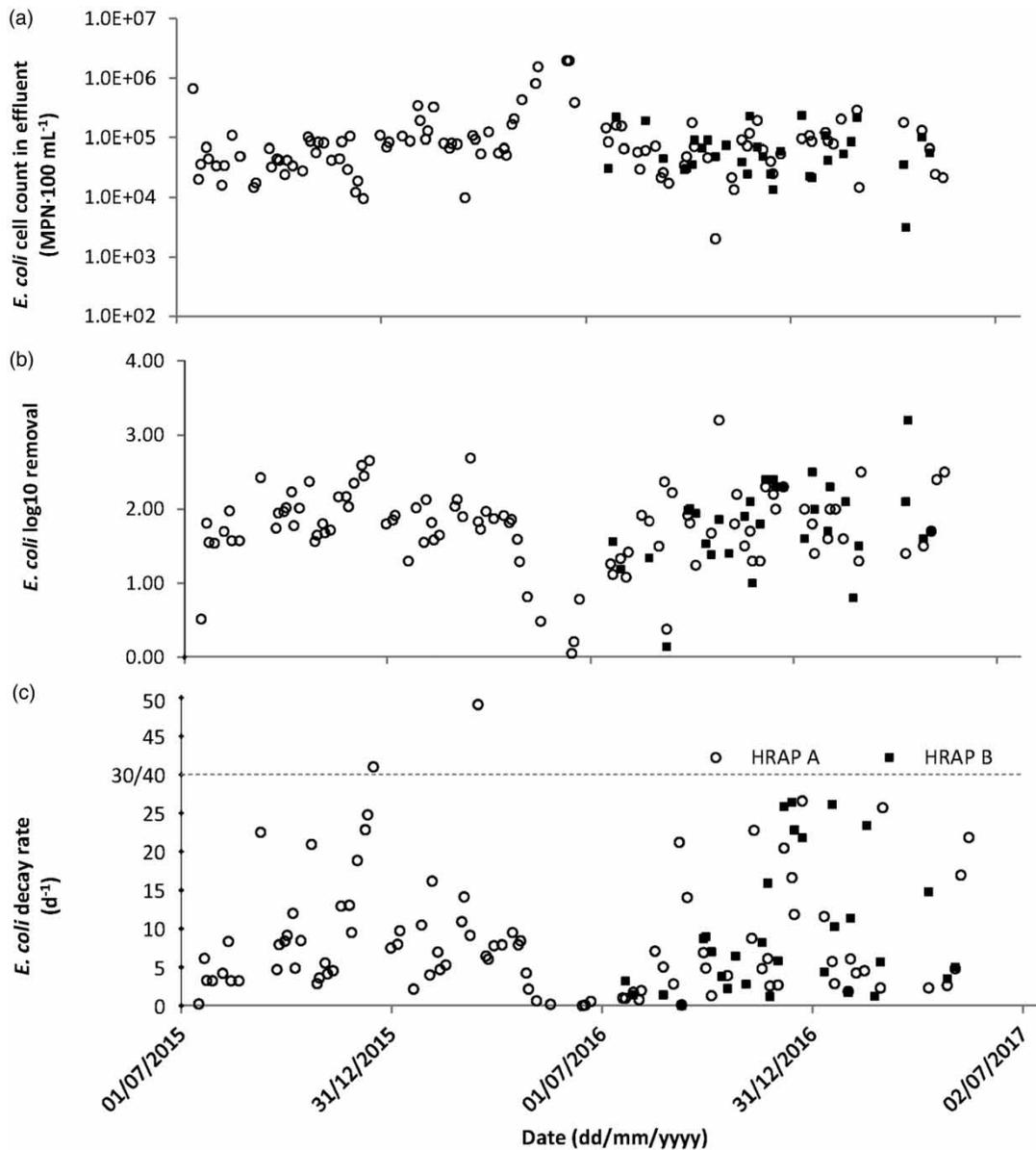
The wastewater fed to the HRAPs had the typical characteristics of medium-strength primary domestic wastewater (Metcalf & Eddy Inc. 2003; see SI-D in the Supplementary information for detailed data). The removal efficiencies of the main pollutants (SI-D) were also in agreement with typical full-scale HRAP performance reported during secondary wastewater treatment (Craggs et al. 2012; Sutherland et al. 2014). These results show that the HRAPs performed under conditions relevant to full-scale HRAPs treating primary wastewater, which provides confidence that *E. coli* removal performance can be regarded as relevant to full-scale systems. Temperature, pH and DO concentration in the algal broth were highly variable (Figure 1). High DO concentrations (>20 mg·L<sup>-1</sup>) and pH (>10) levels were frequently recorded over 7 months of the year.

