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.

Water disinfection with UV-LEDs

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Abstract

This thesis presents an investigation into an alternative method for disinfecting microorganisms in wastewater treatment plants. The current methods of disinfection, such as the use of chemicals like chlorine, are dangerous and require large quantities for the disinfection process. Another method, using Ultraviolet lamps, is also used but has numerous drawbacks such as short lifetime, toxic substance like mercury, high voltage setup, long startup time, fragile, and available in only one wavelength, which makes it unsuitable to disinfect all types of microorganisms. Additionally, when the lamps break, the mercury can mix with the water, and special treatment is needed to remove it. Therefore, this thesis proposes the use of ultraviolet Light Emitting Diodes (LEDs) as an alternative method to replace traditional ultraviolet lamps. Ultraviolet LEDs have several advantages over traditional lamps, such as availability in multiple wavelengths, no toxic substances, no need for high voltage, and no startup time. This research aims to investigate the potential of ultraviolet LEDs as a safer and more efficient method of disinfection in wastewater treatment plants. The disinfection experiments were conducted using real wastewater from a wastewater treatment plant in Linares-Spain and three different microorganisms were chosen for the experiments.

The work presented in this thesis includes the selection and characterization of various ultraviolet light-emitting diodes (UV-LEDs) with different wavelengths and optical powers. Printed circuit boards (PCBs) were designed for each type of UV-LED, taking into account the specific dimensions and footprints of the LEDs. The UV-LEDs were then placed and soldered onto the PCBs in a way that ensured optimal irradiation of a wastewater sample container (Petri dish). A reactor was constructed for disinfection experiments, and a monitoring system was developed to control and record parameters such as voltage, current, temperature, and output optical power during the experiments. Additionally, the experiments' data were saved for analysis.

The results of the initial study indicate that the utilization of ultraviolet LEDs with low irradiance at various wavelengths effectively activates the inactivation of three strains of bacteria (*Escherichia coli*, *Enterococcus faecalis*, *Clostridium perfringens*) present in wastewater effluent. The data suggests that the wavelength of 268 nm was the most efficient and rapid in terms of bacterial inactivation, followed by 279 nm. However, the inactivation rate was significantly diminished when utilizing the 310 nm wavelength, making it less viable for disinfecting wastewater through the use of ultraviolet LEDs due to the high energy consumption in comparison to the other two wavelengths.

The second study revealed the validity of the reciprocity law in ultraviolet disinfection utilizing two wavelengths of ultraviolet LEDs with varying optical powers. The study employed the same microorganisms as the initial study. The results indicate that the inactivation rates were comparable for both optical powers. However, additional research is required to confirm these findings and account should be taken of the impact

of turbidity and UV transmittance on the results as a 2 cm water depth resulted in a significant decrease.

List of publications

The following publications (Article I & II) are presented a part of this thesis. They are already published in international journals

[1] Kamel A; Fuentes M; Palacios AM; Rodrigo MJ; Vivar M; “Deactivating environmental strains of escherichia coli, enterococcus faecalis and clostridium perfringens from a real wastewater effluent using UV-leds”, Heliyon. U.S. National Library of Medicine. Available at: https://pubmed.ncbi.nlm.nih.gov/36636203/ (Accessed: January 22, 2023).

[2] A. Kamel, A. Palacios, M. Fuentes, and M. Vivar, “Analysing the Reciprocity Law for UV-LEDs in Water Disinfection of Escherichia coli, Enterococcus faecalis, and Clostridium perfringens,” *Water*, vol. 15, no. 2, p. 352, Jan. 2023, doi: 10.3390/w15020352.

List of conferences attended

[1] 3rd International Conference on Disinfection and DBPs, IWA DDBPs 2022, June 27th – July 1st, 2022 – Milan, Italy.

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Chapter 1: Introduction

Wastewater plant's main function is the removal of harmful substances from wastewater before discharging into the environment. From larger objects such as stones or sticks (and other similar objects) to the final microorganisms as bacteria, which are reduced or completely eliminated from the water. One important issue in wastewater plants operation is the large electrical consumption, and more importantly, the fact that around 30 % to 50 % of the total consumption belongs to the operation and maintenance process, which are permanent processes.

Figure 1 - Wastewater plant in Linares

Generally, a wastewater treatment facility consists of various stages. The first stage is called "Pretreatment" and it is where large-size components that can cause operating and maintenance problems are removed, such as stones, branches, plastics, rags, etc. Sand and fat removal processes are done afterwards. Secondly, the "Primary treatment" comes next, with the objective of eliminating 50 % of suspended solids and 20 % of biochemical oxygen demand (BODs). It uses Sedimentation and a Physical-chemical treatment (Coagulation + Flocculation). Sedimentation is conducted in a cylindrical tank separated into three zones: first is the influent zone where water enters the sedimentation tank from the center, second comes the settling zone where the solid sludge settles down in the bottom of the tank and then it is collected by the sludge collector, and lastly is the effluent zone that allows the treated water reaches the next treatment stage (Figure (2)).

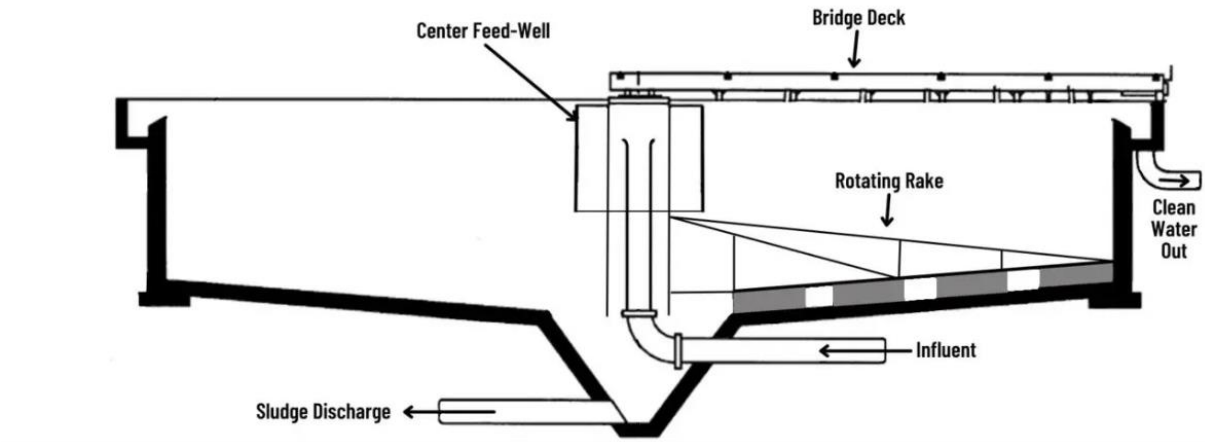


Figure 2 - Sedimentation blue print [32]

After sedimentation, a physical-chemical treatment follows, which consists of a coagulation/flocculation process. This process main objective is to reduce the turbidity in the water as lower turbidity allows higher rates of disinfection. By including Coagulant chemical to water, small particles and impurities group in to form larger particles that can settle down in the tank due to gravity as shown in figure (3).

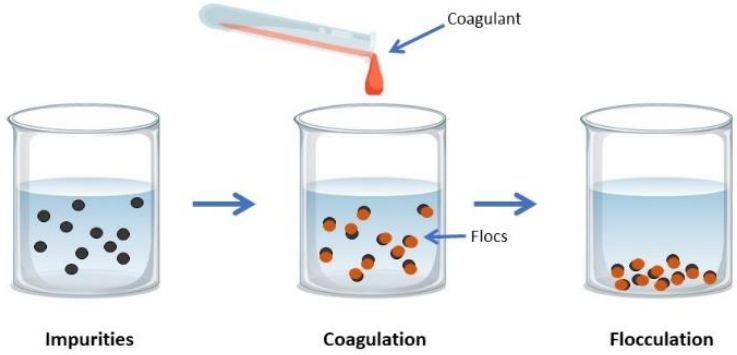


Figure 3 - Coagulation/Flocculation process explanation

Then, the "Secondly treatment" discards 90 % of suspended solids, 70 % - 90 % of DOB_5 (25 mg/l) and COD must reach a limit of 125 mg/l [33]. Ultimately, the "Tertiary treatment" which consists of a filtering and disinfection stage using Ultra violet light (UV) or chlorination. Figure (4) illustrates all the treatment stages.

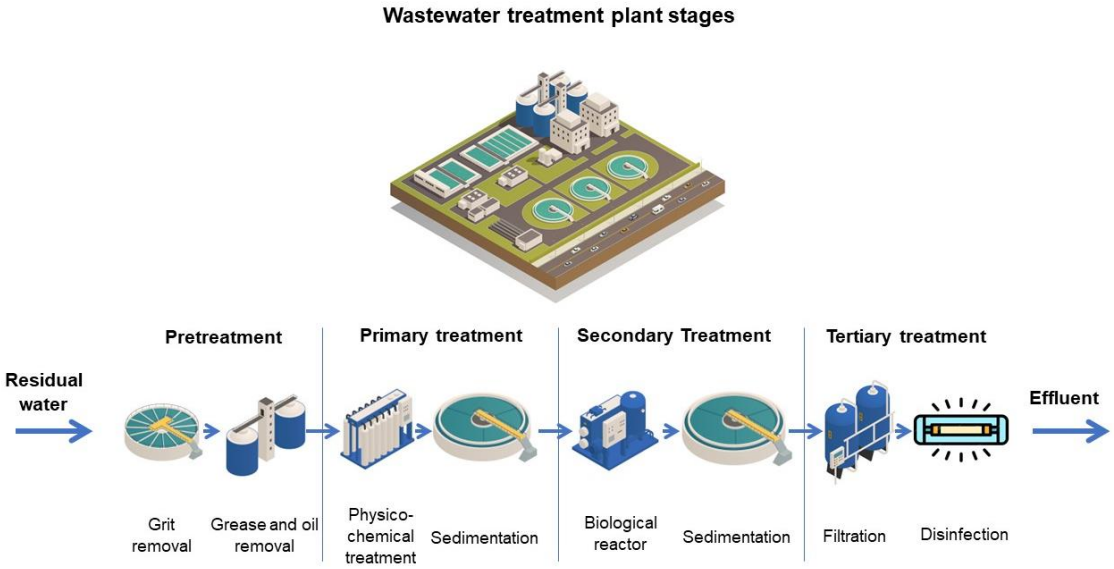


Figure 4 - Wastewater treatment stages

As a result of the overall high-power consumption, the "Tertiary treatment", which is responsible of improving the water quality and making it useable again is not always implemented due to the cost of investment, operation and maintenance (mainly electricity). In Spain it is only mandatory in the so-called "sensitive areas" according to the Spanish law [34]. But the European directive (EU directives 2000/60/EC and 91/271/EEC) [35-36] requires the best possible treatment of water until "zero" discharge. The fact that the water is not properly purified produces a high environmental impact of discharge on surfaces, as well as a loss of the opportunity of reusing water which is a scarce source.

There are two main or common methods currently used in the "Tertiary treatment", the first one is "chlorination", which is widely used due to its effectiveness. It consists of a chlorine concentration mixed with the treated water in a chamber where it has to go through a long path. The water gets disinfected during that time and after this process is ready to get cleanly discharged to the stream. However, it is required a continuous chemical supply and this process can also produce a significant number of toxic gases if not properly handed. The alternative method is to use conventional artificial ultraviolet lamps, which despite being very effective and fast, they have several drawbacks: high energy consumption, a spectral peak response centered in 255nm wavelength only, require high voltage to be functional, and they have a relatively slow starting time which makes it unsuitable to run discontinuously. The other main problem is the need of replacement every 6 or 12 months, which increase the cost of operation. They also contain mercury, which is toxic and needs to follow a special recycling process in order to extract the mercury and do not discharge it into the environment [2,3,4,7,9,11,12,13,14,19,22,23,24,29,30]. Hence, a new effective solution for this water disinfection stage that has low energy consumption and low environmental impact is required. Figure (5) indicates the main disadvantages of UV lamps.

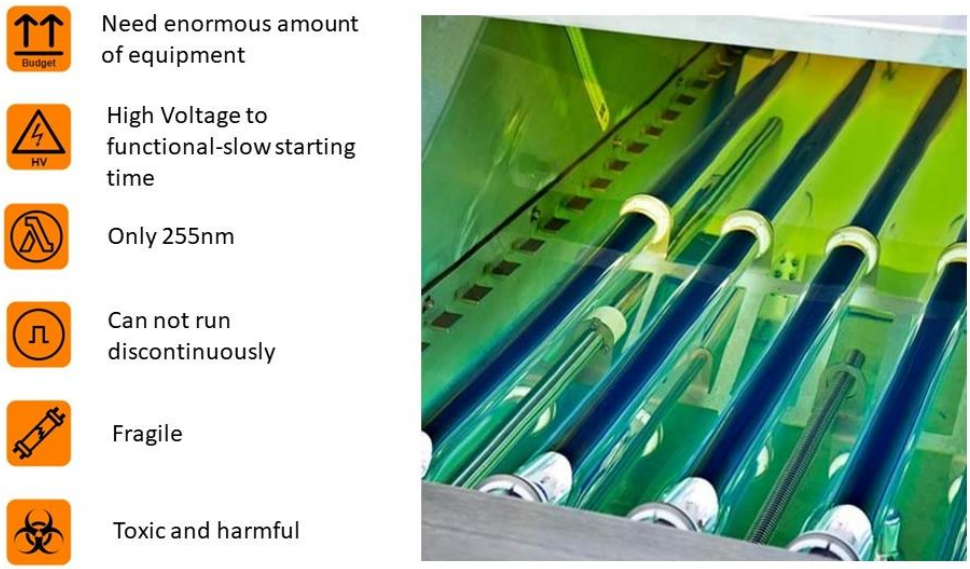


Figure 5 - Some of the disadvantages of UV Lamps

On the contrary, UV LEDs have many different small sizes which makes it suitable for designing any type of reactor as showed in figure (6), besides other interesting benefits they provide such as the low operating temperature, lower voltage needed compared to the traditional UV lamps or the instantaneous switching that leads to a new different technique of operation to reduce the power consumed during the operation (pulse illumination). Furthermore, they are produced in different wavelengths which make them the most optimal solution to disinfect a various type of bacteria since every single bacterium is sensitive to a particular wavelength in contrast with UV lamp which is only centered at 255nm wavelength. Lastly and most important, they are environmentally friendly as they do not contain mercury or any type of toxic chemicals as UV lamps [2,3,4,7,9,11,12,13,14,19,22,23,24,29,30].

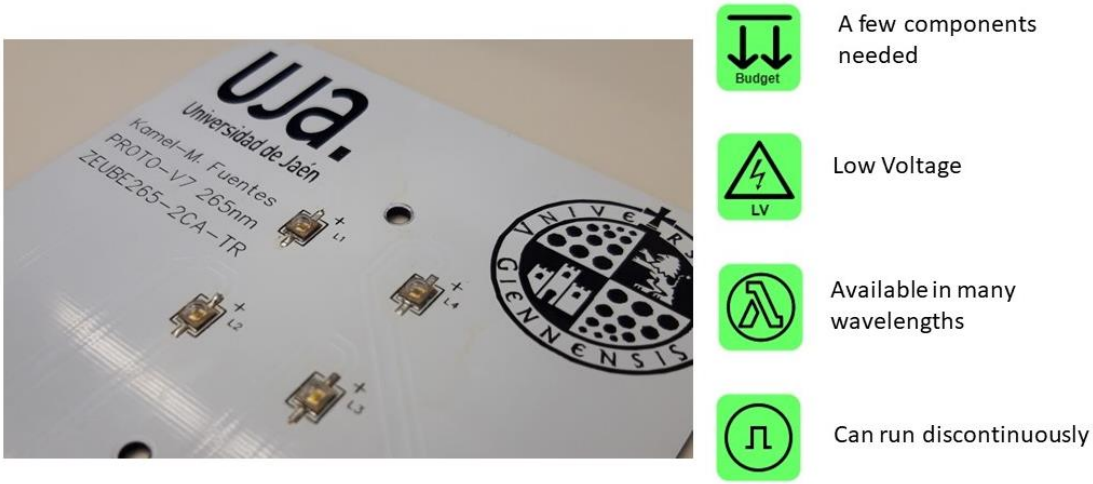


Figure 6 - Advantages of UV LEDs

In the next sections UV-LEDs and their use in water disinfection treatment will be described in detail.

1.1 LEDs structure and operation

LED stands for Light Emitting Diode, and as the name indicates it is based on a diode. Normally diodes consist of P-N junction and they have two certain operation modes. First one is the “forward bias” when the positive terminal of the diode is connected to the positive of the power supply and similarly the negative terminals of the diode and the supply power are connected, this mode narrows the depletion region between the P-N junction and allows free electrons to cross the junction and recombined with the free holes. This movement of electrons releases energy in terms of heat, but in the case of LEDs the energy releases as light. The other mode is the “reverse bias” where the supply terminals of the diode are connected the opposite way compared with supply power terminals, and simply in this case the diode or the LEDs are unfunctional and acting as open circuit.

In figure (7) a graphical illustration for the LED structure from the outer layout to the P-N junction is shown.

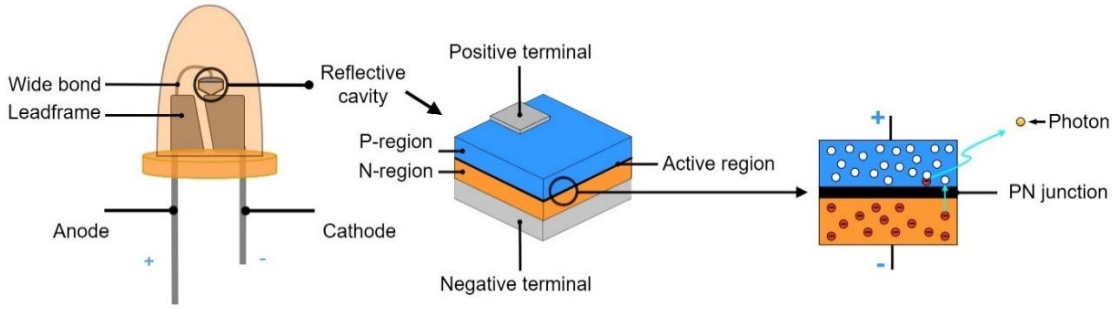


Figure 7 - LED structure

1.2 UV light properties

The Ultraviolet light can be found in the light spectrum as shown figure (8) in the range of 200 nm to 400 nm, and it is then divided into three sub-subsection called UV-A, UV-B and UV-C [16]. This light is usually invisible to the human eyes and some of its wavelengths are harmful to human skin and eyes [17]. The sun is the largest source of that UV light, however most of it is being blocked by the ozone layer in the atmosphere. UV-A is not being absorbed or filtered through the atmosphere as neither the UV-B is, but the UV-C which is playing an important role in the disinfection process does not make it through the atmosphere.

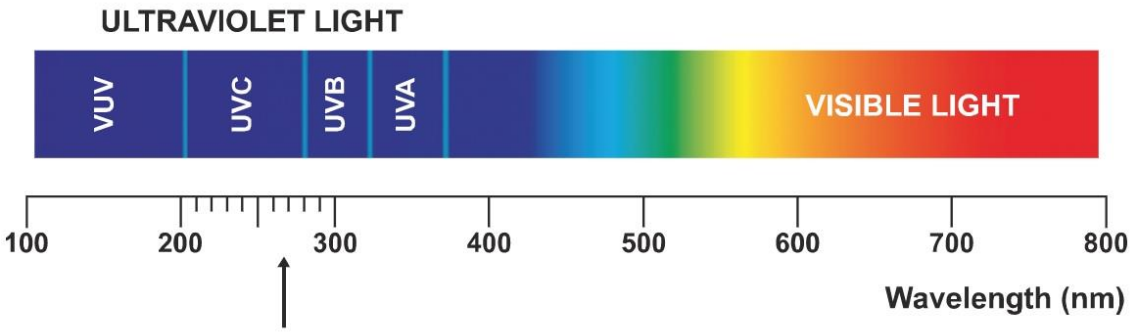


Figure 8 - Light spectrum

The UV-A ranges from 315 nm to 400 nm in the wavelength spectrum, next is the UV-B from 280 nm to 315 nm, and finally the UV-C from 200 nm to 280 nm. Conventionally the optimum peak for killing bacteria and viruses is 254 nm (peak of mercury-based lamps). And as mentioned previously, none of the UV-C light passes through the atmosphere, that is why it is being artificially produced for various applications including water disinfection. According to numerous studies, the UV-C is the ideal range for killing viruses, bacteria, mold spores and other contamination [8,10,19,21,23,27,28,30].

This type of UV-C can be emitted using mercury-based UV lamps that can be found in the water disinfection systems. But due to its drawbacks (high operating voltage, fragile, warm up time, cannot be operated in pulsed mode operation, contain toxic substance such as mercury), scientific research is trying to find a solution to replace the UV lamps by other cleaner technologies such as UV LEDs.

1.3 DNA and deactivation

DNA stands for “Deoxy riboNucleic Acid” which is a molecular substance carrying the genetic information for functioning and reproducing in any organism. It consists of subunits called nucleotides, each subunit composed of three different parts: deoxyribose, phosphate and one of four nitrogenous bases which are thymine(T), adenine (A), cytosine(C) and guanine(G). These four bases form pairs of either thymine bonded to adenine or cytosine bonded to guanine. UV wavelengths found to have a strong effect in stopping the effect of microorganism such as bacteria by breaking the bonds and deactivating them and preventing them from being produced [31].

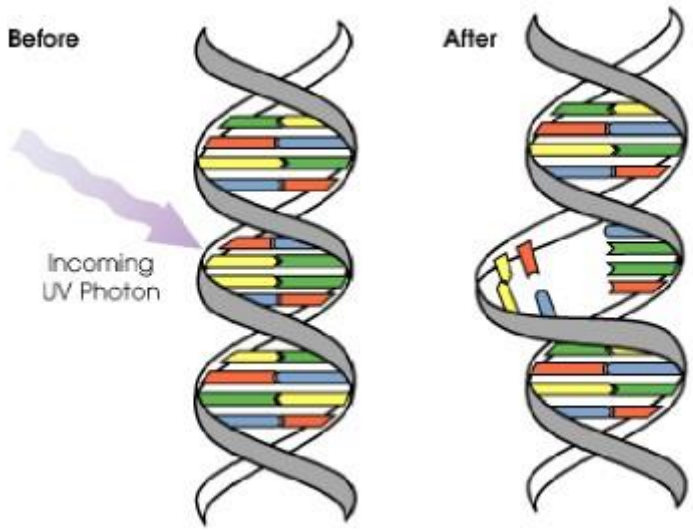


Figure 9 - DNA deactivation using UV light [18]

1.4 Previous work on water treatment using UV LEDs

Scientists have centered their attention in the last ten years on different ways of deactivating some certain microorganism in wastewater in order to make it usable using UV LEDs. Most experimental set-ups consist of a PCB board containing the UV-LEDs attached to a cooling system that illuminate a petri dish which contains the water to be treated.

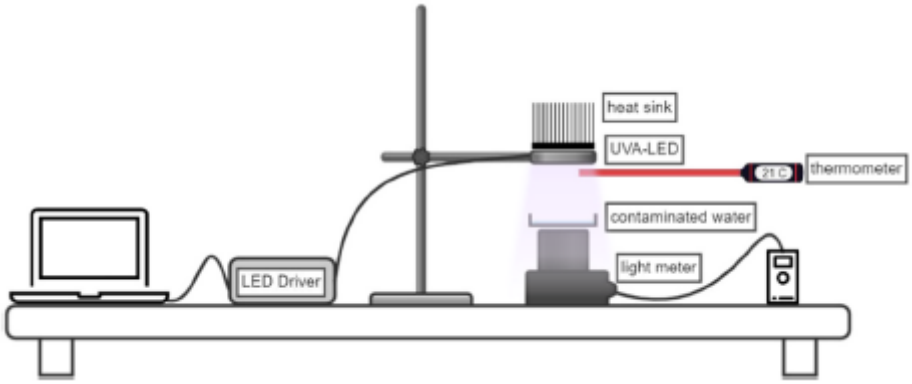


Figure 10 - Experimental setup [25]

1.4.1 Standard (continuous) illumination – Effect of different wavelengths

A group of researchers from the University of Tokyo led by K. Oguma in 2018 working on disinfecting *Escherichia coli*, *Bacillus subtilis spores* and *bacteriophage* using both the conventional mercury-based low-pressure UV and; 265 nm, 280 nm and 300 nm UV LEDs. Results showed that 265 nm was the most effective wavelength in reducing the effect of most of the bacteria, but also the 280 nm LED presented the lower energy consumption compared by the rest light sources [1]. Another article by Matsumoto, Tatsuno and Hasegawa in 2019 shows that the 265 nm wavelength LED could disinfect

many types of bacteria that had been artificially prepared in the laboratory such as *E. coli*, *V. cholerae* and *C. parvum* [2].

Sari, Heikki and Mike also mentioned a unique setup followed in their experiments in 2009, they created two reactors with a 120° viewing angle, one for 269 nm and the other was 276 nm, ten LEDs in each reactor, they were attached to a black plastic tube with 7 cm diameter and 20 cm height above the 25 ml sample water in the Petri dish, also a magnetic stirrer provided proper mixing. Furthermore, different type of water like Ultrapure water, nutrient water and nutrient water with humic acids were tested beside the *artificial E. coli.* The experiment concluded that the lower wavelength was more effective, although the slight difference in the results, even though the one emitting higher wavelength had doubled optical power compared to the other [7].

Würtele et al. from Berlin institute of technology were working on disinfecting laboratory cultured *Bacillus subtilies spores* using 269 nm and 282 nm LEDs in 2010. Two modules had been made, the first which is a static one consists of 269 nm 33 LEDs with 0.33 mW emission power at 20 mA per LED and was arranged in a grid of 1 LED/cm^2. The second module was an aluminum milled flow-through with 6 mm wide 5 mm depth channel covered by a 2 mm thick Suprasil® window which allows 90 % of the UV light to pass through, it is created using 282 nm 33 LEDs and 12 ml/min flow rate and were placed in three concentric circles with diameters of 1.5 cm, 3.5 cm and 5.2 cm, with the same current of 20 mA, the output optical power of the 282 nm LEDs was almost the double compared to the 269 nm module. The first model experiment setup was a little bit different since they placed the modules under the 6 cm diameter Petri dishes, and they put over the modules a 2 mm thick Suprasil® base which reduced the UV light by 10 %. All the LEDs in this article were almost similar in the current- voltage characteristics, at 20 mA the 269 nm LEDs took 5.8 voltage drop and the 6.3 volts for the 282nm LEDs. During the experiments samples of 1.5 ml were taken after 372, 248, 155, 62 and 0 seconds for the 269 nm LEDs, and 255, 170, 106, 43 and 0 seconds for the 282 nm LEDs. The 269 nm LEDs reached a higher level of inactivation than the 282 nm LEDs for the same applied fluence, and lower inactivation achieved by the 282nm LEDs was compensated by their higher photon flux. The team also observed a reduction by 40 % in the emitted optical power of the 269 nm LED after 100 hours of emission but with no change in the wavelength [8].

1.4.2 Pulsed vs. Continuous illumination

Also, Song, Taghipoir and Mohseni in 2018 used a different mode of operation with the UV-LEDs, using pulsed and continuous illumination to deactivate lab-cultured *E. coli* and *coliphage MS2* under 265 nm wavelength. A high output 265 nm LED was attached to a heatsink with a fan for heat dissipation and was connected to a thermocouple for temperature monitoring, the UV-LED was located above a 9 cm diameter glass Petri dish with 50 mL water sample and the distance between the UV LED and the water surface

was 2 cm. Their analysis indicates that the pulsed irradiation at 90 % and 75 % duty cycle produced slightly lower inactivation compared to the continuous mode [3].

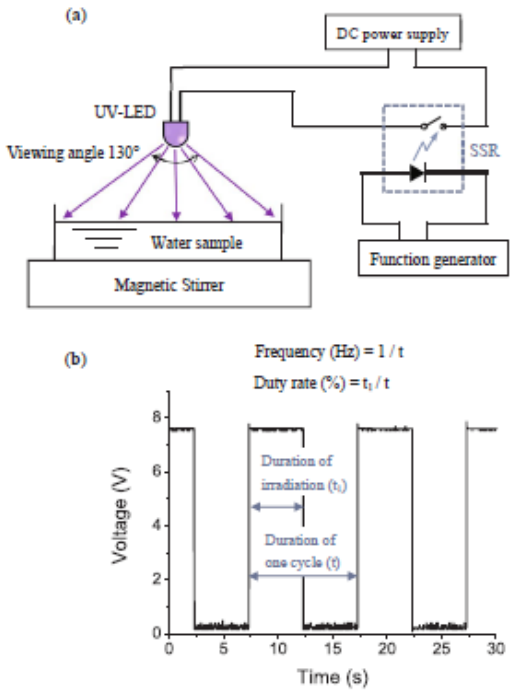


Figure 11 - Experiment setup for Song et al., (a) Schematic diagram of pulsed UV-LED circuits, (b) illustration of voltage waveform for pulsed UV-LED irradiation at 0.1 HZ frequency and 50 % duty cycle [3]

Similar to the previous technique, pulsed and continuous operation modes were used for *E. coli* and *MS-2* using a 15-watt low pressure mercury-arc lamps with 253 nm and various UV LEDs with different wavelengths (255 nm, 265 nm and 285 nm) by Sholter and Linden in 2019. Five duty cycles (10, 25, 50, 75 and 90 %) and three frequencies (1, 10 and 100 Hz) were examined. Results showed that there was no difference found between the *E. coli* inactivation at all frequencies for comparable duty rates and wavelengths. Besides, pulsed technique did not provide a significant benefit over continuous mode [5].

P.O. Nyangaresi, Yi and Goulong in 2019 did the same comparison between pulsed and continuous irradiation over artificial *E. coli*, and studied the effect of driving the LEDs with different currents and monitored temperature of the LED by measuring the solder temperature of the cathode terminal of the LED during the experiments. Nine UV LEDs with 265 nm and 280 nm with 0.88, 1.01 mW respectively were used in this experiment, with a voltage drop around 5.7 and 5.4 v with 20 mA for each LED. All nine UV LEDs were mounted on an aluminum PCB in order to boost the heat dissipation. The PCBs reactor was above a 60 mm diameter dish by 30 mm. 1 kHz, 5 kHz and 10 kHz pulse frequency were applied with 10 %, 25 %, 50 %, 75 % and 90 % duty cycles. As expected, the pulse radiation kept the LEDs temperature lower compared by the continuous radiation, while continuous irradiation caused the LEDs to increase and by rising up the driving current and a negative impact on wavelength and optical power was observed. And regarding

to the *E. coli* inactivation, the observed difference in the log inactivation of *E. coli* was only in the wavelength not the technique used, which the 265 nm UV LED had a 4.4 log inactivation which was slightly higher than 4.0 for the 275 nm, and there was no difference between pulsed mode and continuous mode [19].

Using the same previous wavelengths, 265 nm and 280 nm, Zou et al. at the University of Nanjing in 2019 applied the irradiation of low-power 1 mW 265 nm and 280 nm LEDs, as well as a high-power 30 mW 285 nm LED on lab-cultured *E. coli*. Three modules were created, the first for the only high-power LED, and the second and third modules have 9 LEDs each for the 265 nm and 280 nm wavelength. The modules were placed over an 8.6 cm diameter Petri dish, and the current was 20 mA for 265 nm and 280 nm for each LED, and 350 mA for the high-power 285 nm LED. Five different duty cycles were tested in this study including 5 %, 10 %, 20 %, 50 % and 100 % at 1 Hz, 10 Hz, 100 Hz and 1000 Hz. Surprisingly, the inactivation efficiency increased substantially as the duty cycle decreased from 100 % to 5 % within the same UV dose. The log inactivation enhanced using the pulsed 265 nm and 280 nm LEDs, and remarkably increased using the high-power 280 nm LEDs [21].

1.4.3 Simultaneous and sequential illumination modes

Another illumination technique used is combining more than ultraviolet wavelength (UV-C, UV-B and UV-A) to purify water. Song, Taghipour and Mohseni in 2018 used laboratory prepared *E. coli* and *Coliphage MS2* [4] and exposed to UV-LED irradiance using the two different operation mode: simultaneous and sequential modes. In the simultaneous mode, two UV LEDs with different wavelengths were turned on at the same time to expose their light over the water sample, while the sequential irradiation is functioning by turning on one UV LED for a period, and then turned off while other UV-LED with a different wavelength was turned on for another period of time. Three UV-LED chips with 265 nm, 285 nm and 365 nm wavelengths were used in this study over a 9 cm diameter Petri dish by 2 cm distance from the module. Moreover, a black box was covering the reactor during the experiment to prevent any potential photoreactivation by ambient light. Conclusions demonstrate that combining UV-C and UV-B always achieved additive effect on microorganism inactivation, nonetheless combining UV-A with UV-C/UV-B simultaneously or applying UV-A after UV-C/UV-B reduced the inactivation rate of *E. coli* due to the DNA repair effect of UV-A.

1.4.4 Combination of wavelength

Regarding combination of wavelengths, Li et al. from Tsinghua University in 2017 examined the effect of the combination of 265 nm LEDs using lab culture *E. coli* UV LEDs were compared to a mercury-based low pressure. Four LED units were used: 265 nm, 280 nm, the combination of 265 nm (50 %) + 280 nm (50 %), and 265 nm (25 %) + 280 nm (75 %). For the combination of 265 nm, two LED PCBs were connected in parallel. The PCBs where over a 2.6 cm diameter petri dish, while the low-pressure lamp was set

by 40 cm over an 8.6 cm Petri dish. It had been observed that the 265 nm LEDs were more effective than 280 nm LEDs and the low-pressure lamp in inactivation, and no significant effect was observed using the combination of 265 nm and 280 nm. Also, there was no reactivation performance seen after using 265 nm LEDs and low-pressure lamp, while the photoreactivation and dark repair appeared after using the 280 nm LEDs at low irradiation intensity [20].

By real wastewater, Chevremont et al. in 2012 studied the effect of single and coupled UV-A and UV-C LEDs. Four different wavelengths UV-LEDs were tested, 254 nm, 280 nm, 365 nm and 405 nm and combinations of 254/365 nm, 254/405 nm, 280/405 nm. The current was varying between the different LEDs from 46 mA up to 115 mA, and the optical power was 0.29, 0.55, 1.69 and 10.23 mW for the LEDs 254 nm, 280 nm 365 nm and 405 nm respectively. The 55 mm diameter Petri dish was placed 1 cm below the LEDs and filled by 10 mL of water and the exposure started from stayed for 60 seconds. *Mesophilic bacteria*, *Fecal streptococci*, *total coliforms* and *Fecal coliforms* had been selected to be disinfected. The results showed that combining 280/365 nm or 280/405 nm can achieve a remarkable reduction in *Mesophilic bacteria*, and they were more effective than the single irradiation for *fecal streptococci*, *total coliforms* and *fecal coliforms* [12].

