

Article



# Effects of Environmental Factors on the Disinfection Performance of a Wastewater Stabilization Pond Operated in a Temperate Climate

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**Abstract:** Treatment in a wastewater stabilization pond (WSP) relies on natural purification processes, which can be sensitive to both location and climate. This study investigated the effects of three environmental factors, pH, dissolved oxygen (DO) and temperature, on disinfection efficiency in a WSP system consisting of three facultative cells, and operated in a temperate climate region, in Eastern Ontario, Canada. Indicator organism (*Escherichia coli* (*E. coli*)) removal in WSP systems is driven by a combination of different factors. Elevated pH and DO concentrations, which are attributed to the presence of algae, are important factors for effective disinfection. Therefore, the presence of algae in natural wastewater treatment systems can contribute appreciably to disinfection. Consequently, based on algal concentrations, removal efficiencies of pathogenic microorganisms during wastewater treatment over the course of a year can be highly variable, where higher removal efficiencies would be expected in summer and fall seasons.

**Keywords:** disinfection; wastewater treatment; environmental factors; pH; dissolved oxygen; temperature; *E. coli* 

# 1. Introduction

Pressured by water scarcity and increases in water demand worldwide, treated wastewater has been reused for a variety of purposes over the last few decades, including agricultural irrigation and other industrial, environmental and municipal uses [1,2]. The potential for water reuse is dependent on effective pathogen removal. The disinfection process, generally the last step in wastewater treatment, aims to minimize the risk of pathogen exposure in receiving environments in order to protect public health [2]. However, the removal efficiency of pathogenic organisms can vary greatly and depends on the type of treatment process.

Wastewater stabilization ponds (WSPs) are often considered to be the most environmentally and economically sustainable technology for small, rural or remote communities that require low-cost and low-maintenance wastewater treatment systems [3]. WSPs have the capability to effectively attenuate organic and nutrient concentrations, as well as bacteria and pathogen levels, present in municipal wastewater [4,5]. The removal of a wide range of pathogenic organisms, such as bacterial, viral, protozoan and helminthic pathogens, is commonly achieved in WSP systems. Conversely, disinfection methods (UV irradiation, chlorination and ozone) applied in conventional treatments often only target the removal of pathogenic bacteria and viruses, as helminth eggs and protozoan (oo)cysts are resistant to these disinfection methods [6–8]. Some studies have suggested that WSP systems may remove up to 6 log units of bacteria and practically all protozoan and helminth eggs, producing final effluents that meet World Health Organization (WHO) guidelines for the use of treated wastewater in unrestricted

agricultural irrigation [3]. The reported performance of WSP systems is generally superior to that of conventional treatment processes, such as activated sludge or primary treatments, for which reductions of 1 to 2 log units for bacteria and 70%–99% for protozoan and helminth eggs have been noted [9].

Indicator organisms are often employed to reflect pathogen levels in a particular wastewater or its effluent, as it would be costly and time-consuming to monitor each pathogenic organism that could be present in the treated wastewater. It is expected that indicator organisms can be detected and quantified easily and cost-effectively. The presence, behavior, and population of indicator organisms and other pathogens are generally assumed to be correlated [10,11]. For the past few decades, bacterial indicator organisms, such as Escherichia coli (E. coli), fecal coliforms and total coliforms, have been commonly used for monitoring and regulating pathogen levels in treated wastewater. However, the limitations of bacterial indicator organisms have been recognized in recent publications. In some studies, E. coli populations were not found to be well correlated with pathogenic bacteria including Vibrio cholera (V. cholera) and Enterococcus faecalis (E. faecalis). Populations of V. cholera and E. faecalis increased with increasing pH and temperature, while E. coli populations decreased [12–14]. Burkhardt III et al. [15], and Len et al. [16], also challenged the reliability of E. coli as a pathogen indicator. Nascimento et al. [17], recommended using multiple organisms as indictors, because the removal kinetics of various pathogens differ. A number of municipalities, such as the one in this study, continue to employ E. coli as indicator organisms because the wastewater treatment plants are required to meet the effluent standards specified in their Certificate of Approval issued by the Ontario Ministry of the Environment, as well as the newer Canadian Wastewater Effluent Regulation Guidelines, which stipulate minimum E. coli concentrations.

## 2. Materials and Methods

#### 2.1. System Overview

In this study, water samples were collected from the effluents of three WSP cells at a Water Pollution Control Plant (WPCP) located in Eastern Ontario (Canada), with a rated average daily flow capacity of 5700 m<sup>3</sup>/day and rated peak capacity of 16,000 m<sup>3</sup>/day. The treatment system consists of primary treatment, biological treatment via an extended aeration activated sludge process, secondary clarification, followed by effluent polishing WSPs. The flow diagram of the WPCP is presented in Figure 1.



