



Effect of turbidity on ultraviolet disinfection of domestic wastewater for agricultural reuse

ARTICLES doi:10.4136/ambi-agua.2766

Received: 05 Jul. 2021; Accepted: 10 Nov. 2021

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ABSTRACT

Water treatment and reuse are fundamental because of the increasing demand for freshwater, especially in agriculture. Accordingly, this study evaluated the effects of turbidity of wastewater processed at the Effluent Treatment Station (ETE) of the UFSCar/Araras and of UV dose on microbial inactivation. The ETE treats up to 2000 L of wastewater daily from toilets and a university restaurant and has five components (grease box, septic tank, microalgae tank, upflow anaerobic filter, and wetlands). Pretreated effluents were used in the experiments, and sampling sites consisted of inspection boxes located after the wetlands. Sample collection, inspection, preservation, and analyses were performed according to standard methods. Sample turbidity was adjusted to 5, 50, 100, 200, and 300 nephelometric turbidity units (NTU), and UV doses of 7.2–28.8 mWs cm⁻² were used. A 5 x 5 factorial design (five turbidity levels and five radiation doses) was used, totaling 25 treatments. Each treatment was performed in triplicate. The data were submitted to analysis of variance and Tukey's test. The results showed that the increase in turbidity significantly decreased disinfection efficiency in samples with turbidity levels higher than 50 NTU. The microbial inactivation coefficients obtained here can be extrapolated to disinfection of wastewater with turbidity up to 300 NTU to eliminate thermotolerant coliforms. The UV sterilizer is feasible for wastewater treatment and its reuse in agriculture.

Keywords: domestic effluent, sustainability, ultraviolet radiation, water reuse.

Efeito da turbidez na desinfecção ultravioleta de águas residuais domésticas para reutilização agrícola

RESUMO

O tratamento e o reúso da água são fundamentais devido à crescente demanda por água doce, principalmente na agricultura. Nesse sentido, este estudo teve como objetivo avaliar os



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efeitos da turbidez de águas residuárias tratada na Estação de Tratamento de Efluentes (ETE) da UFSCar/Araras e da dose de UV na inativação microbiana. A ETE trata até 2.000 litros de águas residuais diariamente de banheiros e um restaurante universitário e tem cinco componentes (caixa de graxa, fossa séptica, tanque de microalgas, filtro anaeróbio de fluxo ascendente e *wetlands*). Efluentes pré-tratados foram usados nos experimentos, e os locais de amostragem consistiram em caixas de inspeção localizadas após os *wetlands*. A coleta de amostras, inspeção, preservação e análises foram realizadas de acordo com métodos padrão. A turbidez da amostra foi ajustada para 5, 50, 100, 200 e 300 unidades de turbidez nefelométrica (NTU) e doses de UV de 7,2-28,8 mWs cm⁻² foram usadas. Foi utilizado um planejamento fatorial 5 x 5 (cinco níveis de turbidez e cinco doses de radiação), totalizando 25 tratamentos. Cada tratamento foi realizado em triplicata. Os dados foram submetidos à análise de variância e teste de Tukey. Os resultados mostraram que o aumento da turbidez diminuiu significativamente a eficiência da desinfecção em amostras com níveis de turbidez superiores a 50 NTU. Os coeficientes de inativação microbiana aqui obtidos podem ser extrapolados para a desinfecção de águas residuais com turbidez de até 300 NTU para eliminar coliformes termotolerantes. O esterilizador UV é viável para o tratamento de efluentes e sua reutilização na agricultura.

Palavras-chave: efluente doméstico, radiação ultravioleta, reúso de água, sustentabilidade.