Wan et al. in 2019 discussed the effect of the traditional mercury-based lamp versus the 280 nm and 265 nm LEDs and their combination. Laboratory cultured *Aspergillus niger*, *Penicilium polonicum* and *Trichoderma harzianum* which are three water-borne fungal species were tested in this study. 2 cm was the distance between the UV LEDs modules and the surface of the water inside the 4 cm diameter petri dish. For the LP UV lamp, 50 cm was the distance over Petri dish with a 12 cm diameter. The irradiance was 0.215 mW/cm^2 for the 265 nm LEDs, 0.214 mW/cm^2 for the 280 nm LEDs, 0.185 mW/cm^2 for the 265/280 nm combination UV-LEDs and 0.120 mW/cm^2 for the 254 nm for the lamp. Before the experiments, the UV LEDs modules were conducted to warm-up and reach a stable emission phase for 5 min and the 254 nm lamp went through the same process for 30 min. The results indicated that the inactivation performance of UV LEDs and their combination were more effective for fungal spores compared with the 254 nm LP UV lamp, while there were no significant differences observed among UV-LEDs, specially 280 nm and 265/280 nm which caused more damage for the *fungal spores* more than the LP lamp. However, the electrical energy consumption of UV LEDs was higher than that of the LP lamp [24].

In 2011, Chevremont, Farnet and Sergent concluded experiments in order to study the effect of the UV-A and UV-C over artificial *E. coli* and *Enterococcus faecalis* in wastewater. Four LEDs emitting 255 nm, 280 nm, 365 nm and 405 nm with 115 mA ,77 mA, 22 mA and 46 mA forward current respectively. Four parameters had taken place in their experiments: pH, bacterial density, exposure time and wavelength. Many different combinations were tried, but combining 280 nm and 365 nm or 280 nm and

405 nm effect over the bacteria were remarkable significant than using 365 nm alone, and after 60 seconds the absence of the bacteria was observed, the experiment design was almost identical to the researchers, setting the LEDs over the 55 mm diameter Petri dishes by a 1 cm distance [9].

The same way Kumiko et al. in 2013 performed many tests with single and various combinations of UV-LEDs using 265 nm, 280 nm and 310 nm wavelengths in 2013. The voltage drops for the UV-LEDs were 9.5, 8 and 6.1 v and the optical power was 0.7 mW, 1.3 mW and 1.1 mW for the 265 nm, 280 nm and 310 nm respectively at 20 mA. The experiments were done to deactivate lab culture *E. coli* microorganism using two different reactors, batch reactor and flow-through reactor. In the batch reactor, which contained an array for nine LEDs, all nine LEDs emitted a single wavelength, either 265 nm, 280 nm or 310 nm. The batch reactor was above a 34.5 mm diameter Petri dish by 17 mm. While the flow-through contains of three arrays and every single array had 10 similar LEDs. The three arrays were bounded together in a triangular shape and placed inside a 16 mm diameter cylinder. The emission of 265 nm achieved over 6 log deactivation rates with 10 mJ/cm^2 dose in the batch reactor, and 16.4 mJ/cm^2 in the flow-through. Similarly, the 280 nm achieved almost 4 log deactivation rates at 13.8 and 25.5 mJ/cm^2 in the batch reactor and the flow-through reactor respectively. The 310 nm required 56.9 mJ/cm^2 for 0.6 log inactivation in the batch reactor which was less effective than the emission of 268 nm and 280 nm. And finally, the combined emissions were less efficient compared by the single emission [10].

Following Nyangaresi et al. in 2018 with the same approach of comparing single and combined irradiation of UV-LEDs, the group from the Xiamen university in China picked the 267 nm, 275 nm and 310 nm UV peak emission and combined 267 nm/275 nm, 267 nm/310 nm and 275 nm/310 nm to applied their emission on a batch water disinfection system and evaluate the process by using lab culture *E. coli* as a bacterium model. The output optical power for the LEDs was 1.8 mW, 1.6 mW and 1.3 mW for 265 nm, 275 nm and 310 nm respectively, and the voltage drop was between 3.9 v and 6 v at 20 mA. 16 mL was placed in a 60 mm diameter petri dish and over it by 2.2 cm was the UV-LEDs. The 267 nm had the highest inactivation efficiency than other UV-LEDs and 310 nm was the least, nevertheless reactivation observed after 267, 275, 267/275 and 275/310 nm irradiations [11].

Sara et al. from Colorado university performed an experiment using a commercial reactor from AquiSense Technologies system to compare the irradiation effect of 260 nm, 280 nm LEDs and the combination of them against a medium-pressure and low-pressure UV mercury lamp. Four lab culture microorganisms were tested: *E. coli*, *MS2 coliphage*, *human adenovirus type 2* and *Bacillus pumilus spores*. A 3.5 cm diameter Petri dish filled with 5 mL sample was placed by 4 cm distance from the UV source which had a varied irradiance from 0.19 mW/cm^2 to 0.55 mW/cm^2 for the UV LEDs, and from 0.35 mW/cm^2 to 1.17 mW/cm^2 for the MP UV lamp and 0.3 to 0.75 mW/cm^2 for the LP UV.

The data proved that the five UV sources inactivation rate of *E. coli* was high, but the 260 nm wavelength was the most effective. For *HAdV2* and *B. pumilus*, the MP UV lamps was the most powerful. Regarding to the electrical efficiency, the LP UV lamp was the most efficient for inactivating *E. coli* and *MS2*, the LP and MP UV mercury lamps were equally efficient for *HAdV2* and *B. pumilas spores* [23].

Figure 12 - AquiSense Technologies system [6]

1.4.5 Turbidity

Kristina, Dena and Christopher in 2013 is fully justifying the effect of the turbidity of water during the UV disinfection process. They observed that the more contaminated in water, the more UV light power needed for the disinfection process. Two water samples types were used in the experiment, the first was real wastewater and the second was prepared water contained a pure culture of *E. coli*. Seven 260 nm flat lens LEDs were used for the research, they have 6.5 voltage drop and 20 mA per LED connected in series to a current-limiting resistor ranged from 100 Ω to 380 Ω, and 9 v alkaline battery. The optical power ranged from 45 mW to 182 mW and the average was 130 mW, this fluctuation corresponding to the battery age. Each LED was connected to an independent battery. The same common technique was used except that they did not set any distance between the LEDs surface and the water sample, in other words they lowered the LEDs until the optical lens made contact with the water sample surface. The leads of the LEDs were protected by extending these an extra 100 cm using 0.25 mm 30 AWG blue wrapping wire, the insulation of the wrapping wire was stripped by about 3 cm to cover the slot producing a proper fit. Also, a plastic o-ring was placed around the lip of each UV LED to complete the water-tight seal. The samples volume ranged from

10 ml to 100 ml and the time period of the experiments was between 20 min and 50 min. The analysis illustrated that water quality has a significant impact on disinfection efficiency. For the waste water results, a minimal reduction of bacteria was measured from 20 min to 40 min period, however the artificial samples showed a notable reduction with the *E. coli* [13].

1.4.6 Commercial prototypes

In 2019, Peter et al. of Cranfield University conducted a study on the efficacy of using Typhon Treatment Systems, consisting of 1000 UV LED lights with 275 nm wavelength and 100 mW optical power, for disinfecting wastewater at a treatment plant in the north of England. This full-scale reactor was able to disinfect 6 mega-liters of water per day, and its performance was evaluated using the microorganisms *Male-specific bacteriophage (MS2)* and *E. coli*. The results indicated that this UV-LED reactor was at least as effective as traditional mercury UV reactors under the specific water quality and operational conditions examined. This study represents the first successful implementation of a full-scale UV-LED reactor for disinfecting pathogenic microorganisms in drinking water at a municipal treatment facility [14].

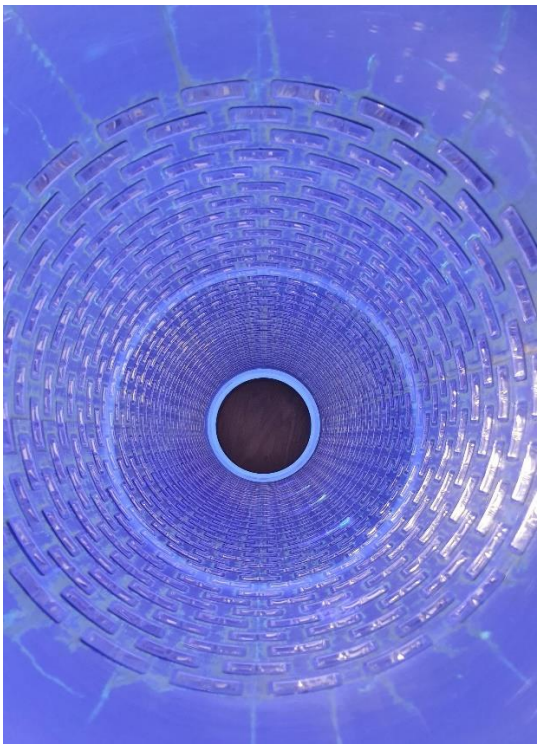


Figure 13 - Typhon treatment system inner tube [22]

Figure 14 - Typhon treatment system reactor [14]

1.4.7 Cost efficiency

In terms of cost efficiency, Elaheh el at. From Napoli university in 2022, investigated the deactivation of *Legionella pneumophila* and *Lengionella dumoffii* using a UV-A wavelength since it is significantly cheaper than the UV-B and UV-C. A 0.3 A with a 22.516±0.436 mW/cm^2 output of density LED was mounted to a heatsink to dissipate the generated heat, even so it has a rating of 4.5 A and 20 W/cm^2. The distance between the UV-A LED and the 35 mm diameter Petri dish was 38 mm, The results illustrated that by applying 1700 mJ/cm^2 UV dose, the inactivation rate for *Legionella pneumophila* 99.89 % and 99.1 % for *Lengionella dumoffii* [25].

1.4.8 PCB temperature effect

As already known that the biggest opposition for the LEDs performance is the temperature, the more temperature rises the lower optical output can be obtained. That is why Huang el al. in 2021 focused their study on the different ways to improve the thermal dissipation of the LEDs while they are functional. Therefore, 280 nm LEDs were chosen in this topic and driven using many different currents from 20 mA up till 200 mA, not only that but also the thickness of the substrate materials. *E. coli* was the tested microorganism in this experiment, including two different materials where the LEDs were placed on: Copper (Cu) and Aluminum (AIN). Various different thicknesses for the copper layer were used: 70 µm, 100 µm ,130 µm and 200 µm, and 500 µm for Aluminum. All these modules where 1 cm over the 8.5 cm diameter Petri dish. The results revealed that the thickness of the metal is not playing a significant role in the heat dissipation. However, the large area of the LED evidently exhibits high thermal dissipation. Also, the 280 nm LEDs were able to deactivate 95 % of the *E. coli* in just 60 seconds [26].

1.4.9 Stirring speed and exposure time

Different aspects had been taken by Seul el al. in 2021 from Korea university such as the exposure time, stirring speed and volume of water during conducting a study of disinfecting prepared bacteria like *E. coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella Typhimurium* and *Listeria monocytogenes*. A 278 nm UC-C LED with a forward voltage and current of 7.5 v and 20 mA, respectively was fixed at the bottom of a cylindrical tube with a 15.2 cm diameter and 8.4 cm height. The time of exposure was from 15 to 60 minutes, and a 10 mL sample was withdrawn every different interval. The analysis demonstrated that the higher stirring speed and longer exposure time the more inactivation rate for all the bacteria [27].

1.4.10 3D printed reactors

Variety of reactors had been designed and created by the university of Taipei in Taiwan [28]. Wang and Lin in 2021 created four reactors were made using a 3D printer, three of them with internal tubes with different pitches (10 mm, 20 mm and 30 mm) surrounding a quartz tube which contained the LEDs mounted on an aluminum rod to provide sufficient heat dissipation, and the last reactor was just without a tube. 24 278 nm LEDs with 10 mW optical power at 100 mA current each were their choice, and in each side of the aluminum rode were soldered 6 LEDs. A pump and water flow meter were installed to monitor the flow of the water inside the reactors. This study focused on the effect of the flow rate of the water and spiral pitch in order to disinfect the laboratory prepared *E. coli*. The analysis suggested that the 10 mm tube reactor achieved the highest inactivation rate among the other tube reactors, besides the tubeless reactor was the most ineffective one to deactivate the bacteria compared by the other three reactors with tubes due to the lack of mixing effect of fluid particles inside it. Besides reducing the flow rate from 120 mL/min to 60 mL/min increased the inactivation under the UV LEDs.

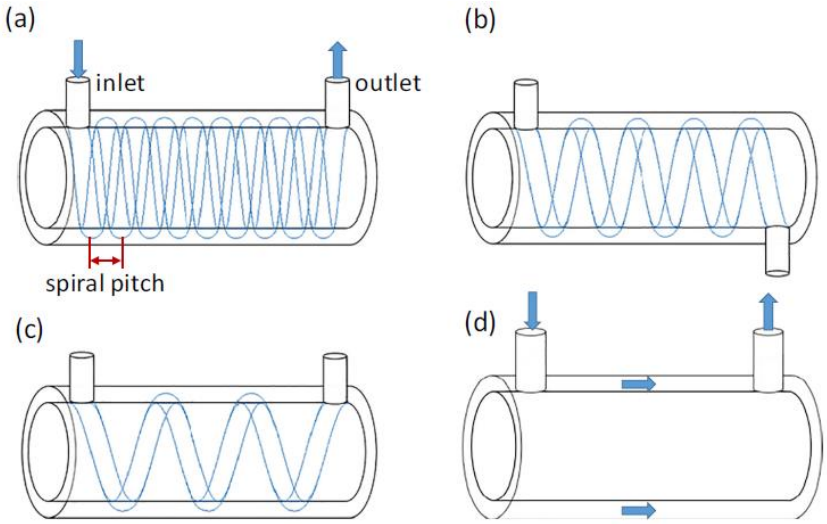


Figure 15 Flow reactor with different spiral pitches (a) 10 mm, (b) 20 mm, (c) 30 mm and (d) flow-through reactor [28]

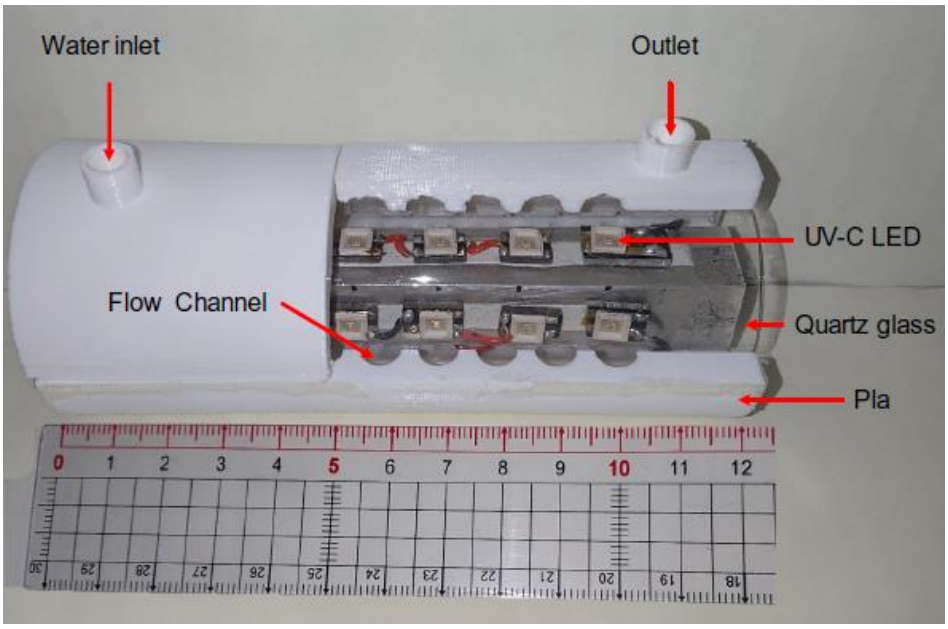


Figure 16 - Image of a flow reactor with a spiral channel using UV-C LEDs [28]

1.4.11 UV light combined with visual light

Using the UV-A and blue light, the center of food and bioconvergence in Korea led by Kim and Kang in 2021 employed 395 nm, 405 nm, 415 nm and 425 nm over artificial *E. coli*, in addition to checking the reactive oxygen species (ROS). Therefore, they created a black coated chamber from aluminum to block the unnecessary external light and blue light reflection. A single LED with a heatsink was attached in the upper part of the black chamber, 4 cm was distance between the LED and the 60 mm diameter Petri dish. A 500 mA constant current driving each LED. All the wavelengths used in this experiment were able to stop the effect of the *E. coli* after 70 mJ/cm^2 at 81 minutes [29]

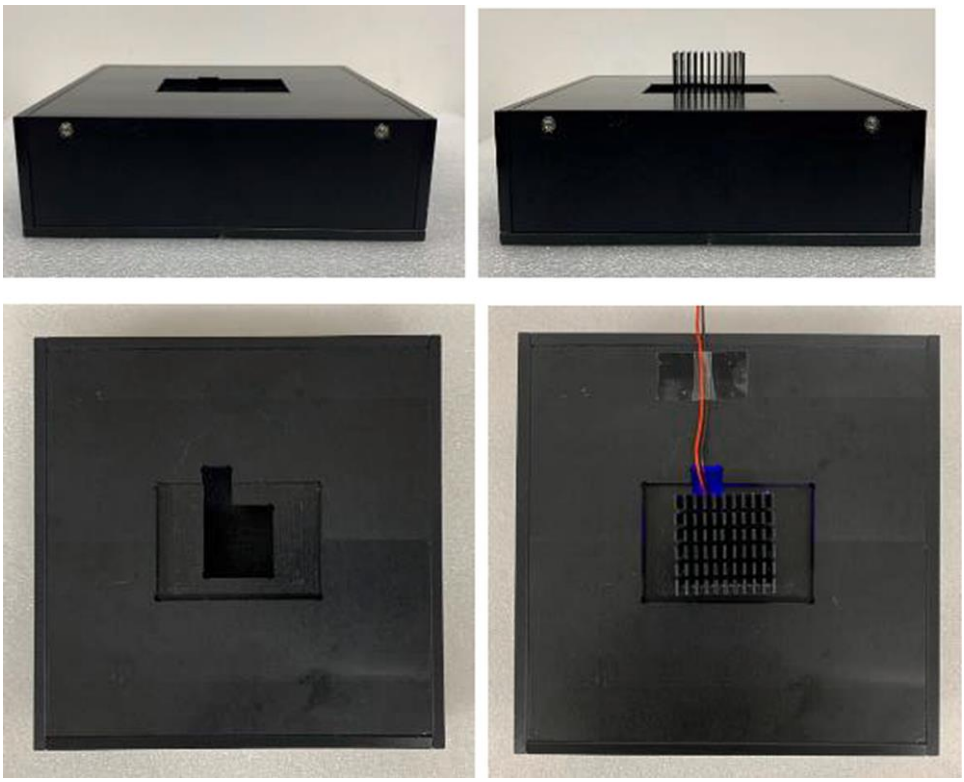
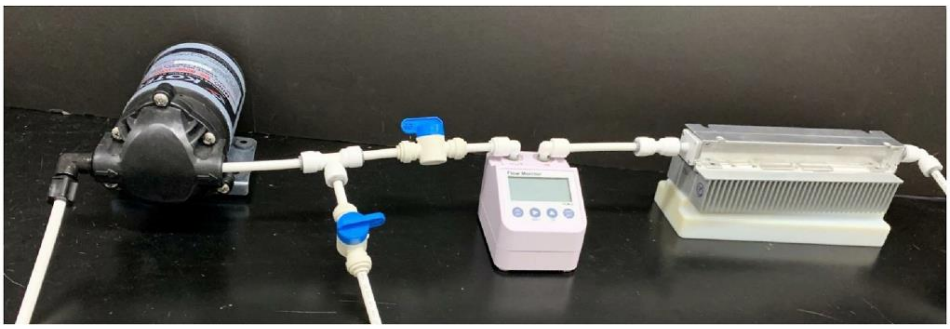


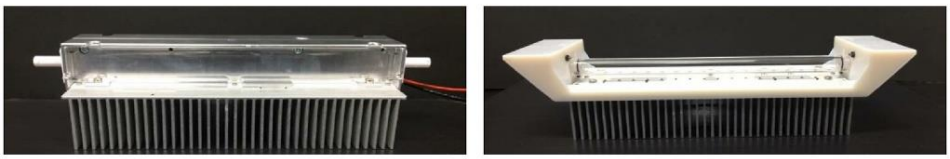
Figure 17 - A picture of the aluminum reactor used by Kim and Kang in their experiments [29]

Kim and Kang also conducted another study in 2020 by creating a different water flow reactor to disinfect lab prepared *E. coli*. Various numbers of the same LED chip had been tested 18, 21 and 33 with 280 nm wavelength with a range between 150 mA to 900 mA operating current. The LEDs PCB were mounted over a heatsink with the dimensions of 6.5 x 19 cm. Over the PCB by 1 cm was placed a quartz tube connected to a water pump and water flow sensor. The experiment was conducted in two ways, first without placing anything over the PCB and the quartz tube, and second by adding an aluminum reflector of over the PCB and the quartz tube. The data revealed that the change of number of LEDs didn't make a significant difference, whereas adding the aluminum reflector raised up the inactivation process 1.5 times compared to the one without the reflector [30].

(a)



(b)



(c)

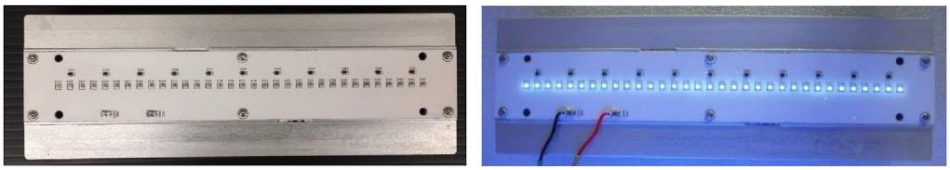


Figure 18 - The reactor used by Kim and Kang in their second study. (a) the entire system, (b) UV-C LEDs array module with or without the aluminum reflector, (C) 33 UV-C LEDs array [30]

As mentioned, the researchers are trying to replace the traditional UV mercury lamp with UV LEDs, applying variety of different wavelengths over different microorganisms. Different techniques are being used to archive the highest efficiency in terms of inactivation the duration of the inactivation process. As a result from the articles, the most effective wavelengths are in the range of the UV-C and the least is the UV-A for most of the bacteria. Also, the techniques used and the experiments setup have a crucial effect on the results, for instance the most powerful technique is the continuous mode for almost all the bacteria, however the pulsed mode was only effective electrically and not suitable for all kinds of bacteria. Followed by the combination techniques which was effective with some microorganisms. On top of that, the distance between the UV light source and the water has a significant impact on the duration of the disinfection process, the wider distance between the LEDs and the surface of the water, the longer time needed to eliminate the effect of the bacteria. And lastly but not least, mixing the water during the deactivation process leads to decrease in the disinfection process time. Finally, the turbidity is one of the most important parameters in the disinfecting process and it can make the disinfection process takes much longer time or even make the inactivation process unreachable.

1.5 Reference

[1] S. Rattanakul and K. Oguma, “Inactivation kinetics and efficiencies of UV-LEDs against Pseudomonas aeruginosa, Legionella pneumophila, and surrogate microorganisms,” *Water Res.*, vol. 130, pp. 31–37, Mar. 2018.

[2] T. Matsumoto, I. Tatsuno, and T. Hasegawa, “Instantaneous Water Purification by Deep Ultraviolet Light in Water Waveguide: Escherichia Coli Bacteria Disinfection,” *Water*, vol. 11, no. 5, p. 968, May 2019.

[3] K. Song, F. Taghipour, and M. Mohseni, “Microorganisms inactivation by continuous and pulsed irradiation of ultraviolet light-emitting diodes (UV-LEDs),” *Chem. Eng. J.*, vol. 343, pp. 362–370, Jul. 2018.

[4] K. Song, F. Taghipour, and M. Mohseni, “Microorganisms inactivation by wavelength combinations of ultraviolet light-emitting diodes (UV-LEDs),” *Sci. Total Environ.*, vol. 665, pp. 1103–1110, May 2019.

[5] K. Sholtes and K. G. Linden, “Pulsed and continuous light UV LED: microbial inactivation, electrical, and time efficiency,” *Water Res.*, vol. 165, p. 114965, Nov. 2019.

[6] https://aquisense.com/products/research/pearllab-beam/

[7] S. Vilhunen, H. Särkkä, and M. Sillanpää, “Ultraviolet light-emitting diodes in water disinfection,” *Environ. Sci. Pollut. Res. Int.*, vol. 16, no. 4, pp. 439–442, Jun. 2009.

[8] M. A. Würtele *et al.*, “Application of GaN-based ultraviolet-C light emitting diodes--UV LEDs--for water disinfection,” *Water Res.*, vol. 45, no. 3, pp. 1481–1489, Jan. 2011.

[9] A.-C. Chevremont, A.-M. Farnet, M. Sergent, B. Coulomb, and J.-L. Boudenne, “Multivariate optimization of fecal bioindicator inactivation by coupling UV-A and UV-C LEDs,” *Desalination*, vol. 285, pp. 219–225, Jan. 2012.

[10] K. Oguma, R. Kita, H. Sakai, M. Murakami, and S. Takizawa, “Application of UV light emitting diodes to batch and flow-through water disinfection systems,” *Desalination*, vol. 328, pp. 24–30, Nov. 2013.

[11] P. O. Nyangaresi, Y. Qin, G. Chen, B. Zhang, Y. Lu, and L. Shen, “Effects of single and combined UV-LEDs on inactivation and subsequent reactivation of E. coli in water disinfection,” *Water Res.*, vol. 147, pp. 331–341, Dec. 2018.

[12] A.-C. Chevremont, A.-M. Farnet, B. Coulomb, and J.-L. Boudenne, “Effect of coupled UV-A and UV-C LEDs on both microbiological and chemical pollution of urban wastewaters,” *Sci. Total Environ.*, vol. 426, pp. 304–310, Jun. 2012.

[13] K. Y. Nelson, D. W. McMartin, C. K. Yost, K. J. Runtz, and T. Ono, “Point-of-use water disinfection using UV light-emitting diodes to reduce bacterial contamination,” *Environ. Sci. Pollut. Res. Int.*, vol. 20, no. 8, pp. 5441–5448, Aug. 2013.

[14] Jarvis, P., Autin, O., Goslan, E. H., & Hassard, F. (2019). Application of ultraviolet light-emitting diodes (UV-LED) to full-scale drinking-water disinfection. *Water (Switzerland)*, *11*(9). https://doi.org/10.3390/w11091894

[15] https://www.uvsystem.in/what-is-uv/

[16] https://www.larsonelectronics.com/images/product/LightingFacts/266985.PDF

[17] https://www.larsonelectronics.com/images/product/EyeSafety/272823.PDF

[18] G. Ali Mansoori, Pirooz Mohazzabi, Percival McCormack, Siavash Jabbari, "Nanotechnology in cancer prevention, detection & treatment bright future lies ahead," *World Review of Science, Technology and Sustainable Development, Vol. 4*, 2007.

[19] P. O. Nyangaresi, Y. Qin, G. Chen, B. Zhang, Y. Lu, and L. Shen, "Comparison of the performance of pulsed and continuous UVC-LED irradiation in the inactivation of bacteria," *Water Res.*, vol. 157, pp. 218–227, Jun. 2019.

[20] G.-Q. Li, W.-L. Wang, Z.-Y. Huo, Y. Lu, and H.-Y. Hu, "Comparison of UV-LED and low pressure UV for water disinfection: Photoreactivation and dark repair of Escherichia coli," *Water Res.*, vol. 126, pp. 134–143, Dec. 2017.

[21] X.-Y. Zou *et al.*, "Enhanced inactivation of E. coli by pulsed UV-LED irradiation during water disinfection," *Sci. Total Environ.*, vol. 650, no. Pt 1, pp. 210–215, Feb. 2019.

[22] https://www.typhontreatment.com/

[23] S. E. Beck *et al.*, "Evaluating UV-C LED disinfection performance and investigating potential dual-wavelength synergy," *Water Res.*, vol. 109, pp. 207–216, Feb. 2017.

[24] Q. Wan *et al.*, "Comparison of UV-LEDs and LPUV on inactivation and subsequent reactivation of waterborne fungal spores," *Water Res.*, vol. 173, p. 115553, Apr. 2020.

[25] E. Allahyari *et al.*, "A sequential utilization of the UV-A (365 nm) fluence rate for disinfection of water, contaminated with Legionella pneumophila and Legionella dumoffii," *Environ. Pollut.*, vol. 304, p. 119224, Jul. 2022.

[26] S.-Y. Huang, J.-C. Lin, X.-Q. Huang, and W.-K. Wang, "Large-area 280 nm LED flexible sterilization light source with improved thermal performance," *Optik* , vol. 248, p. 168109, Dec. 2021.

[27] S.-K. Park *et al.*, "Bactericidal effect of ultraviolet C light-emitting diodes: Optimization of efficacy toward foodborne pathogens in water," *J. Photochem. Photobiol. B*, vol. 222, p. 112277, Sep. 2021.

[28] C.-P. Wang and W.-C. Lin, "Combination of ultraviolet-C light-emitting diodes and a spiral-channel configuration in a water disinfection reactor," *Journal of Water Process Engineering*, vol. 42, p. 102160, Aug. 2021.

[29] D.-K. Kim and D.-H. Kang, “Efficacy of light-emitting diodes emitting 395, 405, 415, and 425 nm blue light for bacterial inactivation and the microbicidal mechanism,” *Food Res. Int.*, vol. 141, p. 110105, Mar. 2021.

[30] D.-K. Kim and D.-H. Kang, “Investigation of a new UVC LEDs array continuous type water disinfection system for inactivating Escherichia coli O157:H7 according to flow rate and electrical energy efficiency analysis,” *Food Control*, vol. 119, p. 107470, Jan. 2021.

[31] Kowalski, W. (2009). Ultraviolet Germicidal Irradiation Handbook. In *Ultraviolet Germicidal Irradiation Handbook*. https://doi.org/10.1007/978-3-642-01999-9

[32] https://clearwaterind.com/how-sedimentation-water-treatment-works-and-how-to-make-it-efficient/

[33] https://www.boe.es/buscar/act.php?id=BOE-A-1996-7159#:~:text=Ayuda-,Real%20Decreto%20509%2F1996%2C%20de%2015%20de%20marzo%2C%20de,de%2029%2F03%2F1996

[34] https://www.google.com/url?client=internal-element-cse&cx=013579175504638608352:lrebgzono2s&q=https://www.miteco.gob.es/es/agua/publicaciones/Manual_para_la_gestion_de_vertidos_tcm30-137170.pdf&sa=U&ved=2ahUKEwic9puoy7f7AhVhWqQEHdVNB3gQFnoECAYQAQ&usg=AOvVaw0Luc6bCUWvcvrJzur6nyUU

[35] https://www.miteco.gob.es/es/agua/publicaciones/03_Manual_Directiva_91_271_CEE_tcm30-214069.pdf

[36] https://eur-lex.europa.eu/resource.html?uri=cellar:5c835afb-2ec6-4577-bdf8-756d3d694eeb.0008.02/DOC_1&format=PDF

Chapter 2: Objectives

General objective

Then main objective of this PhD thesis is **to explore the potential of UV-LEDs to disinfect the real effluent of a wastewater plant by designing, manufacturing and testing a novel prototype based on UV-LEDs**. This prototype could be implemented in the tertiary treatment stage of wastewater plants, allowing a safe reuse of the effluent for new uses according to the Spanish legislation (agricultural, industrial, etc.) thus reducing the environmental impact of conventional technologies.

Specific objectives

1. To design and manufacture novel prototype at laboratory level that disinfects the raw water within a Petri dish of at least 60 mm diameter, studying the optical characterisation of the UV-LEDs, the electronic PCB design and arrangement of UV-LEDs, the cooling system and the total UV irradiance.

2. To determine the optimum UV-LED wavelength for inactivation of each of the studied microorganisms (*Escherichia coli*, *Enterococcus faecalis* and *Clostridium perfringens*) using real environmental strains from the effluent of the municipal wastewater treatment plant of Linares.

3. To determine the UV dose for inactivation for each microorganism and wavelength (time of exposure and UV irradiance).

4. To analyse the transmittance losses of the UV-light from the LEDs through the water to determine a maximum water depth along with the effect of turbidity.

5. To verify the law of reciprocity for water disinfection using UV-LEDs. High power and low power UV-LEDs will be used for treating wastewater with the same UV dose. Microbiological content (*Escherichia coli*, *Enterococcus faecalis* and *Clostridium perfringens*) will be analysed to observe if under the same UV dose but different initial irradiance the bacteria inactivation is the same.

Chapter 3: UV-LEDs reactor prototype: Design, fabrication and set-up

3.1 Prototype design

The UV-LEDs reactor prototype design proposed for this PhD thesis follows the schematics and geometry of the most used reactor type in the scientific literature for starting works on UV-LEDs, as it is flexible and allows for measurements of multiple parameters easily (irradiance, transmittance losses in water, LEDs temperatures, etc.), as well as for variations on the experimental conditions (LEDs wavelengths, optical power, etc.) [1-13]. As it is shown in Figure (1), it consists of a set of UV-LEDs connected in a PCB board that illuminates the area of a Petri dish containing the raw water (water to be treated). Since the power density of the UV-LEDs decreases gradually with the rise of temperature, it is necessary to use a heatsink with active cooling to maintain the temperature and so the stability of the power density. A heatsink with a fan was attached to the LEDs PCB boards using thermal paste for the interface surfaces. For the experimentation, the UV-LEDs are placed at a certain distance above the water surface in the Petri dish.

