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Research article

Deactivating environmental strains of *Escherichia coli*, *Enterococcus faecalis* and *Clostridium perfringens* from a real wastewater effluent using UV-LEDs

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HIGHLIGHTS

- UV LEDs were used to treat a real wastewater effluent containing real bacteria.
- 268 nm was the most effective wavelength for disinfection.
- Higher UV doses were needed to inactivate *C. perfringens* completely.

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ABSTRACT

Environmental bacteria strains are known to be more resistant but studies on UV-LEDs are scarce, especially for *Clostridium perfringens* and *Enterococcus faecalis*. UV-LEDs of different wavelengths (268 nm, 279 nm and 307 nm) have been used for treating real wastewater from the effluent of the municipal plant in Linares (Spain), with real organic matter content, for *E. coli*, *Enterococcus faecalis* and *Clostridium perfringens* disinfection.

Experimental results demonstrate that 268 nm was the most effective wavelength for inactivation of the three different bacteria strains: *E. coli* showed an inactivation rate of 0.561 at 268 nm vs. 0.245 at 279 nm and 0.0029 for 307 nm; *E. faecalis* inactivation rate was 0.313 at 268 nm, 0.231 at 279 nm and 0.0023 at 307 nm; and *C. perfringens* inactivation rate was 0.084 at 268 nm, 0.033 at 279 nm and 6.9e-4 at 307 nm. In general, 307 nm wavelength showed a significantly lower inactivation rate so it would not be recommended for practical applications. *C. Perfringens* required higher UV doses and longer times to achieve complete inactivation.

1. Introduction

UV-LEDs for water disinfection is a promising technology due to several characteristics [1]: possibility of wavelength selection, non-toxicity (vs. UV lamps containing mercury), instantaneous switch-on-off (no need of warming-up times, possibility of use of pulsed illumination, therefore saving energy), low operating working temperatures (25–30 °C), flexible architecture to design reactors, use of continuous current that allows direct connection with photovoltaic systems, longer lifetime when pulsed illumination is used, etc. [2, 3]. But they also face some important constraints that hamper their widespread: economic cost, low efficiency and low power output [4]. These latter characteristics are still in development and will be eventually overcome [5, 6, 7, 8]. In the meantime, the technology is already capable of disinfecting water, but there are various aspects that require more studies.

In terms of disinfection, there are already scientific studies that show that the ability of UV LEDs to select the wavelength and match the peak action spectra of the microorganism can lead to greater inactivation rates than conventional low-pressure (LP) mercury lamps. For example, Rattanakul and Oguma in 2018 demonstrated that 265 nm UV-LEDs achieved higher inactivation rates that LP lamps for *P. aeruginosa*, *Legionella pneumophila* and other surrogates [9]. Li et al. in 2017 also showed that 265 nm was more effective to inactivate *E. coli* than LP lamps and that no reactivation occurred [10].

In this regard, studies show how matching the UV-LED peak with the spectral peak of the action spectrum of each microorganism (or spectral sensitivity of the microorganism) leads to a more effective disinfection. The response of each microorganism to the different wavelengths is unique and is given by its unique composition of proteins and acids nucleic [11]. For the case of bacteria, DNA damage tends to dominate the

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Table 1. UV-LEDs characteristics from manufacturers datasheet.

Peak wavelength emission (nm)	Current (mA)	Voltage (V)	Optical Power (mW) Radiant Flux (from datasheet)	Operating temperature (°C)	Angle of emission (°)	Size (mm × mm)	Manufacturer
265	20–40	6.5	2.5	−10 to +50	120	3.5 × 3.5	QT-Brightek
275	20–30	6	1.6	60	125	3 × 3	Seoul Viosys
310	20–30	6.2	1.2	–	120	3.5 × 3.5	Seoul Viosys

inactivation, and the nucleic acid has its peak at 265 nm. When studying viruses, protein damage is more important, being the peak absorbance around the wavelength of 280 nm [12]. For example, *E. coli* has its response peak at 266 nm, and it has been shown that with UV LEDs of 255–285 nm, inactivation has been obtained with doses of 0.15–0.81 mJ/cm^2 [11,13,14]. Other microorganisms studied have been *P. aeruginosa* (whose peak is at 258 nm), inactivating the microorganism with doses of 0.51–0.74 mJ/cm^2.

More recently, Jing et al. in 2022 have demonstrated that for 265 nm UV LEDs, the inactivation efficiency of chlorine-resistant bacteria (CRB) is higher than for LP lamps and MP lamps, providing empirical evidence for the reasonable application of UV disinfection technology in the treatment of water and wastewater and their conduits and delivery systems [15].

Regarding viruses, Kojima et al. in 2020 also showed that 260 nm UV-LEDs were more effective to inactivate influenza A viruses than LP lamps [16]. Oguma, Rattanakul and Bolton also showed that 285 nm UV-LEDs have higher performance than LP UV lamps for adenoviruses and therefore 285 nm UV-LEDs could be a good option to inactivate adenoviruses in water [17].

Regarding the aspects that require further in-depth studies, thermal management of UV-LEDs is crucial as they need to be refrigerated adequately so the power output does not drop drastically [18] and so the illumination power does [19, 20, 21, 22]. According to the UV-LEDs datasheet, they need to operate below 30 °C, so their cooling system is a key part of the entire disinfection reactor, as it is a very low operating temperature.

Another aspect that needs to be covered is the studies with water containing organic matter, as most scientific studies work with water that do not contain any organic matter [23]. Environmental strains may change the response to UV-LED light [24, 25, 26]. On the other hand, there are highly-resistant pathogens that need further studies, such as *Clostidrium perfringens* whose spores are particularly difficult to eliminate [27, 28]. Only with data from real water sources the technology can advance to practical applications in water treatment, including both drinking water and wastewater.

Most studies have analysed several wavelengths to evaluate the effectiveness in inactivation of pathogens in water [13, 23, 29, 30, 31, 32]. They usually include 255 nm, 265 nm, and 275 nm, as they match the action spectra peaks of most bacteria, which are in the range of 260 nm–270 nm [33]. For example, *E. coli* peak is at 266 nm and *P. aeruginosa* is at 258 nm. 310 nm is also used widely in the literature although inactivation rates are up to 6 times lower than for 260–280 nm. Various studies also include combinations with visible LEDs, for example a common one is 365 nm (UVA) with 405 nm (visible), but the performance is insufficient to produce effective inactivation. In general, they work with *E. coli* and some also include *E. faecalis*. 265 nm is the reported wavelength with a relative higher inactivation than others [34].