1. INTRODUCTION

Increases in world population, urbanization, and water use for irrigation have led to irregular and disordered water supply, hence limiting water availability. This has promoted indirect reuse of wastewater without prior planning or treatment (Oliveira *et al.*, 2019). However, after proper treatments, the reuse of effluents is an alternative to meet water demands of the agricultural sector, in which large amounts of freshwater are used (Zewde *et al.*, 2019). Disinfection is considered a tertiary treatment, with emphasis on chlorination, ozonation, reverse osmosis, membrane filtration, and ultraviolet (UV) radiation (Collivignarelli *et al.*, 2018). However, these treatments are expensive and increase the toxicity of effluents (Chai *et al.*, 2018).

The purpose of UV sterilization is eliminating or inactivating microorganisms such as bacteria and viruses (Chhetri *et al.*, 2018). UV radiation has been increasingly used in wastewater treatment in the last 25 years because of little contact time and high antimicrobial activity (Masschelein *et al.*, 1989; Guo *et al.*, 2009; Zewde *et al.*, 2019).

UV disinfection of water and wastewater normally uses low-pressure mercury lamps at the 254 nm wavelength (Zewde *et al.*, 2019). Short UV radiation (100 - 280 nm) causes changes in DNA and RNA molecules, which absorb radiation at wavelengths between 200 and 300 nm, especially at about 260 nm. This radiation range alters the structure and function of nucleic acids, inhibiting DNA replication and inactivating microbial cells (Gibson *et al.*, 2017). More recently, Silva *et al.* (2020) evaluated the inactivation of *Escherichia coli* in effluent from an urban wastewater treatment plant subjected to UV-LED (Ultraviolet-Light Emitting Diodes) radiation, emitting UVA (365 and 405 nm) or UVC (255 and 280 nm). The authors indicated that 280 nm UV-LED appears to be crucial for the success of the disinfection process, with a 4-log reduction being obtained at this wavelength.

The advantages of UV radiation include absence of dangerous co-products or chemicals that could change water composition, in addition to short contact time and inactivation of viruses (Guo *et al.*, 2012; Gibson *et al.*, 2017; Bolyard *et al.*, 2019). After UV radiation treatment, wastewater could be used in agriculture if legal requirements are met and its use does not alter microbial ecosystems in soils (Chevremont *et al.*, 2013).

The degree of microbial cell inactivation is directly proportional to the UV dose applied. The dose is the amount of UV light emitted by the lamp and is calculated as the radiation intensity multiplied by the duration of exposure, as well as the dose of chemical disinfectants (Gibson *et al.*, 2017; Zewde *et al.*, 2019). Therefore, the use of adequate doses during disinfection is essential for the complete inhibition of microbial growth.

UV radiation intensity decreases when penetrating materials due to its absorption (Artichowicz *et al.*, 2020). Therefore, physicochemical properties of wastewater may affect UV light transmittance, and hence treatment efficiency. In this sense, the efficiency of UV radiation can be affected by water turbidity, suspended solid concentrations, microorganisms, cellular aggregates, leachate, and fluid density, thus requiring higher doses for disinfection process (Hassen *et al.*, 2000; Brahmi *et al.*, 2010; Uslu *et al.*, 2015; Azimi *et al.*, 2017; Bolyard *et al.*, 2019). Of these, turbidity, which is determined by the presence of suspended particles that reflect or absorb radiation, strongly affect UV transmittance and hence disinfection efficiency (Nourmoradi *et al.*, 2012); in addition, colloidal particles tend to favor microbial activity (Burch and Thomas, 1998).

The Center for Agricultural Sciences of the Federal University of São Carlos (CCA-UFSCar) manages a Pilot Effluent Treatment Station (ETE), in which a UV sterilizer was used to remove microbial loads; however, the equipment has not performed steadily due to variable wastewater turbidity (Oliveira *et al.*, 2019). Thus, in this study, we assessed at a bench scale the effect of wastewater turbidity and radiation dose on microbial inactivation using a UV sterilizer for effluent treatment.