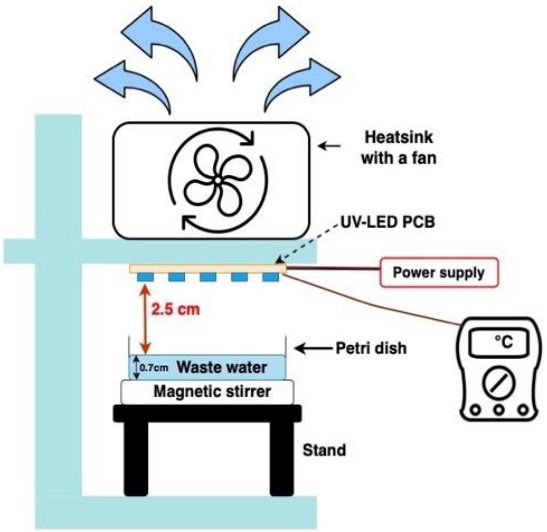


Figure 1 - Experiment set-up showing the scheme for wastewater exposure to UV-LEDs.

The key point of the prototype design is the electronic interconnection of the UV-LEDs in the PCB board and its soldering. In the next section we describe it thoroughly.

3.1.1 UV-LEDs characteristics and PCB design for interconnection

The conventional industrial mercury-based UV lamps are easy to install and operate, since they come with their terminal connectors or can be brought separately. However, this is not the case with the UV LEDs as they need to be placed on an electronic board

designed according to the electronic specifications of the LED itself and the designed interconnection in a group.

Regarding the material of the printed circuit board or PCB for short, a wide variety of electronic boards are available from different materials, but the most common ones are FR4 which contain fiber glass epoxy panel between its conductive layers for isolation. Another option is the aluminum PCB, which has larger thermal heat dissipation but with higher costs and only one conductive layer. On the contrary to the FR4 that can be built with up to 12 or 14 conductive copper layers. In figure (2), a clear demonstration for the cross section of the FR4 board(left) and the Aluminum(right).

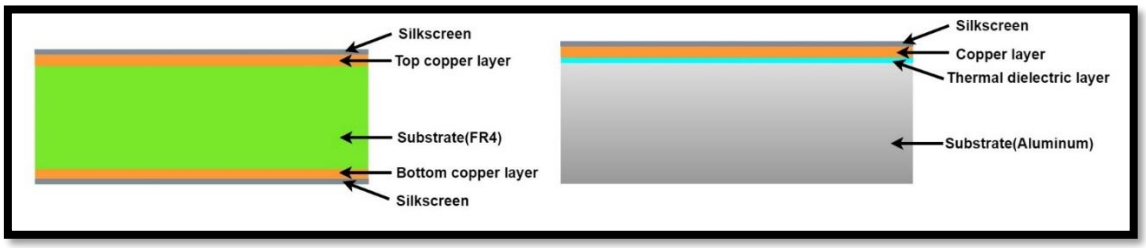


Figure 2 - PCBs cross section

Regarding the UV-LEDs, they can present two different structures: first is the LED with through hole leads (TH), where these leads should be soldered from the other conductive layer of the electronic board. The second type is the Surface Mounted Device (SMD) which is soldered directly on the copper layer where it is laying on.

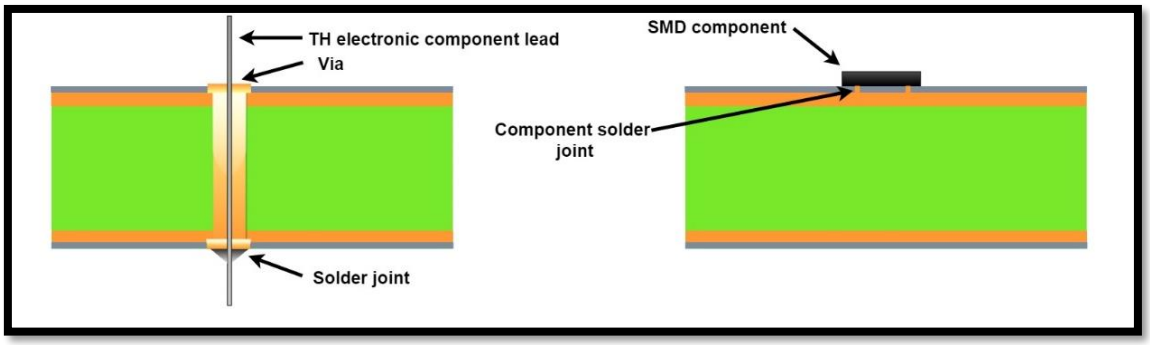


Figure 3 - Through hole technology vs. SMD technology

SMD components come in many different footprints, so this is why a special PCB design is needed for each type of SMD LED. In every single LED datasheet there is a recommended footprint to eliminate the possibility of short circuit the LED during the soldering and then operation. For that reason, LEDs footprints are a crucial part during designing the PCBs. In figure (4) we can observe the actual footprint of a SMD LED (back view), and the recommended footprint for the same SMD LED in the datasheet in figure (5).

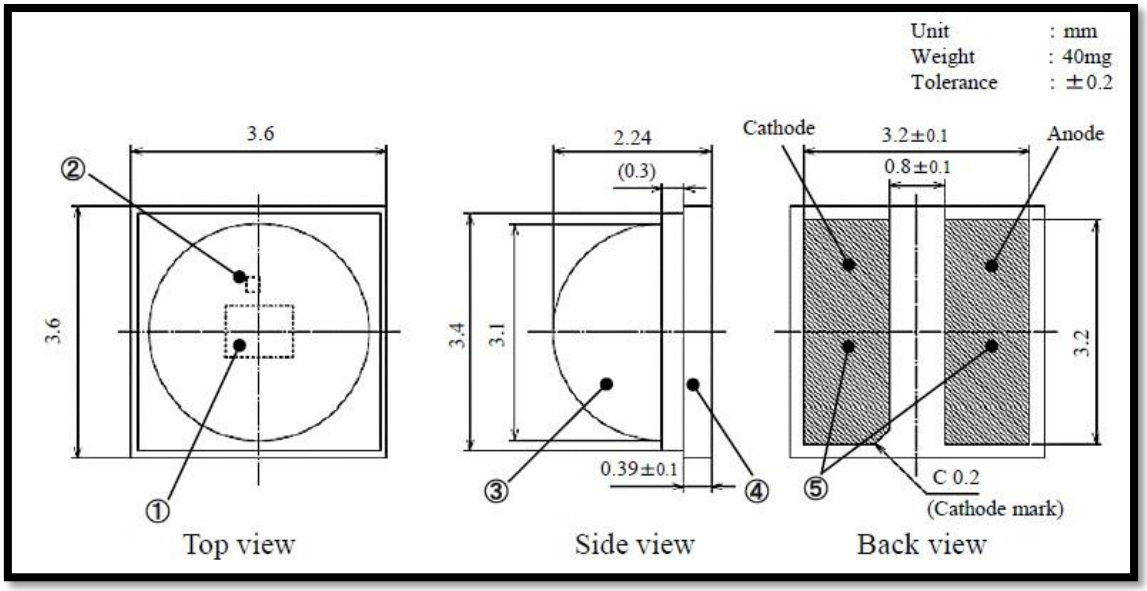


Figure 4 - The 3-views of a SMD LED

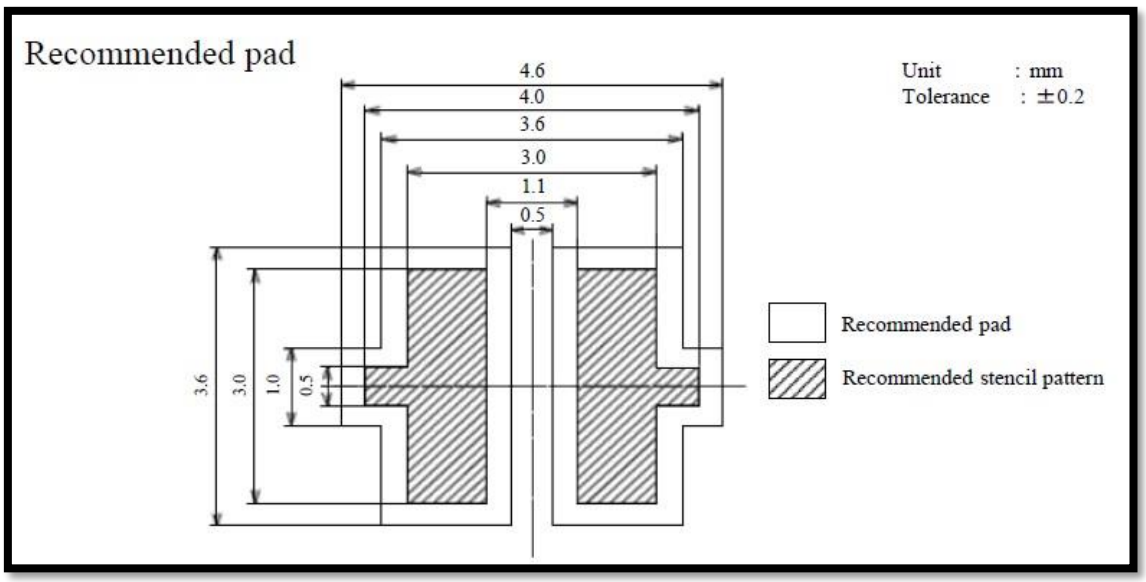


Figure 5 - The recommended footprint of a SMD LED datasheet

The main objective for designing the UV-LEDs configuration within the PCB is that they are arranged in the board according to their optical characteristics so they ensure homogeneous illumination on the Petri dish that contains the water to be treated. The Petri dish diameter is 55 mm, so depending on the type of LED (size, optical power) a different number can be required as well as a specific arrangement to comply with the needed illumination characteristics. Figure (6) show different possible arrangements for low-power UV-LEDs (1-2 mW) and medium-power UV-LEDs (50-60 mW):

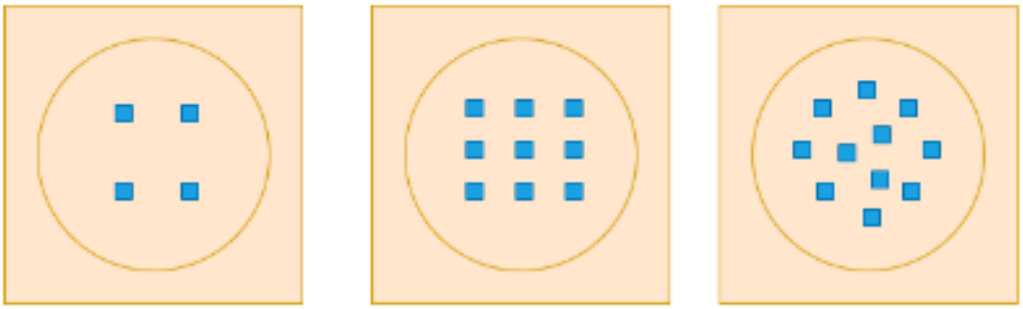


Figure 6 - LED arrangement according to how many they are and the diameter of the Petri dish

Design of the PCB using electronic design software: Orcad and Easyeda.

Both Orcad and Easyeda PCB design programs were used to design the footprints and the PCBs of the project. Here we are describing and illustrating an example of design using Easyeda for designing the board containing four UV-LEds of 50 mW at 275 nm.

Easyeda "www.easyeda.com" is an online platform for designing PCBs. By opening the website, signing up and logging in, the welcome screen will show up as in figure (7).

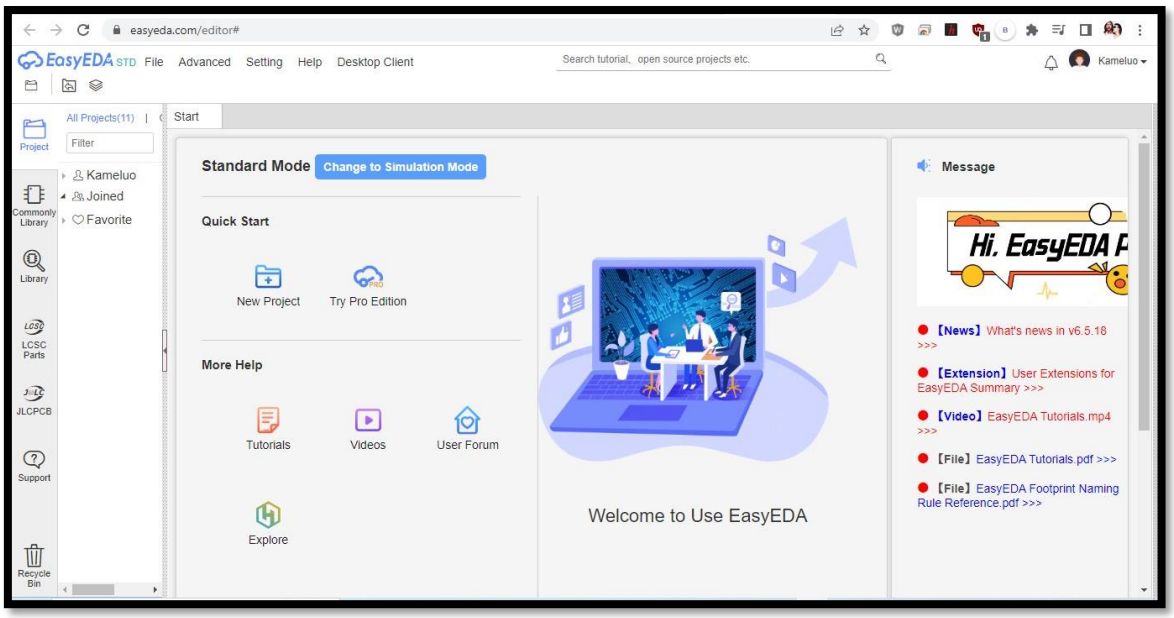


Figure 7 - Welcome screen of Easyeda online platform

Now, a new project should be created, from the upper left menu bar in **File>New>Project** or by right click the account name and select **New Project**.

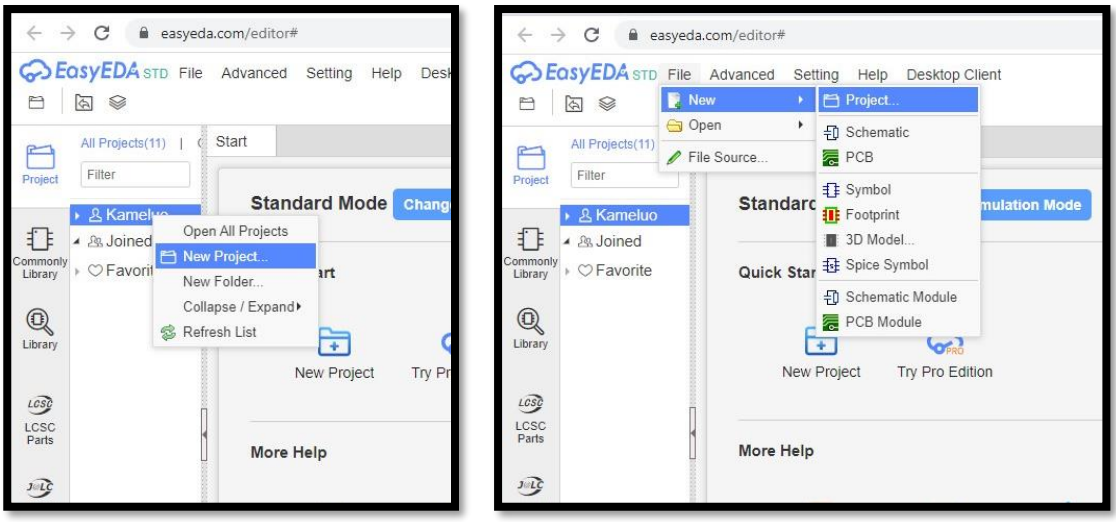


Figure 8 - Creating new project

A creating new project window will pop up where the name and the description of the project can be written and saved like in figure (8), then a schematic page will appear, by right click the project folder on the left menu **New PCB** we can create a layout page as in figure (9).

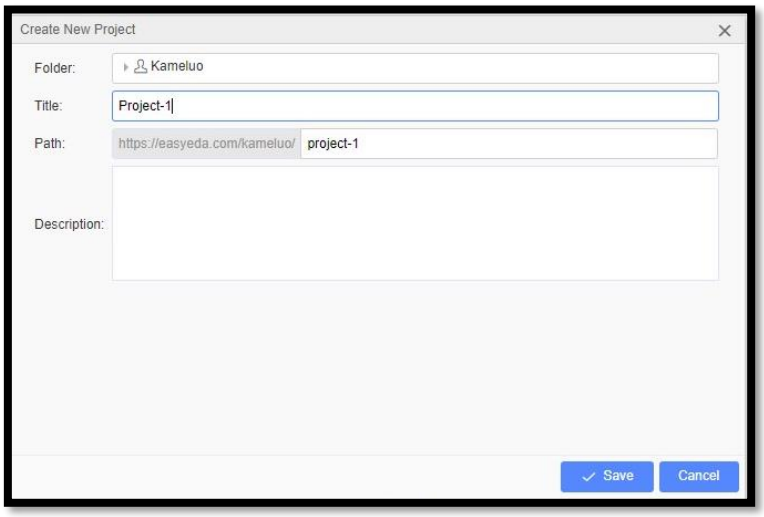


Figure 9 - Naming the saving the project

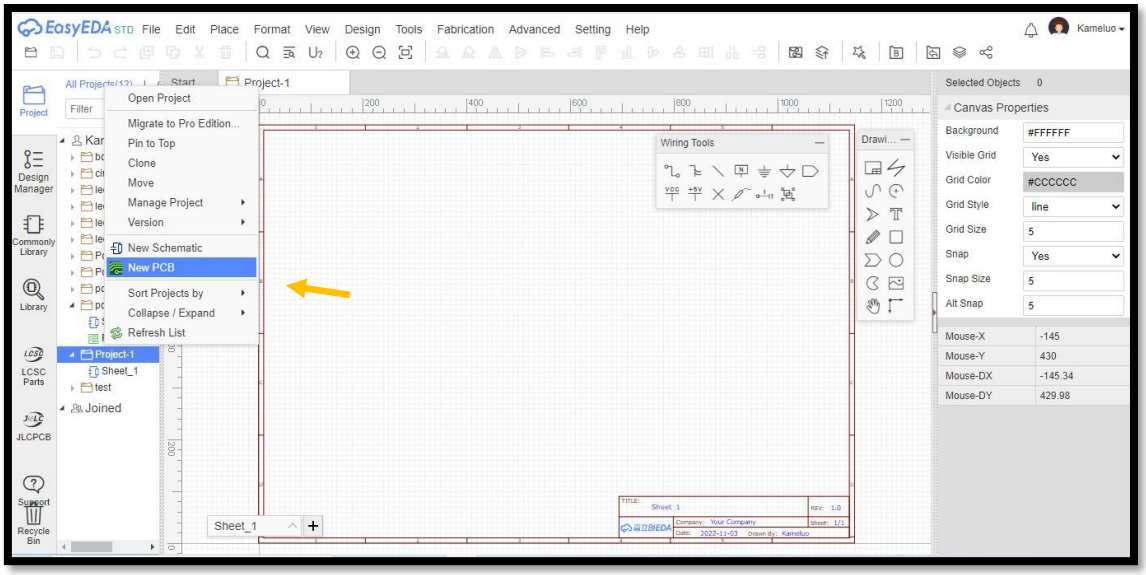


Figure 10 - Creating the layout page

The layout page will appear and a window will ask for the dimenstion of the PCB, but we will leave it for now like it is and click **Apply**.

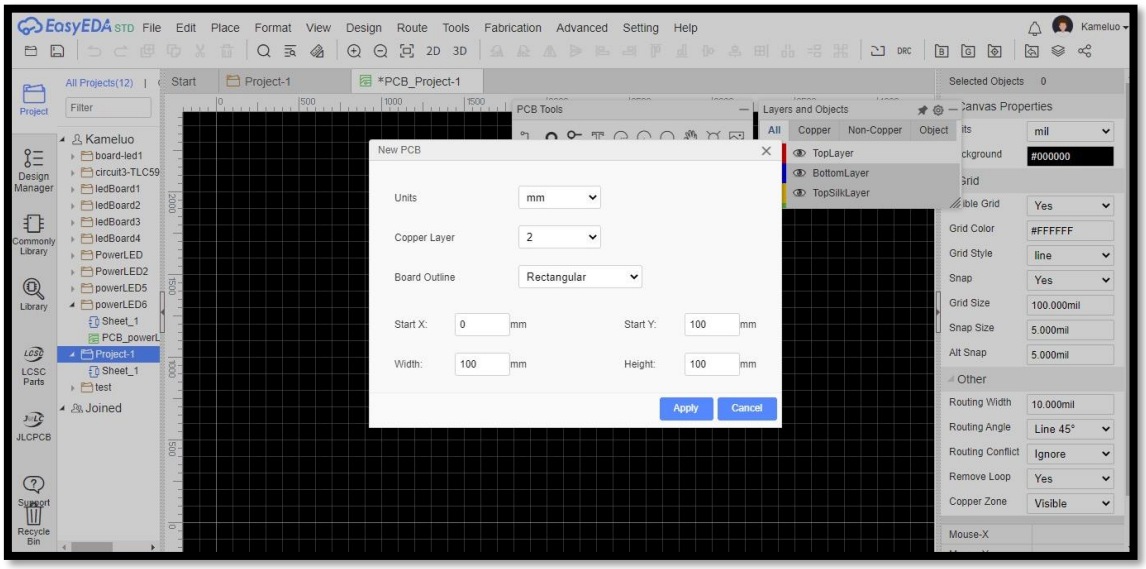


Figure 11 - Setting the PCB dimension window

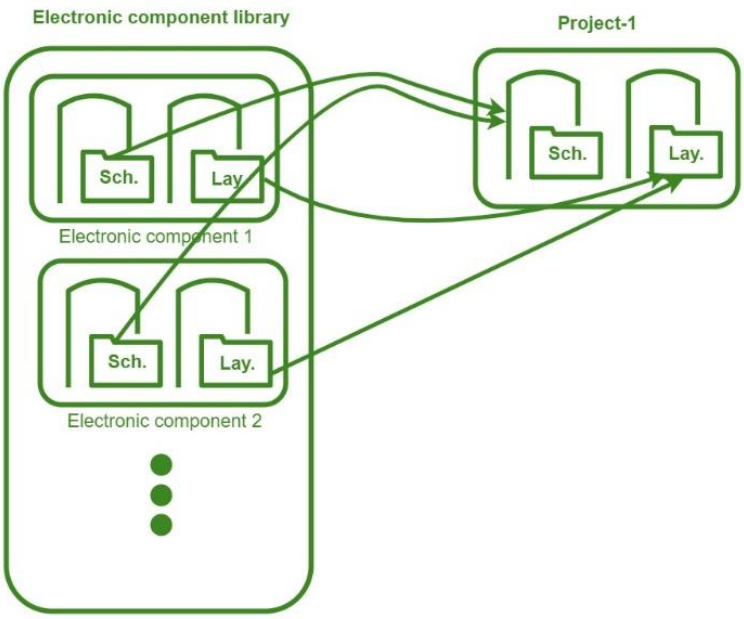


Figure 12 - The file structure in Easyeda

Before we create the PCB schematic and layout, the schematic and layout of every electronic component should be created and saved in the library, so the library of any component can be called from any project. So, to create a new component schematic and layout which in our case is the SMD LED, we go to the **File>New>Symbol**. New symbol page shows up as shown in figure (13) and by using the Drawing Tools we can create the symbol of the LEDs.

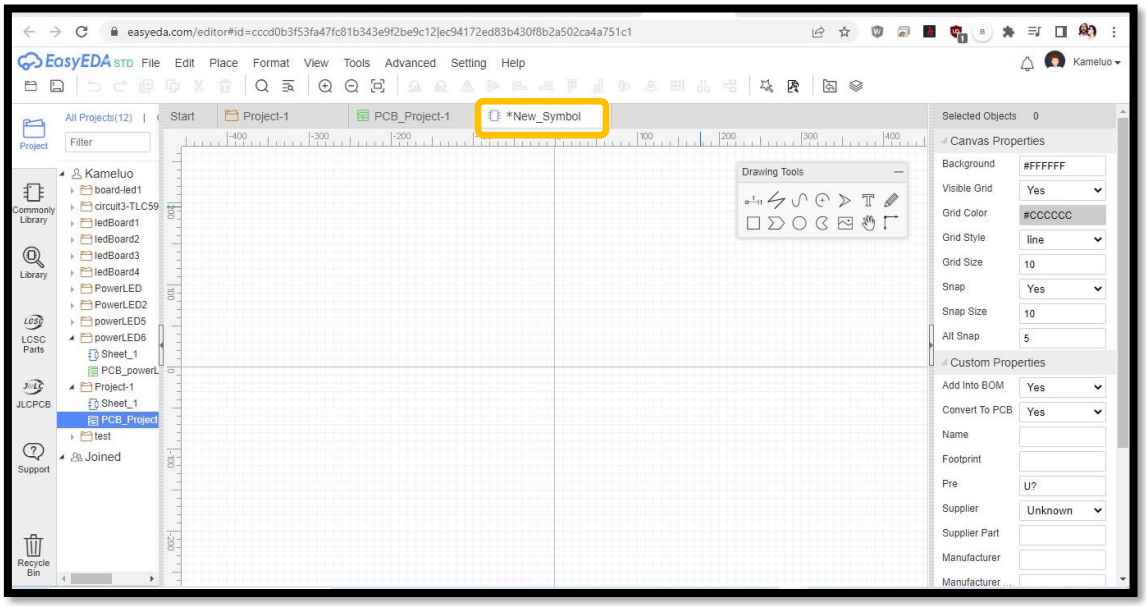


Figure 13 - The symbol page where the LED symbol will be drawn

After we create the symbol of the LED the lead must be numbered to map the anode and cathode of the schematic symbol to the anode and cathode of the layout symbol.

Then the right menu should be full filled since the symbol page will be connected later to the layout page of this specific component.

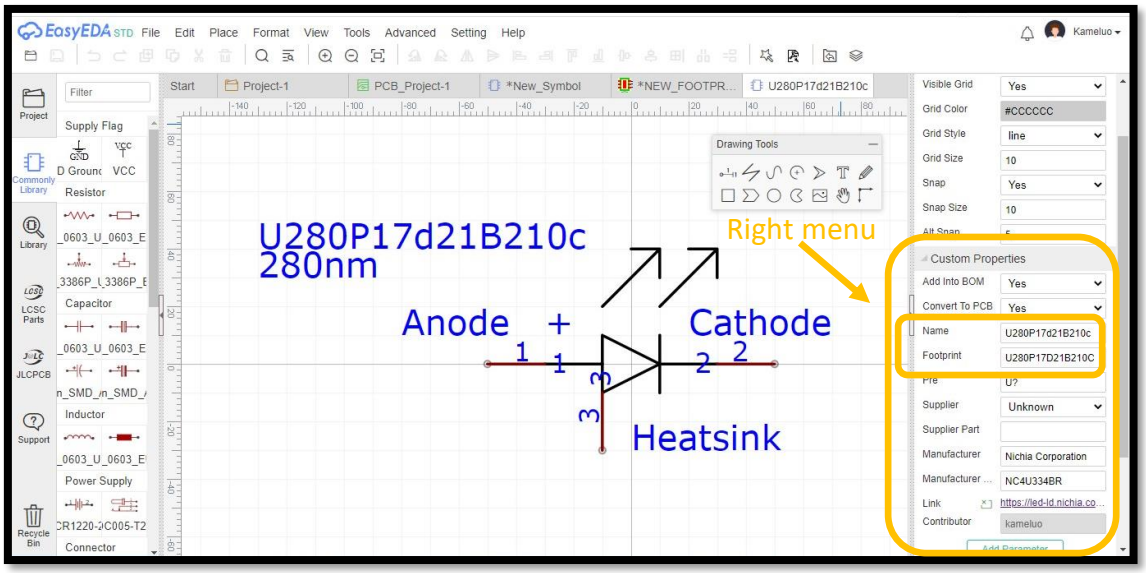


Figure 19 - After creating one of the SMD LED symbols

After that we have to create the LED footprint by going to the same menu **File>New>Footprint**. And by following the recommended dimensions in the datasheet of the LED like in figure (15), we create the footprint. Also, the right menu should be filled by the needed data and the most important is the name as it will be connected to the symbol page as mention previously.

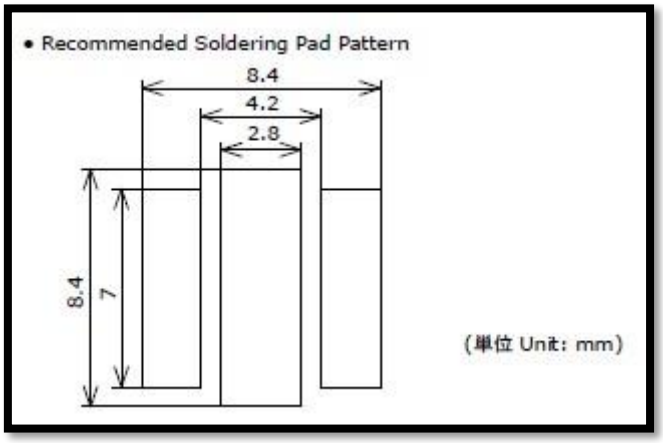


Figure 15 - Recommended footprint from Stanly SMD LED

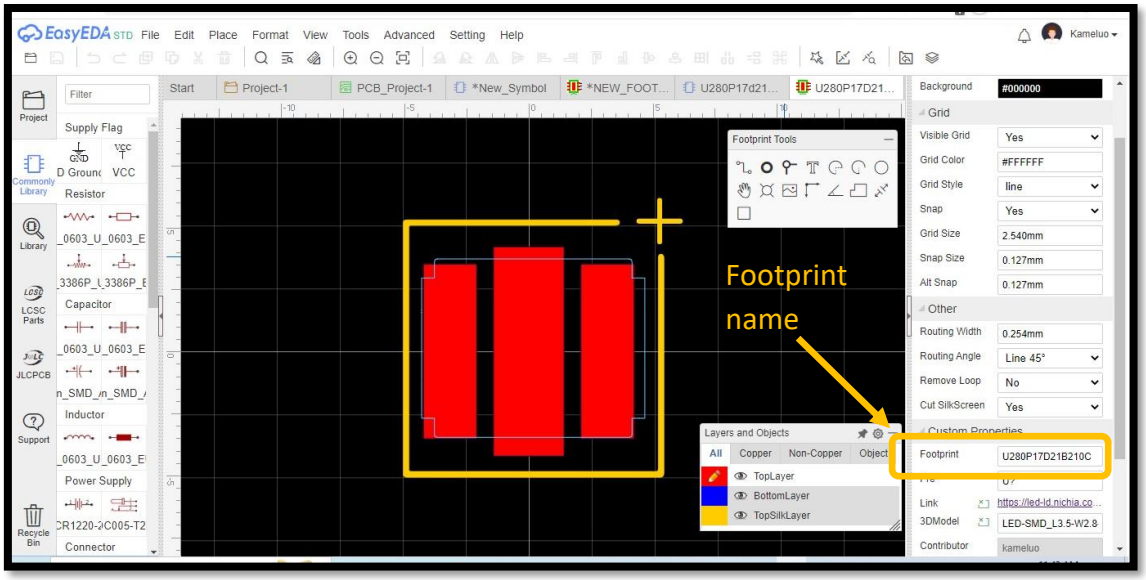


Figure 16 - Stanly SMD LED footprint

By getting back to the symbol page, in the right menu the footprint field should be filled by the layout name to connect the LED symbol to the LED footprint, there will appear a really important window which is called Footprint manager, this window is responsible to map the terminals of the symbols to the terminals of the footprint of the LED. In other words, the anode of the symbol must be the anode of the footprint and the same for the cathode terminal.

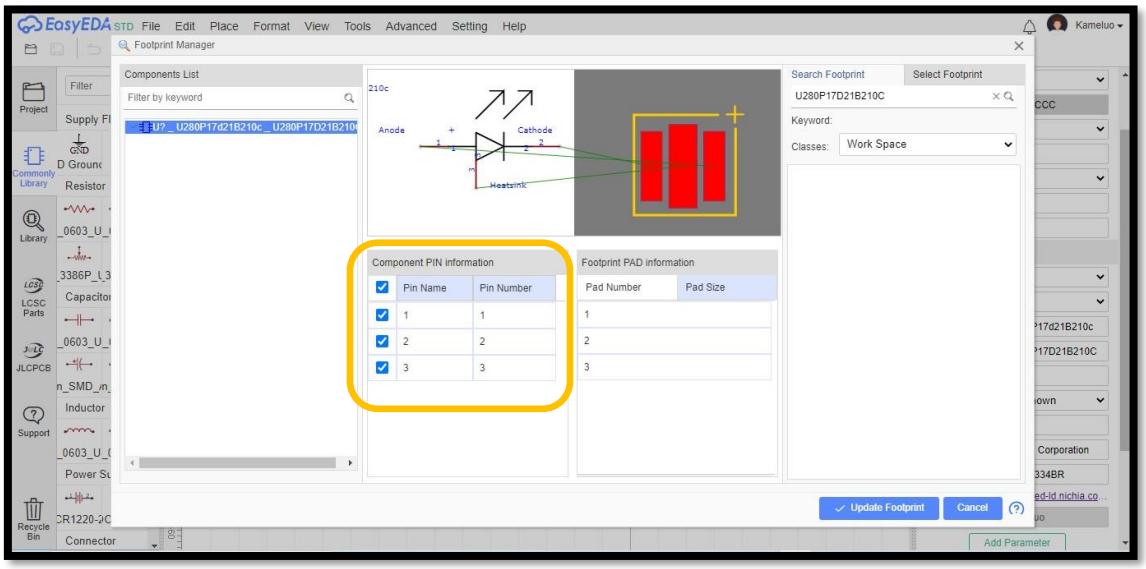


Figure 17 - Footprint manger window

After saving the LED symbol and footprint pages in the library, the LED can be used in any project. And according to the design, the schematic of the PCB will be drawn as seen in figure (18).