The objective of this study is to test the efficacy of UV-LEDs of different wavelengths (nominal peaks of 265 nm, 275 nm and 310 nm) for treating real wastewater from the effluent of a wastewater treatment plant in Linares (Spain). As a real effluent, it contains environmental bacteria strains in water with organic matter content that makes inactivation and disinfection more difficult. The three microorganisms analysed are *E. coli*, *Enterococcus faecalis* and *Clostridium perfringens* disinfection.

The reclaimed water standard in Spain allows the reuse of the water for various uses (Royal Decree 1620/2007 [35]) based on several parameters, including the *E. coli* microbial population. Depending on its value (<10.000 CFU/100 mL, <1000 CFU/100 mL, etc.), the water could be reused for agricultural use (different water qualities and applications), industrial use (process and cleaning waters), recreational use (ponds, bodies of water and ornamental circulating flows, in which public access to water is prevented) or environmental use (recharge of aquifers, irrigation of forests, green areas and of another type not accessible to the public).

On the other hand, the EU regulation (Regulation (EU) 2020/741 [36]) only includes agricultural uses: 1) food crops that are consumed

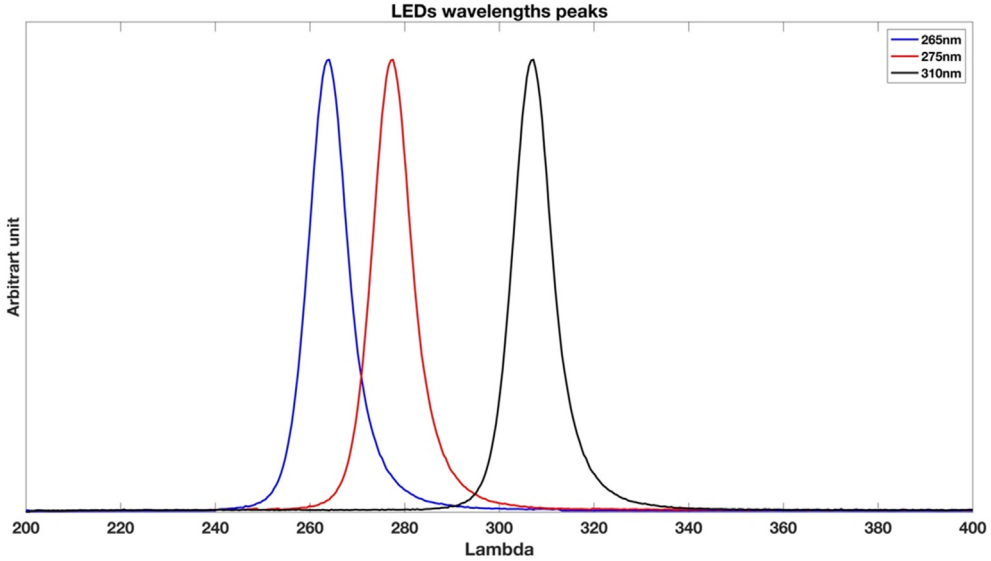
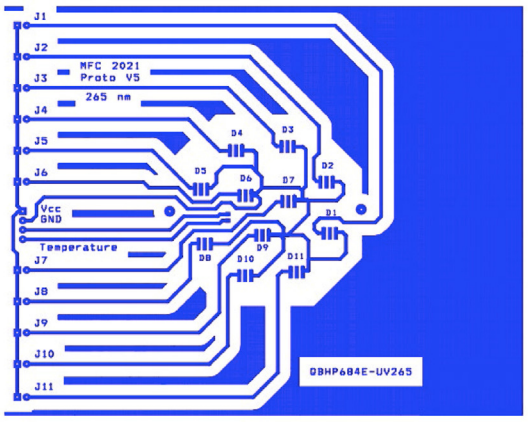
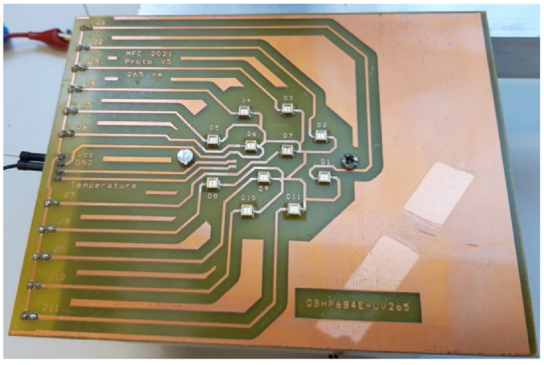


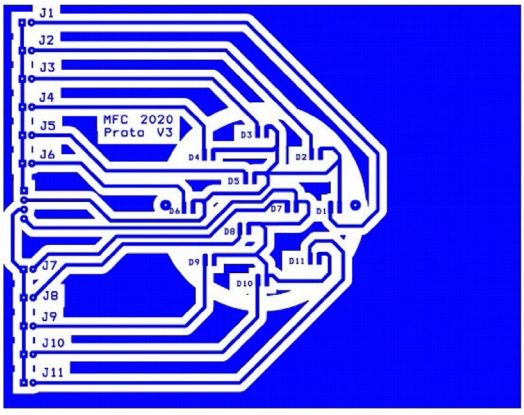
Figure 1. UV-LEDs wavelengths measurement (265 nm, 275 nm and 310 nm).



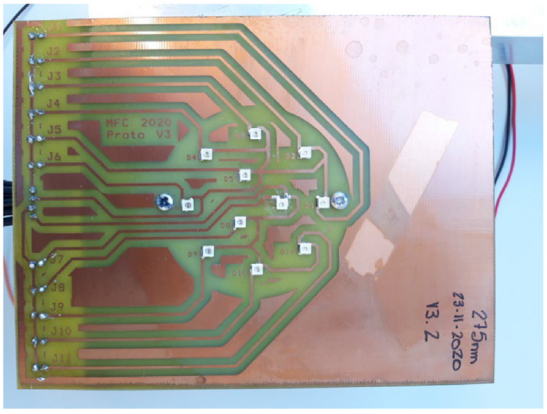
268 nm PCB layout



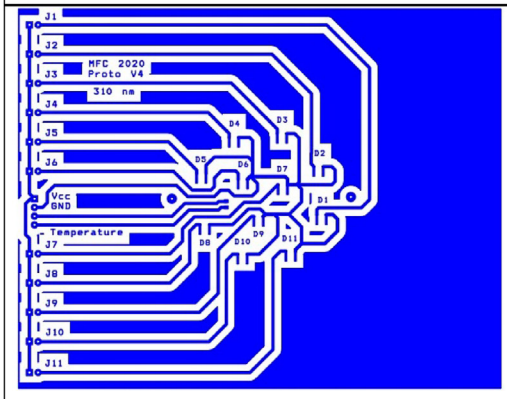
268 nm real manufactured PCB including the UV-LEDs