2. MATERIAL AND METHODS

2.1. Study site

The study was conducted at the ETE of CCA-UFSCar, located in Araras, São Paulo State, Brazil. The ETE treats up to 2000 liters of wastewater daily from toilets and a university restaurant and has five components (grease box, septic tank, microalgae tank, upflow anaerobic filter, and wetlands) (Figure 1).

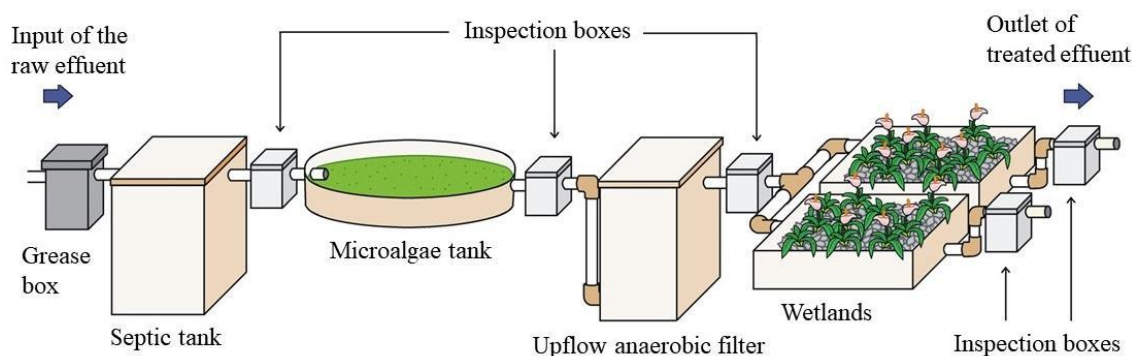


Figure 1. Schematic diagram of the effluent treatment station. (Source: Souza *et al.*, 2015).

2.2. Wastewater

Pretreated effluents were used in the experiments, and sampling sites consisted of inspection boxes located after the wetlands (Figure 1). The effluents were characterized weekly from May to June (Table 1). Sample collection, inspection, preservation, and analyses were performed according to standard methods (APHA *et al.*, 2012).

Turbidity was adjusted to 5, 50, 100, 200, and 300 nephelometric turbidity units (NTU). Average turbidity was 20 NTU and was normalized to 5 NTU by diluting the samples in water. To increase turbidity, a sample from the microalgae tank was centrifuged at 3000 rpm for 5 min

and resuspended to obtain a suspension with turbidity higher than 1000 NTU. Aliquots were added to diluted samples until the desired turbidity was achieved.

Table 1. Physicochemical and microbiological characteristics of wastewater.

Variable	Values
pH	7.50 ± 0.16
Turbidity (NTU)	20.00 ± 12.16
EC (µS cm ⁻¹)	1030.00 ± 47.36
DO (mg L ⁻¹)	3.40 ± 0.90
TOC (mg L ⁻¹)	26.00 ± 10.59
TN (mg L ⁻¹)	46.00 ± 6.88
TP (mg L ⁻¹)	19.00 ± 6.13
TC (× 10 ⁶ MPN 100 mL ⁻¹)	5.39
<i>E. coli</i> (× 10 ⁵ MPN 100 mL ⁻¹)	3.53

EC: electrical conductivity; DO: dissolved oxygen; TOC: total organic carbon; TN: total nitrogen; TP: total phosphorus; TC: total coliforms.

2.3. Experimental procedure

Figure 2 shows a schematic of the UV reactor used in the experiments. This reactor was built using a PVC tube and contained a UV germicidal lamp (Philips TUV 75W HO G75 T8 UV 254nm) protected by a quartz cylinder. The average radiation intensity was 0.24 mW cm⁻². The water depth inside the reactor was 1 cm.