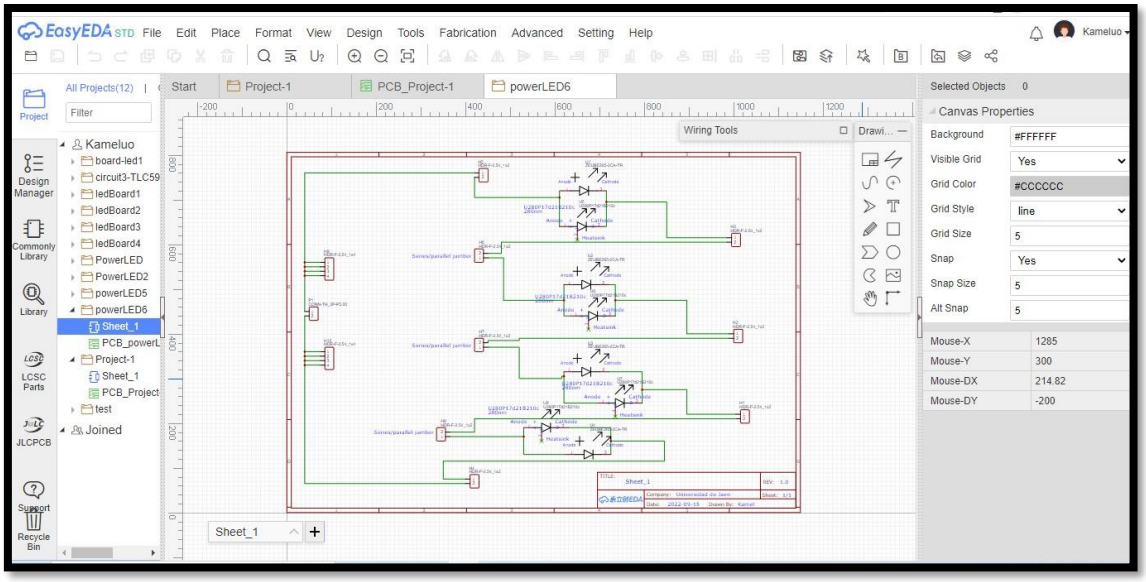


Figure 18 - PCB schematic design

In the layout page we place and align the electronic components according to our needs, for example we set the dimension of the PCB bigger than the dimensions of the heatsink in order to have a free space to solder the connectors and jumpers. Also, the track thickness in the PCBs must be designed to carry the needed current going through it. There are many different rules and programs to calculate this, but the most common tool is “Saturn” and by selecting the **Conductor properties tab** the track thickness can be checked by entering the desired current as shown in figure (19).

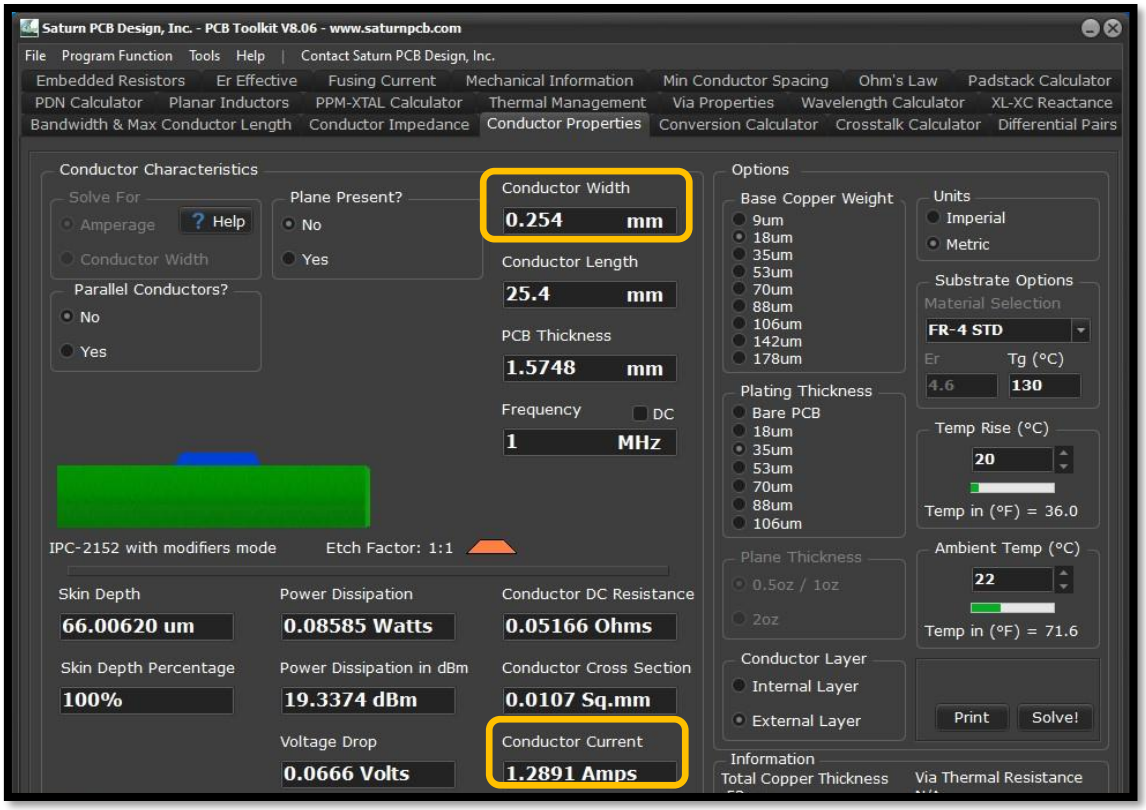


Figure 19 - Checking the track thickness using Saturn program

After finishing the layout design of the PCB, the Gerber file should be created and sent to any PCB manufacture or it can be printed to create the PCB manually in the laboratory using the chemical method.

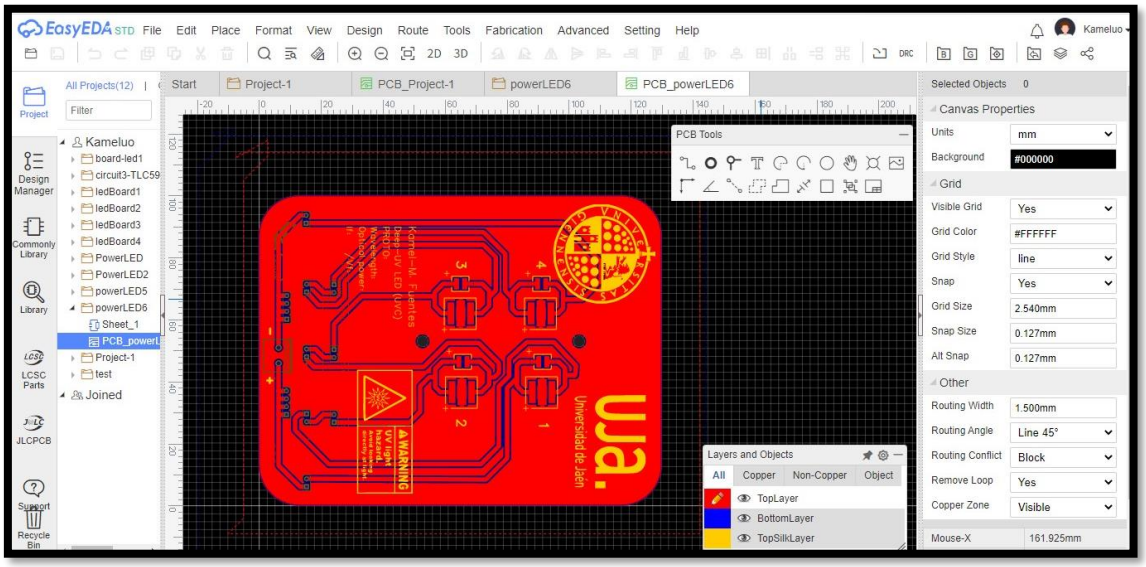


Figure 20 - PCB layout design

Also, the program has the feature of viewing the PCB in 3D in order to have a visual inspection of the components over the PCB.

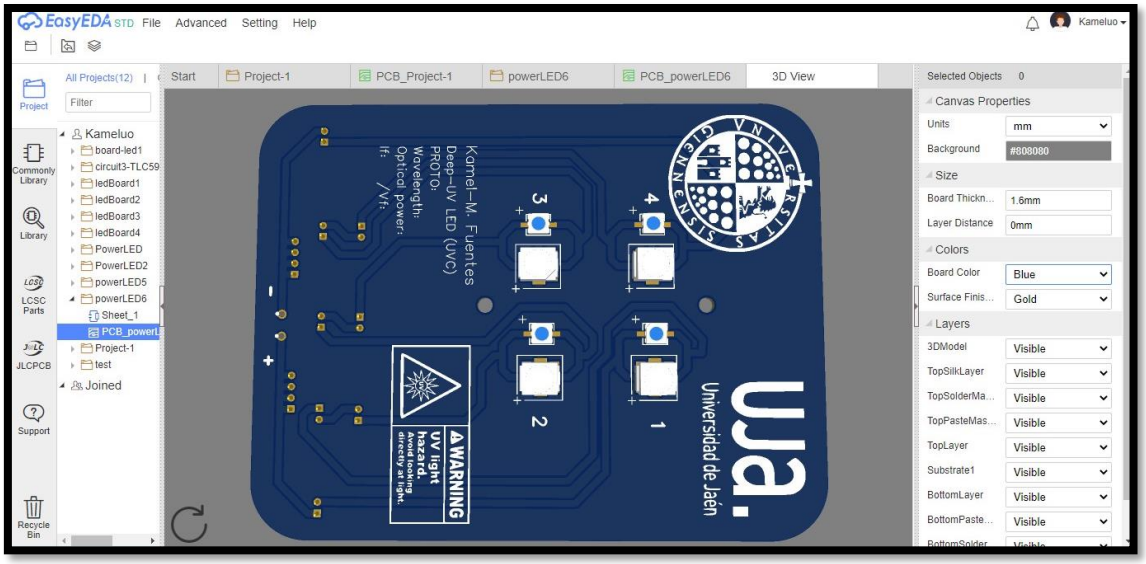


Figure 21 - 3D view of the PCB (front side)

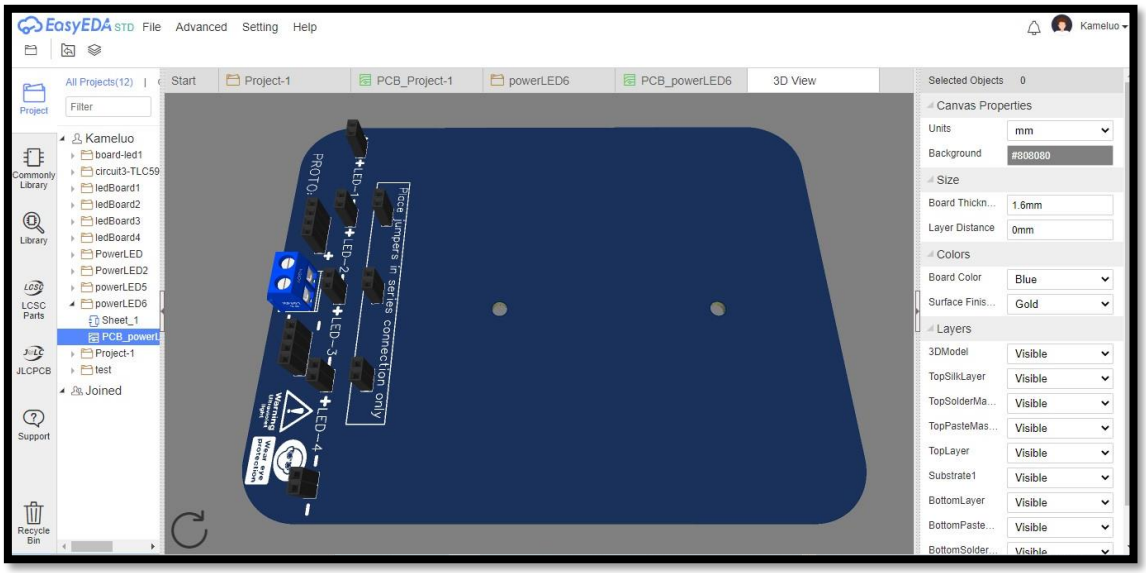
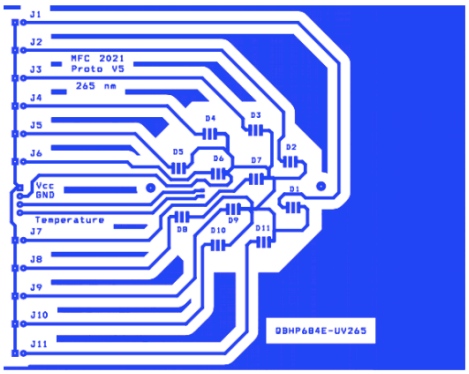
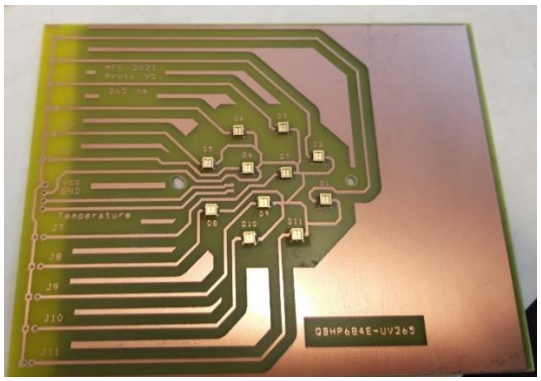


Figure 22 - 3D view of the PCB (back side)

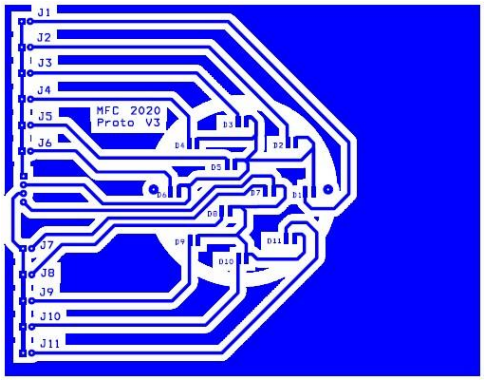
Finally, a total of 5 PCB boards were designed:

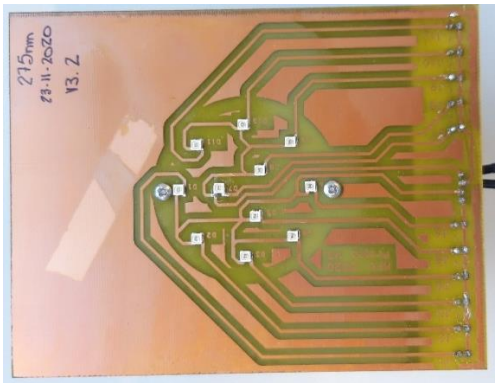
1) Board containing 11 UV-LEDS of 265 nm and 1.6 mW optical power



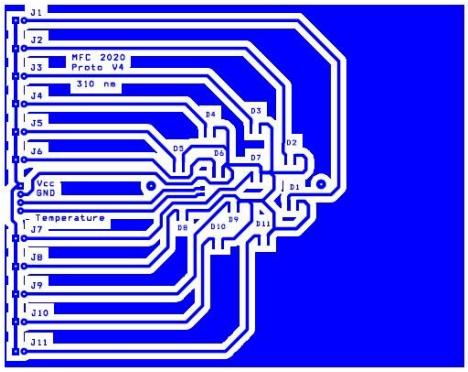


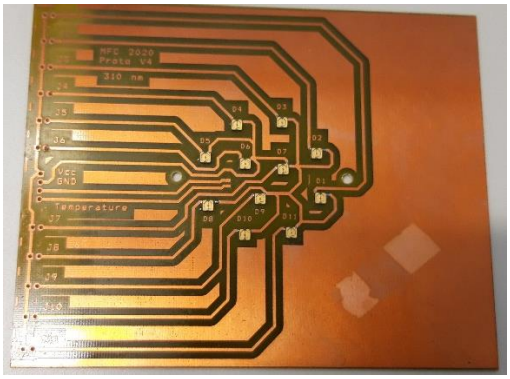
2) Board containing 11 UV-LEDs of 275 nm and 1.6 mW optical power



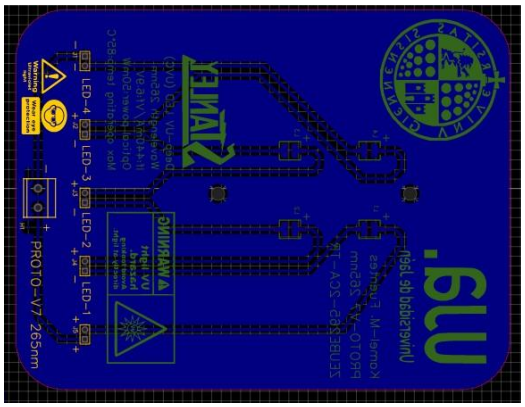


3) Board containing 11 UV-LEDs of 310 nm 1.6 mW optical power

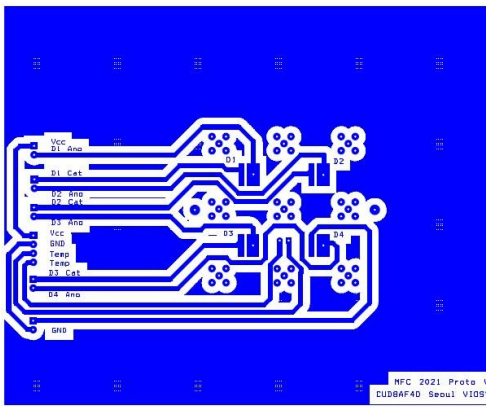


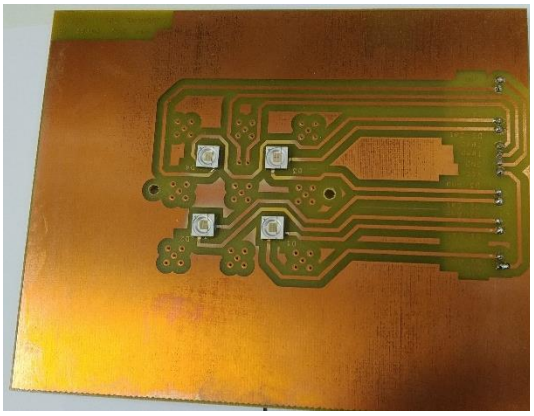


4) Board containing 4 UV-LEDs of 265 nm 50 mW optical power



5) Board containing 4 UV-LEDs of 275 nm 50 mW optical power





3.1.2 Manufacturing: UV-LEDs soldering process onto the PCB board

To manufacture the UV-LEDs PCB boards, first it is required to manufacture the PCB board and then to solder the UV-LEDs onto the board. The final PCB with UV-LEDs is then attached to a heatsink and ready to be mounted on the final set-up.

3.1.2.1 Manufacturing of the PCB board using the chemical method

Once the design is finished, the layout of the board must be saved in PDF format with the actual size and printed on a special paper. Then the PCB should be cut according to the dimension of the design. By aligning the paper on the PCB and applying UV light on top of it, the ink in the paper will be transferred to the PCB. Finally, the etching process which main purpose it removing the unnecessary copper on the PCB by placing the PCB in a chemical solution (ferric chloride).

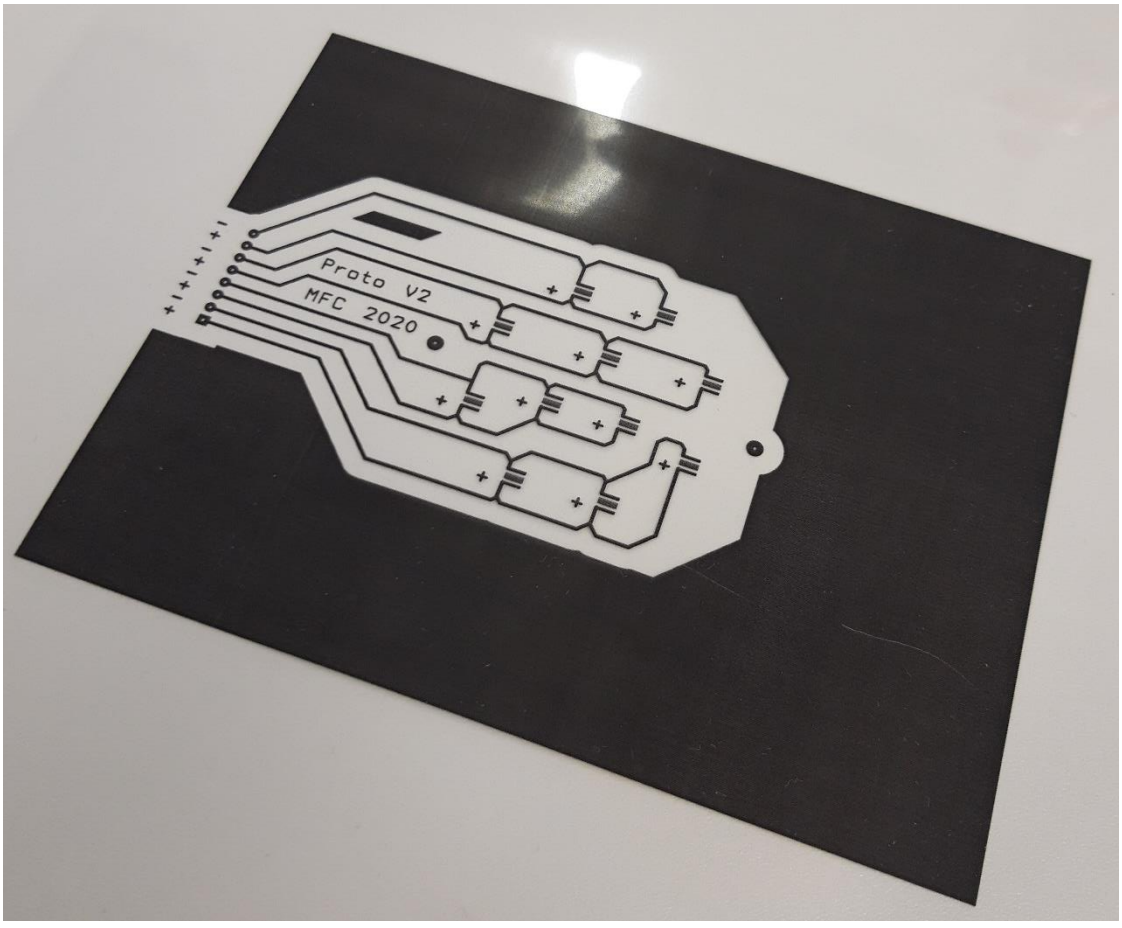


Figure 23 - Printed design on Glossy paper

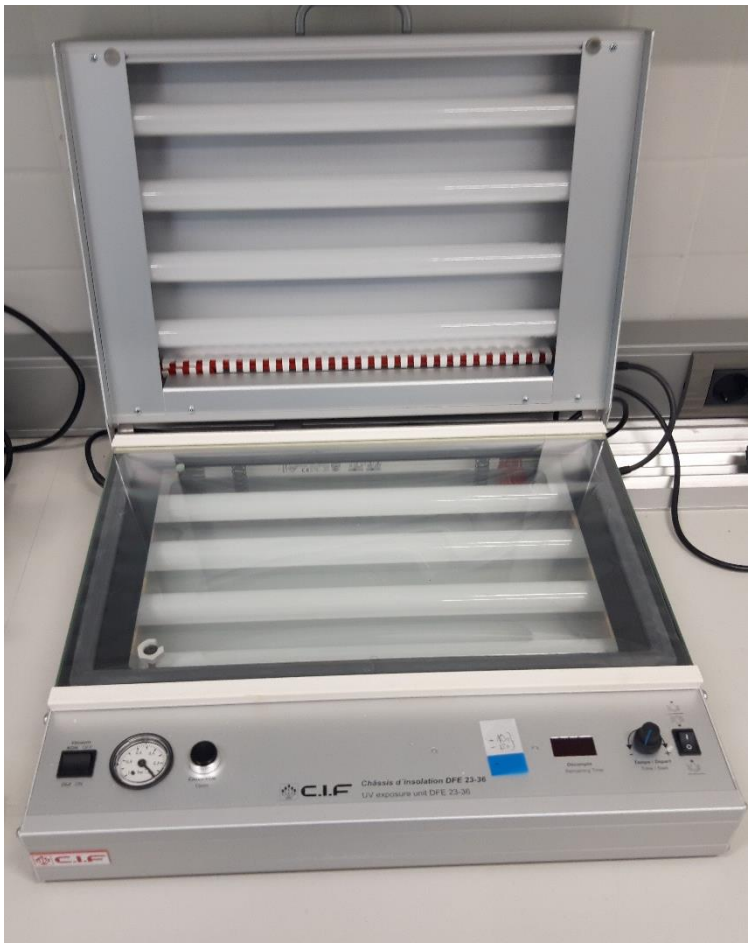


Figure 24 - UV light exposure device

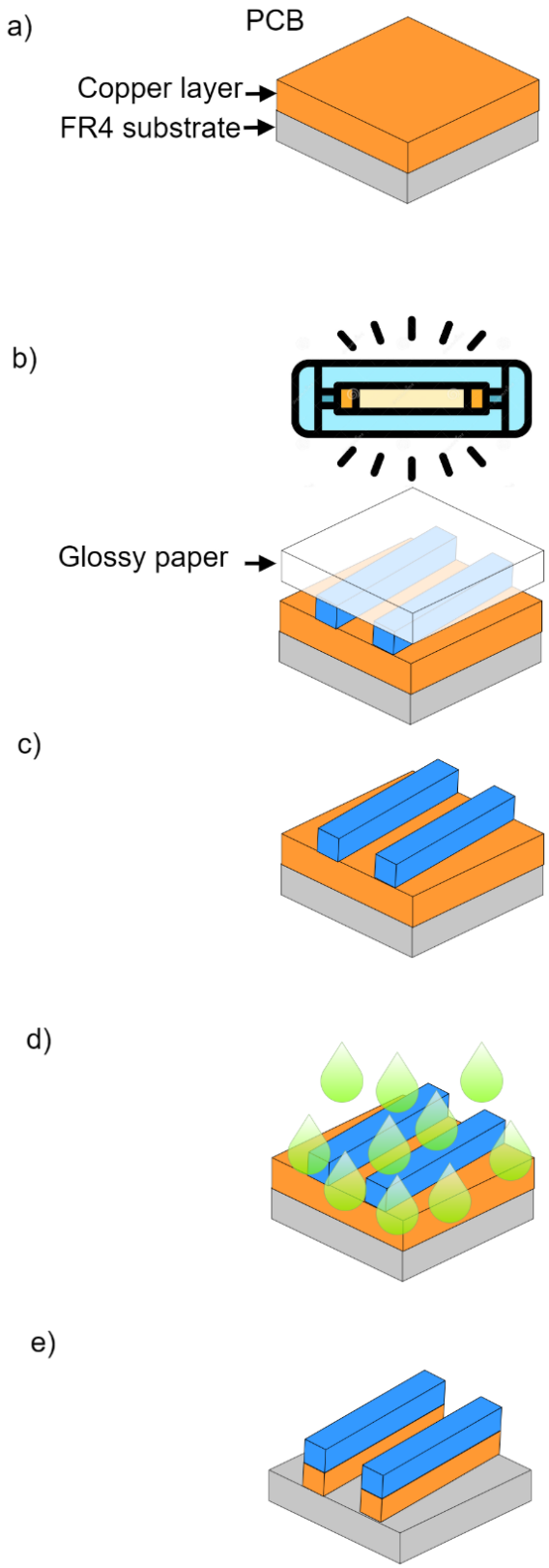


Figure 25 - Fabricating the PCB using the chemical method. A) PCB, b) Applying UV light over the glossy paper where the design is printed, c) Transferred design over the copper layer, d) Applying ferric chloride to remove unnecessary copper(etching), e) Final PCB with tracks on it

3.1.2.2 Manufacturing of the PCB board by a manufacture

If we want to fabricate the PCB in a PCB manufacture, the Gerber file has to be sent to the manufacture website. In our case we sent the Gerber file to a Chinese manufacture called “JLCPCB”. By following the demonstrated example using EasyEDA, we generate the Gerber file by going to **Fabrication menu>PCB Fabrication file (Gerber)** as shown in the figure.

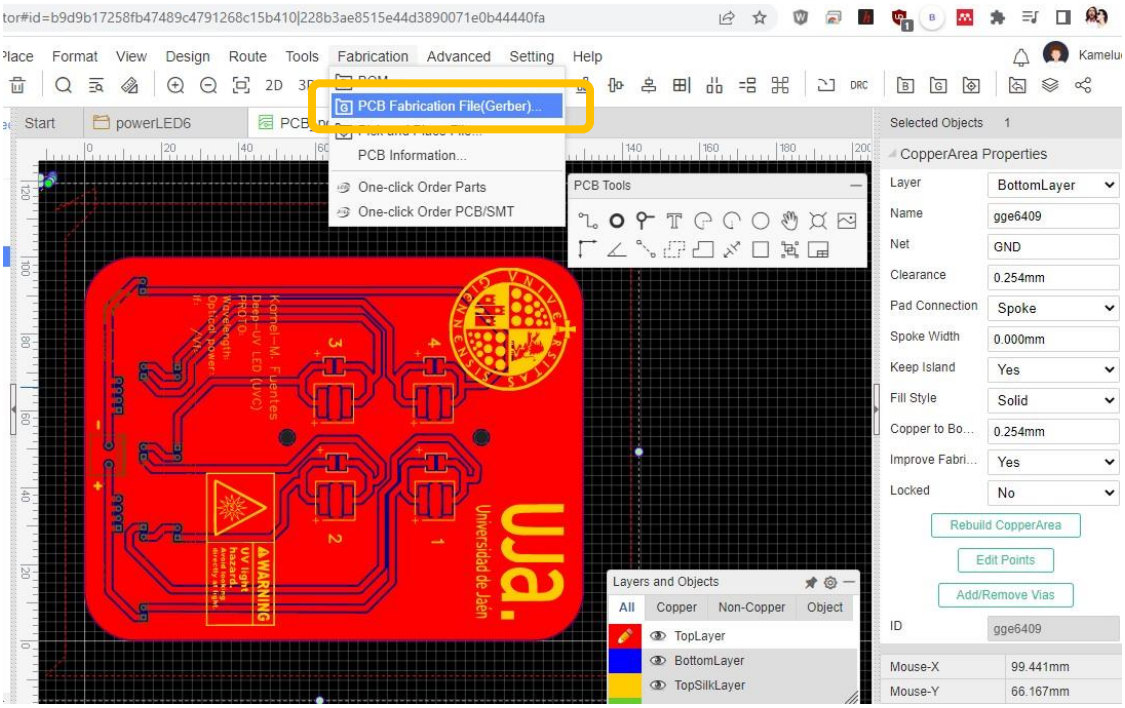


Figure 26 - Generating the Gerber file for the circuit

A window will appear to confirm the action, and by clicking **Generate Gerber** the Gerber file will be downloaded on the PC.

Figure 27 - Downloading Gerber file

By opening the JLCPCB website www.jlcpcb.com and clicking **Instant Quote**, the Gerber file can be added.

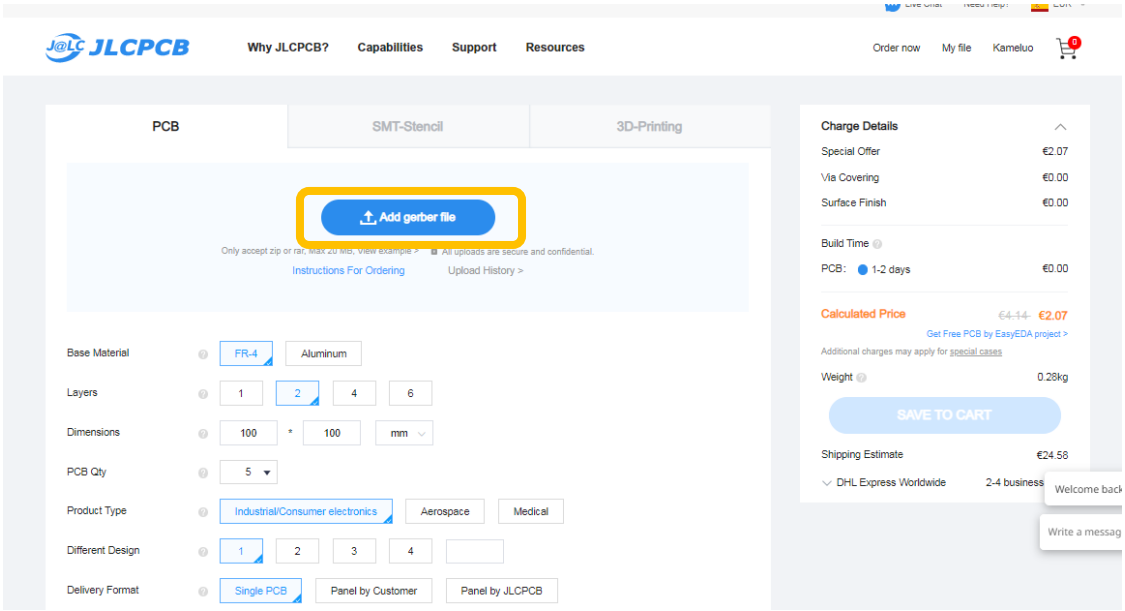


Figure 28 - Adding the Gerber file to the website

After adding the Gerber file to the website, the desired features should be chosen, such as **Base material** which is the substrate type, the quantity of the boards wanted from **PCB QTY**, **PCB thickness**, **PCB Color**, **Silkscreen**, thickness of the copper layer form **Outer Copper Weight**, and much more features.