### *E. coli* removal performance

As can be seen in Figure 2, *E. coli* cell counts in the effluent and log10 removal were relatively consistent over the duration of this study (Figure 2(a) and 2(b)), with mean values of  $1.32 \cdot 10^5 \pm 2.81 \cdot 10^5$  MPN·100 mL<sup>-1</sup> ( $N = 137$ ) and  $1.77 \pm 0.54$  ( $N = 128$ ), respectively. In contrast, *E. coli* first-order decay rates varied significantly (Figure 2(c)) and followed a non-normal distribution (shown in SI-F). Consequently, this performance can be summarized (for better comparison with values given in the literature) by a first-order decay rate range of 3.34–11.9 d<sup>-1</sup>, representing the 25–75% data range ( $N = 128$ ). A two-sample  $t$ -test did not reject the hypothesis that *E. coli* cell counts (log10-transformed) in the effluent of the two HRAPs had equal mean and variances at the 99% significance level ( $p = 0.725$ ,  $N = 41$  for HRAP-A,  $N = 32$  for HRAP-B,  $t$ -test including



**Figure 1** | Monthly distributions of temperature, pH and DO concentration in each HRAP. The data shown represent the 5, 25, 50, 75 and 95 percentiles (the number of observations for each distribution shown is given in SI-E, Supplementary information).

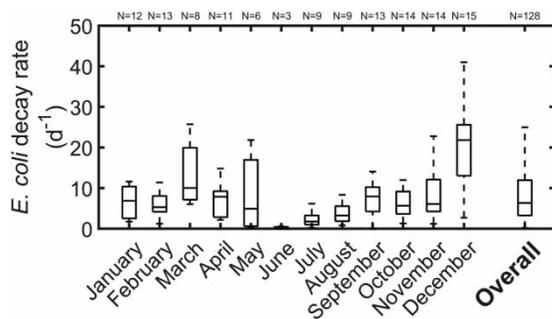


**Figure 2** | *E. coli* removal performances in HRAP-A (open circle) and HRAP-B (closed squares) quantified as: (a) *E. coli* MPN·100 mL<sup>-1</sup>; (b) log<sub>10</sub> removal; (c) first-order *E. coli* decay rate (d<sup>-1</sup>). The data shown were obtained from single measurements.

only the data recorded when the two HRAPs were simultaneously operated), confirming both HRAPs displayed similar behaviour.

Besides a drop in performance during winter (May–June), there was little temporal variation in *E. coli* cell count over the period monitored (Figure 2). This was unexpected because *E. coli* removal during algae-based wastewater treatment has been associated repeatedly with sunlight-mediated mechanisms in the literature (Davies-Colley et al. 1999; Nelson et al. 2018), meaning a higher removal performance should be recorded in summer

(December–February) than in winter (June–August). The influent *E. coli* cell density was consistently higher in summer ( $6.69 \cdot 10^6 \pm 4.50 \cdot 10^6$  MPN·100 mL<sup>-1</sup>,  $N = 40$ ) than in winter ( $2.47 \cdot 10^6 \pm 1.13 \cdot 10^6$  MPN·100 mL<sup>-1</sup>,  $N = 21$ , see SI-G for statistical analysis). Since the HRAPs were operated at a lower HRT in summer (approximately 9 d) than in winter (approximately 11 d), these differences caused the *E. coli* input in the HRAPs to be on average 3.6-fold higher in summer than in winter. The first-order decay rate (shown in Figure 2(c) for the whole data set and Figure 3 for monthly and overall whisker plots), however, allowed



**Figure 3** | Monthly and overall distribution of *E. coli* decay rate accounting for both HRAP. The data shown represent the 5, 25, 50, 75 and 95 percentiles (the numbers of observations are shown on top).