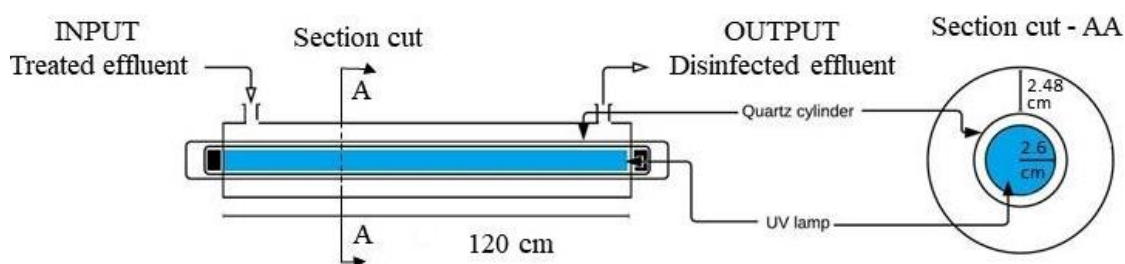


Figure 2. Diagram of the ultraviolet sterilizer.

Sterilization tests were carried out in batches by adding 1500 mL of a sample with known turbidity. The lamp remained on for 120 s. Samples were collected at 0, 30, 60, 90, and 120 s, and heterotrophic bacteria were counted. At each time point, the radiation dose was calculated by $D = I \times t$, where D is the radiation dose (mWs cm⁻²), I is the radiation intensity (mW cm⁻²), and t is the duration of exposure (s) (Zewde *et al.*, 2019). The doses used were 0.0, 7.2, 14.4, 21.6, and 28.8 mWs cm⁻².

The collected samples were serially diluted, and 1 mL aliquots were transferred to 3M Petrifilm Aqua plates and incubated at $36 \pm 2^\circ\text{C}$ for 44 ± 4 hours. Bacterial counts were expressed as colony-forming units (CFU) per mL.

A 5×5 factorial design (five turbidity levels and five radiation doses) was used, totaling 25 treatments. Each treatment was performed in triplicate. The data were submitted to analysis of variance and Tukey's test. The disinfection model was evaluated by the relationship between the logarithmic change in the number of inactivated bacterial cells and the applied dose (Zhou *et al.*, 2016) and followed first-order Chick-Watson kinetics according to Equation 1 (Hijnen *et al.*, 2006):

$$\log\left(\frac{N_0}{N}\right) = k * D \quad (1)$$

N_0 - pre-treatment bacterial count, CFU mL⁻¹;

N - post-treatment bacterial count, CFU mL⁻¹;

k - inactivation coefficient, cm² mWs⁻¹;

D - radiation dose, mWs cm⁻².

3. RESULTS AND DISCUSSION

The effect of turbidity was evaluated as a function of transmittance measures of samples. Transmittance is the amount of radiation passing through a sample relative to the emitted radiation. The lower the transmittance, the greater the amount of particles impairing disinfection (Nguyen *et al.*, 2019). Transmittance decreased as turbidity increased (Figure 3).

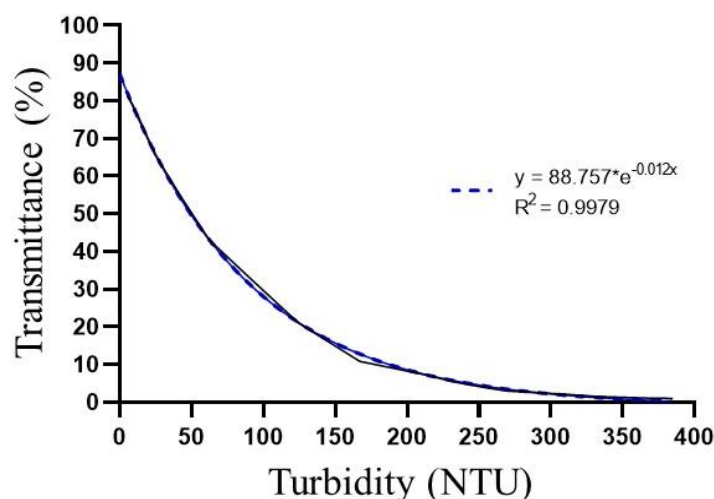


Figure 3. Transmittance profile as a function of turbidity.