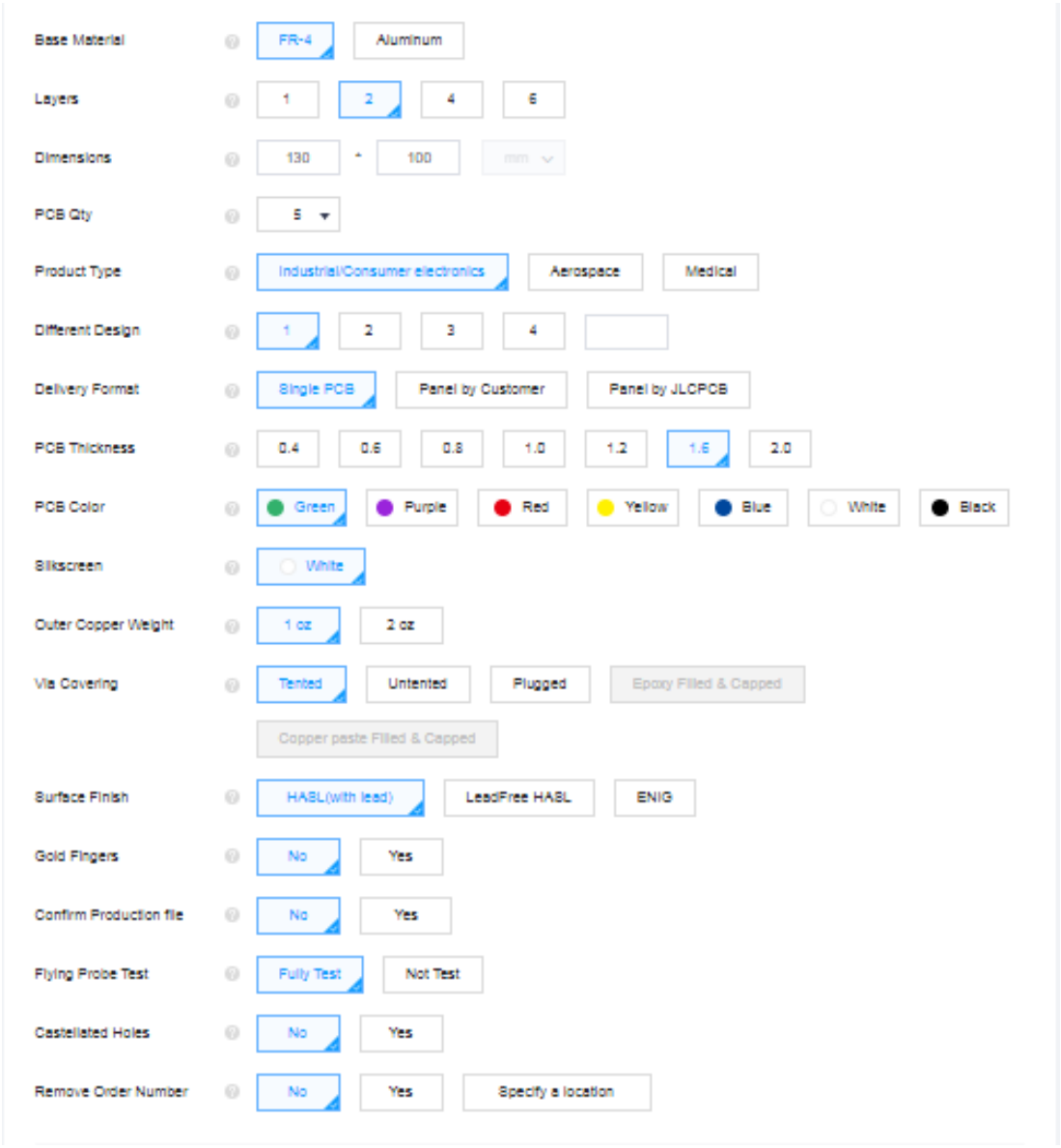


Figure 29 - JLCPCB fabrication features

After selecting the wanted features, the design should be saved in the cart and ordered.

Figure 30 - The actual PCB

3.1.3 UV-LEDs soldering process onto the PCB boards

In the datasheet of every LEDs there is a recommended soldering pattern that should be followed by the user using a PCB soldering oven. That pattern consists mostly from 3 different stages, first stage is the preheating where the PCB gets warm for around 100 to 130 seconds at a range of 150°C to 180°C. Second stage is the soldering stage which is responsible of the actual soldering process of the LED over the pad in the PCB itself at around 260°C. Lastly is the cooling down stage.

In figure (31), a solder pattern from one of the LEDs used in our research. As it can be seen the different stages with the recommended temperature for every one of them.

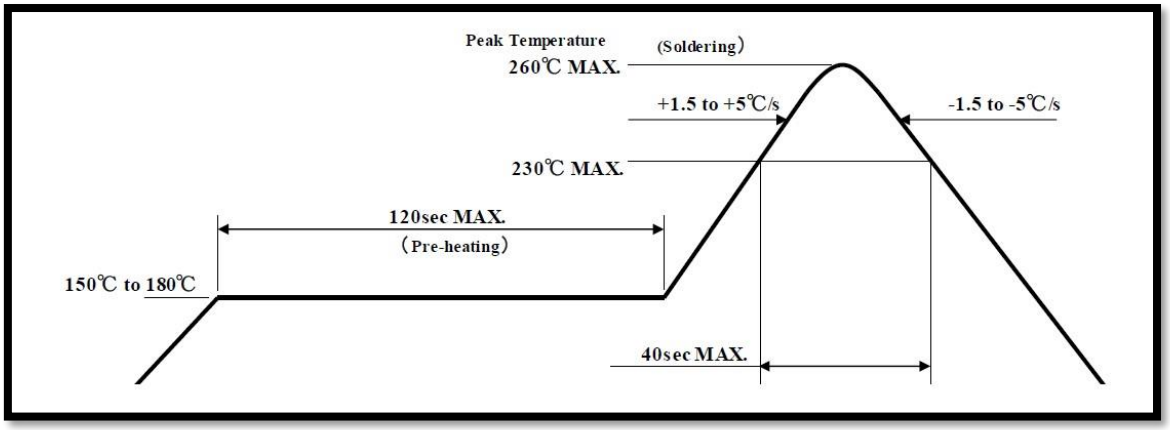


Figure 31 - Soldering pattern for a Stanly LED

Another consideration needs be taken is the soldering paste used in the process, there are two common types of solder paste, one contains lead and the other is lead-free. The lead-free solder paste is environmentally friendly, however it needs more temperature and time to be liquified compared to the one with lead, around 50°C more. Soldering with lead-free solder paste is more challenging and for these first prototypes conventional soldering has been used, although the objective is to move to lead-free soldering in the next stages. As displayed in figure (32), there is a difference between the soldering pastes and the recommended temperature should be followed during the process.

Profile Feature	Sn-Pb Eutectic Assembly	Pb-Free Assembly
Average ramp-up rate (Tsmax to Tp)	3° C/second max.	3° C/second max.
Preheat - Temperature Min (Tsmin) - Temperature Max (Tsmax) - Time (Tsmin to Tsmax) (ts)	 100 °C 150 °C 60-120 seconds	 150 °C 200 °C 60-180 seconds
Time maintained above: - Temperature (TL) - Time (tL)	 183 °C 60-150 seconds	 217 °C 60-150 seconds
Peak Temperature (Tp)	215℃	260℃
Time within 5°C of actual Peak Temperature (tp)2	10-30 seconds	20-40 seconds
Ramp-down Rate	6 °C/second max.	6 °C/second max.
Time 25°C to Peak Temperature	6 minutes max.	8 minutes max.

Figure 32 - Solder paste with lead vs. lead-free from SeoulViosys LED manufacturer

Therefore, a PCB oven was used in the LED soldering process in the project. NeoDen IN6 PCB reflow oven with three stages and internal temperature sensor to ensure full control of the heating chamber. It can reach the optimal temperatures in just 15 minutes.

Figure 33 - NeoDen IN6 PCB oven

In order to start the process, the PCB oven should be switched on and prepared by leaving it till the green strip indicator turns on.

Figure 34 - Green strip indicator

Then the 3 stages of the PCB oven should be set and saved in the oven system, every single stage has an upper and lower heater. The soldering temperature of every stage and the time needed had been set using the datasheets of the LEDs and the practice.

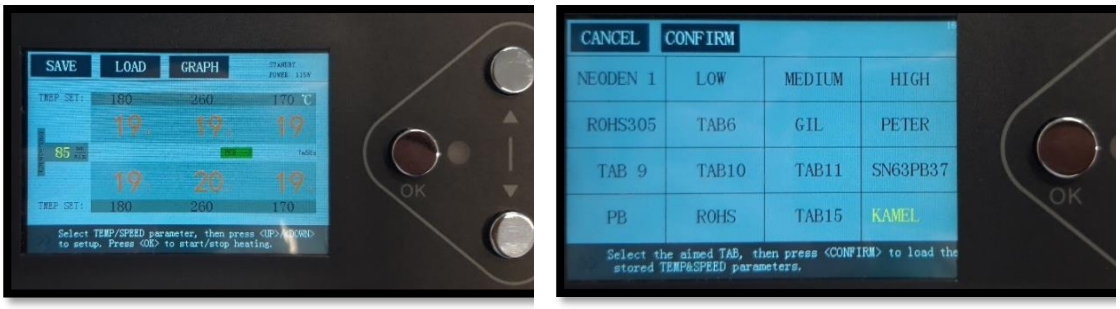


Figure 35 - Temperature stages setup and saving profile

Once the saved profile has been selected, the confirm icon should be clicked using the OK button, and the white LED turns on indicating that the oven is heating up the three stages. This process can take up to 5 minutes and once the oven is ready, it will turn on the green strip and give a beeping sound.

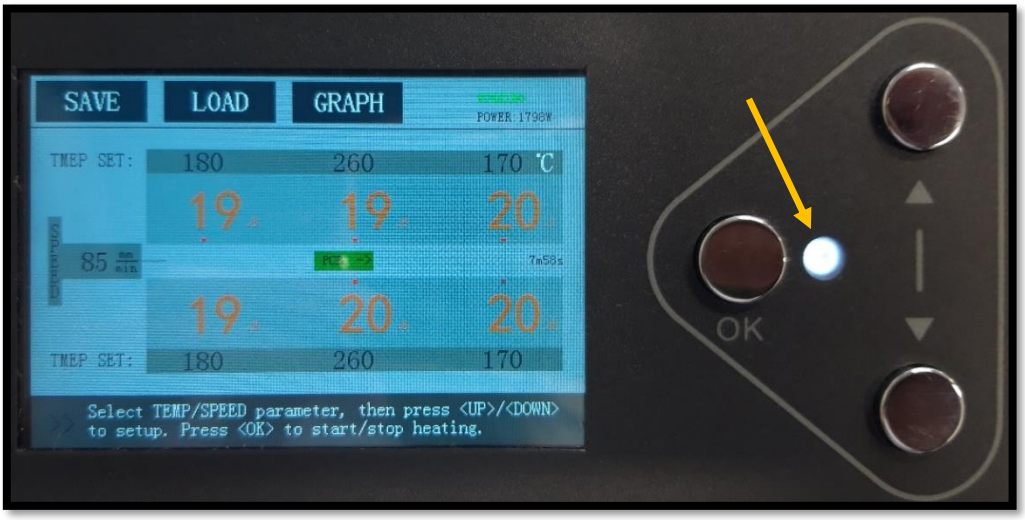


Figure 36 - The heating process started

One of the features that this oven has is that can display the live temperature of the PCB during the soldering process. It comes with a thermocouple temperature sensor which can be placed over the PCB using Kapton tape and its terminal must be connected to the temperature sensor socket next to the opening.

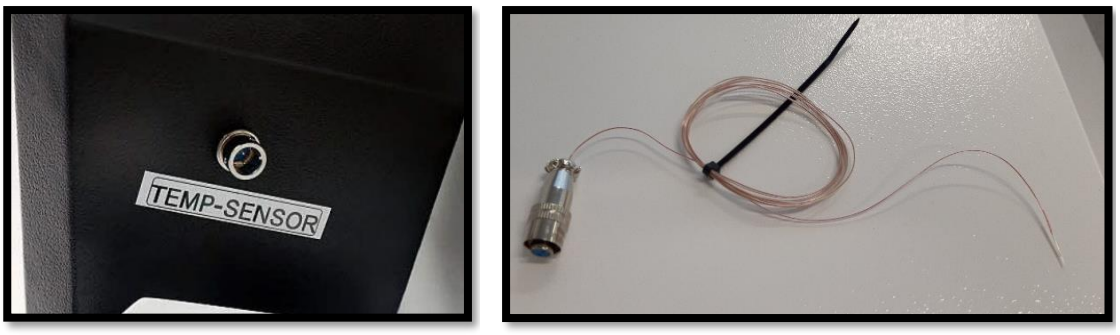


Figure 37 - Temperature sensor socket and the thermocouple temperature sensor

After applying the leaded solder paste and placing the LEDs, a thermal kapton tape used to fix the thermocouple sensor over the PCB. And by clicking the GRAPH icon on the screen, the real temperature of the soldering process can bee seen.

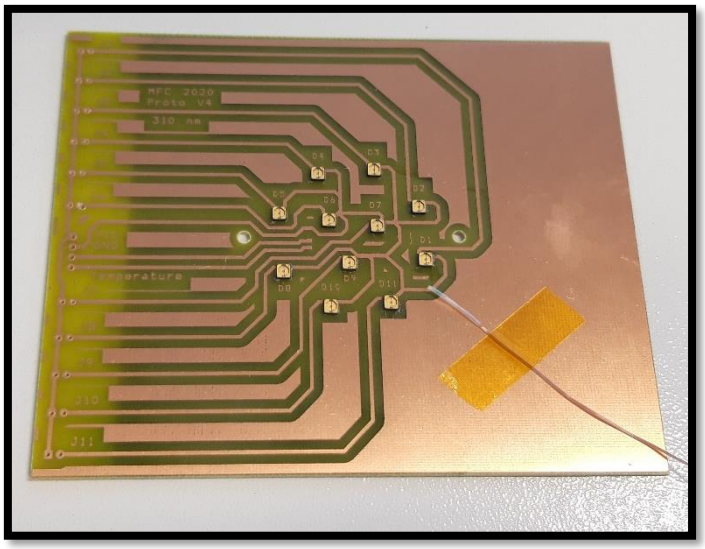


Figure 38 - Placing the thermocouple sensor on the PCB

By checking the datasheet soldering pattern and the actual graph from the oven we can observe that they are similar.

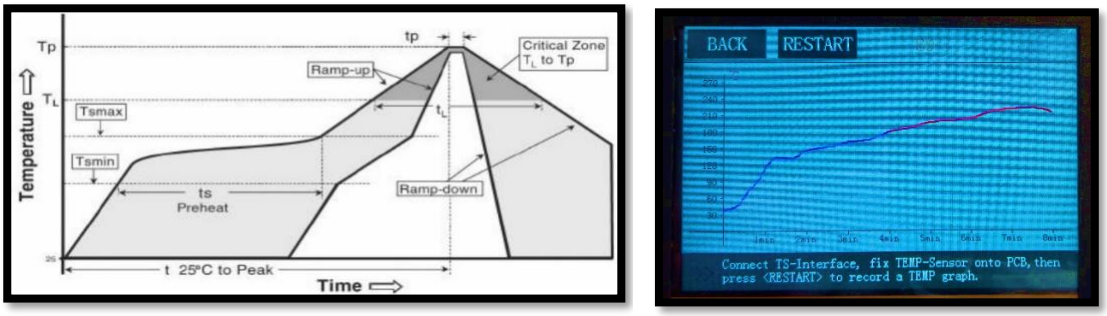


Figure 39 - Compression between the recommended and the oven temperature graph

The soldering quality using the leaded solder paste and the oven was accurate and precise in all the PCBs. After that the rest of the electronic components were soldered by the manual soldering station.

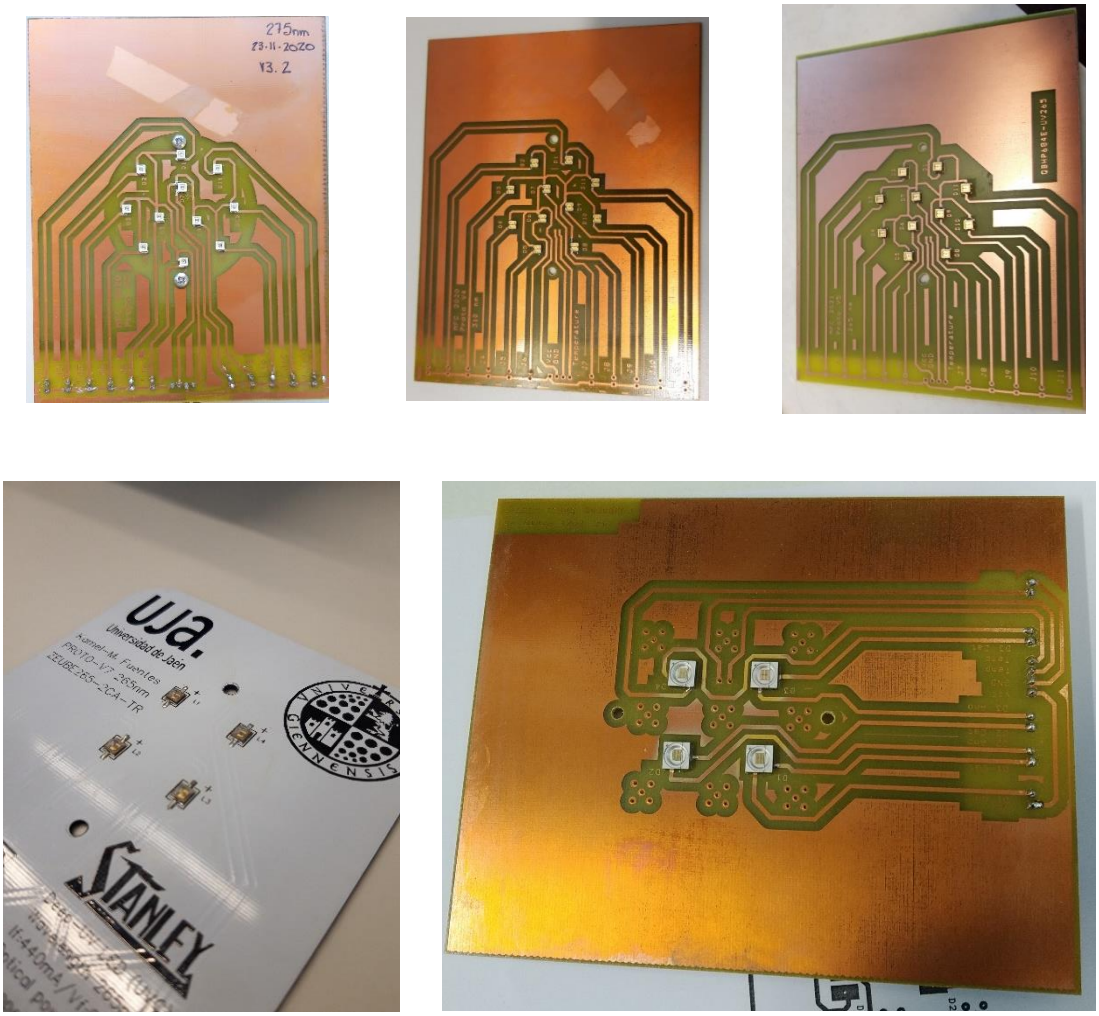


Figure 40 - Some of the PCB of the project, in the first row are the small power modules, (275 nm ,310 nm and 265 nm). In the second row are the medium power modules (265 nm and 275 nm).

3.1.4 Set-up

Once the PCB boards with the UV-LEDs are ready, they are attached to a heatsink with a fan, and the heatsink itself is physically connected to the stand using an aluminum bar to adjust the high of the module over the Petri dishes. Beside the heatsink is the Raspberry-pi to run the experiments and save the data. The PCB terminals were connected to the desktop power supply and also a thermocouple temperature sensor was attached to the PCBs during the experiments to monitor the operating temperature using the Fluke 179 multimeter. Also, a magnetic stirrer was placed under the Petri dish to provide a proper mixing of the sample wastewater during the experiments.

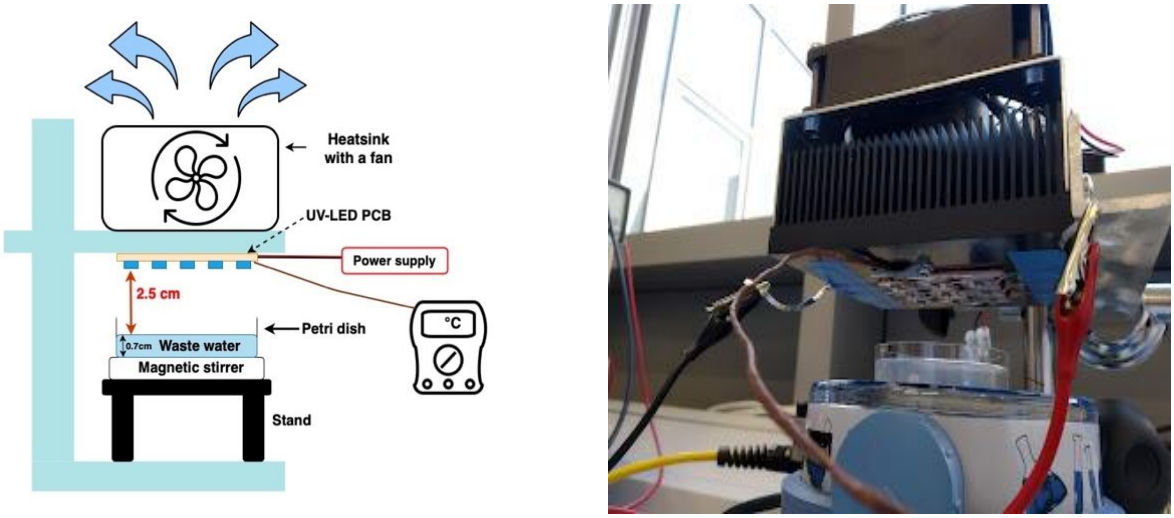


Figure 41 - Experiment set-up showing the scheme for wastewater exposure to UV-LEDs and a real image during experimentation.

3.2 Precautions

UV light specially UC-C can cause temporary or permanent loss of vision, and redness or ulceration (mild to severe sun burn) to exposed skin [14-15]. That is why precautions was followed during testing the PCBs and the conducting the experiments. A special face mask was used to protect the eyes and the entire face, and also gloves.

Figure 42 - Protection gloves and face mask used during the entire project

3.3 Reference

[1] E. Allahyari *et al.*, "A sequential utilization of the UV-A (365 nm) fluence rate for disinfection of water, contaminated with Legionella pneumophila and Legionella dumoffii," *Environmental Pollution*, vol. 304, Jul. 2022, doi: 10.1016/j.envpol.2022.119224.

[2] P. O. Nyangaresi, Y. Qin, G. Chen, B. Zhang, Y. Lu, and L. Shen, "Comparison of the performance of pulsed and continuous UVC-LED irradiation in the inactivation of bacteria," *Water Res*, vol. 157, pp. 218–227, Jun. 2019, doi: 10.1016/j.watres.2019.03.080.

[3] G. Q. Li, W. L. Wang, Z. Y. Huo, Y. Lu, and H. Y. Hu, "Comparison of UV-LED and low pressure UV for water disinfection: Photoreactivation and dark repair of Escherichia coli," *Water Res*, vol. 126, pp. 134–143, 2017, doi: 10.1016/j.watres.2017.09.030.

[4] A. C. Chevremont, A. M. Farnet, B. Coulomb, and J. L. Boudenne, "Effect of coupled UV-A and UV-C LEDs on both microbiological and chemical pollution of urban wastewaters," *Science of the Total Environment*, vol. 426, pp. 304–310, Jun. 2012, doi: 10.1016/j.scitotenv.2012.03.043.

[5] P. O. Nyangaresi, Y. Qin, G. Chen, B. Zhang, Y. Lu, and L. Shen, "Effects of single and combined UV-LEDs on inactivation and subsequent reactivation of E. coli in water disinfection," *Water Res*, vol. 147, pp. 331–341, Dec. 2018, doi: 10.1016/j.watres.2018.10.014.

[6] X. Y. Zou *et al.*, "Enhanced inactivation of E. coli by pulsed UV-LED irradiation during water disinfection," *Science of the Total Environment*, vol. 650, pp. 210–215, Feb. 2019, doi: 10.1016/j.scitotenv.2018.08.367.

[7] S. Rattanakul and K. Oguma, "Inactivation kinetics and efficiencies of UV-LEDs against Pseudomonas aeruginosa, Legionella pneumophila, and surrogate microorganisms," *Water Res*, vol. 130, pp. 31–37, Mar. 2018, doi: 10.1016/j.watres.2017.11.047.

[8] K. Song, F. Taghipour, and M. Mohseni, "Microorganisms inactivation by continuous and pulsed irradiation of ultraviolet light-emitting diodes (UV-LEDs)," *Chemical Engineering Journal*, vol. 343, pp. 362–370, Jul. 2018, doi: 10.1016/j.cej.2018.03.020.

[9] K. Song, F. Taghipour, and M. Mohseni, "Microorganisms inactivation by wavelength combinations of ultraviolet light-emitting diodes (UV-LEDs)," *Science of the Total Environment*, vol. 665, pp. 1103–1110, May 2019, doi: 10.1016/j.scitotenv.2019.02.041.

[10] A. C. Chevremont, A. M. Farnet, M. Sergent, B. Coulomb, and J. L. Boudenne, "Multivariate optimization of fecal bioindicator inactivation by coupling UV-A and UV-C

LEDs," *Desalination*, vol. 285, pp. 219–225, Jan. 2012, doi: 10.1016/j.desal.2011.10.006.

[11] K. Y. Nelson, D. W. McMartin, C. K. Yost, K. J. Runtz, and T. Ono, "Point-of-use water disinfection using UV light-emitting diodes to reduce bacterial contamination," *Environmental Science and Pollution Research*, vol. 20, no. 8, pp. 5441–5448, Aug. 2013, doi: 10.1007/s11356-013-1564-6.

[12] K. Sholtes and K. G. Linden, "Pulsed and continuous light UV LED: microbial inactivation, electrical, and time efficiency," *Water Res*, vol. 165, Nov. 2019, doi: 10.1016/j.watres.2019.114965.

[13] D. Pousty, R. Hofmann, Y. Gerchman, and H. Mamane, "Wavelength-dependent time–dose reciprocity and stress mechanism for UV-LED disinfection of Escherichia coli," *J Photochem Photobiol B*, vol. 217, Apr. 2021, doi: 10.1016/j.jphotobiol.2021.112129.

[14] https://www.larsonelectronics.com/images/product/EyeSafety/272823.PDF

[15] https://www.larsonelectronics.com/images/product/LightingFacts/266985.PDF

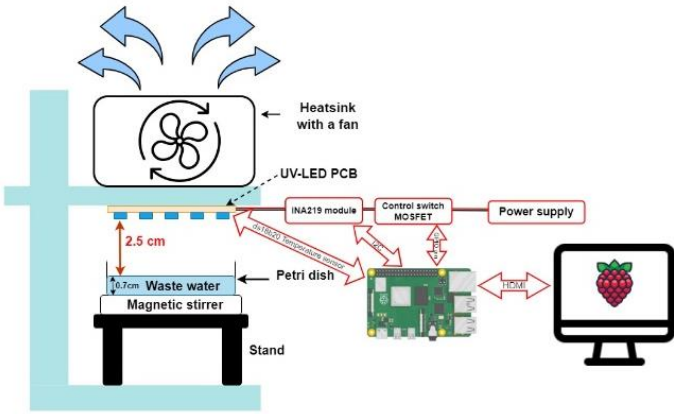
Chapter 4: Summary of results

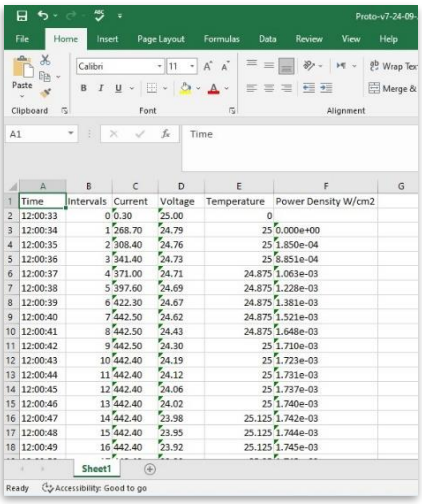
In this chapter we present a summary of the main results and their connection with the PhD specific objectives.

Objective 1. To design and manufacture novel prototype at laboratory level that disinfects the raw water within a Petri dish of at least 60 mm diameter, studying the optical characterisation of the UV-LEDs, the electronic PCB design and arrangement of UV-LEDs, the cooling system and the total UV irradiance.

This work was conducted mainly under Chapter 3 and the publication 1 ('Deactivating environmental strains of *Escherichia coli*, *Enterococcus faecalis* and *Clostridium perfringens* from a real wastewater effluent using UV-LEDs'), and was improved in Annex B and the publication 2 ('Analysing the reciprocity law for UV-LEDs in water disinfection of *Escherichia coli*, *Enterococcus faecalis* and *Clostridium perfringens*'). In the first publication, it is explained the design of the experiment reactor which consists of the propriate UV-LED board attached to heat sink and fan for heat dissipation using an aluminium bar to adjust the hight over the sterile plastic Petri dish containing the raw water to be treated. The readings of the forward current, voltage, optical power density and PCBs temperature were recorded manually.

In the second publication, a raspberry-pi-3b development board was used with a current-voltage module, optical power density and temperature sensor to control and monitor the experiment automatically by setting the duration of the experiment and saving the measurements in an excel file (figure (1)).





	A	B	C	D	E	F
1	Time	Intervals	Current	Voltage	Temperature	Power Density W/cm2
2	12:00:33	0	0.30	25.00	0	
3	12:00:34	1	268.70	24.79	25	0.000e+00
4	12:00:35	2	308.40	24.76	25	1.850e-04
5	12:00:36	3	341.40	24.73	25	8.851e-04
6	12:00:37	4	371.00	24.71	24.875	1.063e-03
7	12:00:38	5	397.60	24.69	24.875	1.228e-03
8	12:00:39	6	422.30	24.67	24.875	1.381e-03
9	12:00:40	7	442.50	24.62	24.875	1.521e-03
10	12:00:41	8	442.50	24.43	24.875	1.648e-03
11	12:00:42	9	442.50	24.30	25	1.710e-03
12	12:00:43	10	442.40	24.19	25	1.723e-03
13	12:00:44	11	442.40	24.12	25	1.731e-03
14	12:00:45	12	442.40	24.06	25	1.737e-03
15	12:00:46	13	442.40	24.02	25	1.740e-03
16	12:00:47	14	442.40	23.98	25.125	1.742e-03
17	12:00:48	15	442.40	23.95	25.125	1.744e-03
18	12:00:49	16	442.40	23.92	25.125	1.745e-03

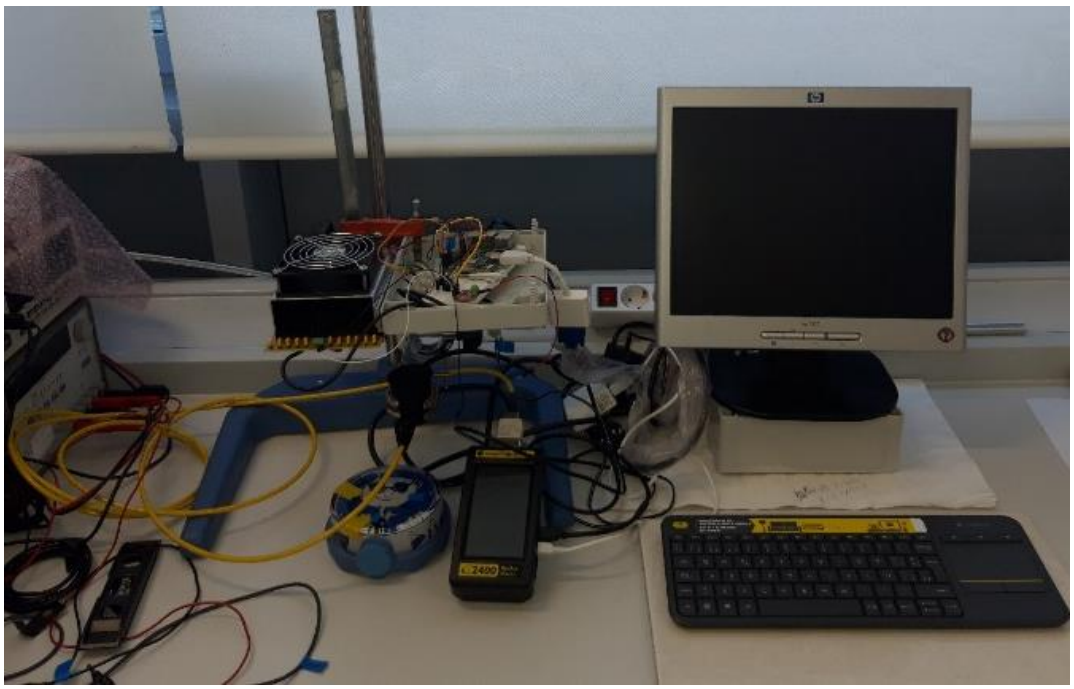


Figure 1 - Experiment setup and saved measurements

Objective 2. To determine the optimum UV-LED wavelength for inactivation of each of the studied microorganisms (*Escherichia coli*, *Enterococcus faecalis* and *Clostridium perfringens*) using real environmental strains from the effluent of the municipal wastewater treatment plant of Linares.

This work was conducted in the publication 1. Here the novel manufacture prototypes are used to treat wastewater from the effluent of the Linares wastewater plant at three different wavelengths 265 nm, 275 nm and 310 nm. And as observed from the experiments, the lower the wavelength, the faster the disinfection process as publication 1 mentioned, *Escherichia coli*, *Enterococcus faecalis* and *Clostridium perfringens* are sensitive to 265 nm and 275 nm wavelengths, and on contrast with 310 nm wavelength which takes higher UV doses and longer time in order to disinfect the bacteria. In addition, every microorganism is sensitive to particular wavelengths rather than others, for instance 275 nm wavelength was slightly effective to *Escherichia coli* and *Enterococcus faecalis*, but *Clostridium perfringens* was deactivated by a higher rate using the 265 nm wavelength compared to the 275 nm wavelength as shown in the graphs.

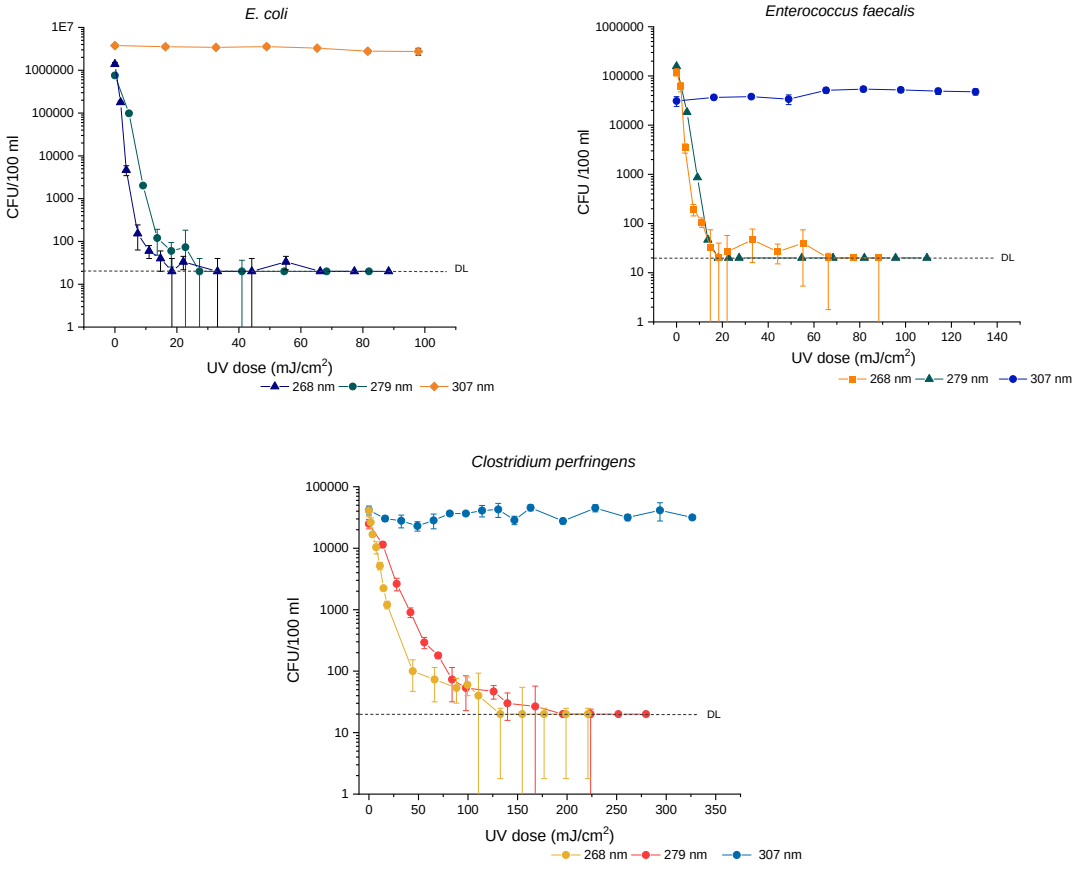


Figure 2 - UV dose vs. CFU/100 ml graphs for E. col, Enterococcus faecalis and Clostridium perfringens from publication 1

Objective 3. To determine the UV dose for inactivation for each microorganism and wavelength (time of exposure and UV irradiance).