Figure 1. The configuration of the wastewater treatment plant [18].

The main wastewater treatment plant feeds into WSP Pond 1, flowed by Pond 2, and then Pond 3. The three facultative WSPs are operated in series, provide a total volume of 154,794 m<sup>3</sup> and primarily facilitate disinfection prior to discharge. Table 1 summarizes the area, volume, and depth of the WSPs based on field measurements. The hydraulic retention time of the WSP system is approximately 27 days, which is anticipated to be sufficient for disinfection of secondary effluent. The discharge limit for this plant is 200 CFU/100 mL and the design objective is 100 CFU/100 mL.

WSP	Surface Area (m <sup>2</sup> )	Volume (m <sup>3</sup> )	Depth (m)
1	48,600	78,246	1.61
2	28,200	40,044	1.42
3	31,200	36,504	1.17
Total	108,000	154,794	4.2

Table 1. Area, volume and depth of the wastewater stabilization ponds (WSPs) [19].

#### 2.2. Field Monitoring and Sampling

Eastern Ontario has a humid continental climate with four distinct seasons: winter, spring, summer and fall. Over the course of a year, the temperature typically varies from -15 to  $26 \degree C$  and is rarely below  $-24 \degree C$  or above  $31 \degree C$ . The relative humidity typically ranges from 40% (comfortable) to 94% (very humid), rarely dropping below 22% (dry) and reaching as high as 100% (very humid).

Monitoring was carried out from 10 May 2011 to 24 February 2015 and comprised of periods of cold climate (January–March), warm weather (April–June), the hottest season (July–September) and the return of cooler weather (October–December). pH, dissolved oxygen (DO) and temperature were measured at the effluent weirs of the three ponds between 10 am and 12 pm at weekly intervals. pH and temperature were recorded using a HQ40d (Hach, Loveland, CO), a portable pH, conductivity, DO, ORP and ISE multi-parameter meter, while DO was monitored using a Model 3100 portable dissolved oxygen analyzer (Insite IG, Slidell, LA, USA). At the same time, effluent samples were collected for *E. coli* analysis. The culture and enumeration of *E. coli* were carried out using the membrane filtration method according to Standard Methods for the Examination of Water and Wastewater [20].

#### 2.3. Statistical Analysis

Because the data could not be assumed to be normally distributed, the non-parametric Spearman  $\rho$  correlation coefficient was evaluated. MATLAB codes were developed to calculate the correlation coefficient. Spearman's rank correlation coefficient is a measure of statistical dependence between two variables. A Spearman correlation coefficient of 1 or -1 results when the two variables being compared are monotonically related. If one variable tends to increase when the other variable increases, the Spearman correlation coefficient is positive. Conversely, if one variable tends to decrease when the other variable increases, the Spearman correlation coefficient is become closer to being perfect monotone functions of each other. An absolute value of Spearman correlation coefficient larger than 0.5 suggests that the two variables are well correlated, a value between 0.25 and 0.5 indicates the two variables are moderately correlated, while a value below 0.1 suggests the two variables are not correlated.

### 3. Results and Discussion

The influent to Pond 1 typically contains over 2000 CFU/100 mL of *E. coli*. The four-year average effluent *E. coli* of Pond 1, Pond 2 and Pond 3 were 84, 26, 28 CFU/100 mL respectively. More than 98.6% of disinfection efficiency was achieved throughout this WSP system. Spikes in *E. coli* concentrations were observed in the effluent of the first pond, some of which exceeded the dischargeable microbiological level of 200 CFU/100 mL of *E. coli* (Figure 2). Figure 3 shows the average, median, minimum and maximum value of *E. coli* concentrations in the effluents of the three WSP cells

over the monitoring period. The maximum *E. coli* concentration observed in Pond 1 was more than seven times that of the discharge criteria. Most of the spikes were noted to occur during the colder seasons (November–March). The next two ponds (Pond 2 and Pond 3) provided further reduction in *E. coli* concentration to lower levels. *E. coli* concentrations above 200 CFU/100 mL were rarely observed, and at no time did the monthly geometric mean density of *E. coli* exceeded 200 CFU/100 mL.



Figure 2. E. coli concentrations in the effluents of Pond 1, Pond 2 and Pond 3 of the Amherstview WPCP.



**Figure 3.** Average, median, minimum and maximum value of *E. coli* concentrations in the WSP effluents over a four year monitoring period.