The samples with a turbidity of 5, 50, 100, 200, and 300 NTU showed an approximate transmittance of 84%, 49%, 27%, 8%, and 2%, respectively. Figure 2 shows that UV transmittance decreased exponentially with turbidity; therefore, transmittance differences between samples with 5 and 50 NTU were greater than those of samples with 200 and 300 NTU. Similar results were obtained by Nguyen *et al.* (2019), wherein treatments that reduced effluent turbidity increased UV transmittance.

The dose of radiation absorbed by microorganisms is hard to be measured and varies with emitted light and period of exposure (Gayan *et al.*, 2011). Given that decimal reduction times are longer (lower microbial inactivation) for heterotrophic bacteria in treated effluents, it is possible to estimate the duration of exposure for samples with turbidity levels of up to 300 NTU. Faily (1994) recommended a minimum dose of 30 mWs cm⁻² for wastewater, although the dose depends on the type of installation and the physicochemical characteristics of the effluent (Hassen *et al.*, 2000).

Turbidity may increase or transmittance decrease with the addition of several compounds (Bolyard *et al.*, 2019; Azimi *et al.*, 2012; Carré *et al.*, 2018). Depending on the material used, the efficiency of UV disinfection in inactivating microorganisms varies (Farrell *et al.*, 2018). For instance, ultrasound treatment did not decrease effluent turbidity but increased the efficiency of UV disinfection (Zhou *et al.*, 2016).

Microbial aggregates are formed during biological or secondary treatments through

incorporation or adsorption of microorganisms to suspended particles. These aggregates help protect microorganisms against UV radiation and decrease disinfection efficiency (Azimi *et al.*, 2017). Therefore, the use of a microalgae suspension to increase turbidity may have decreased the efficiency of disinfection by decreasing transmittance and promoting the formation of bacterial aggregates.

To evaluate the effect of turbidity and radiation dose on disinfection, doses of 0, 7.2, 14.4, 21.6, and 28.8 mWs cm⁻² were applied for 30, 60, 90, and 120 s, respectively, for effluents with a turbidity of 5, 50, 100, 200, and 300 NTU. The results showed that the samples with a turbidity of 5 and 50 NTU presented the highest levels of disinfection. Inactivation was higher than 99.97% for all turbidity levels at a dose of 28.8 mWs cm⁻². These results agree with those of USEPA (2006), which indicated that the dose required for killing 99.99% of coliforms in wastewater was at least 20 mWs cm⁻².

The analysis of variance showed significant differences due to interaction between factors ($p < 0.05$). Therefore, the efficiency of disinfection was analyzed according to turbidity levels and radiation doses (Table 2). All turbidity levels tested showed significant disinfection efficiency differences among radiation doses. The analysis also proved that 95% of the variation was caused by radiation doses. Thus, dose variations had a greater impact on bacterial inactivation than did turbidity changes, which agrees with a previous study (Farrell *et al.*, 2018).

Table 2. Log-cycle reduction for the inactivation of heterotrophic bacteria as a function of the duration of exposure, radiation dose, and sample turbidity.

Duration of exposure (s)	Radiation dose (mWs cm ⁻²)	Turbidity (NTU)				
		5	50	100	200	300
Log-cycle reduction						
0 s	0	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Aa
30 s	7.2	1.56 Ba	1.40 Bab	1.18 Bbc	0.99 Bcd	0.84 Bd
60 s	14.4	2.62 Ca	2.64 Ca	2.26 Cb	1.96 Cc	1.88 Cc
90 s	21.6	3.59 Da	3.74 Da	3.29 Db	2.97 Dc	2.81 Dc
120 s	28.8	5.11 Ea	4.88 Ea	4.59 Eb	4.32 Ec	3.58 Ed

Means with the same uppercase letters in each column and the same lowercase letters in each line were not significantly different from each other by the Tukey's test at a significance level of 5%.