these differences in input loads to be discounted. One-way ANOVA analysis based on *E. coli* decay rates grouped by month showed that March and December were the only months when the average decay rate was significantly different from the rest of the year (see SI-H), despite the fact that solar radiation was highest in December and January, and temperatures were highest in January and February (see SI-C). When removing the March and December data from the analysis, one-way ANOVA did not reject the hypothesis of equal means between the groups ( $p = 0.109$ ), despite lower solar radiation during the winter months than the rest of the year. Regression analysis evidenced significant positive correlations between *E. coli* decay rate and both sunlight irradiance received within the 24 h before the sampling ( $R^2 = 0.137$ ,  $p = 2.19 \cdot 10^{-5}$ ) and the maxima hourly sunlight intensity recorded within the 24 h before the sampling ( $R^2 = 0.149$ ,  $p = 8.53 \cdot 10^{-6}$ ), but the associated  $R^2$  values were low ( $< 0.15$ ). Results from the individual linear regressions performed are shown in SI-I. Both ANOVA and regression analyses therefore suggest that sunlight-mediated decay mechanisms due to direct exposure to sunlight radiations (including direct DNA damage by UVB and/or indirect damage by ROS formed from endogenous photosensitizers) were not the main cause of *E. coli* decay in HRAPs on a long-term basis, despite some level of covariation. The lack of significance of the relationship between *E. coli* removal and sunlight irradiation may be explained by the combination of high algae concentration and good mixing conditions causing efficient ‘shielding’ to *E. coli* compared to MPs, which are typically not well mixed and where volatile suspended solids concentration is typically lower (Norvill et al. 2016).

Additional correlation analyses were conducted to identify possible decay mechanisms causing *E. coli* removal in HRAPs (see detailed results in SI-I). Significant ( $p < 0.05$ ) positive correlations with  $R^2 > 0.1$  were found between

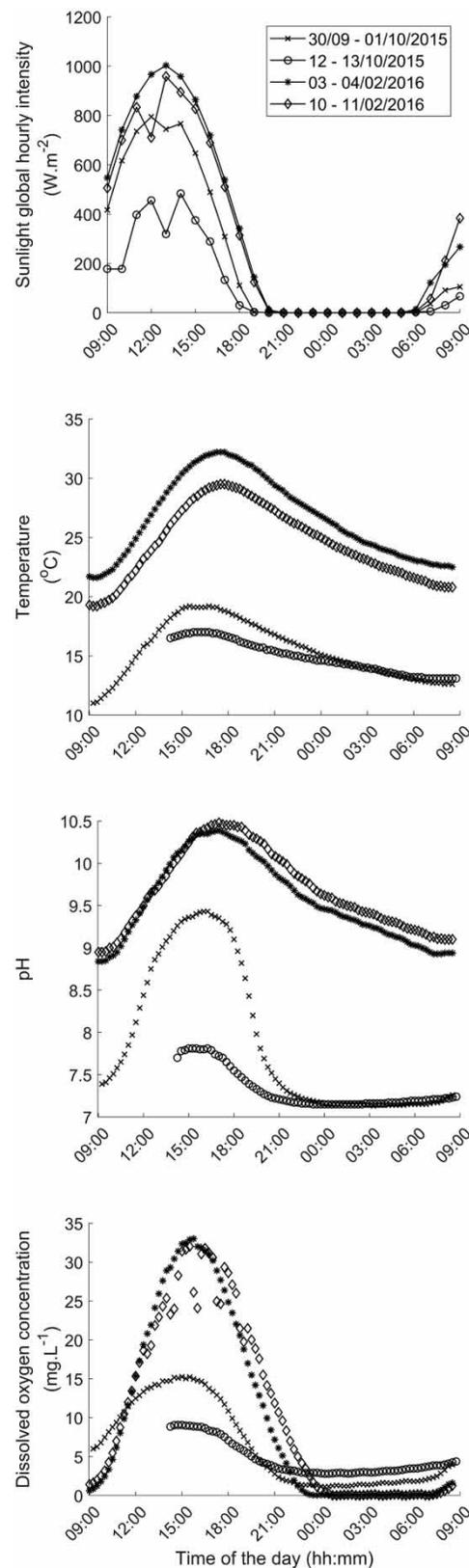
*E. coli* decay rate and TSS concentration in the HRAPs ( $R^2 = 0.170$ ,  $p = 1.94 \cdot 10^{-6}$ ), TSS productivity ( $R^2 = 0.109$ ,  $p = 1.82 \cdot 10^{-4}$ ), COD concentration in the wastewater ( $R^2 = 0.132$ ,  $p = 0.0035$ ), chloride concentration in the HRAPs ( $R^2 = 0.192$ ,  $p = 5.43 \cdot 10^{-7}$ ) and *E. coli* cell counts in the wastewater ( $R^2 = 0.124$ ,  $p = 5.38 \cdot 10^{-5}$ ). All calculated  $R^2$  values were, however, below 0.2, evidencing a lack of clear correlation that may be explained by differences in the time-scales variability of parameters tested and/or interacting effects between these parameters. No significant negative correlations were found.