The treatment and disinfection of wastewater in WSPs mainly rely on natural purification processes. Hence, treatment and disinfection efficiency is expected to be influenced by environmental factors such as sunlight, temperature, pH and dissolved oxygen (DO). High pH levels (>9) and over-saturated DO concentrations often coincided with the occurrence of excessive algal growth. The pH in the WSP cells ranged from 6.55 to 10.89, with an average of 8.90 during the monitoring period (mid 2011 to beginning of 2015) as shown in Figure 4. The three WSP cells exhibited similar trends in annual pH fluctuations, where pH started to increase in January, reached a maximum in May, dropped down to below 8 in July, increased again to above 10 in September and then started to decrease in September as ambient temperatures decreased and the daylight hours shortened. The influent pH was

between 6.5 and 7.5 during monitoring period, which suggested that the pH fluctuation within WSPs were likely due to algal activity.



Figure 4. pH fluctuation in the effluents of Pond 1, Pond 2 and Pond 3.

Figure 5 displays the DO concentrations in the effluents of each of the three cells. DO levels fluctuated within a range of 0 to 25 mg/L. Elevated DO concentrations could be attributed to algal growth.



**Figure 5.** Changes in DO concentrations in the effluents of Pond 1, Pond 2 and Pond 3 over the four-year monitoring period.

Elevated pH and DO in WSPs resulting from algal growth is generally associated with the extensive consumption of dissolved  $CO_2$  by algae. Through photosynthesis, algae utilize dissolved inorganic carbon to produce organic matter, as shown in Equation (1). Oxygen is generated as a photosynthetic byproduct.

$$6CO_2 + 12H_2O \xrightarrow{light, pigment \ receptor} C_6H_{12}O_6 + 6H_2O + 6O_2 \tag{1}$$

When sufficient light is available and nutrients are not limiting, algae in WSPs remove  $CO_2$  from wastewater more rapidly than heterotrophic microorganisms can produce respiratory  $CO_2$ . The uptake of  $CO_2$  causes a shift in the equilibrium concentrations of dissolved  $CO_2$ , carbonic acid (H<sub>2</sub>CO<sub>3</sub>), bicarbonate ion (HCO<sub>3</sub><sup>-</sup>) and carbonate ion (CO<sub>3</sub><sup>2-</sup>), the equilibrium relationships can be described by Equations (2)–(4) [21,22].

$$H_2CO_3^* \leftrightarrow CO_2 + H_2O$$
 (2)

$$HCO_3^- + H_2O \leftrightarrow H_2CO_3^* + OH^-$$
(3)

$$CO_3^{2-} + H_2O \leftrightarrow HCO_3^- + OH^-$$
(4)

When  $CO_2$  is removed from the system, to maintain equilibrium, Equations (2)–(4) will shift to the right to produce more  $CO_2$ . As a result, hydroxide ions will be released, increasing the pH. Therefore, high pH and DO levels are often observed in WSPs containing algae. This effect is more pronounced during warmer weather and, particularly, during daylight hours [23].

Figure 6 displays the relationship between *E. coli* concentrations and pH in Pond 1. As the three WSP cells exhibited similar trends in pH fluctuation, Pond 1 data was selected to compare the relationship between *E. coli* concentrations and pH, DO and temperature. As can be seen, the spikes in *E. coli* concentrations tended to occur when the pH was below 8. As shown in Table 2, the statistical analysis indicated that *E. coli* concentrations were well correlated with pH values ( $\rho = -0.54$ ), where elevated pH coincided with the decreases in *E. coli* concentrations. Hence, these observations would suggest that pH levels higher than 8 could be considered effective in *E. coli* inactivation under these temperate climatic conditions.



Figure 6. The relationship between E. coli population and pH in the effluent of Pond 1.

**Table 2.** Spearman rank correlation coefficients between *E. coli* population and pH, DO and temperature.

	E. coli	
pН	-0.54	
DO	-0.11	
Temperature	-0.5	

The observed correlation between *E. coli* removal and pH is consistent with other studies, which reported increased *E. coli* inactivation with increases in pH [24,25]. A neutral to slightly acidic pH has been reported as optimal for fecal bacteria growth [26], while pH levels higher than 9 have been

reported to be effective in pathogen removal [9,26–28]. Both the terms "removal" and "inactivation" are intended to refer to the conditions where indicator organisms lose their ability to be cultured using Standard Methods.