At each radiation dose, there were no significant differences in bacterial elimination between samples with 5 and 50 NTU. Besides, at the exposure times of 30, 60, and 90 s, there were no significant differences in bacterial elimination between samples with 200 and 300 NTU. However, above 50 NTU, higher turbidity levels decreased microbial inactivation significantly. Thus, despite applying the same doses, turbidity had a negative effect on UV disinfection, and as dose increases, such differences become greater.

The inactivation of heterotrophic microorganisms using a contact reactor was greater than that obtained by Chhetri *et al.* (2018), who used similar doses in a reactor without direct contact, indicating that the type of reactor strongly affected disinfection efficiency. Sanctis *et al.* (2016) found that total coliforms in domestic wastewater decreased by 2.8 logs using a dose of 40 mWs cm⁻². In our study, heterotrophic bacteria counts were reduced by 3.0 logs at a dose of 20 mWs cm⁻² and by 5 logs at a dose of 28.8 mWs cm⁻² in samples with a turbidity of 5 NTU. Nguyen *et al.* (2019) reported that the total count of *E. coli* was reduced by 5 logs in domestic wastewater pretreated with a UV dose of 69.4 mWs cm⁻² in samples with an initial turbidity of 4 NTU.

Our results were similar to those obtained by Guo *et al.* (2011), in which counts of *E. coli*

and fecal coliforms were reduced by 5 logs, and total counts of *B. subtilis* were reduced by 4 logs using 40 mWs cm⁻². However, other studies found that killing efficiency was higher using UV doses lower than 40 mWs cm⁻² (Lazarova *et al.*, 1999; Beck *et al.*, 2016; Nyangaresi *et al.* (2018). Silva *et al.* (2020) obtained similar results with much lower irradiance intensity using 280 nm UV-LED (0.019 mW cm⁻²) with a 4 log reduction of 15 min *E. coli*. This wavelength range from 280 to 200 nm, called UVC and used in our experiments, appears to cause more effective and irreversible damage to DNA. Therefore, it is also important to consider this aspect combined with the physicochemical characteristics of the effluent and irradiance intensity.

Previous studies demonstrated that increases in turbidity have a negative effect on UV disinfection effectiveness. Nourmoradi *et al.* (2012) used a UV reactor like that used in our study and reported that an increase in turbidity from 1 to 5 NTU reduced inactivation effectiveness by 0.2-0.5 log. Wu and Doan (2005) also reported a decrease in inactivation efficiency as turbidity increased from 1 to 5 NTU, and Gullian *et al.* (2012) observed that microbial inactivation decreased as turbidity was raised from 9 to 28 NTU. Likewise, Zhou *et al.* (2016) reported that increases in turbidity and UV absorbance reduce efficiency of disinfection.

In the above studies, small changes in turbidity had great impacts on disinfection efficiency. However, in ours, only major changes impacted the treatment, even though we expected greater differences after analyzing Figure 3. This discrepancy might have been due to the composition of microalgae used to increase sample turbidity. The phosphate concentration in microalgae is high, and these microorganisms absorb phosphorus from the medium during wastewater treatment (Sajjadi *et al.*, 2018).

Azimi *et al.* (2014a) reported that flocc-forming during biological treatment with polyphosphates would enhance UV-radiation disinfection by acting as photoactive agents and producing oxidative hydroxyl radicals. In addition, effluents with low phosphorus concentrations are more resistant to UV disinfection than effluents with higher phosphorus levels (Azimi *et al.*, 2014b). Thus, the presence of microalgae biomass may have benefited disinfection since, despite the increase in turbidity and reduction in transmittance, its efficiency has not dropped dramatically.

The k value indicates the sensitivity of microorganisms to UV radiation (Hijnen *et al.*, 2006) and was calculated using Equation 1 (Figure 4). The k values (slopes) varied from 0.1266 to 0.1759 cm² (mWs)⁻¹.