As noted above, a significant drop in *E. coli* removal performance was recorded during the coldest months of the year. In particular, decay rates in the lower 5 percentile of the data set ( $< 0.83 \text{ d}^{-1}$ ,  $n = 7$ ) were all recorded between May and September. The drop of *E. coli* removal in May 2016 was associated with the exceptional rainfalls observed during the month (238 mm, of which 47.2 mm fell over 3 h on 5 May 2016). High rainfall during cold seasons therefore seems to reduce pathogen removal performance, although no clear mechanism could be identified from our data analysis to explain this impact. Conversely, looking at seven events of highest *E. coli* removal efficiencies representing the 95 percentile of the data sets, two outliers (decay rates of 189 and 191  $\text{d}^{-1}$  on 25 October 2016 and 13 April 2017, respectively) potentially explained by grazing (see discussion in SI-J) were excluded from Figure 2(c). With one exception, all other high decay rates were recorded during summer following sunny days (total sunlight energy received in the previous 24 h above  $22.3 \text{ MJ} \cdot \text{m}^{-2}$ ) exhibiting high pH (maxima recorded above 9.88), DO concentration (maxima recorded above  $15.7 \text{ mg} \cdot \text{L}^{-1}$ ) and relatively elevated broth temperature (maxima recorded above  $21.2^\circ \text{C}$ ). One last high decay rate was recorded on 15 March 2016, without clear explanation for this performance. Based on the yearly monitoring and the ‘peak events’ analysis, this study evidenced a relatively constant yearly performance with a higher occurrence of ‘low performance’ events during cold rainy days and a higher occurrence of ‘high performance’ events during sunny summer day. This pattern suggests the existence of a relatively weather-independent ‘background’ decay mechanism(s) supported at times by weather-dependent secondary mechanisms. The fact that poor *E. coli* removal performances were only found at times of extreme weather conditions (e.g. low temperature combined with high rainfall) also suggests HRAPs is particularly suited to places where good effluent quality is mainly needed in summer, as this is often the case when receiving aqueous bodies have recreational use.