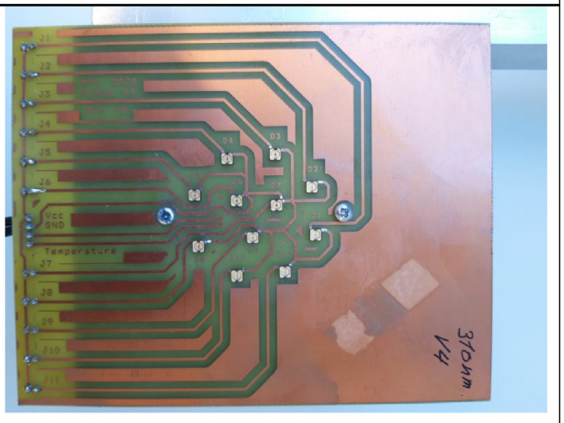
279 nm PCB layout



279 nm real manufactured PCB including the UV-LEDs

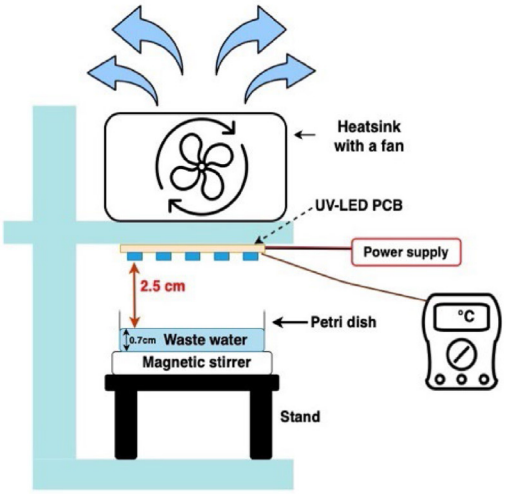


307 nm PCB Layout

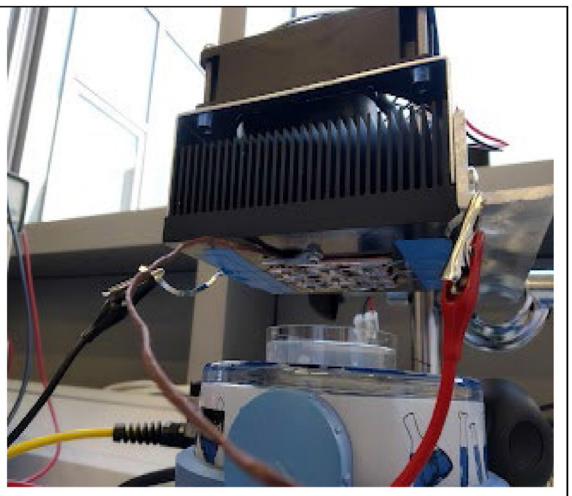


307 nm real manufactured PCB including the UV-LEDs

Figure 2. PCBs layouts for the three configurations of UV-LEDs on the left panel, one for each wavelength (268 nm, 279 nm and 307 nm; and real manufactured PCBs including the soldered UV-LEDs on the right panel.



Schematic experimental set-up for UV irradiation of wastewater contained in a Petri dish.



Real experimental set-up including the PCB with the UV-LEDs, the heat sink, the Petri dish containing the wastewater and the magnetic stirrer.

Figure 3. Experiment set-up showing the scheme for wastewater exposure to UV-LEDs on the left panel and a real image during experimentation on the right panel.

raw when the edible part is grown above ground level and is not in direct contact with reclaimed water, processed food crops, and non-food crops, including animals and milk, and 2) crops for industry and for the production of energy and seeds. It also includes *C. perfringens* for class A water reuse (highest quality class requirements for agricultural irrigation). Finally, although *Enterococcus* is not currently included in wastewater standards, it is recommended as an indicator of faecal contamination because it is used for recreational waters worldwide [37].

2. Material and methods

2.1. UV-LEDs set-up and characteristics

Three different types of UV-LEDs were used, corresponding to three different wavelengths: 265 nm, 275 nm and 310 nm Table 1 shows their main characteristics. The forward voltages were varying between 6 V to 6.5 V for all LEDs, the current was around 20 mA–30 mA for 275 nm and 310 nm LEDs, and around 20 mA–40 mA for 265 nm LEDs. The highest radiant power was 2.5 mW for the 265 nm LEDs from QT-Brightek; and 1.6 mW and 1.2 mW for 275 nm and 310 nm, respectively, from Seoul Viosys as seen in Table 1.

The spectra of each UV-LED was measured by a spectrophotometer from Ocean Insight (Maya 2000 Pro). Figure 1 gives the relative intensity in arbitraty units vs. wavelength in nm, where we can observe that the peak emission was of 267.7 nm for the 265 nm, 278.8 nm for the 275 nm and 306.6 nm for the 310 nm; and the full width at half-maximum (FWHM) was of 12.61 nm, 11.17 nm and 10.38 nm, respectively.

2.1.1. UV-LEDs electronic board design

The printed circuit boards (PCBs) which contain the surface mount device (SMD) LEDs were designed using Orcad PCB designer software (Figure 2). PCB dimensions are 140 mm × 110 mm, placing the LEDs in a 55 mm diameter circular shape in the center of the PCB, ensuring the LEDs irradiation covers the Petri dishes neatly. The boards contained 11 UV-LEDs connected in parallel. They were soldered using a reflow PCB oven from Hangzhou NeoDen Technology "NeoDen IN6" with a lead-solder paste, controlling the actual soldering temperature in real time. The forward current for the three modules was the same; 330 mA for 11 LEDs, and the voltage were 6.5 V, 6 V and 6 V for the 268 nm, 279 nm and 307 nm PCB circuits, respectively.

The UV-LEDs board was then attached to a heat sink with fan for thermal dissipation. A thermocouple for monitoring temperature was inserted in a specific designed and drilled hole in the PCB to monitor temperature using a Fluke 179 multimeter.

2.1.2. Irradiance measurement

The irradiance provided by the UV-LEDs group was measured with an ILT 2400 radiometer from International Light using the ILT-SUD005-10/U SUD detector. It was measured at 25 mm (minimum distance required by the detector), which is also the selected distance from the UV-LEDs to the water surface layer.

2.1.2.1. Thermal control. The power density of the UV-LEDs decreases gradually with the rise of temperature, so it is necessary to use a heat sink with a fan to maintain the stability of the power density. The temperature of the UV-LED is critical, and increasing the temperature can lead to degradation in the power density of the LEDs and may overheat or even break them. A heat sink with a fan was attached to the LEDs PCB boards using thermal paste for the interface surfaces. The PCB board was designed so a thermocouple was inserted next to the UV-LEDs, so temperature could be measured and controlled in the proximity of the LED.