This work was done in publication 1 and publication 2. The UV dose used for inactivating the microorganisms are influenced by diverse potential factors which are the turbidity of the wastewater, the wavelength used in the disinfection method, the initial concentration of the microorganism and the depth of the wastewater container during the experiment (which is a 60 mm diameter Petri dish in our case).

For instance, in publication 1 the initial population and the turbidity level for *Escherichia coli* using 265 nm, 279 nm and 310 nm wavelengths were 1.4 x 10^6 CFU/100 ml (12.4 NTU), 7.6 x 10^5 CFU/100 ml (6.3 NTU) and 3.8 x 10^6 CFU/100 ml (8.9 NTU) respectively, and the inactivation UV doses in these cases were 18.4 mJ/cm^2 UV dose for 50 seconds for 265 nm, 27.3 mJ/cm^2 of UV dose for 60 seconds for 275 nm and 97.9 mJ/cm^2 after 180 seconds for 310 nm. The 265 nm and 279 nm have similar effect on deactivating *Escherichia coli,* however the higher inactivation for 265 nm wavelength was suggested to be resulted by the different turbidity values of the raw water during the 265 nm experiment (12.4 NTU for 265 nm vs. 6.3 NTU for 275 nm).

Similarly in publication 2, the initial concentration of *Escherichia coli* was varying between 4.4 x 10^5 and 4.6 x 10^5 with 8 NTU turbidity level for 50 mW optical power UV-LED. The UV dose required to deactivate the *Escherichia coli* was 58 mJ/cm^2 using the 50 mW 265 nm UV-LEDs for 34 seconds. Whereas 13 mJ/cm^2 of UV dose for 11 seconds were provided using 50 mW 275 nm UV-LEDs with 8 NTU turbidity level.

For *Enterococcus faecalis* in publication 1, the initial population was from 1.2 x 10^4 CFU/100ml with 12.4 NTU turbidity level and reached the detection line after 50 seconds using 18 mJ/cm^2 UV dose by the 268 nm wavelength. Under the 279 nm wavelength the initial population was 1.6 x 10^5 CFU/100 ml and 6.3 NTU, 40 seconds and 18.2 mJ/cm^2 UV dose was needed to reach the detection line. Finally, the initial population was 3.1 x 10^4 CFU/100 ml and 5.4 NTU in the case of using 307 nm wavelength, the final population of the disinfection process was 4.8 x 10^4 CFU/100 ml which indicates the negligible effect of this wavelength in deactivating the *Enterococcus faecalis* microorganism.

In publication 2 the initial concentration for *Enterococcus faecalis* was ranged between 5.7 x 10^3 CFU/100 ml and 6.9 x 10^4 CFU/100 ml (8 NTC) using 1.6 mW and 50 mW-265 nm UV-LEDs. The UV dose was slightly similar, 31 mJ/cm^2 UV dose for 80 seconds and 34 mJ/cm^2 UV dose for 21 seconds for the 265 nm low-power and medium-power LEDs respectively. For the 275 nm wavelength, 17 mJ/cm^2 UV dose (40 seconds) and 13 mJ/cm^2 UV dose (11 seconds) for low-power and medium power UV LEDs respectively.

The total inactivation of *Clostridium perfringens* in publication 1 using 268 nm was reached after 360 seconds and 133 mJ/cm^2 UV dose, starting at 4.1 x 104 CFU/100 ml (7.3 NTU). For 279 nm the initial concentration was 2.5 x 10^4 CFU/100 ml (4.8 NTU), the reached the detection line after 420 seconds and 196 mJ/cm^2 UV dose. Finally, under 307 nm irradiation and with 4.2 x 10^4 CFU/100 ml (9.5 NTU), after 600 seconds (10

minutes) and 326 mJ/cm^2 UV dose and final concentration was 3.2 x 10^4 CFU/100 ml (31% inactivation).

In the publication 2, the initial concentration for *Clostridium perfringens* was ranged between 3.2 x 10^4 CFU/100 ml and 4.9 x 10^4 CFU/100 ml (4.9 NTU) using 1.6 mW and 50 mW-265 nm UV-LEDs, the inactivation was reached by applying 100 mJ/cm^2 UV dose (240 seconds) and 124 mJ/cm^2 UV dose (70 seconds) for the 265 nm wavelength low-powered and medium-powered LEDs. For the 275 nm and 10 NTU turbidity level, 159 mJ/cm^2 UV dose (360 seconds) using the 1.6 mW UV LEDs and the same UV dose with the 50 mW UV LEDs after 221 seconds.

Objective 4. To analyse the transmittance losses of the UV-light from the LEDs through the water to determine a maximum water depth along with the effect of turbidity.

This was done in publication 2 by testing UV irradiance of two different wavelengths 265 nm and 275 nm with two different optical power 1.6 mW and 50 mW versus the turbidity and the depth of the water significantly. The results illustrate that these two variables affect the disinfection process, the UV irradiance losses increases by the high turbidity and the depth of the container containing the wastewater. 45.6 % maximum UV irradiance losses was observed using the 275 nm 50 mW UV-LEDs with 0 NTU turbidity level at 2 cm depth, and 73.7 % using the same UV-LEDs with 8 NTU turbidity level at the same depth.

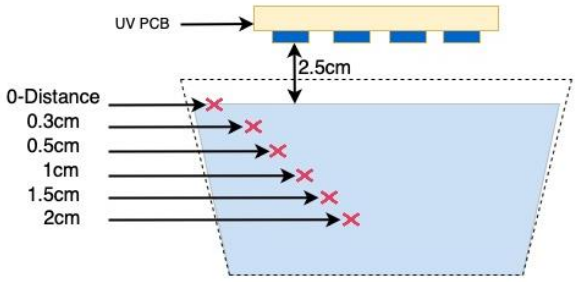


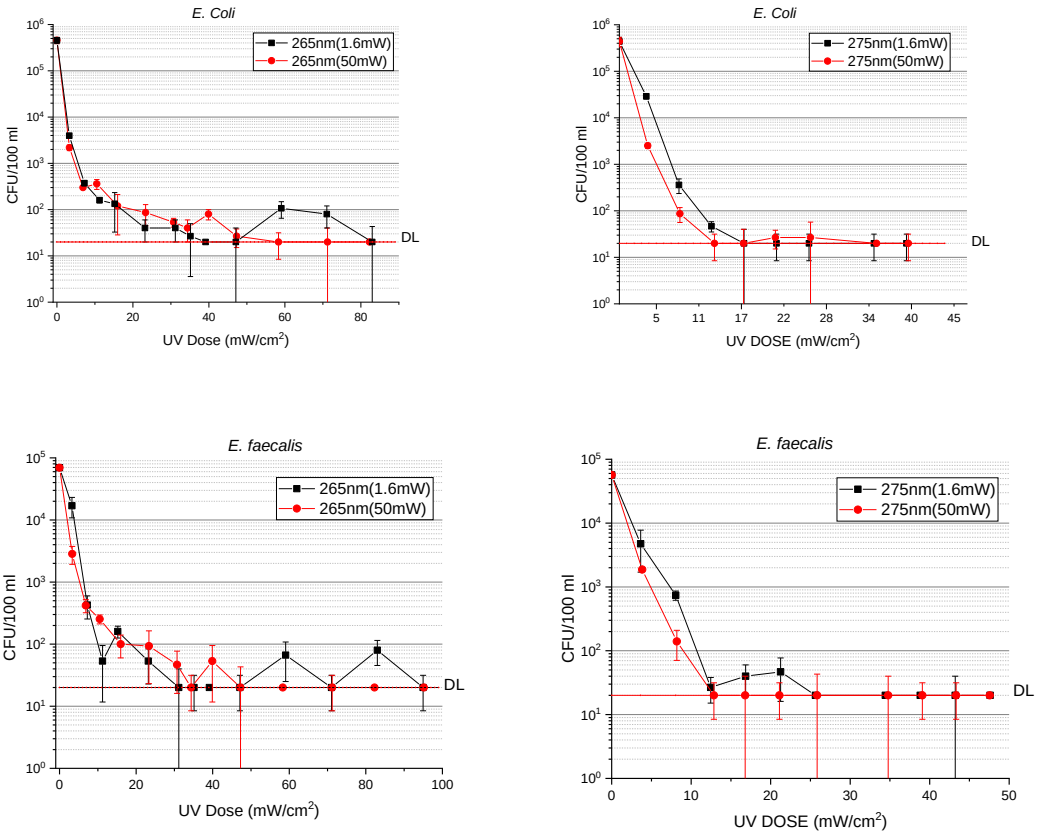
Figure 3 Illustration for the measurement of the optical power of the UV-LEDs and the depth of the water

	UV Irradiance losses (%) with 0 NTU				UV Irradiance losses (%) with 8 NTU			
Water depth (cm)	265 nm (2.5 mW)	265 nm (50 mW)	275 nm (1.6 mW)	275 nm (50 mW)	265 nm (2.5 mW)	265 nm (50 mW)	275 nm (1.6 mW)	275 nm (50 mW)
0.3	11.6	9	8.7	8.7	10.5	16.6	16.8	20.5
0.5	16	13.7	13.1	15.9	19.6	26.8	20	29.6
1	26.4	24.9	23.6	27.9	41.4	45	41.3	49.9
1.5	36.2	34.8	32.5	37.3	57.1	62.5	57.5	63.3
2	44.5	41.9	40.2	**45.6**	69.9	73.3	68.5	**73.7**

Table 1 - Losses percentage due to the depth of the water and the turbidity level

Objective 5. **To verify the law of reciprocity for water disinfection using UV-LEDs. High power and low power UV-LEDs** will be used for treating wastewater with the same UV dose. Microbiological content (*Escherichia coli*, *Enterococcus faecalis* and *Clostridium perfringens*) will be analysed to observe if under the same UV dose but different initial irradiance the bacteria inactivation is the same.

In publication 2 reciprocity law for wastewater disinfection was examined using two UV wavelengths (265 nm and 275 nm) with two different optical power (1.6 mW, 50 mW) to disinfect three microorganisms (*Escherichia coli*, *Enterococcus faecalis* and *Clostridium perfringens*). Similar inactivation rates have been calculated for the two optical powers, with the exception of 275 nm UV-LEDs that showed slightly superior values for the 50 mW LEDs for the case of *Escherichia coli* and *Enterococcus faecalis*



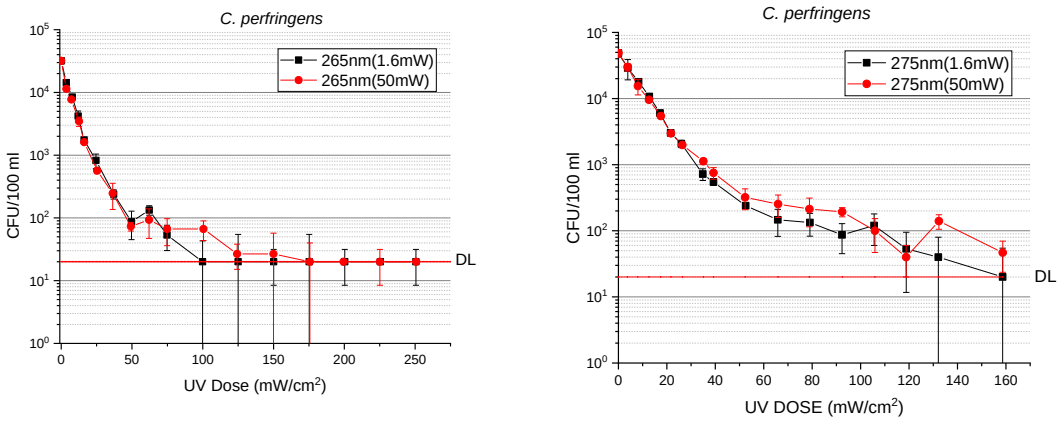


Figure 4 - UV dose vs. CFU/100 ml for the 3 different microorganisms using 265 nm and 275 nm with different optical powers (1.6 mW and 50 mW)

Chapter 5: Conclusion

The conclusion chapter of this thesis presents the findings of the research on the use of ultraviolet LEDs as an alternative method for disinfecting wastewater. The research demonstrates that ultraviolet LEDs are a viable alternative to traditional ultraviolet lamps, as they offer a number of advantages such as being less fragile, the absence of toxic substances, and being available in a variety of wavelengths. Additionally, ultraviolet LEDs require lower voltage to function, making them easy to use in pulsation mode which has the potential to lead to lower costs, although this method was not implemented in the current study. Overall, this research suggests that ultraviolet LEDs have the potential to be a more efficient and effective in the future, as the costs per UV-LED decrease.

In publication 1, The experimental findings reveal the efficacy of using UV-LEDs of low irradiance at various wavelengths (268 nm, 279 nm, and 307 nm) in inactivating diverse strains of bacteria present in real wastewater effluent. The results indicate that the wavelength of 268 nm was the most efficient and rapid in terms of bacterial inactivation, followed by 279 nm. However, the inactivation rate was significantly lower when using a 307 nm wavelength. As such, it is not recommended for use in disinfecting wastewater with UV-LEDs due to its high energy consumption compared to the other two wavelengths.

The data obtained from the experiments indicated the ability to inactivate the three different bacteria strains (*Escherichia coli*, *Enterococcus faecalis*, *Clostridium perfringens*) using UV-LEDs at different wavelengths. However, it was observed that the required UV dose and corresponding time of exposure varied among the different bacteria strains. Specifically, *Escherichia coli* and *Enterococcus faecalis* were inactivated with a minimal UV dose (18.4 mJ/cm^2) and a corresponding time exposure of just 1 minute. On the other hand, *C. perfringens* required higher UV doses and longer time exposures of up to 6 minutes. While it is still possible to disinfect this bacteria strain, further research is necessary to investigate the use of higher powered UV-LEDs which can reduce time exposures, and thus facilitate the scaling-up of small prototype reactors to full-size reactors that can be implemented in wastewater treatment facilities.

In publication 2, The reciprocity law has been found to be valid for UV-LED disinfection using LEDs with wavelengths of 265 nm and 275 nm and two different optical powers of 1.6-2.5 mW and 50 mW. This was determined through testing on three microorganisms present in the effluent of the Linares wastewater plant, including *Escherichia coli*, *Enterococcus faecalis*, *Clostridium perfringens*. The inactivation rates were similar for both optical powers, with the exception of 275 nm LEDs showing slightly better results with 50 mW power for *Escherichia coli* and *Enterococcus faecalis*. However, further investigation is needed to confirm these findings and consideration should be given to

the impact of turbidity and UV transmittance on the results, as a 2 cm water depth resulted in a significant decrease in transmittance.

Future work:

In the future, the scope of the project is planned to be expanded by

- Increasing the volumes of wastewater in the disinfection experiments. This increase will require the development of new printed circuit boards to cover the container holding the wastewater and a temperature management system to accommodate the new printed circuit boards. Additionally, a new electronic system will be designed to control and monitor the new reactor for efficient and effective disinfection.
- And testing the effectiveness of UV-LED disinfection on a wider range of microorganisms, including *Salmonella* and *Legionella*, which are specifically targeted by Spanish regulations in regards to their presence in wastewater treatment systems. This would provide a more comprehensive understanding of UV-LED disinfection's capabilities in this context and help to ensure compliance with relevant regulations (Royal Decree 1620/2007 of 7 December).
- Additionally, I intend to investigate the use of more powerful UV-LEDs, such as those with a power output of 200 mW as they have not been extensively examined in the field of wastewater disinfection.
- Furthermore, in the field of wastewater disinfection utilizing UV-LEDs, there are instances where bacteria re-emerge post-treatment, so the plan is to delve deeper into the underlying causes of bacteria reactivation to understand how to mitigate this phenomenon and develop strategies to mitigate it.
- Another area of focus will be to implement the use of Pulsed Width Modulation (PWM) as an alternative to the continuous mode. PWM involves supplying the UV LEDs with voltage signals that are pulsed at a specific frequency and duty cycle. By employing this technique, the effectiveness in deactivating microorganisms will be determined and also the power consumption during the disinfection process will be reduced.
- Finally, based on the results and insights gained from these experiments, a portable reactor will be designed and developed that utilizes the most effective UV-LEDs and is powered by a solar panel system, making it suitable for use in remote areas where access to electricity may be limited.

Annex A. Publications

A. 1 Publication 1

Ref: Deactivating environmental strains of *Escherichia coli*, *Enterococcus faecalis* and *Clostridium perfringens* from a real wastewater effluent using UV-leds, Heliyon. U.S. National Library of Medicine. Available at: https://pubmed.ncbi.nlm.nih.gov/36636203/ (Accessed: January 22, 2023).

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.

DOI: 10.1016/j.heliyon.2022.e12628

A. 2 Publication 2

Ref: Analysing the Reciprocity Law for UV-LEDs in Water Disinfection of *Escherichia coli*, *Enterococcus faecalis*, and *Clostridium perfringens*, *Water*, vol. 15, no. 2, p. 352, Jan. 2023.

Abstract: The aim of this study is to verify the reciprocity law in the wastewater disinfection processusing UV light. The optical power UV-LEDs used were 1.6 mW and 50 mW, and the wavelengths were265 nm and 275 nm. E. coli, Enterococcus faecalis, and Clostridium perfringens were the three microorgan-isms analysed in the study. The results showed lower inactivation rates around 0.063–0.065 cm2/mJfor 265 nm and 0.047–0.049 cm2/mJ for 275 nm for the Clostridium perfringens compared with theother two bacteria. For E. coli and Enterococcus faecalis, the inactivation rate was almost identical; 0.28and 0.21 cm2/mJ, respectively, using 265 nm wavelength. There was a slightly better inactivationperformance using the medium-power 275 nm UV-LEDs of 0.39 cm2/mJ and 0.29 cm2/mJ for E. coliand Enterococcus faecalis, respectively, and 0.33 cm2/mJ and 0.26 cm2/mJ using the low-power 275 nmUV-LEDs. The analysed data justify the reciprocity law for UV-LEDs disinfection using 265 nm and275 nm UV-LEDs with two optical powers of 1.6 mW and 50 mW.

DOI: 10.3390/w15020352

Annex B. Monitoring system using open hardware and software – Raspberry Pi

A Raspberry-pi-3B was used to control the entire system and also to save the experiments data on an Excel file as seen in Figure (5), like voltage, current, time and temperature. A power switch “N-MOSFET(IRF3205)” was driven by the Raspberry-pi to start and end the experiment in a certain time using python code. In addition to a module “INA219” was measuring the voltage and current of the PCBs during the experiments.

Also, the optical power density was measured using “LT2400” optical sensor and was connected to the Raspberry-pi via USB.

As shown on Figure (1), The “INA219” was connected using the SDA and SCL pins (pin-3 and pin-5) in the raspberry pi and finally the radiometer was connected using the USB connection as it can be seen in Figure (3). In addition of controlling a N-MOSFET(IRF3205-55V-75A) to be able to switch on and off our load in the experiment using pin-33. The pin out of the raspberry-pi can be checked in Figure (2) and the whole system can be check in Figure (3) and Figure (4).

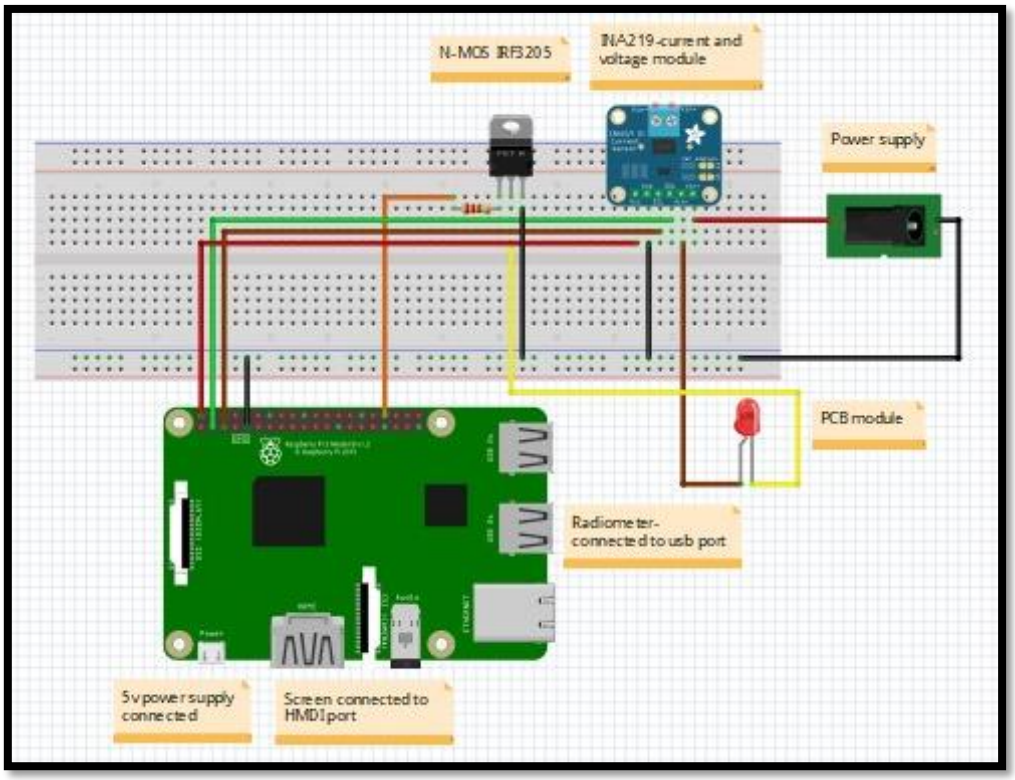


Figure 1 - Monitoring system schematic

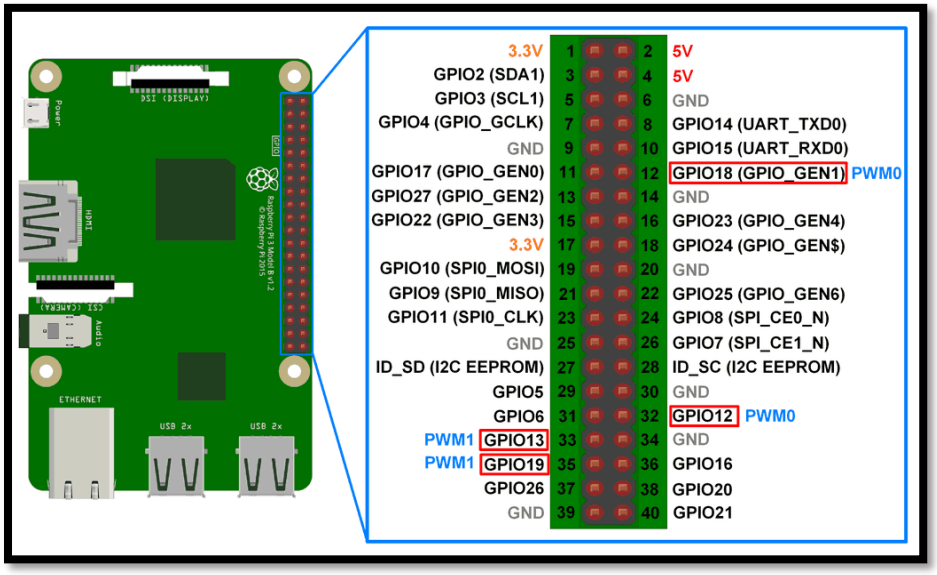


Figure 2 - Raspberry-pi pin out

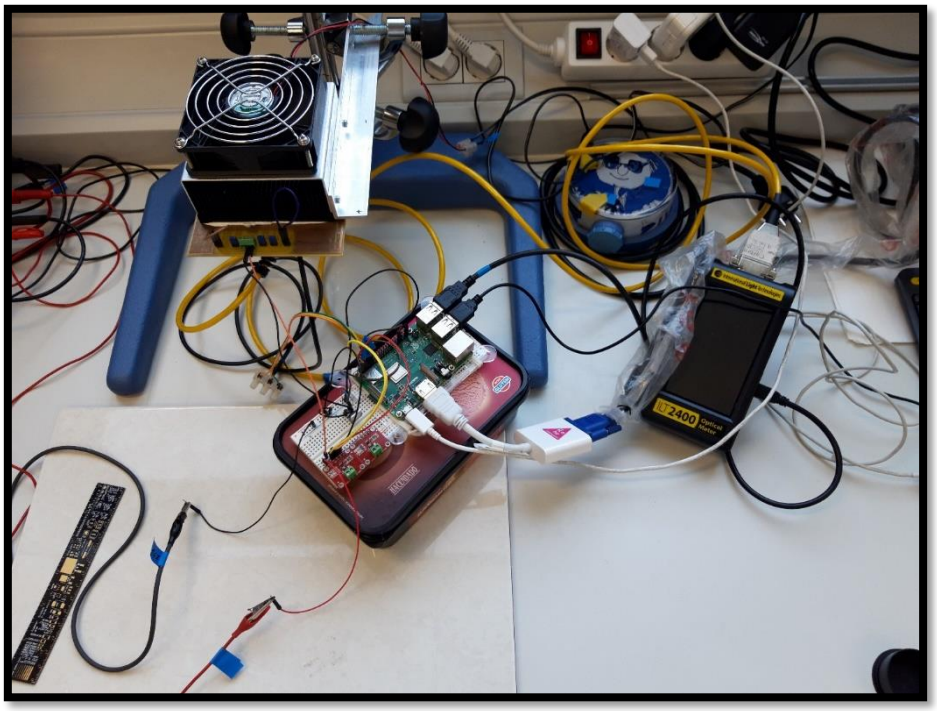


Figure 3 - The monitoring system

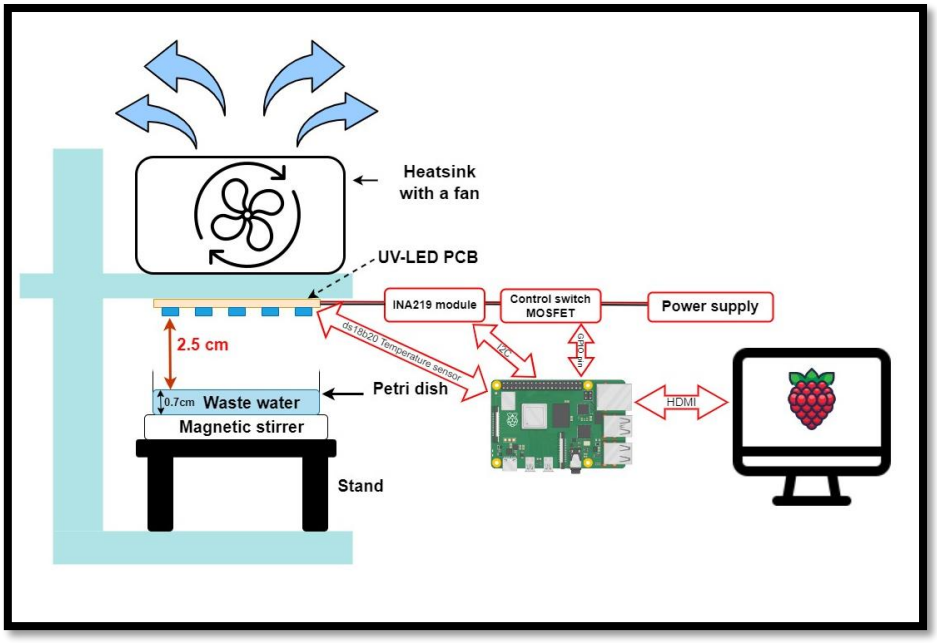
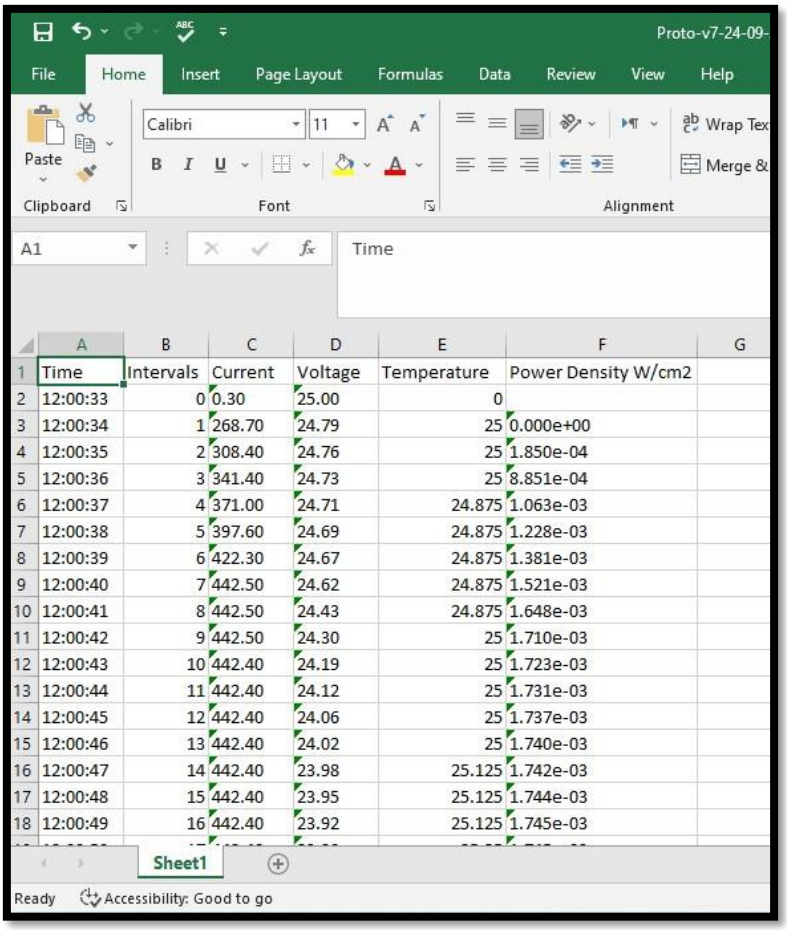


Figure 4 - The graph of the monitoring system



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	A	B	C	D	E	F	G
1	Time	Intervals	Current	Voltage	Temperature	Power Density W/cm2	
2	12:00:33	0	0.30	25.00	0		
3	12:00:34	1	268.70	24.79	25	0.000e+00	
4	12:00:35	2	308.40	24.76	25	1.850e-04	
5	12:00:36	3	341.40	24.73	25	8.851e-04	
6	12:00:37	4	371.00	24.71	24.875	1.063e-03	
7	12:00:38	5	397.60	24.69	24.875	1.228e-03	
8	12:00:39	6	422.30	24.67	24.875	1.381e-03	
9	12:00:40	7	442.50	24.62	24.875	1.521e-03	
10	12:00:41	8	442.50	24.43	24.875	1.648e-03	
11	12:00:42	9	442.50	24.30	25	1.710e-03	
12	12:00:43	10	442.40	24.19	25	1.723e-03	
13	12:00:44	11	442.40	24.12	25	1.731e-03	
14	12:00:45	12	442.40	24.06	25	1.737e-03	
15	12:00:46	13	442.40	24.02	25	1.740e-03	
16	12:00:47	14	442.40	23.98	25.125	1.742e-03	
17	12:00:48	15	442.40	23.95	25.125	1.744e-03	
18	12:00:49	16	442.40	23.92	25.125	1.745e-03	

Sheet1

Ready Accessibility: Good to go

Figure 5 - Example of the output data

B. 1 The coding part

A Python code was written in order to control all the hardware and save the sensors data on an Excel file on the Raspberry-pi. The code is separated to libraries, variables and functions.

B.1.1. Libraries

Many python libraires were used in order to run the experiments without experiencing any delay from the code execution.

```
from _datetime import datetime
import time
import schedule
from schedule import every, repeat, run_pending
import sys
```

"**_datetime**" and "**time**" libraries were used to set the date and time during the experiment. "**schedule**" and "**sys**" used to run, repeat and stop executing the code during the experiments.

```
import busio
```

"**busio**" library was used to activate the 1-wire protocol in order to obtain the temperature measurements from the digital temperature sensor.

```
from board import *
```

"**board**" library used to control the hardware as well as the communication protocols on the Raspberry-pi such as the I2C.

```
import RPi.GPIO as GPIO
```

"**RPi.GPIO**" was used to control the OUTPUT GPIO pins of the raspberry-pi, in our case it was needed to control the N_MOSFET "IRF3205", the OUTPUT voltage of the pin is 3.3V.

```
from adafruit_ina219 import ADCResolution, BusVoltageRange, INA219
```

The "**adafruit_ina219**" was used in obtain the voltage and current from the "INA219" module connected to the Raspberry-pi.

```
import xlsxwriter as xw
```

"**xlsxwriter**" was used to create and insert all the sensors measurements in an EXCEL file.

```
import serial, string
```

"**serial**" and "**string**" were used to make the serial communication with the radiometer "".

```
import _thread
```

"**_thread**" library was really important in order to communicate with the temperature sensor and the radiometer on 2 different cores since they are slow. 3 cores were used in the code. The Raspberry-pi has 4 cores.

```
from w1thermsensor import W1ThermSensor, Sensor
```

"**w1thermsensor**" was used to communicate with the temperature sensor "DS18B20" and to change the resolution if needed.