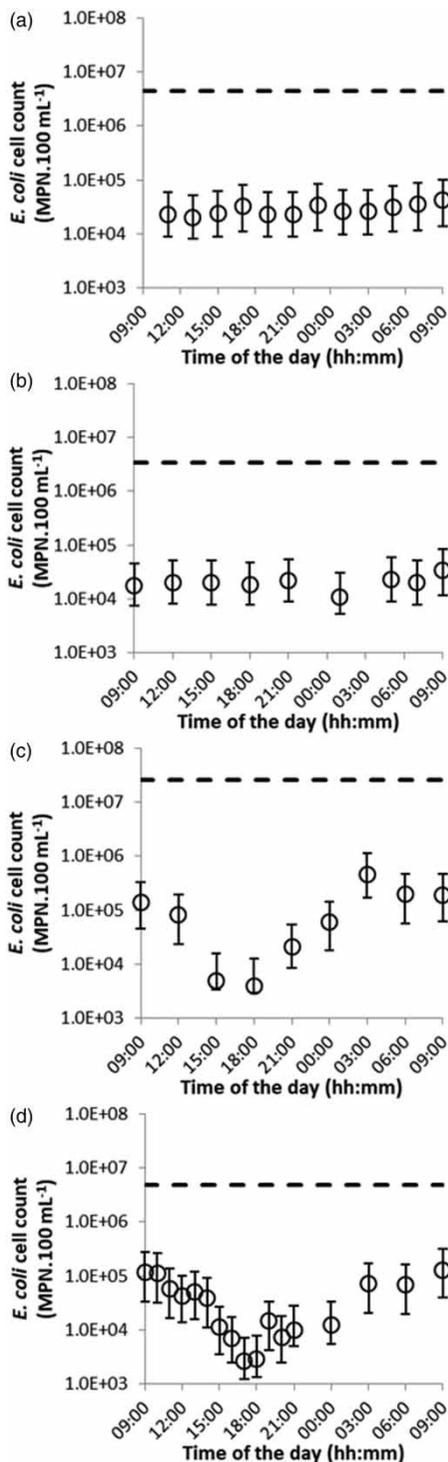
## Daily variations

As can be seen in Figure 4, the amount of sunlight radiation received since dawn was strongly correlated with the daytime changes in pH ( $R^2 = 0.342$ ,  $p = 1.47 \cdot 10^{-34}$ ), DO concentration ( $R^2 = 0.407$ ,  $p = 1.06 \cdot 10^{-42}$ ) and temperature ( $R^2 = 0.355$ ,  $p = 3.84 \cdot 10^{-56}$ ) recorded in the HRAPs. These correlations are expected because sunlight radiation powers heat accumulation and photosynthesis activity during daytime (radiative and conductive heat losses are slower than radiative heat gains, Béchet et al. 2011) and because photosynthesis subsequently drives increases in pH and DO (as the rates of  $O_2$  and  $CO_2$  mass transfer at the air–water interface are typically slower than the rate of production/consumption through photosynthesis at high sunlight intensities in HRAPs). Interestingly, pH, DO concentration and temperature peaked later than sunlight intensity, and this time lag is due to the differences in the rates of the various heat and mass transfer mechanisms involved. The corresponding changes in *E. coli* cell counts for the same days as shown in Figure 4 are shown in Figure 5, and two ‘typical’ profiles can be seen:

- (1) During the two summer days (3 February 2016 and 10 February 2016) characterized by high light intensities ( $>900 \text{ W}\cdot\text{m}^{-2}$ ), pH, DO concentration and temperature increased sharply to reach high values in late afternoon (pH  $> 10.3$ , DO  $> 32.0 \text{ mg}\cdot\text{L}^{-1}$ ,  $T > 27^\circ\text{C}$ ). On both days, *E. coli* cell counts dropped significantly during daytime, reaching minima late in the afternoon, when pH, DO concentration and temperature reached their peak values. The significant time lag between the sunlight intensity peaks (958 and  $914 \text{ W}\cdot\text{m}^{-2}$  were reached at 1 and 12 pm respectively) and the *E. coli* decay rate peaks (the peaks were reached around 6 pm) suggests that direct sunlight-mediated decay mechanisms did not cause the high daytime decay recorded. Instead, the similarities between the *E. coli* decay profiles and those of pH, DO concentration and temperature suggest that indirect sunlight-mediated mechanisms, and/or a dark mechanism, influenced by pH, DO concentration and/or temperature, significantly enhanced *E. coli* decay during daytime. *E. coli* cell counts rapidly increased during night-time to stabilize at levels corresponding to decay rates around 15 and  $8 \text{ d}^{-1}$ , respectively, which is likely to be representative of ‘dark’ mechanisms decay rates under the experimental conditions experienced.
- (2) During the two spring days (30 September 2015 and 12 October 2015), when milder environmental parameters variations were experienced, *E. coli* cell counts



**Figure 4** | Hourly variations of sunlight intensity, temperature, pH and DO concentration in HRAP-A. The data shown were obtained from single measurements.



**Figure 5** | Changes in *E. coli* cell counts recorded on (a) 30 September–1 October 2015, (b) 12–13 October 2015, (c) 3–4 February 2016 and (d) 10–11 February 2016. The data shown (o) represent single measurements and 95% confidence interval relative to the MPN calculation associated to Quanti-Tray measurements; dashed lines represent the *E. coli* cell count in the influent of the corresponding day (single measurement).