2.2. Water source and physico-chemical and microbiological analyses

The water source for all experiments came directly from the effluent of the wastewater treatment plant of Linares (Jaén, Spain) after the secondary treatment. It is real water with organic content.

2.2.1. Physico-chemical analyses

Turbidity was analysed with a Lovibond TB 211 IR turbidimeter. pH and conductivity were measured with HACH Sension + MM374 Multimeter +5014 electrode (pH) + 5070 cell (electrical conductivity).

Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Nitrogen, nitrates (NO_{3-}), phosphates (PO^{4}_{3-}), sulphates (SO^{4}_{2-}), chromium (Cr^{6+}), ammonium (NH^{4+}), copper (Cu), zinc (Zn), aluminium (Al), iron (Fe), and nitrites (NO_{2-}) were measured with a Spectroquant Prove 100 spectrometer from Merck and the corresponding reactive tests. Total Suspended Solids (TSS) and Sedimentable Solids (S. Sed.) were analysed at the wastewater plant following the APHA standards [38].

2.2.1.1. Microbiological analyses. Three different microorganisms were studied, *E. coli*, *Enterococcus faecalis* and *Clostridium perfringens*. For all of them, the membrane filtration method was used followed by an incubation in the appropriate culture medium [39]. For *E. coli*, Chromogenic Colinstant agar (Scharlau 01-695-500) prepared with the selective supplement CV coliforms (Scharlau 06-140LYO1). After incubation of 18–24 h at 36 °C ± 2 °C, deep blue to violet colonies were considered as *E. coli* bacteria, and red colonies were identified as other coliform bacteria (UNE-EN ISO 9308-1: 2014 [40]). *Enterococcus faecalis* were identified after two stages: first, the samples were incubated in Slanetz & Bartley agar (Scharlau 01-579-500) with sterile 1% TTC solution (Scharlau 06–023) at 36 ± 2 °C for 44 ± 4 h, and the colonies red or purple were considered as possible Enterococci; and second, the samples were subjected to a confirmation stage where they were incubated in biliary esculin azide agar, preheated to 44 °C, at 44 °C ± 0.5 °C for 2 h. Those colonies turning to black spots surrounded by a brown shadow were finally identified as *Enterococcys faecalis* (UNE-EN ISO 7899-2: 2000 [41]). Finally, *Clostridium perfringens* were identified using the ChromAgar™ Chromogenic *Clostridium perfringens* culture medium [42]. Petri dishes were incubated anaerobically at 37 °C ± 1 °C for 21 h ± 3 h, and orange colonies were counted as *Clostridium perfringens*.

2.3. Experimental set-up

For each UV-LED wavelength, the experimentation consisted in placing the appropriate UV-LED board with its heat sink and fan over the sterile plastic Petri dish containing the raw water to be treated (see Figure 3).

In all the experiments the UV-LEDs were placed 25 mm above the water surface. Sterile plastic Petri dishes, with 55 mm diameter were filled with 15 ml of wastewater from the effluent of the Linares wastewater treatment plant (WWTP). A 2.5 mm × 2.5 mm magnetic stirrer bar was used to stir the water and ensure homogeneous illumination. Figure 3 shows the entire set-up for the experimentation.

Different intervals of time were used to analyse the inactivation kinetics, with at least 6 points per experiment. One Petri dish was used per time interval, filled with a volume of 15 ml (with a water layer height of 7.5 mm) and placed over the magnetic stirrer without a cover, at 25 mm from the UV-LED board. Microbial analyses used triplicates of 5 ml each, so the detection limit (DL) was 1 CFU/5 ml, or the equivalent 20 CFU/100 ml. Water temperature was measured in the Petri dish before and after the exposure process. The UV-LED boards were power supplied with an Agilent E3631A power supply and the board temperature was measured by a FLUKE 179 multimeter.

3. Results and discussion

Three wavelengths were tested: 268 nm, 279 nm and 307 nm. A complete set of experiments (up to 12) were conducted, and here we present the most representative ones for each bacteria and UV-LED wavelength, which are those reaching or almost reaching the microbiological detection limit and that have microbiological analysis triplicates.

The measured irradiance was of 0.370 mW/cm^2, 0.456 mW/cm^2 and 0.443 mW/cm^2 for the 266 nm, 279 nm and 307 nm modules, respectively.

Table 2. Raw water physico-chemical range results.

Parameter	Minimum	Maximum
pH	7.62	7.74
Conductivity (μS/cm)	533	1123
Turbidity (NTU)	4.74	13.47
BDO (mg/L)	8.4	26
COD (mg/L)	33	66
TSS (mg/L)	13	28
Total nitrogen (mg/L)	36	49
Nitrates (mg/L)	<2	2.6
Nitrites (mg/L)	<0.07	0.09
Phosphates (mg/L)	1.36	6.32
Sulphates (mg/L)	68	99
Iron (mg/L)	<0.05	0.1
Aluminum (mg/L)	<0.02	<0.1
Copper (mg/L)	0.05	0.11
Amonium (mg/L)	35.7	58.1
Zinc (mg/L)	0.05	3.18
Chromium (mg/L)	<0.05	0.05

Table 3. Initial concentration of the bacteria in the water.

Initial CFU/100ml	Minimum	Maximum
E.coli	1.7×10^4	3.8×10^6
Enterococcus faecalis	1.2×10^4	1.6×10^5
Clostridium perfringens	2.3×10^4	5×10^4

3.1. Raw water quality

Raw water physico-chemical quality analyses are shown in Table 2. The table shows a summary with the minimum and maximum values from the 12 experiments conducted.

It can be observed that pH ranged from 7.62 to 7.74, conductivity from 533 to 1123 μS/cm, turbidity varied from 4.74 to 13.47 NTU, BOD from 8.4 to 26 mg/L, COD from 33 to 66 mg/L, total nitrogen between 36 to 49 mg/L, nitrates for all the experiments was under 2.6 mg/L, phosphates from 1.36 to 6.32 mg/L, sulphates from 68 to 99 mg/L, iron was below 0.1 mg/L for all the experiments, similarly with aluminium was under 0.1 mg/L for all experiments, copper from 0.05 to 0.11 mg/L, ammonium from 35.7 to 58.1 mg/L, zinc from 0.05 to 3.18, chromates was almost 0.05 for all the experiments.