B.1.2. Variables declarations

In order to save the measurements continuously from the sensors, variables were declared.

```
RadioOutput=""
```

Declaration for the radiometer OUTPUT value.

```
RadioFlag=True
```

Boolean Flag to check if the radiometer will be used in the experiment or not.

```
testDuration=0
```

The test duration entered by the user in seconds.

```
testIntervals=1
```

Setting the time interval to get the measurements every 1 second.

```
i2c_bus = board.I2C()
ina219 = INA219(i2c_bus,0x41)

ina219.bus_adc_resolution = ADCResolution.ADCRES_12BIT_32S
ina219.shunt_adc_resolution = ADCResolution.ADCRES_12BIT_32S

ina219.bus_voltage_range = BusVoltageRange.RANGE_32V
```

Declaring the instance variable of the voltage-current module "INA219" and setting its address, resolution and voltage range so that the raspberry-pi communicate with it through the I2C communication protocol. I2C is a synchronous communication protocol, so the output of bits is synchronized to the sampling of bits by a clock signal between the master (Raspberry pi) and the slave (INA219 module), and the clock signal is always controlled by the master.

```
sensor1 = W1ThermSensor(sensor_type=Sensor.DS18B20, sensor_id="041770da4dff")
sensor1.set_resolution(10)
temperatureThreadOutput=0.0
```

Declaring the variable of digital temperature sensor “DS18B20” and setting its resolution and initial value.

```
GPIO.setmode(GPIO.BCM)
GPIO.setup(13,GPIO.OUT)
GPIO.setwarnings(False)
```

Setting the GPIO pins mode to control the GPIO pins, setting the pin13 as an OUTPUT pin to control the N-MOSFET and lastly setting the warning of the GPIO pins off because if it is on the warnings will stop the execution of the code during the experiment.

```
outputMeasurmentsList=[]
```

experiment creating the list used to save the measured data.

```
finishFlag=False
```

Creating a finishing Boolean flag in order to stop the execution of the code after finishing the experiment.

```
excelFileName=""
```

Declaring a variable to set the name of the EXCEL file where the measurements data will be saved. This variable will be set by the used.

B.1.3. Functions

Functions are written to specify the functionality for every single part in the experiments.

```
def temperatureThread():#core2--->check the thread function down
    temperatureThreadOutput=sensor1.get_temperature()
```

This function used for setting the **temperatureThreadOutput** variable, and to obtain the temperature measurement from the digital temperature sensor “DS18B20” and save it in the **temperatureThreadOutput** variable.

```
def radioThread():#core3--->check the thread function down
    ser=serial.Serial('/dev/ttyUSB1',115200,8,'N',1,timeout=1)
    ser.write(b'gi\r')
    time.sleep(0.5)#giving the radiometer 0.5 seconds to get the response
    output=ser.readline()
    RadioOutput=str(output,'utf-8')
    ser.flush()
    ser.close()
```

“**radioThread**” function was used to setup the “**RadioOutput**” as a variable, set the serial communication parameters such as the name of the USB port, communication speed

and timeout which are recommended in the radiometer service manual. By sending "**gi\r**" to the radiometer and waiting for 0.5 seconds, the measurement can be read by the raspberry-pi and saved in the "**RadioOutput**" variable, and at the end the serial channel should be flushed and closed to get ready for the next call.

```
def createAndAddDataToExcelFile(excelFileName, outputMeasurmentsList):
    # Createing Excel file
    workbook = xw.Workbook(excelFileName+ "-" +
datetime.now().date().strftime("%d-%m-%Y") +".xlsx")
    worksheet1 = workbook.add_worksheet()
    worksheet1.write(0,0,"Time")
    worksheet1.write(0,1,"Intervals")
    worksheet1.write(0,2,"Current")
    worksheet1.write(0,3,"Voltage")
    worksheet1.write(0,4,"Temperature")
    worksheet1.write(0,5,"Power Density W/cm2")
    # worksheet1.write(0,6,"Power Density mW/cm2")

    # Inserting data to the Excel file
    existingWorkingSheet = workbook.get_worksheet_by_name("Sheet1")
    itemCounter=0
    while itemCounter < len(outputMeasurmentsList):
        measurmentValue=0
        while measurmentValue < len(outputMeasurmentsList[itemCounter]):
            existingWorkingSheet.write(itemCounter+1, measurmentValue,
outputMeasurmentsList[itemCounter][measurmentValue])
            measurmentValue += 1
        itemCounter += 1
    workbook.close()
```

"**createAndAddDataToExcelFile**" function was used to save all the measured data during the experiment in the **outMeasurementsList** and save it in an excel file named by the user.

```
def startButtonFunction():
    global RadioOutput
    global temperatureThreadOutput
    global testDuration
    global finishFlag
    global excelFileName
    global testIntervals

    #startingTestTime=datetime.now()#.replace(microsecond=0)

    GPIO.output(13,GPIO.HIGH)

@repeat(every().second)
def runCode():
   _thread.start_new_thread(temperatureThread, ()) #--> Starting the second
thread of by calling the temperature sensor function

   #if RadioFlag==True:
   #  _thread.start_new_thread(radioThread, ()) #--> Starting the third
thread of by calling the radiometer sensor function, if selected

   _thread.start_new_thread(radioThread, ()) if RadioFlag==True else None

   bus_voltage = ina219.bus_voltage  # voltage on V- (load side)
   shunt_voltage = ina219.shunt_voltage  # voltage between V+ and V- across
the shunt
   current = ina219.current  # current in mA
   Voltage = "{:.2f}".format(bus_voltage + shunt_voltage)
   Current = "{:.2f}".format(current)

   outputMeasurmentsList.append([datetime.now().strftime("%I:%M:%S"),
len(outputMeasurmentsList), Current, Voltage, temperatureThreadOutput,
RadioOutput.replace('\n','').replace('\r','').replace("'","").replace('
','')])

   if len(outputMeasurmentsList) >= testDuration:
      print("-----------LED off-----------")
      finishFlag=True
      GPIO.setmode(GPIO.BCM) #--> Preparing the mode used in controlling the
GPIO pins of the raspberry-pi
      GPIO.setup(13,GPIO.OUT) #--> setting pin 13 as an OUTPUT pin
      GPIO.setwarnings(False) #--> setting the warnings off because it is not
needed in our application
      GPIO.output(13,GPIO.LOW)
      GPIO.cleanup()
      createAndAddDataToExcelFile(excelFileName, outputMeasurmentsList)
      outputMeasurmentsList.clear()
      schedule.cancel_job(runCode)
      schedule.clear()
      print("End test")
      sys.exit()
```

"**startButtonFunction**" is the function which sets the time, starts executing the code, turns on and off the GPIO, takes the measurements form the sensors, saves the measurements in the list and after finishing the experiment it calls the "**createAndAddToExcelFile**" function and finally stops the execution.

```
while finishFlag==False:

   excelFileName = input("Input the excel file name: ")

   answer = input("Are you going to use the radiometer? yes or no: ")
   if answer=="no":
      RadioFlag=False
      RadioOutput="Not-Connected"
   elif answer == "yes":
      RadioFlag=True

   testDuration = int(input("Input the test duration in seconds: "))

   startButtonFunction()
   while finishFlag==False:
      schedule.run_pending() #--> start running the scheduled function every
1 second
```

And lastly, the main function in the entire code, it asks the user about the name of the Excel file, if the radiometer will be used or not and the duration of the experiment in seconds. After that it calls the "**startButtonFunction**" to run the experiment.

In some experiments which had high voltage LEDs "more than 32 volts", the voltage current module was discarded from the experiment due to its limitation and the voltage and current reading were obtained from the power supply manually.

Chapter 6: Introducción

La función principal de las depuradoras es eliminar las sustancias nocivas de las aguas residuales antes de verterlas al medio ambiente. Desde objetos más grandes como piedras o palos (u otros objetos similares) hasta los microorganismos finales como bacterias, que se reducen o eliminan por completo del agua. Una cuestión importante en el funcionamiento de las plantas de aguas residuales es el gran consumo eléctrico y, lo que es más importante, el hecho de que alrededor del 30 % al 50 % del consumo total corresponde a las operaciones de funcionamiento y mantenimiento, que son procesos permanentes.

Figura 1 - Depuradora de Linares

En general, las instalaciones de tratamiento de agua de una depuradora constan de varias etapas. La primera se llama “Pretratamiento” y es donde los objetos más grandes, como piedras, ramas, plásticos, ropa, etc., que pueden producir problemas de funcionamiento y mantenimiento en la maquinaria, son eliminados. Posteriormente se realiza el proceso de eliminación de arenas y grasas. El siguiente paso es el denominado “Tratamiento primario”, cuyo objetivo principal es la eliminación del 50 % de los sólidos suspendidos y del 20 % de la demanda de oxígeno bioquímico (DBO). Para este tratamiento se utiliza la sedimentación y un tratamiento físico-químico (coagulación + floculación).

La sedimentación se realiza en un tanque cilíndrico dividido en tres zonas: la primera es la zona de afluencia donde el agua entra al tanque de sedimentación por el centro, en segundo lugar, la zona de sedimentación, donde los lodos sólidos se depositan en el fondo del tanque y, a continuación, son recogidos por el colector de lodos, y finalmente la zona efluente que permite al agua tratada continuar a la siguiente etapa del tratamiento (Figura 2).

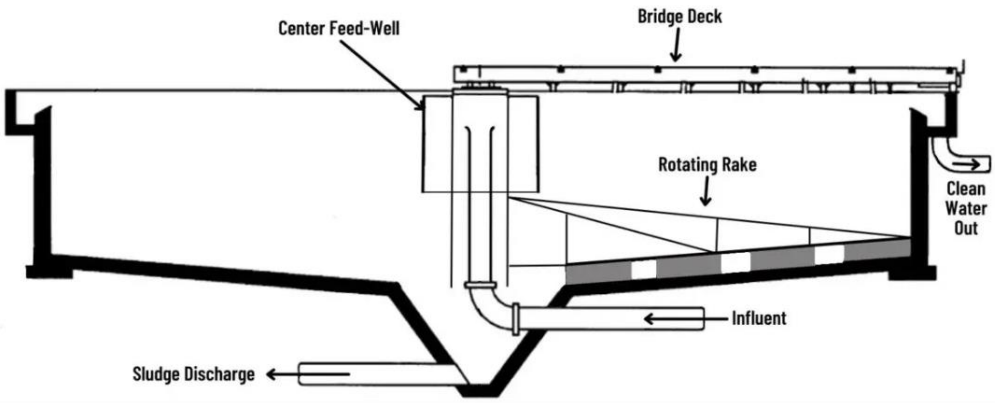


Figura 2 – Modelo de sedimentación.

Después de la sedimentación, sigue el tratamiento físico-químico, que consiste en un proceso de coagulación/floculación. El objetivo principal de este proceso es reducir la turbidez en el agua, ya que una turbidez menor permite un mayor porcentaje de desinfección. Al añadir un coagulante químico al agua, las pequeñas partículas y grupos de impurezas se agrupan para formar partículas más grandes que permiten asentarse en el tanque por gravedad, como se muestra en la Figura 3.

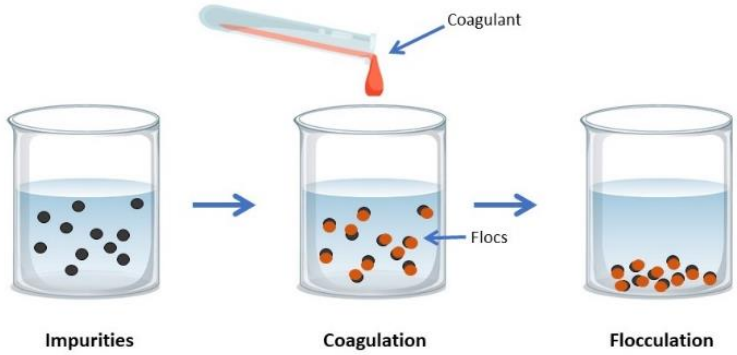


Figura 3 – Evolución del proceso Coagulación/Floculación.

A continuación, el "Tratamiento secundario" elimina el 90% de las partículas suspendidas, el 70 – 90 % de la DOB_5 (25 mg/L) y la Demanda Química de Oxígeno (DQO) debe alcanzar el límite de 125 mg/L [33]. Por último, el "Tratamiento Terciario" es una etapa de filtrado y desinfección usando normalmente luz ultravioleta (UV) o cloración. La Figura 4 muestra todas las etapas del tratamiento de aguas residuales en una depuradora.

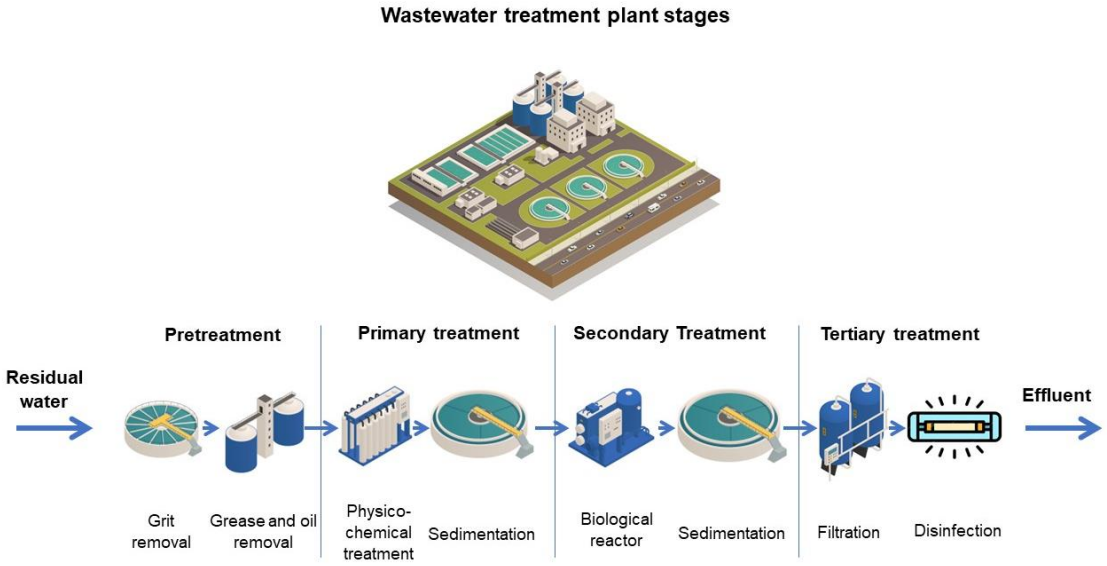


Figura 4 – Etapas del tratamiento de una depuradora de agua.

Como resultado del alto consumo energético, el "Tratamiento Terciario", que es el responsable de la mejora final de la calidad del agua hasta su posible reutilización, normalmente no se implementa en las depuradoras debido al coste de inversión, operación y mantenimiento (principalmente electricidad). En España sólo es obligatoria su implementación en las llamadas "zonas sensibles" de acuerdo a la ley española [34]. Pero la directiva europea (2000/60/EC y 91/271/ECC [35 – 36]) requiere el mejor tratamiento de aguas posible hasta llegar al vertido "cero", es decir, con una muy alta calidad. El hecho de que el agua no esté adecuadamente purificada produce un alto impacto medioambiental de vertidos en superficies, además de la perdida de la oportunidad de reutilizar el agua que es una fuente escasa.

Los dos métodos principales que se utilizan actualmente en el "Tratamiento Terciario" son la cloración y la desinfección con lámparas ultravioleta artificiales. En cuanto al primero, la "cloración", ésta es muy utilizada por su eficacia. Consiste en una concentración de cloro mezclado con el agua tratada en una cámara donde tiene que recorrer un largo camino. El agua se desinfecta durante ese tiempo y después de este proceso está lista para ser vertida. Sin embargo, se requiere un suministro continuo de productos químicos y este proceso también puede producir un número significativo de gases tóxicos si no se maneja adecuadamente. El método alternativo es el uso de lámparas ultravioletas artificiales convencionales, que, a pesar de ser muy eficaces y rápidas, presentan varios inconvenientes: alto consumo energético, un pico espectral de respuesta centrado únicamente en la longitud de onda de 255 nm, altos valores de voltaje para su funcionamiento, y tiempos de arranque relativamente lentos lo que las hace inadecuadas para funcionar de forma discontinua. El otro problema principal es la necesidad de sustituir las lámparas cada 6 o 12 meses, lo que aumenta el coste de funcionamiento. Por otra parte, las lámparas convencionales contienen mercurio, que es muy tóxico. Las lámparas necesitan seguir un proceso especial de reciclaje para

extraer el mercurio y no liberarlo al medioambiente [2,3,4,7,9,11,12,13,14,19,22,23,24,29,30]. Por tanto, es necesario buscar nuevas soluciones efectivas para la etapa de desinfección del agua que sea de bajo consumo eléctrico y bajo impacto medioambiental. La Figura 5 señala las principales desventajas de las lámparas ultravioletas de mercurio.

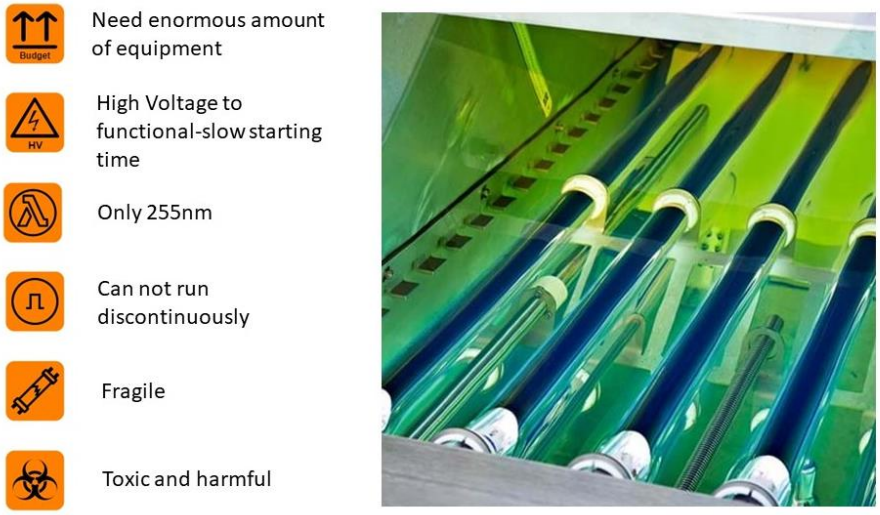


Figura 5 – Algunas desventajas de las lámparas UV.

Una de las tecnologías que presentan gran potencial para la desinfección del agua con bajo impacto ambiental y gran eficacia son los LED ultravioleta. Tienen grandes ventajas frente a las lámparas convencionales de mercurios como son el pequeño tamaño de los mismos, que los hacen adecuados para el diseño de cualquier tipo de reactor como se muestra en la Figura 6, baja temperatura de funcionamiento, menor voltaje necesario en comparación con las lámparas UV tradicionales o el encendido/apagado instantáneo que permite el modo de operación como luz pulsada y la reducción de consumo energético asociada. Por otro lado, se fabrican en diferentes longitudes de onda, lo que los convierte en una solución óptima para desinfectar diversos tipos de microorganismos, ya que cada uno de ellos es sensible a una longitud de onda concreta, a diferencia de las lámparas UV, que sólo se centran en la longitud de onda de 255 nm. Por último, y lo más importante, son respetuosas con el medio ambiente, ya que no contienen mercurio ni ningún tipo de sustancia química tóxica como las lámparas UV [2,3,4,7,9,11,12,13,14,19,22,23,24,29,30].

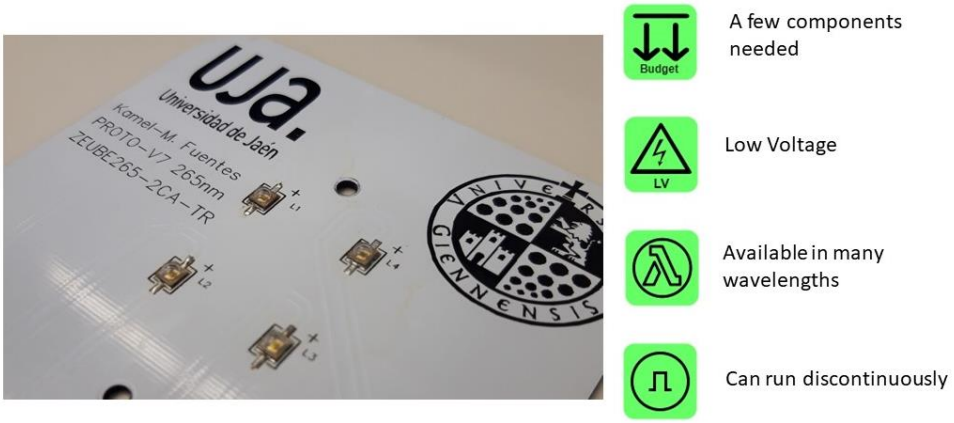


Figura 6 – Ventajas de LEDs UV.

En las siguientes secciones se describirán en detalle los LED UV y su uso en el tratamiento de desinfección del agua.

6.1 Estructura y funcionamiento de los LED

LED son las siglas de Light Emitting Diode (diodo emisor de luz) y, como su nombre indica, se basa en un diodo. Normalmente, los diodos están formados por uniones P-N y tienen dos modos de funcionamiento. El primero es la "polarización directa", cuando el terminal positivo del diodo está conectado al positivo de la fuente de alimentación y, del mismo modo, los terminales negativos del diodo y de la fuente de alimentación están conectados, este modo estrecha la región de agotamiento entre la unión P-N y permite que los electrones libres crucen la unión y se recombinen con los huecos libres. Este movimiento de electrones libera energía en forma de calor, pero en el caso de los LED la energía se libera en forma de luz. El otro modo es el de "polarización inversa", en el que los terminales de alimentación del diodo están conectados al revés que los terminales de alimentación, y simplemente en este caso el diodo o los LED no conducen y actúan como circuito abierto.

En la se muestra una ilustración gráfica de la estructura del LED desde el diseño exterior hasta la unión P-N.

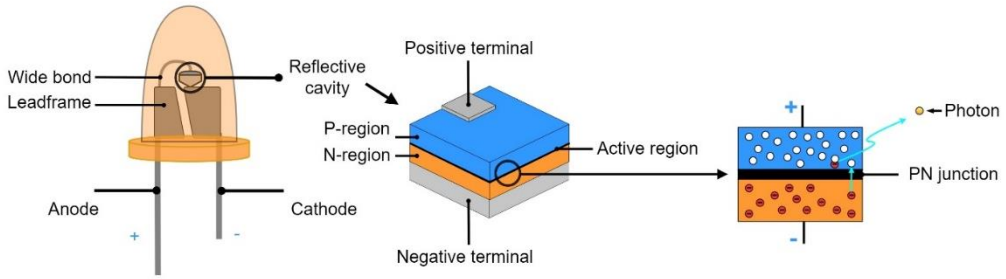


Figura 7 – Estructura del LED.

6.2 Propiedades de la luz ultravioleta

La luz ultravioleta se puede encontrar en el espectro de la luz como se muestra en la Figura 8 en el rango de 200 nm a 400 nm, y se divide en tres subsecciones llamadas UV-A, UV-B y UV-C [16]. Esta luz es invisible a los ojos humanos y algunas de sus longitudes de onda son nocivas para la piel y la vista [17]. El sol es la mayor fuente de luz ultravioleta, pero la mayor parte queda bloqueada por la capa de ozono de la atmósfera. La UV-A no es absorbida ni filtrada por la atmósfera, como tampoco lo es la UV-B, pero la UV-C, que desempeña un papel importante en el proceso de desinfección, no atraviesa la atmósfera.

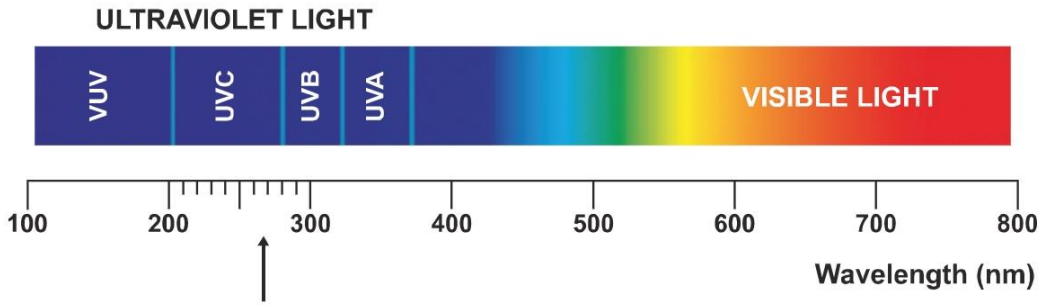


Figura 8 – Espectro de la luz.

El espectro de longitudes de onda de los rayos UV-A comprende las longitudes de onda de 315 nm a 400 nm, el de los rayos UV-B de 280 nm a 315 nm y el de los rayos UV-C de 200 nm a 280 nm. Convencionalmente, el pico óptimo para eliminar bacterias y virus se ha considerado el 254 nm (pico de las lámparas de mercurio). Y como ya se ha mencionado, ninguna luz UV-C atraviesa la atmósfera, por eso se produce artificialmente para diversas aplicaciones, incluida la desinfección del agua. Según numerosos estudios, el UV-C es el rango ideal para matar virus, bacterias, esporas de moho y otros tipos de contaminación [8,10,19,21,23,27,28,30].

Este tipo de UV-C puede emitirse mediante lámparas UV de mercurio que se encuentran en los sistemas de desinfección del agua. Pero debido a sus inconvenientes (alto voltaje de funcionamiento, frágiles, tiempo de calentamiento, no pueden funcionar en modo pulsado, contienen sustancias tóxicas como el mercurio), la investigación científica está tratando de encontrar una solución para sustituir a las lámparas UV por otras tecnologías más limpias como los LED UV.

6.3 ADN y desactivación

ADN significa "ácido desoxirribonucleico", que es una sustancia molecular portadora de la información genética para el funcionamiento y la reproducción de cualquier organismo. Está formado por subunidades denominadas nucleótidos, cada una de ellas compuesta por tres partes diferentes: desoxirribosa, fosfato y una de las cuatro bases nitrogenadas que son la timina (T), la adenina (A), la citosina (C) y la guanina (G). Estas cuatro bases forman pares de timina unida a la adenina o de citosina unida a la guanina.

Se ha descubierto que las longitudes de onda UV tienen unos fuertes efectos para detener los microorganismos como las bacterias rompiendo los enlaces y neutralizarlos y prevenir que se vuelva a generar [31].

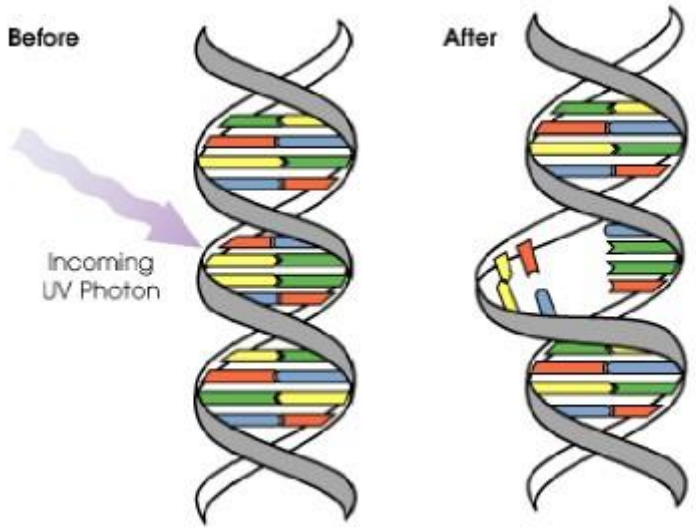


Figura 9 – Desactivación del ADN mediante luz ultravioleta [18].

6.4 Trabajos anteriores sobre el tratamiento de aguas mediante LED UV

En los últimos diez años, los científicos han centrado su atención en diferentes formas de desactivar algunos microorganismos de las aguas residuales para hacerlas utilizables utilizando LED UV. La mayoría de los montajes experimentales consisten en una placa de circuito impreso que contiene los LED UV conectados a un sistema de refrigeración que ilumina una placa de Petri que contiene el agua que se va a tratar.

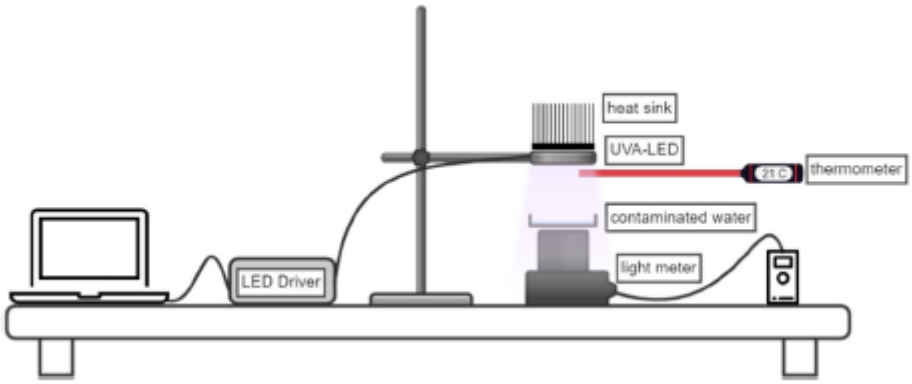


Figura 10 – Configuración del experimento.

6.4.1 Iluminación estándar (continua) - Efecto de diferentes longitudes de onda

Un grupo de investigadores de la Universidad de Tokio dirigido por K. Oguma en 2018 trabajaron en la desinfección de *Escherichia coli*, esporas de *Bacillus subtilis* y bacteriófagos utilizando tanto la UV convencional de baja presión basada en mercurio como LEDs UV de 265 nm, 280 nm y 300 nm. Los resultados mostraron que la longitud

de onda de 265 nm era la más eficaz para reducir la mayoría de las bacterias, pero también que el LED de 280 nm presentaba el menor consumo de energía en comparación con el resto de fuentes de luz [1]. Otro artículo de Matsumoto, Tatsuno y Hasegawa en 2019 muestra que el LED de 265 nm de longitud de onda podía desinfectar muchos tipos de bacterias que habían sido preparadas artificialmente en el laboratorio, como *E. coli*, *V. cholerae* y *C. parvum* [2].

Sari, Heikki y Mike también trabajaron con una configuración única de iluminación continua en sus experimentos en 2009, creando dos reactores con un ángulo de visión de 120°, uno para 269 nm y el otro para 276 nm Cada reactor contenía diez LEDs UV que estaban unidos a un tubo de plástico negro de 7 cm de diámetro y 20 cm de altura, situados por encima del agua de muestra de 25 ml en la placa Petri. Utilizaban un agitador magnético que proporcionaba una mezcla adecuada del agua evitando sedimentación. Además, probaron diferentes tipos de agua como agua ultrapura, agua con nutrientes y agua con nutrientes y con ácidos húmicos junto a la *E. coli* artificial. El experimento concluyó que la longitud de onda más baja, 269 nm, era más eficaz, a pesar de la ligera diferencia en los resultados, aunque la que emitía una longitud de onda más alta, 276 nm, tenía el doble de potencia óptica en comparación con la otra [7].

Würtele et al., del Instituto de Tecnología de Berlín, trabajaron en 2010 en la desinfección de esporas de *Bacillus subtilies* cultivadas en laboratorio utilizando LED de 269 nm y 282 nm. Se fabricaron dos módulos: el primero, estático, consistía en 33 LED de 269 nm con una potencia de emisión de 0,33 mW y 20 mA por LED, dispuestos en una rejilla de 1 LED/cm^2. El segundo módulo era un canal de flujo fresado de aluminio de 6 mm de ancho y 5 mm de profundidad cubierto por una pieza de cristal Suprasil® de 2 mm de grosor, creado utilizando 33 LED de 282 nm y un caudal de 12 ml/min. Se colocaron en tres círculos concéntricos con diámetros de 1,5 cm, 3,5 cm y 5,2 cm, con la misma corriente de 20 mA, la potencia óptica de salida de los LED de 282 nm era casi el doble en comparación con el módulo de 269 nm. La configuración del experimento del primer modelo era relativamente diferente, ya que colocaron los módulos bajo placas de Petri de 6 cm de diámetro y pusieron sobre los módulos una base de Suprasil® de 2 mm de grosor que reducía la luz UV en un 10%. Todos los LEDs de este artículo fueron similares en las características de corriente-voltaje, todos presentaban 20 mA de corriente y los valores de voltaje fueron de 5,8 V y 6,3 V para los LEDs de 269 nm y 282 nm, respectivamente. Durante los experimentos se tomaron muestras de 1,5 ml tras 372, 248, 155, 62 y 0 segundos de exposición para los LED de 269 nm, y tras 255, 170, 106, 43 y 0 segundos para los LED de 282 nm. Los LED de 269 nm alcanzaron un mayor nivel de inactivación que los LED de 282 nm para la misma fluencia aplicada. El equipo también observó una reducción del 40 % en la potencia óptica emitida del LED de 269 nm tras 100 horas de emisión, pero sin cambios en la longitud de onda [8].

6.4.2 Iluminación pulsada frente a iluminación continua

Por otro lado, Song, Taghipoir y Mohseni en 2018 utilizaron un modo de funcionamiento diferente de los LED UV, utilizando iluminación pulsada y comparando con continua para desactivar *E. coli* cultivada en laboratorio y el *colifago MS2* bajo una longitud de onda de 265 nm. Un LED de 265 nm de alta potencia se acopló a un disipador térmico con ventilador para disipar el calor y se conectó a un termopar para controlar la temperatura; el LED UV se colocó sobre una placa Petri de vidrio de 9 cm de diámetro con 50 mL de muestra de agua. La distancia entre el LED UV y la superficie del agua era de 2 cm. Su análisis indica que la irradiación pulsada al 90% y al 75% del ciclo de trabajo produjo una inactivación ligeramente inferior en comparación con el modo continuo [3].

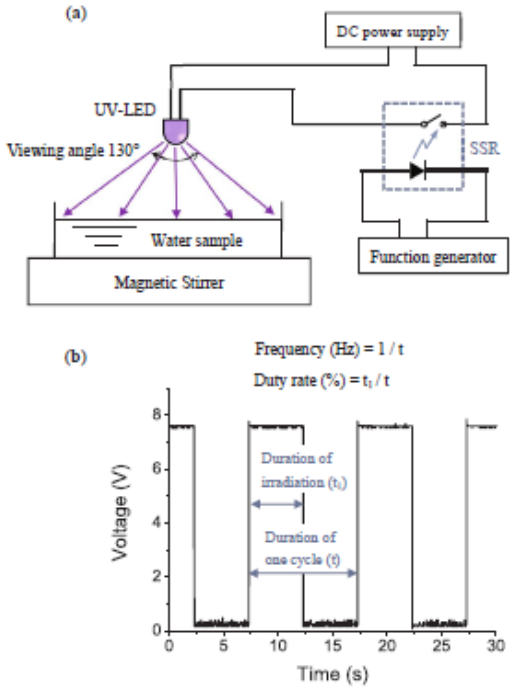


Figura 11 - Montaje del experimento de Song et al., (a) Diagrama esquemático de los circuitos UV-LED pulsados, (b) ilustración de la forma de onda del voltaje para la irradiación UV-LED pulsada a una frecuencia de 0,1 HZ y un ciclo de trabajo del 50 % [3].