A number of explanations for the effects of pH on disinfection have been proposed. A number of studies have reported that light inactivation of *E. coli* is dependent on pH [24,25,29]. Under elevated pH conditions (pH > 8.5), *E. coli* would be inactivated by exogenous mechanisms, while endogenous mechanisms would inactivate *E. coli* more slowly under more moderate pH conditions [24,29]. These mechanisms could explain why pH levels above 8 were found to be effective in *E. coli* inactivation. The effects of pH on *E. coli* have also been attributed to conformational changes in the membrane of the bacteria [30,31]. Bacterial inactivation can result from respiratory chain damage due to a physical breakdown in the membrane, which exposes nucleic acids to environmental stresses [32].

Figure 7 shows the relationship between *E. coli* concentrations and DO levels. Light inactivation of *E. coli* and enterococci increased with increasing levels of DO [25]. As previously noted, oxygen is produced as a by-product of algal photosynthesis, which is also a source of oxygen in WSP systems. Elevated DO concentrations, as high as 23 mg/L, were observed in Pond 1. Photo-oxidation, which is a disinfection mechanism, requires the presence of oxygen. Photo-oxidation is a process where endogenous or exogenous sensitizers absorb light and transfer this energy to other molecules leading to the formation of reactive oxygen species (ROS), which can react with microorganisms and cause damage. Therefore, an increase in DO concentration would be expected to increase the effect of photo-oxidation. However, DO concentrations were not correlated with *E. coli* concentrations as the spearman's coefficient is low ( $\rho = -0.11$ ). This might be because photo-oxidation induced disinfection is not only dependent on DO concentrations, but also impacted significantly by sunlight intensity.



Figure 7. The relationship between E. coli population and DO concentration in the effluent of Pond 1.

Figure 8 illustrates the relationship between *E. coli* concentration and temperature, while Figure 9 indicates the average, median, minimum and maximum values of *E. coli* concentration for the four different seasons. The statistical analysis indicated that *E. coli* concentrations were well correlated with temperature ( $\rho = -0.50$ ), where the negative correlation suggested that at lower temperatures, *E. coli* concentrations were higher than during the warmer seasons. Notably lower concentrations of *E. coli* were observed during the warm seasons. The *E. coli* concentrations during the warmer seasons (April–September) were found to be significantly different from the *E. coli* concentrations in the colder seasons (October–March) (p < 0.05). These findings suggest that temperature may play a role in the survival of *E. coli*. *E. coli* survived better in cold weather (T < 5 °C), possibly because algae growth

was inhibited due to reduced sunlight intensity, shorter daylight period and lower temperature [33]. Reduced algal activity resulted in a pH decrease to  $\leq 8$ , and a consequently lower disinfection efficiency in the studied WSP system. On the other hand, although optimal bacterial growth rates are typically restricted to small temperature ranges, bacterial organisms are generally able to survive within broader temperature ranges. In this four-year full-scale WSP study, the removal efficiencies of *E. coli* may increase with increasing temperature. This observation is consistent with a number of studies that have reported increased removal efficiencies of fecal coliforms with increasing temperature [25,34–38].



Figure 8. The relationship between E. coli population and temperature in the effluent of Pond 1.



**Figure 9.** Seasonal (Spring: April–June; Summer: July–September; Fall: October–December; Winter: January–March) *E. coli* concentrations in Pond 1 during the monitoring period.

It should be noted that effects of hydraulic retention [39,40], hydraulic efficiency [39,40], sunlight [3], attachment/sedimentation [9], predation and nutrient availability may all contribute to disinfection in WSPs. This study was focused on the environmental factors associated with algae growth, which is commonly present in facultative and maturation ponds due to their long hydraulic retention time.

## 4. Conclusions

Pathogen removal in WSP systems is driven by a combination of mechanisms and factors. Environmental factors, such as pH, DO and temperature, all play a role in the inactivation of indicator organisms. Elevated pH, due to the presence of algae, and temperature are considered important factors because they were statistically correlated well with *E. coli* concentrations. pH values higher than 8 in this study were noted to be effective in disinfection. Temperatures lower than 5 °C seemed to inhibit algal activity, but *E. coli* were able to remain active under the same temperature conditions. Inhibited algal activity may lead to a decrease in pH to below 8, which is not considered to be effective to achieve disinfection. DO concentration was not well correlated with *E. coli* removal, possibly due to the influence of other factors, such as sunlight penetration and intensity. Pathogen removal efficiencies can be highly variable in WSPs, and exhibit seasonal patterns, such as observations of lower *E. coli* concentration during the warm season. This would imply that WSPs performance is sensitive to location and climate, making wastewater treatment using natural systems more challenging. The last two WSP cells at the WPCP were noted to further reduce *E. coli* populations, suggesting that multiple WSP cells in series could provide a more reliable indicator organism (*E. coli*) removal performance than a single WSP system.

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