Regarding initial microbiological water quality, *E. coli* ranged from 1.7×10^4 CFU/100 ml to 3.8×10^6 CFU/100ml, *Enterococcus faecalis* content varied from 1.2×10^4 CFU/100 ml to 1.6×10^5 CFU/100 ml; and *Clostridium perfringens* ranged from 2.3×10^4 CFU/100 ml to 5×10^4 CFU/100 ml (Table 3).

3.2. UV transmittance in the water (%)

The percentage of UV transmittance in the water (UVT) has been measured for the different water qualities used across the different experiments and for the different wavelengths used. For each experiment, UVT was measured using the water from the wastewater plant that was used for disinfection and for the three UV wavelengths used: 268 nm, 279 nm and 307 nm. Water was placed into a clean 1-cm path length quartz cuvette and then the cuvette was placed in the spectrophotometer to measure transmittance at the three studied wavelengths. Results are shown in Table 4.

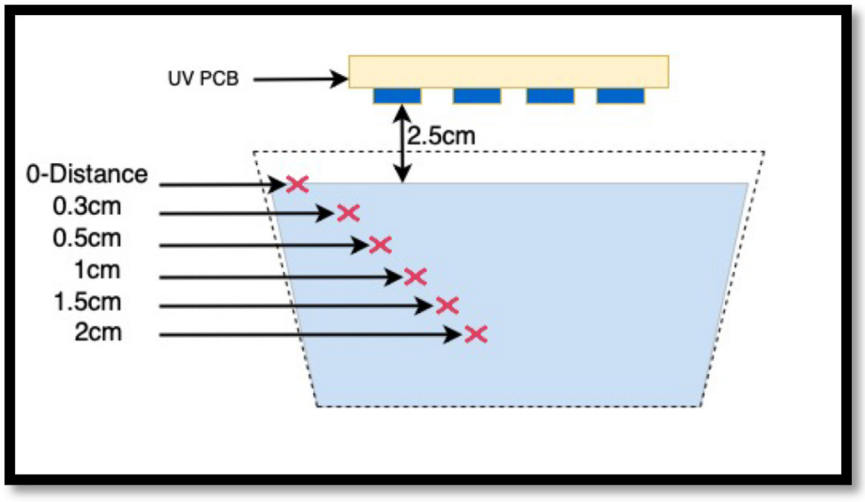
Table 4. UVT (%) for the three different wavelengths in water of different turbidity values.

UV LED wavelength (nm)	UVT (%) 4.7 NTU	UVT (%) 6.3 NTU	UVT (%) 7.3 NTU	UVT (%) 13 NTU
268	76.1	76.1	74.2	75.7
279	78.1	78.2	76.2	77.7
307	85.8	85.9	83.8	85.4

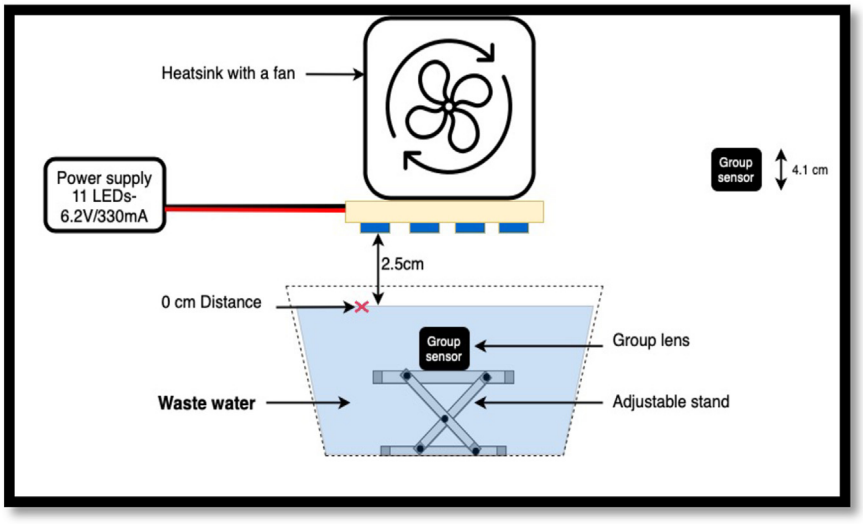
3.3. Effect of turbidity on UV light transmission in water

Regarding the raw water sample quality, the main parameter that directly affects the disinfection process is the turbidity as it reduces the UV light transmission in the water and therefore can slow the disinfection process. In our experiments, UV light transmission was measured at different distances under the water, as it is shown in Figure 4. An underwater UV irradiance sensor is used (SED005/WBS320/WU underwater detector with from International Light) placed on an adjustable stand to vary the water depth. The sensor and the adjustable stand are located within a water tank containing the water. Two types of water were used for the measurement: Milli-Q water, with 0 NTU, and raw wastewater from the plant (effluent) with 8 NTU.

The three UV-LEDs boards were used: 268 nm, 279 nm and 307 nm (Table 5). Results with the Milli-Q water at 0 NTU show that transmittance losses in the water reached about 25% at 1 cm depth and about 43% at 2 cm depth. At 8 NTU, the losses increase up to 40% at 1 cm and 65% at 2 cm. When making a comparison between the three wavelengths, they all perform very similar, with a slight better transmission of the 279 nm board at 0 NTU and 268 nm at 8 NTU. As the water depth of



(a)



(b)

Figure 4. Experimental set-up for measuring the UV light transmission from the LEDs under the water: a) Different water depths under test and b) Complete experimental set-up including the UV-LEDs, the water tank, the underwater sensor and the adjustable stand to move the sensor at various distances.

Table 5. –UV irradiance losses at various water depths at 0 NTU and 8 NTU.

	UV Irradiance losses (%) with 0 NTU			UV Irradiance losses (%) with 8 NTU		
Water depth (cm)	268 nm	279 nm	307 nm	268 nm	279 nm	307 nm
0.3	11.6	8.7	10.9	10.5	16.8	11.8
0.5	16	13.1	15.7	19.6	20	21.7
1	26.4	23.6	26.8	41.4	41.3	39.2
1.5	36.2	32.5	35.4	57.1	57.5	52.8
2	44.5	40.2	43	69.9	68.5	63.5

the experimental set-up in the disinfection experiments is 0.7 nm, the losses would be on the 1 cm water depth losses range.

3.4. Escherichia coli

Figure 5 shows the disinfection of *E. coli* using the 268 nm, 279 nm, and 307 nm wavelengths versus the UV-dose. In the case of 268 nm, the initial population was 1.4×10^6 CFU/100 ml (12.4 NTU) and complete inactivation was reached after 50 s, corresponding to an 18.4 mJ/cm^2 UV dose. For the 279 nm UV LEDs the initial bacteria population was 7.6×10^5 CFU/100 ml (6.3 NTU) and a considerable decrease happened after less than 20 s followed by reaching the detection limit after almost 60 s, corresponding to a 27.3 mJ/cm^2 of UV dose. Meanwhile, in the 307 nm experiment, the starting population was 3.8×10^6 CFU/100 ml (8.9 NTU), however the inactivation rate was negligible, the microbiological population being 2.8×10^6 CFU/100 ml after 180 s, corresponding to a 97.9 mJ/cm^2 UV dose (26.59% inactivation).