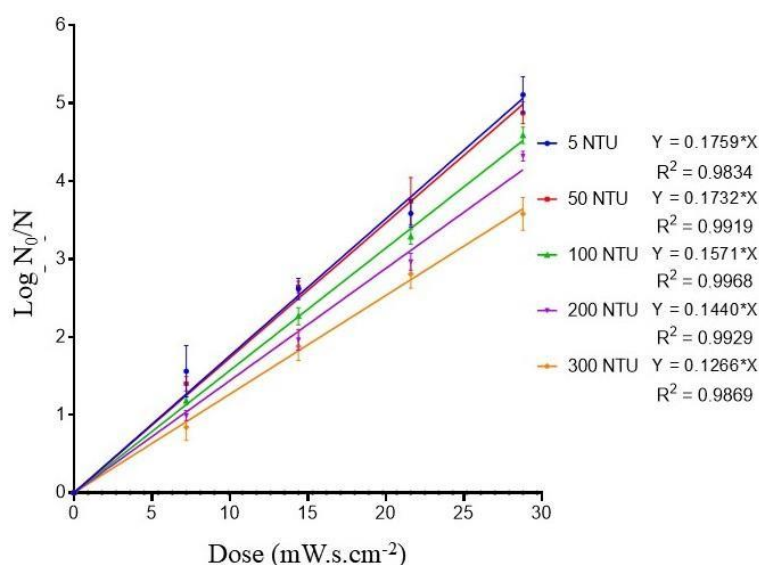


Figure 4. Microbial inactivation profile ($\text{Log } N_0/N$) for each turbidity level as a function of UV dose.

The k values vary depending on the microorganism, UV radiation system, and the type and physicochemical characteristics of wastewater. The obtained K values agree with the literature. Zhou *et al.* (2016) reported that k values for the UV inactivation of *E. coli* in municipal wastewater varied between 0.1027 and 0.1887 cm² mWs⁻¹, and Oguma *et al.* (2016) found that the k value for the inactivation of *E. coli* in synthetic media was 0.157 cm² mJ⁻¹. In contrast, Beck *et al.* (2016) reported that the k value for killing *E. coli* using a low-pressure UV lamp was 0.31 cm² mWs⁻¹.

Torres-Palma *et al.* (2017) observed that pretreatment increased the efficiency of UV disinfection, and the k value for inactivating fecal coliforms in secondary effluents pretreated with hexanol and ultrasound was 0.3 cm² mWs⁻¹.

The results indicate that the k values estimated here can be extrapolated to disinfection of effluents at the ETE of CCA/UFSCar, with turbidity values below 300 NTU. This is because thermotolerant coliforms are classified as heterotrophic bacteria.

According to CONAMA Resolution 357/05 (CONAMA, 2005), the maximum concentration of thermotolerant coliforms estimated by the technique of the most probable number (MPN) of CFU per 100 mL in reuse water should be 100 (Classes 1 and 2) and 2000 (Class 3). The MPN of *E. coli* in the effluents was 3.53×10^5 (Table 1).

A disinfection efficiency of 99.999% (5 log reduction) would be necessary to obtain a bacterial count of fewer than 2000 coliforms. The time required to achieve this goal can be calculated from the k value at a given turbidity, with the number of log cycles required being obtained by the product k versus dose of UV radiation (D).

4. CONCLUSION

Our results indicated that turbidity in wastewater samples influence bacterial inactivation by ultraviolet light. Inactivation coefficients between 0.1266 and 0.1759 cm² (mWs⁻¹) reduce heterotrophic bacteria counts by more than 99.999%, i.e., more than 5 log cycles. This efficiency could be improved by increasing the time of exposure to ultraviolet.

5. ACKNOWLEDGEMENTS

The authors are grateful to CAPES, FAPESP - 2013/14893-7 and CNPq.

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