remained relatively constant. The lack of significant day-time variations suggests that, on these days, indirect sunlight-mediated mechanisms were not significant and that only ‘background’ dark mechanisms caused significant *E. coli* removal. Interestingly, the 30 September 2016 was a sunny day with a sunlight peak intensity around  $800 \text{ W}\cdot\text{m}^{-2}$  and was milder than during summer, yet had significant variations of pH (7.13–9.43), DO ( $1.12\text{--}15.27 \text{ mg}\cdot\text{L}^{-1}$ ) and temperature ( $11.0\text{--}19.2 \text{ }^\circ\text{C}$ ). To explain why no significant increase in *E. coli* removal was reported on 30 September 2015 (in contrast with 3 February 2016 and 10 February 2016, Figure 5), we hypothesize that indirect sunlight-mediated decay is significant only when pH, temperature, and/or DO concentration reach certain thresholds (perhaps due to synergistic effects). The literature suggests the existence of a pH threshold of 9–9.5, above which *E. coli* decay in the dark becomes significant (Parhad & Rao 1974; Maynard et al. 1999).

A linear regression analysis performed over the entire daily variation data set (shown in Figures 4 and 5) evidenced an absence of correlation between sunlight direct radiation and *E. coli* decay rate ( $R^2 = 0.0335$ ,  $p = 0.907$ ), but showed significant relationships between *E. coli* decay rate and temperature ( $R^2 = 0.320$ ,  $p = 6.35\cdot 10^{-5}$ ), DO concentration ( $R^2 = 0.324$ ,  $p = 5.46\cdot 10^{-5}$ ) and pH ( $R^2 = 0.186$ ,  $p = 3.46\cdot 10^{-3}$ ). *E. coli* decay rate was also significantly correlated to the cumulative sunlight energy received since dawn ( $R^2 = 0.197$ ,  $p = 2.54\cdot 10^{-3}$ ), but this correlation could be indirect as the cumulative sunlight energy was also correlated with pH, temperature and DO. Linear regression analysis therefore provides further evidence that direct sunlight disinfection was not a significant disinfection mechanism in this study, and that *E. coli* decay was primarily mediated by indirect sunlight mechanisms and dark mechanisms enhanced by high pH, high DO concentration and/or high temperature. Moreover, the stability of *E. coli* cell count during a sunny spring day (30 September 2015) suggests dark mechanisms sustain the bulk of HRAP disinfection performance.

Although consistent with the findings from the analyses of yearly monitoring and peak events, the conclusions from the hourly monitoring study must be considered cautiously. First, the influent *E. coli* cell count was only measured at 9 am at the end of the 24 h sampling, and so temporal variations in this parameter could explain the changes (or lack of) in removal performance herein recorded. A ‘daily profile’

performed on the wastewater influent, however, did not evidence any significant variation in *E. coli* cell count over 24 h (SI-K). Second, variations in the influent flow rate may also explain the daily changes in *E. coli* cell counts reported above. However, the flow rate was controlled at the start and end of each 24-h monitoring period and there is no reason to believe a drop in daytime flow explaining an apparent increase in removal performance would be repeatedly synchronized with pH, DO and temperature, and repeatedly revert at night-time. Third, differences in the duration of samples storage before counting could explain variability in the 24-h profile. However, this is most unlikely because the *E. coli* cell count remained stable during spring and ‘bounced’ back twice to their initial morning values during summer.

## CONCLUSIONS

Long-term (2 years) and short-term (24 h) monitoring of outdoor HRAPs treating primary domestic wastewater evidenced that *E. coli* removal was probably primarily driven by dark mechanisms. Sunlight irradiation may have enhanced daytime removal by driving increases in pH, DO concentration and temperature, but this indirect impact was only evidenced during sunny summer days under the conditions studied (temperate weather, relatively high HRT). This study therefore suggests that future research should focus on elucidating the dark mechanisms involved, as this knowledge may be key to optimizing pond design and operation for disinfection despite having been seldom investigated.

Due to the lack of clear relationship between any of the monitored processes or environmental parameters and *E. coli* removal performance, this study also suggests that the study of a comprehensive (e.g. outdoor, treating real wastewater) HRAP set-up, as performed for this study, will probably fail to evidence single significant disinfection mechanisms: this is probably because of the high variability of the conditions experienced by pathogens and/or because of interacting effects between broth parameters.

Regardless of the mechanisms involved, this study demonstrates HRAP can provide consistent and efficient wastewater disinfection under temperate climatic conditions.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this paper is available online at <https://dx.doi.org/10.2166/wst.2020.233>.

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