Both wavelengths 268 nm and 279 nm have a similar effect on deactivating *E. coli*, although the UV dose needed to eliminate *E. coli* was 18 mJ/cm^2 for 50 s for 268 nm and 28 mJ/cm^2 for 60 s for 279 nm. This suggest a higher inactivation for the 268 nm wavelength despite the higher turbidity value of the raw water during the 268 nm experiment (12.4 NTU for 268 nm vs. 6.3 NTU for 279 nm). 307 UV-LEDs shows a considerable slower effect on the bacteria disinfection.

These results agree with current scientific literature. For example, Nyangaresi et at (2018) [23] in 2018 used different UV-LEDs (265 nm, 275 nm and 310 nm) to disinfect water containing *E. coli* (synthetic water, not environmental strains), with similar power outputs for the LEDs (1.5 mW, 20 mA, 6V) and an irradiance of 0.384 mW/cm^2. They had a good temperature control so temperature stayed below 31.4 °C and also use Petri dishes at 2.2 cm distance. Results showed that the 267 nm UV-LED had the highest inactivation efficiency, therefore results agree with this despite using water with organic content and with turbidity levels between 4.8 and 13.5 NTU. In the same line, the 310 nm UV-LEDs also showed a slower performance.

Oguma et al. in 2013 [13] used the same configuration using 265 nm, 275 nm and 310 nm with pure culture of *E. coli* K12 IFO 3301, observing as well that 265 nm achieved the highest inactivation based on fluence-based efficiency. They are also in agreement with the study from Chatterley and Linden in 2010 [43], which used 265 nm UV-LEDs in a collimated beam to inactivate *E. coli* K12 (ATCC #29425). The UV-LEDs

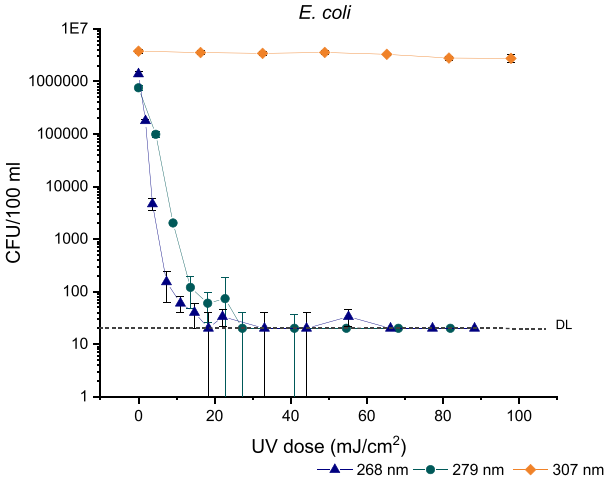


Figure 5. UV-LED disinfection results for *E. coli* under three different wavelengths: 268 nm, 279 nm and 307 nm in CFU/100 ml vs. UV dose (mJ/cm^2), where it can be observed that 307 nm is not sufficient and 268 nm and 279 nm are more effective to disinfect water. * DL: Detection limit: 20 CFU/100 ml.

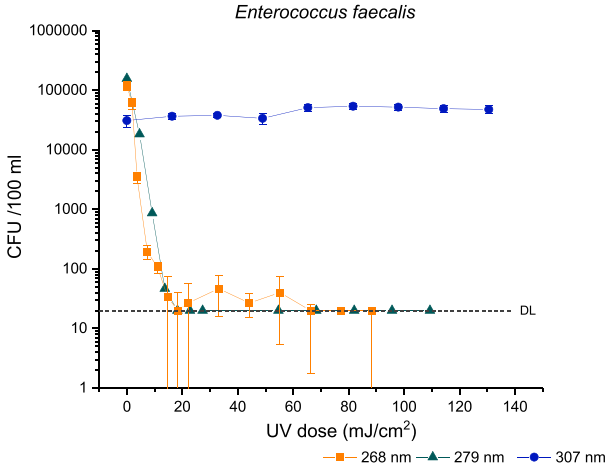


Figure 6. UV-LED disinfection results for *Enterococcus faecalis* under three different wavelengths: 268 nm, 279 nm and 307 nm in CFU/100 ml vs. UV dose (mJ/cm^2). * DL: Detection limit: 20 CFU/100 ml.

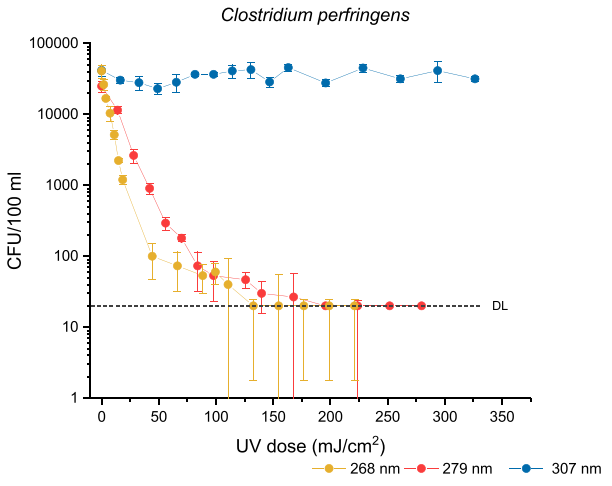
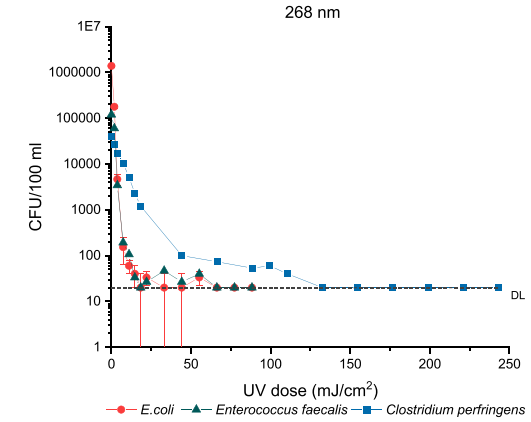
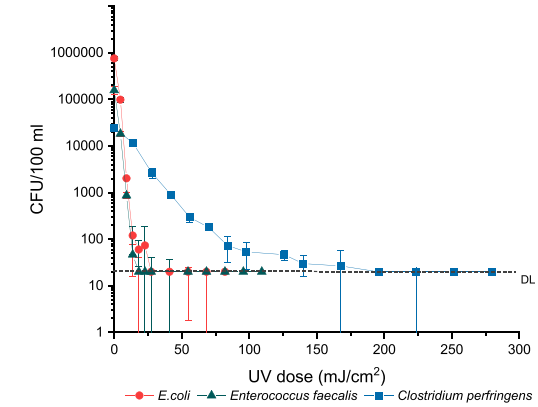


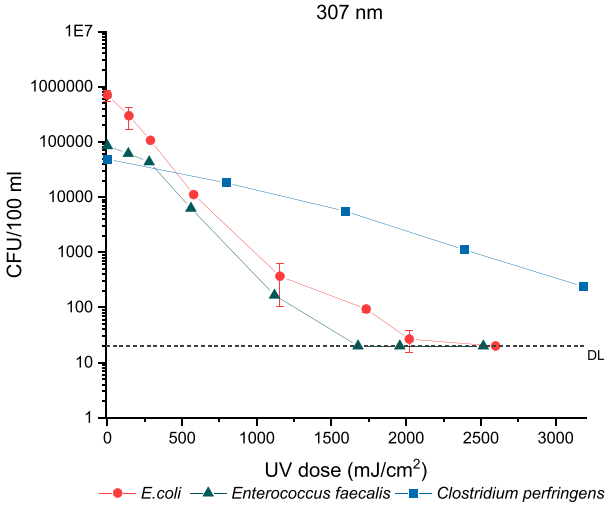
Figure 7. UV-LED disinfection results for *Clostridium perfringens* under three different wavelengths: 268 nm, 279 nm and 307 nm in CFU/100 ml vs. UV dose (mJ/cm^2). * DL: Detection limit: 20 CFU/100 ml.



(a)



(b)



(c)

Figure 8. UV-LED disinfection results for the three bacteria under different illumination wavelengths: a) 268 nm, b) 279 nm, and c) 307 nm (longer experiment: 120 min). * DL: Detection limit: 20 CFU/100 ml.

(6V, 20 mA) were at a distance of 1 mm above water surface, receiving an irradiance of 0.059 mW/cm^2. After inducing an UV dose of 20 mJ/cm^2, the log-reduction was of 3.4.

Bowker et al. in 2011 [44] also used 275 nm UV-LEDs, this time in comparison with 255 nm, and their results suggested that 275 nm could be more effective for *E. coli* than 255 nm. However, Sholtes and Linden in 2019 [11] used 255 nm, 275 nm and 285 nm UV-LEDs to disinfect water with *E. coli* K-12 (ATCC(R) 29425) and in this case, 265 nm was the most effective wavelength to inactive the microorganism, even better than 255 nm. Between 255 nm and 285 nm, 255 nm was more effective. In this same line, Silva, Leonel and Tonetti [31] also compared the performance of 255 nm and 280 nm UV-LEDs to inactivate *E. coli*. Their results show that 280 nm was more effective than 255 nm, supporting the results from Bowker et al. Finally, Betzalel et al. [45] compared 265 nm and 285 nm, with 265 nm UV-LEDs showing higher performance.

3.5. *Enterococcus faecalis*

Figure 6 illustrate the disinfection of *Enterococcus faecalis* under different UV-LEDs wavelengths versus UV dose. Under 268 nm UV-LEDs illumination, the microbiological content varied from an initial population of 1.2 $\times$ 10^5 CFU/100 ml (12.4 NTU) to reaching DL after 50 s and a corresponding 18 mJ/cm^2 UV dose. Under 279 nm, the disinfection process was similar, from an initial 1.6 $\times$ 10^5 CFU/100 ml (6.3 NTU) it reached DL after 40 s and a UV dose of 18.2 mJ/cm^2. Finally, for the case of 307 nm, with an initial population of 3.1 $\times$ 10^4 CFU/100 ml (5.4 NTU) and after 240 s and a UV dose of 131 mJ/cm^2 the disinfection effect was negligible, reaching a final population of 4.8 $\times$ 10^4 CFU/100 ml.

In this case, it is observed that 268 nm and 279 nm wavelengths are also effective vs. 307 nm wavelength, which once again shows little inactivation. 268 nm UV-LED is also more effective to eliminate *E. faecalis* as for the case of *E. coli*.

E. faecalis has not been studied in depth in the scientific literature regarding UV-LED treatment. There are only a few studies, such as the one from Chevremont et al. in 2012 [30] that studied the inactivation of several microorganisms in the effluent of a wastewater treatment plant. Initial levels of fecal enterococci were of 8.3 $\times$ 10^5 CFU/100 mL. The effluent was exposed to 254 nm, 280 nm, 365 nm, 405 nm and combinations of 280/365 nm and 280/405 nm. 254 nm had more bactericidal effect than 280 nm but they noted that the two combinations of 280/365 nm and 280/405 nm had the most important disinfection effect. Another study is the one by Lui et al. (2016) who worked with 270 nm and 310 nm UV-LEDs [32], showing that 310 nm was insufficient for useful inactivation and that 270 nm was effective to disinfect a laboratory strain of *E. faecalis*.

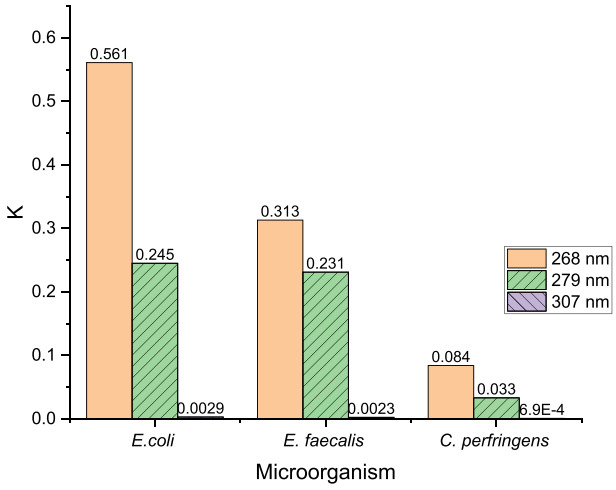


Figure 9. Inactivation rate constant, K (cm^2/mJ) vs. microorganisms and wavelengths.

Table 6. Inactivation rates for the different bacteria and wavelengths.

Microorganism	$K_{268\ nm}$	R^2	NTU	$K_{279\ nm}$	R^2	NTU	$K_{307\ nm}$	R^2	NTU
E. coli	0.561	0.997	12.37	0.245	0.994	6.29	0.0029	0.999	8.85
E. faecalis	0.313	0.991	12.37	0.231	0.995	6.29	0.0023	0.997	5.39
C. perfringens	0.084	0.999	7.30	0.033	0.999	4.74	6.90E − 04	0.999	9.45

3.6. *Clostridium perfringens*

Total inactivation of *Clostridium perfringens* under 268 nm was reached after 360 s and 133 mJ/cm^2 UV dose, starting at 4.1 × 10^4 CFU/100ml (7.3 NTU) (Figure 7). For 279 nm, from an initial concentration of 2.5 × 10^4 CFU/100 ml (4.8 NTU) it reached DL after 420 s and an UV dose of 196 mJ/cm^2. Finally, under 307 nm irradiation and with an initial concentration of 4.2 × 10^4 CFU/100 ml (9.5 NTU), after 600 s (10 min) and 326 mJ/cm^2 the final concentration was of 3.2 × 10^4 CFU/100 ml (31% inactivation).

Once again, 268 nm wavelength shows higher effect on bacteria inactivation, followed by 279 nm. The experiment under 307 nm show a very low inactivation rate.

As for the case of *E. faecalis*, there is a lack of studies in the literature on the efficiency of UV-LED to inactivate *Clostridium perfringens*. Only the work from Thompson and Pasquantonio in 2019 [46] with *Clostridium difficile* spores have been found. In this study, they centred their efforts in analyse the effect of UV LEDs of 275 nm and the combination of 275 nm + 365 nm on the inactivation of spores as their transmission from contaminated surfaces is a continuing problem for health care facilities. They used high intensity UV-LEDs with irradiances up to 491.5 mW/cm^2 for 275 nm, showing that the effectivity in inactivating the spores, with a maximum log reduction of 5.79 for an UV dose of 14.7 J/cm^2. While they were working with a different microorganism and only considering spores, it gives an idea that *Clostridium* disinfection is possible despite their higher resistance in comparison with other bacteria (*E.coli*, *E. faecalis*).

3.7. *Comparison between the three bacteria per wavelength*

Figure 8 shows the disinfection results for all the bacteria per wavelength. First, 269 nm and 279 nm show how *E. coli* and *E. faecalis* exhibit similar behaviour under these wavelengths, with a slightly higher inactivation for 268 nm, vs. *Clostridium perfringens* that is more resistant.

3.7.1. *307 nm UV-LED*

Regarding the inactivation under 307 nm UV-LED illumination, a longer experiment was conducted to investigate the full potential of this wavelength to disinfect water. The initial *E. coli* population was 7 × 10^5 CFU/100 ml. It reached total inactivation after 90 min and 2600 mJ/cm^2 of UV Dose (Figure 8c). *E. faecalis* initial concentration was 8.5 × 10^4 CFU/100 ml, and after 60 min and a UV dose of 1700 mJ/cm^2 it reached DL. *Clostridium perfringens* required more time and UV dose once again, this time it started with 4.9 × 10^4 CFU/100 ml and after 120 min and 3200 mJ/cm^2 the inactivation level reached 99.5% (240 CFU/100 ml), i.e. 2-log reduction only.

3.7.2. *Inactivation rates*

Regarding inactivation rates, they were calculated for each experiment without considering the tailing phase [23]. The following equation (Eq. (1)) was used:

$$Log\ (N_0 / N) = -K_{UV} \cdot\ UV\ \ dose \tag{1}$$

where N_0 and N is the number of colonies (CFU/100 mL) before and after UV exposure, K_{UV} is the inactivation rate and UV dose is the fluence (mJ/cm^2). Figure 9 shows the calculated inactivation rates for the three different bacteria and the different wavelengths, followed by Table 6 that summarises the inactivation rate values, the R^2 and the turbidity value for each experiment.

It can be observed that regarding UV-LED wavelengths, 268 nm has been the most effective one, showing higher rates for all the bacteria, but specially for *E. coli* (0.561 at 268 nm vs. 0.313 at 279 nm despite the higher turbidity value). 307 nm has been very slow, requiring higher doses and longer times to disinfect, which translates into higher energy consumption. For *E. faecalis*, 268 nm and 279 nm exhibit similar inactivation rate values, although as it has been discussed 268 nm has more effect. Regarding the bacteria, *E. coli* is the most sensitive one to UV light exposure, followed by *E. faecalis* and finally by *C. perfringens*. While inactivation rates for *C. perfringens* are significantly lower in comparison with *E. coli* and *E. faecalis*, still is possible to use UV-LEDs to eliminate this microorganism, just requiring higher doses of UV at 268 nm or 279 nm wavelengths. When comparing in time and not in UV dose, for the same initial UV irradiance, *C. perfringens* took approximately 6 min to deactivate vs. 1 min that took *E. coli* or *E. faecalis*.

The 268 nm UV-LED presented a higher value for *E. coli* of 0.561, which is slightly higher than the reported value of 0.42, 0.43 and 0.41 from different scientific studies [13, 23, 47]. For 279 nm and *E. coli* again, the K_{UV} value was 0.245, similar to the 0.292 value reported by Nyangaresi et al [23], and 0.29 from Oguma et al. [13] and 0.30 from Li et al [47]. The inactivation rate value for 307 nm was 0.0029, much lower than the reported value of 0.038 by Nyangaresi et al [23], which could be due to higher turbidity or due to the nature of the environmental strain of the microorganism.

4. Conclusions

Experimental results demonstrate the effective inactivation of the three different bacteria strains from a real wastewater effluent using UV-LEDs of low irradiance at three different wavelengths: 268 nm, 279 nm and 307 nm. In general, 268 nm wavelength was the most effective and rapid, followed closely by 279 nm wavelength. 307 nm wavelength showed a significantly lower inactivation rate so it should be discarded for wastewater disinfection using UV-LEDs as it would consume too much energy in comparison with the other two wavelengths.

Regarding the different bacteria (*E.coli*, *E. faecalis* and *C. perfringens*), it was possible to inactive the three bacteria but with different UV dose. While *E. coli* and *E. faecalis* required a minimum dose and a corresponding time exposure of only 1 min, *C. Perfringens* required higher doses and therefore longer time exposure periods, up to 6 min. While it is still possible to disinfect this latter bacteria, further research should look into higher powered UV-LEDs to reduce the time exposure and to facilitate the growing from small prototypes reactors to full-size reactors that can be utilised in wastewater facilities.

Declarations

Author contribution statement

Marta Vivar: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ahmed Kamel: Performed the experiments; Analyzed and interpreted the data.

Manuel Fuentes: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Ana Palacios: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

M J Rodrigo: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no competing interests.

Additional information

No additional information is available for this paper.

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