De forma similar a la técnica anterior, se utilizaron modos de funcionamiento pulsado y continuo para *E. coli* y *MS-2* utilizando una lámpara de arco de mercurio de baja presión de 15 vatios con 253 nm y varios LED UV con diferentes longitudes de onda (255 nm,265 nm y 285 nm) por Sholter y Linden en 2019. Se examinaron cinco ciclos de trabajo (10, 25, 50, 75 y 90%) y tres frecuencias (1, 10 y 100 Hz). Los resultados mostraron que no se encontraron diferencias significantes entre la inactivación de *E. coli* en todas las frecuencias para tasas de trabajo y longitudes de onda comparables, sin embargo, el modo de pulsación con tasas de funcionamiento del 75 % y del 90 % dio lugar a una tasa de inactivación más elevada en comparación con el modo continuo utilizando los LED UV de 265 nm, así como con las tasas de funcionamiento del 50 % y del 75 % utilizando los LED UV de 285 nm a una dosis UV de 10 mJ/cm^2. [5].

P.O. Nyangaresi, Yi y Goulong en 2019 hicieron la misma comparación entre irradiación pulsada y continua sobre *E. coli* artificial, y estudiaron el efecto de conducir los LED con diferentes corrientes y monitorizaron la temperatura del LED midiendo la temperatura de soldadura del terminal catódico del LED durante los experimentos. En este experimento se utilizaron nueve LED UV de 265 nm y 280 nm con 0,88 y 1,01 mW respectivamente, con voltajes en torno a 5,7 y 5,4 V y valores de corriente de 20 mA por LED. Los nueve LED UV se montaron en una placa de circuito impreso de aluminio para aumentar la disipación del calor. El reactor PCB estaba por encima de un plato de 60 mm de diámetro a 30 mm de altura. Se aplicaron frecuencias de pulso de 1 kHz, 5 kHz y 10 kHz con ciclos de trabajo del 10 %, 25 %,50 %,75 % y 90 %. Como era de esperar, la radiación pulsada mantuvo la temperatura de los LEDs más baja en comparación con la radiación continua, mientras que la irradiación continua provocó el aumento de la temperatura de los LEDs y al aumentar la corriente de conducción se observó un impacto negativo en la longitud de onda y la potencia óptica. En cuanto a la inactivación de *E. coli*, la diferencia observada en la inactivación logarítmica de *E. coli* se debía únicamente a la longitud de onda, no a la técnica utilizada, ya que el LED UV de 265 nm presentaba una inactivación logarítmica de 4,4, ligeramente superior a la del LED de 275 nm, y no había diferencia entre el modo pulsado y el modo continuo [19].

Utilizando las mismas longitudes de onda anteriores, 265 nm y 280 nm, Zou et al. en la Universidad de Nanjing en 2019 aplicaron la irradiación de LEDs de baja potencia de 1 mW 265 nm y 280 nm, así como un LED de alta potencia de 30 mW y 285 nm sobre *E. coli* cultivada en laboratorio. Se crearon tres módulos, el primero para el único LED de alta potencia, y el segundo y el tercero con 9 LED cada uno para la longitud de onda de 265 nm y 280 nm. Los módulos se colocaron sobre una placa de Petri de 8,6 cm de diámetro, y la corriente fue de 20 mA para 265 nm y 280 nm para cada LED, y de 350 mA para el LED de alta potencia de 285 nm. En este estudio se probaron cinco ciclos de trabajo diferentes: 5 %, 10 %, 20 %, 50 % y 100 % a 1 Hz, 10 Hz, 100 Hz y 1000 Hz. Sorprendentemente, la eficacia de inactivación aumentó sustancialmente a medida que el ciclo de trabajo disminuía del 100 % al 5 % con la misma dosis de UV. La inactivación logarítmica mejoró con los LED pulsados de 265 nm y 280 nm, y aumentó notablemente con los LED de alta potencia de 280 nm [21].

6.4.3 Modos de iluminación simultánea y secuencial

Otra técnica de iluminación utilizada consiste en combinar más de una longitud de onda ultravioleta (UV-C, UV-B y UV-A) para purificar el agua. Song, Taghipour y Mohseni en 2018 utilizaron *E. coli* preparada en laboratorio y *Coliphage MS2* [4] y la expusieron a la irradiancia UV-LED siguiendo dos modos de operación diferentes: modos simultáneos y secuenciales. En el modo simultáneo, dos LED UV con diferentes longitudes de onda se encendieron al mismo tiempo para exponer su luz sobre la muestra de agua, mientras que la irradiación secuencial funciona encendiendo un LED UV durante un período, y luego se apaga mientras que otro LED UV con una longitud de onda diferente se

encendió durante otro período de tiempo. En este estudio se utilizaron tres chips de LED UV con longitudes de onda de 265 nm, 285 nm y 365 nm sobre una placa Petri de 9 cm de diámetro a 2 cm de distancia del módulo. Además, una caja negra cubría el reactor durante el experimento para evitar cualquier posible foto reactivación por la luz ambiental. Las conclusiones demuestran que la combinación de UV-C y UV-B siempre lograba un efecto aditivo sobre la inactivación de los microorganismos, sin embargo, la combinación de UV-A con UV-C/UV-B simultáneamente o la aplicación de UVA después de UV-C/UV-B reducía la tasa de inactivación de *E. coli* debido al efecto de reparación del ADN de UVA.

6.4.4 Combinación de longitudes de onda

En cuanto a la combinación de longitudes de onda, Li et al. de la Universidad de Tsinghua en 2017 examinaron el efecto de la combinación de LEDs de 265 nm y 280 nm utilizando LED UV de cultivo de laboratorio de *E. coli* comparando una lámpara de baja presión de mercurio. Se utilizaron cuatro unidades de LED: 265 nm, 280 nm, la combinación de 265 nm (50 %) + 280 nm (50 %), y 265 nm (25 %) + 280 nm (75 %). Para la combinación de 265 nm, se conectaron dos PCB de LED en paralelo. Los PCB se colocaron sobre una placa de Petri de 2,6 cm de diámetro, mientras que la lámpara de baja presión se colocó a 40 cm sobre una placa de Petri de 8,6 cm. Se observó que los LED de 265 nm eran más eficaces que los LED de 280 nm y la lámpara de baja presión en la inactivación, y no se observó ningún efecto significativo utilizando la combinación de 265 nm y 280 nm. Además, no se observó ningún efecto de reactivación tras utilizar LED de 265 nm y lámpara de baja presión, mientras que la foto reactivación y la reparación oscura aparecieron tras utilizar LED de 280 nm a baja intensidad de irradiación [20].

Mediante aguas residuales reales, Chevremont et al. estudiaron en 2012 el efecto de LED UV-A y UV-C simples y acoplados. Se probaron cuatro longitudes de onda diferentes de LED UV, 254 nm, 280 nm, 280 nm y 405 nm y combinaciones de 254/365 nm, 254/405 nm, 280/405 nm. La corriente variaba entre los distintos LED de 46 mA a 115 mA, y la potencia óptica era de 0,29, 0,55, 1,69 y 10,23 mW para los LED de 254 nm, 280 nm 365 nm y 405 nm respectivamente. La placa de Petri de 55 mm de diámetro se colocó 1 cm por debajo de los LED y se llenó con 10 mL de agua; la exposición se inició desde la permanencia durante 60 segundos. Para la desinfección se seleccionaron *bacterias mesófilas*, *estreptococos fecales*, *coliformes totales* y *coliformes fecales*. Los resultados mostraron que la combinación de 280/365 nm o 280/405 nm puede lograr una notable reducción de *bacterias mesófilas*, y fueron más eficaces que la irradiación única para *enterococos fecales*, *coliformes totales* y *coliformes fecales* [12].

Wan et al. en 2019 analizaron el efecto de la lámpara tradicional a base de mercurio frente a los LED de 280 nm y 265 nm y su combinación. En este estudio se probaron *Aspergillus niger*, *Penicilium polonicum* y *Trichoderma harzianum* cultivados en laboratorio, que son tres especies de hongos transmitidos por el agua. La distancia entre

los módulos LED UV y la superficie del agua dentro de la placa de Petri de 4 cm de diámetro fue de 2 cm. En el caso de la lámpara UV LP, 50 cm fue la distancia sobre la placa de Petri de 12 cm de diámetro. La irradiancia fue de 0,215 mW/cm^2 para los LED de 265 nm, de 0,214 mW/cm^2 para los LED de 280 nm, de 0,185 mW/cm^2 para la combinación de LED UV de 265/280 nm y de 0,120 mW/cm^2 para la lámpara de 254 nm. Antes de los experimentos, los módulos LED UV se calentaron y alcanzaron una fase de emisión estable durante 5 minutos y la lámpara de 254 nm se sometió al mismo proceso durante 30 minutos. Los resultados indicaron que el rendimiento de inactivación de los LED UV y su combinación fueron más eficaces para las esporas fúngicas en comparación con la lámpara UV LP de 254 nm, mientras que no se observaron diferencias significativas entre los LED UV, especialmente los de 280 nm y 265/280 nm, que causaron más daño a las esporas fúngicas que la lámpara LP. Sin embargo, el consumo de energía eléctrica de los LED UV fue mayor que el de la lámpara LP [24].

En 2011, Chevremont, Farnet y Sergent realizaron experimentos para estudiar el efecto de la UV-A y la UV-C sobre *E. coli* y *Enterococcus faecalis* artificiales en aguas residuales. Cuatro LED emitían 255 nm, 280 nm, 365 nm y 405 nm con 115 mA ,77 mA, 22 mA y 46 mA de corriente, respectivamente. En sus experimentos se tuvieron en cuenta cuatro parámetros: pH, densidad bacteriana, tiempo de exposición y longitud de onda. Se probaron muchas combinaciones diferentes, pero combinando 280 nm y 365 nm o 280 nm y 405 nm el efecto sobre las bacterias fue notablemente más significativo que usando 365 nm solo, y después de 60 segundos se observó la ausencia de las bacterias, el diseño del experimento fue casi idéntico al de los investigadores, colocando los LEDs sobre las placas Petri de 55 mm de diámetro a una distancia de 1 cm [9].

Del mismo modo, Kumiko et al. realizaron en 2013 numerosas pruebas con LED UV individuales y varias combinaciones de ellos utilizando longitudes de onda de 265 nm, 280 nm y 310 nm. Los valores de tensión y de potencia óptica para los UV-LED fueron de 9,5, 8 y 6,1 V y la potencia óptica fue de 0,7 mW, 1,3 mW y 1,1 mW para los 265 nm, 280 nm y 310 nm respectivamente. La corriente siempre fue de 20 mA por LED. Los experimentos se realizaron para desactivar el cultivo de laboratorio del microorganismo *E. coli* utilizando dos reactores diferentes, un reactor discontinuo y un reactor de flujo continuo. En el reactor discontinuo, que contenía una matriz de nueve LED, los nueve LED emitían una única longitud de onda, 265 nm, 280 nm o 310 nm. El reactor discontinuo estaba por encima de una placa de Petri de 34,5 mm de diámetro por 17 mm. El reactor de flujo consta de tres matrices y cada una de ellas tiene 10 LED similares. Las tres matrices se unieron en forma triangular y se colocaron dentro de un cilindro de 16 mm de diámetro. La emisión de 265 nm logró tasas de desactivación superiores a 6 log con dosis de 10 mJ/cm^2 en el reactor discontinuo y de 16,4 mJ/cm^2 en el de flujo continuo. Del mismo modo, el de 280 nm alcanzó tasas de desactivación de casi 4 log con dosis de 13,8 y 25,5 mJ/cm^2 en el reactor discontinuo y en el reactor de flujo continuo, respectivamente. La de 310 nm requirió 56,9 mJ/cm^2 para una inactivación de

0,6 log en el reactor discontinuo, que fue menos eficaz que la emisión de 268 nm y 280 nm. Por último, las emisiones combinadas fueron menos eficaces que la emisión única [10].

Siguiendo a Nyangaresi et al. en 2018 con el mismo enfoque de comparar la irradiación simple y combinada de los LED UV, el grupo de la universidad de Xiamen en China eligió el pico de emisión UV de 267 nm, 275 nm y 310 nm y combinó 267 nm/275 nm, 267 nm/310 nm y 275 nm/310 nm para aplicar su emisión en un sistema de desinfección de agua por lotes y evaluar el proceso utilizando el cultivo de laboratorio *E. coli* como modelo de bacteria. La potencia óptica de salida de los LED fue de 1,8 mW, 1,6 mW y 1,3 mW para 265 nm ,275 nm y 310 nm respectivamente, y los voltajes eran de 3,9 V - 6 V, con corrientes de 20mA. Se colocaron 16mL en una placa Petri de 60mm de diámetro y sobre ella, a 2,2 cm, los LEDs-UV. El de 267 nm tuvo la mayor eficiencia de inactivación que los otros UV-LED y el de 310 nm fue el menor, no obstante, se observó reactivación tras irradiaciones de 267, 275, 267/275 y 275/310 nm [11].

Sara et al. de la Universidad de Colorado realizaron un experimento utilizando un reactor comercial del sistema AquiSense Technologies para comparar el efecto de irradiación de los LED de 260 nm, 280 nm y la combinación de ellos frente a una lámpara UV de mercurio de media y baja presión. Se probaron cuatro microorganismos de cultivo de laboratorio: *E. coli*, *colifago MS2*, *adenovirus humano de tipo 2* y esporas de *Bacillus pumilus*. Se colocó una placa de Petri de 3,5 cm de diámetro llena de 5 mL de muestra a 4 cm de distancia de la fuente UV, que tenía una irradiancia variada de 0,19 mW/cm^2 a 0,55 mW/cm^2 para los LED UV, y de 0,35 mW/cm^2 a 1,17 mW/cm^2 para la lámpara UV MP y de 0,3 a 0,75 mW/cm^2 para la UV LP. Los datos demostraron que la tasa de inactivación de *E. coli* por las cinco fuentes UV era elevada, pero la longitud de onda de 260 nm era la más eficaz. Para *HAdV2* y *B. pumilus*, las lámparas MP UV fueron las más potentes. En cuanto a la eficacia eléctrica, la lámpara UV LP fue la más eficaz para inactivar *E. coli* y *MS2*, y las lámparas UV de mercurio LP y MP fueron igual de eficaces para las esporas de *HAdV2* y *B. pumilas* [23].

Figura 12 – Sistema AquiSense Tecnologies.

6.4.5 Turbidez

Kristina, Dena y Christopher en 2013 justifican plenamente el efecto de la turbidez del agua durante el proceso de desinfección UV. Observaron que cuanto más contaminada está el agua, más potencia de luz UV se necesita para el proceso de desinfección. En el experimento se utilizaron dos tipos de muestras de agua, la primera eran aguas residuales reales y la segunda era agua preparada que contenía un cultivo puro de *E. coli*. Para la investigación se utilizaron siete LEDs de lente plana de 260 nm, con tensiones de 6,5 V y corrientes de 20 mA por LED conectados en serie a una resistencia limitadora de corriente de 100 Ω - 380 Ω, y una pila alcalina de 9 V. La potencia óptica osciló entre 45 mW y 182 mW y la media fue de 130 mW, correspondiendo esta fluctuación a la edad de la pila. Cada LED estaba conectado a una pila independiente. Se utilizó la misma técnica común, salvo que no fijaron ninguna distancia entre la superficie de los LED y la muestra de agua, es decir, bajaron los LED hasta que la lente óptica entró en contacto con la superficie de la muestra de agua. El cable de los LED se protegió extendiéndolo 100 cm más con un alambre azul de 0,25 mm y 30 AWG; el aislamiento del alambre se peló unos 3 cm para cubrir la ranura y conseguir un ajuste adecuado. Además, se colocó una junta tórica de plástico alrededor del labio de cada LED UV para completar el cierre hermético. El volumen de las muestras osciló entre 10 ml y 100 ml y el periodo de tiempo de los experimentos fue de entre 20 min y 50 min. El análisis demostró que la calidad del agua influye considerablemente en la eficacia de la desinfección. Para los resultados de las aguas residuales, se midió una reducción mínima

de bacterias desde el periodo de 20 min a 40 min, sin embargo, las muestras artificiales mostraron una reducción notable con la *E. coli* [13].

6.4.6 Prototipos comerciales

En 2019, Peter et al. de la Universidad de Cranfield realizaron un estudio sobre la eficacia del uso de los sistemas de tratamiento Typhon, compuestos por 1000 luces LED UV con una longitud de onda de 275 nm y una potencia óptica de 100 mW, para la desinfección de aguas residuales en una planta de tratamiento del norte de Inglaterra. Este reactor a escala real fue capaz de desinfectar 6 megalitros de agua al día, y su rendimiento se evaluó utilizando los microorganismos bacteriófago *MS2* y *E. coli*. Los resultados indicaron que este reactor UV-LED era al menos tan eficaz como los reactores UV de mercurio tradicionales en las condiciones específicas de calidad del agua y de funcionamiento examinadas. Este estudio representa la primera aplicación con éxito de un reactor UV-LED a escala real para la desinfección de microorganismos patógenos en agua potable en una instalación municipal de tratamiento [14].

Figura 13 – Tubo interior del sistema de tratamiento Typhon [22]

Figura 14 – Reactor del sistema de tratamiento Typhon [14]

6.4.7 Rentabilidad

En términos de eficiencia de costes, Elaheh el at. De la universidad de Nápoles en 2022, investigó la desactivación de *Legionella pneumophila* y *Legionella dumoffii* utilizando una longitud de onda UV-A ya que es significativamente más barata que la UV-B y UV-C. Se montó un LED de densidad de 0,3 A con una potencia de salida de 22,5 mW/cm^2 en un disipador térmico para disipar el calor generado, aunque tiene una potencia nominal de 4,5 A y 20 W/cm^2. La distancia entre el LED UV-A y la placa de Petri de 35 mm de diámetro era de 38 mm. Los resultados mostraron que, aplicando una dosis de UV de 1700 mJ/cm^2, la tasa de inactivación de *Legionella pneumophila* era del 99,89 % y la de *Legionella dumoffii* del 99,1 % [25].

6.4.8 Efecto de la temperatura del PCB

Como ya se sabe, la mayor oposición para el rendimiento de los LED es la temperatura, cuanto más aumenta la temperatura, menor es el rendimiento óptico que se puede obtener. Es por ello que Huang el al. en 2021 centraron su estudio en las diferentes formas de mejorar la disipación térmica de los LEDs UV. Eligieron LEDs de 280 nm y se emplearon utilizando diferentes corrientes, desde 20 mA hasta 200 mA, variando no solo la corriente sino también el grosor de los materiales del sustrato. *E. coli* fue el microorganismo probado en este experimento, incluyendo dos materiales diferentes donde se colocaron los LEDs: Cobre (Cu) y Aluminio (AIN). Se utilizaron diferentes espesores para la capa de cobre: 70 µm, 100 µm ,130µm y 200µm, y 500 µm para el Aluminio. Todos estos módulos se colocaron a 1 cm sobre la placa de Petri de 8,5 cm de diámetro. Los resultados revelaron que el grosor del metal no desempeña un papel significativo en la disipación del calor. Sin embargo, es evidente que la gran superficie

del LED presenta una elevada disipación térmica. Además, los LED de 280 nm fueron capaces de desactivar el 95 % de la *E. coli* en sólo 60 segundos [26].

6.4.9 Velocidad de agitación y tiempo de exposición

Seul el al. en 2021 de la universidad de Corea tomó diferentes aspectos como el tiempo de exposición, la velocidad de agitación y el volumen de agua durante la realización de un estudio de desinfección de bacterias preparadas como *E. coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella Typhimurium* y *Listeria monocytogenes*. En el fondo de un tubo cilíndrico de 15,2 cm de diámetro y 8,4 cm de altura se fijó un LED UC-C de 278 nm con un voltaje y una corriente de 7,5 V y 20 mA, respectivamente. El tiempo de exposición fue de 15 a 60 minutos, y se extrajo una muestra de 10 mL en cada intervalo. El análisis demostró que, a mayor velocidad de agitación y mayor tiempo de exposición, mayor era la tasa de inactivación de todas las bacterias [27].

6.4.10 Reactores impresos en 3D

La Universidad de Taipei en Taiwán diseñó y creó diversos reactores con LEDs-UV [28]. Wang y Lin en 2021 crearon cuatro reactores usando una impresora 3D, tres de ellos con tubos internos con diferentes pasos (10 mm, 20 mm y 30 mm) rodeando un tubo de cuarzo que contenía los LEDs montados en una varilla de aluminio para proporcionar suficiente disipación de calor, y el último reactor era simplemente sin tubo. Se eligieron 24 LED de 278 nm con una potencia óptica de 10 mW a 100 mA de corriente cada uno, y en cada lado de la barra de aluminio se soldaron 6 LED. Se instalaron una bomba y un caudalímetro de agua para controlar el flujo de agua en el interior de los reactores. Este estudio se centró en el efecto del caudal del agua y el paso de la espiral para desinfectar la *E. coli* preparada en el laboratorio. El análisis sugirió que el reactor tubular de 10 mm alcanzó la mayor tasa de inactivación entre los demás reactores tubulares, además de que el reactor sin tubos fue el más ineficaz para desactivar las bacterias en comparación con los otros tres reactores con tubos debido a la falta de efecto de mezcla de las partículas de fluido en su interior. Además, la reducción del caudal de 120 mL/min a 60 mL/min aumentó la inactivación bajo los LED UV.

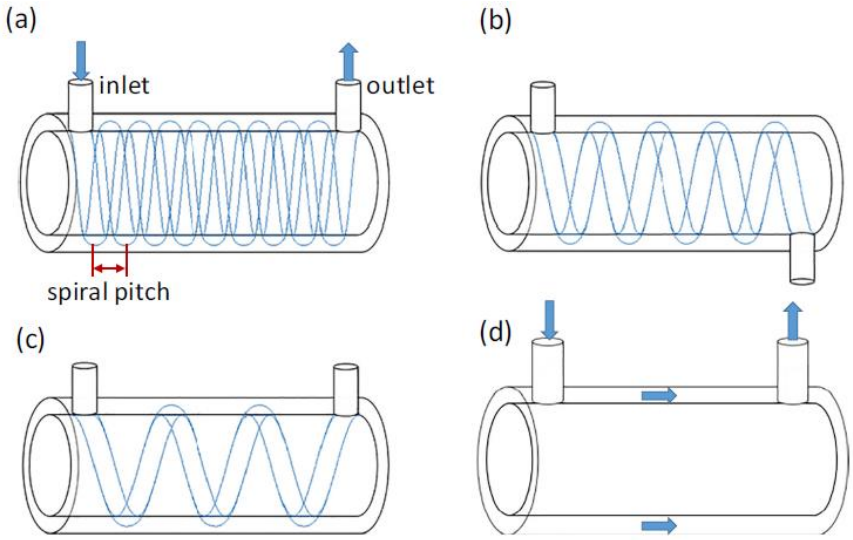


Figura 14 – Reactor de flujo con diferentes pasos de espiral (a) 10 mm, (b) 20 mm, (c) 30 mm y (d) reactor de flujo continuo [28].

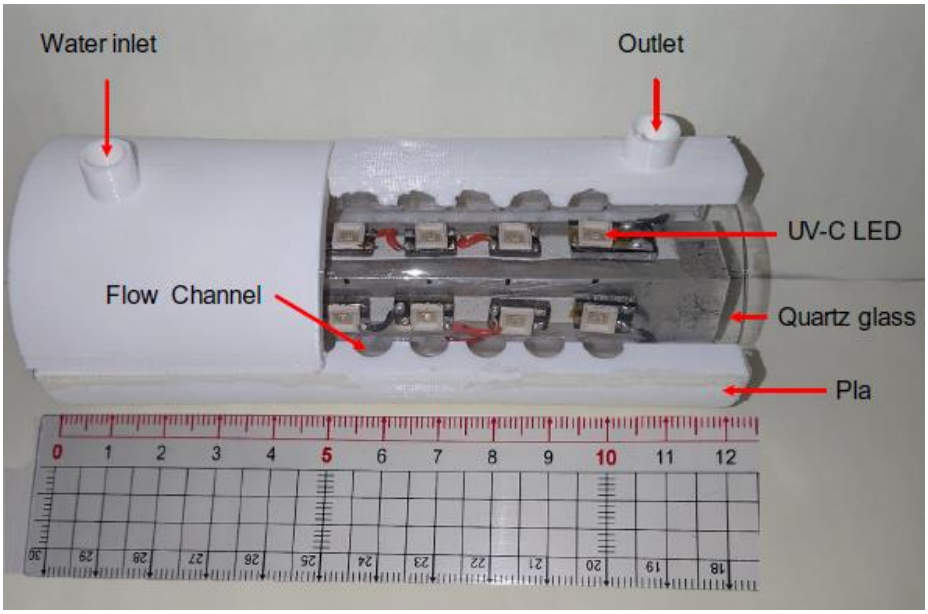


Figura 15 – Imagen de un reactor de flujo con un canal en espiral que utiliza LED UV-C [28]

6.4.11 Luz UV combinada con luz visual

Utilizando la luz UV-A y azul, el centro de alimentación y bioconvergencia de Corea dirigido por Kim y Kang en 2021 empleó 395 nm, 405 nm, 415 nm y 425 nm sobre *E. coli* artificial, además de comprobar las especies reactivas de oxígeno (ROS). Por lo tanto, crearon una cámara recubierta de negro a partir de aluminio para bloquear la luz externa innecesaria y la reflexión de la luz azul. En la parte superior de la cámara negra se colocó un único LED con un disipador térmico, a una distancia de 4 cm entre el LED y la placa Petri de 60 mm de diámetro. Se aplicó una corriente constante de 500 mA a

cada LED. Todas las longitudes de onda utilizadas en este experimento fueron capaces de detener el efecto de la *E. coli* después de 70 mJ/cm^2 a los 81 minutos [29].

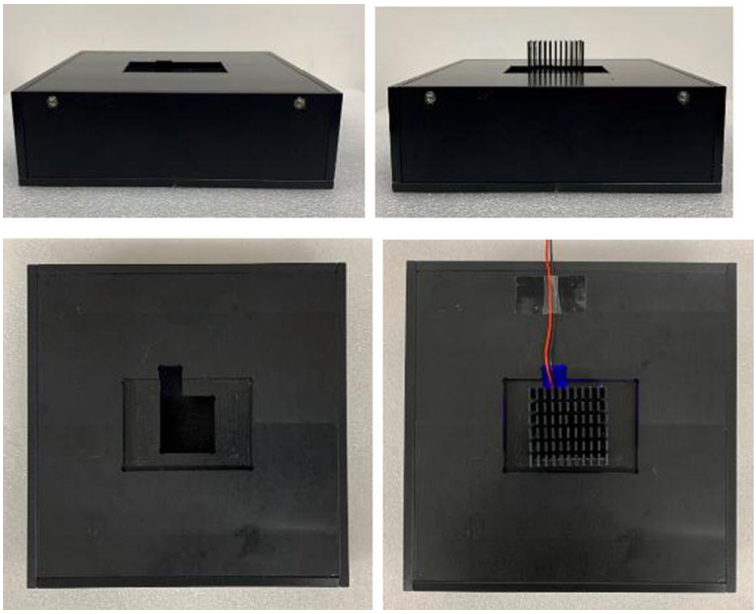
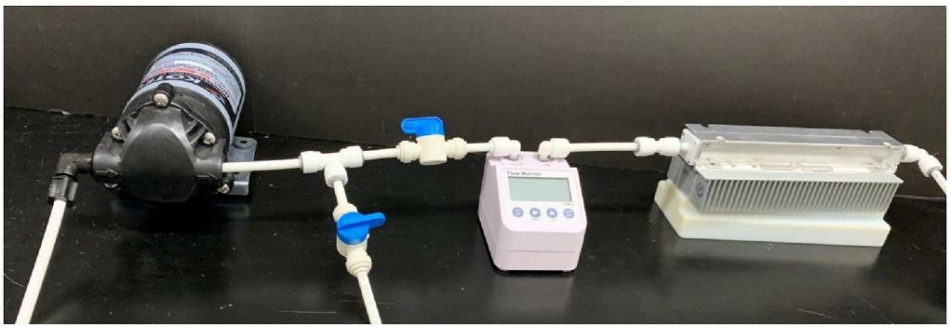


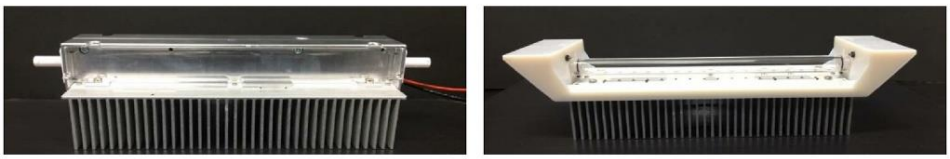
Figura 16 – Imagen del reactor de aluminio utilizado por Kim y Kang en sus experimentos [29].

Kim y Kang también realizaron otro estudio en 2020 creando un reactor de flujo de agua diferente para desinfectar *E. coli* preparada en laboratorio. Se probaron varios números del mismo chip LED 18, 21 y 33 con una longitud de onda de 280 nm con un rango entre 150 mA y 900 mA de corriente operativa. Los LEDs PCB fueron montados sobre un disipador de calor con las dimensiones de 6,5 x19 cm. Sobre el PCB se colocó un tubo de cuarzo de 1 cm conectado a una bomba de agua y a un sensor de flujo de agua. El experimento se realizó de dos formas, la primera sin colocar nada sobre la PCB y el tubo de cuarzo, y la segunda añadiendo un reflector de aluminio sobre la PCB y el tubo de cuarzo. Los datos revelaron que el cambio del número de LEDs no supuso una diferencia significativa, mientras que la adición del reflector de aluminio elevó el proceso de inactivación 1,5 veces en comparación con el proceso sin reflector [30].

(a)



(b)



(c)

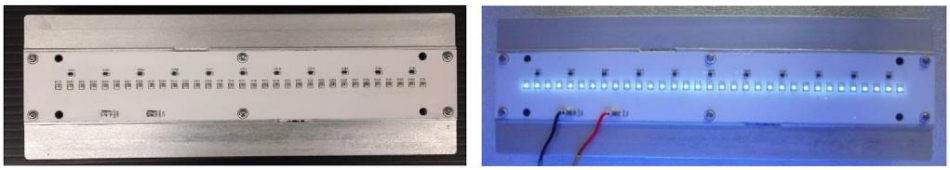


Figura 17 – El reactor utilizado por Kim y Kang en su segundo estudio. (a) el sistema completo, (b) módulo de matriz de LED UV-C con o sin reflector de aluminio, (C) matriz de 33 LED UV-C [30].

Como ya se ha mencionado, los investigadores están intentando sustituir la tradicional lámpara UV de mercurio por LED UV, aplicando una variedad de longitudes de onda diferentes sobre distintos microorganismos. Se están utilizando diferentes técnicas para archivar la mayor eficacia en términos de inactivación la duración del proceso de inactivación. Como resultado de los artículos, las longitudes de onda más eficaces se encuentran en el rango de los UV-C y la menor es la de los UV-A para la mayoría de las bacterias. Además, las técnicas utilizadas y la configuración de los experimentos tienen un efecto crucial en los resultados, por ejemplo, la técnica más potente es el modo continuo para casi todas las bacterias, sin embargo, el modo pulsado sólo fue eficaz eléctricamente y no es adecuado para todos los tipos de bacterias. Le siguieron las técnicas combinadas, que fueron eficaces con algunos microorganismos. Además, la distancia entre la fuente de luz UV y el agua tiene un impacto significativo en la duración del proceso de desinfección, cuanto mayor sea la distancia entre los LED y la superficie del agua, mayor será el tiempo necesario para eliminar el efecto de las bacterias. Y, por último, pero no por ello menos importante, mezclar el agua durante el proceso de desactivación conlleva una disminución del tiempo del proceso de desinfección. Por último, la turbidez es uno de los parámetros más importantes en el proceso de

desinfección y puede hacer que el proceso de desinfección lleve mucho más tiempo o incluso que el proceso de inactivación sea inalcanzable.

6.5 Objetivos de la investigación

Objetivo general

El objetivo principal de esta tesis doctoral es **explorar el potencial de los UV-LEDs para desinfectar el efluente real de una planta de aguas residuales mediante el diseño, fabricación y prueba de un novedoso prototipo basado en UV-LEDs**. Este prototipo podría ser implementado en la etapa de tratamiento terciario de las plantas de aguas residuales, permitiendo una reutilización segura del efluente para nuevos usos de acuerdo con la legislación española (agrícola, industrial, etc.) reduciendo así el impacto ambiental de las tecnologías convencionales.

Objetivos específicos

1. Diseñar y fabricar un prototipo novedoso a nivel de laboratorio que desinfecte el agua bruta dentro de una placa Petri de al menos 60 mm de diámetro, estudiando la caracterización óptica de los UV-LEDs, el diseño de la placa electrónica y disposición de los UV-LEDs, el sistema de refrigeración y la irradiancia UV total.

2. Determinar la longitud de onda UV-LED óptima para la inactivación de cada uno de los microorganismos estudiados (*E.coli*, *Enterococos spp.* y *Clostridium perfringens*) utilizando cepas ambientales reales procedentes del efluente de la depuradora municipal de Linares.

3. Determinar la dosis UV de inactivación para cada microorganismo y la longitud de onda (tiempo de exposición e irradiancia UV).

4. Analizar las pérdidas de transmitancia de la luz UV de los LEDs a través del agua para determinar una profundidad máxima del agua junto con el efecto de la turbidez.

5. Comprobar la ley de reciprocidad para la desinfección del agua mediante LED UV. Se utilizarán LED UV de alta y baja potencia para tratar las aguas residuales con la misma dosis de UV. Se analizará el contenido microbiológico (*E. coli*, *Enterococcus faecalis* y *Clostridium perfringens*) para observar si con la misma dosis UV pero diferente irradiancia inicial la inactivación de las bacterias es la misma.

6.6 Bibliografía

[1] AquiSense PearlLab Beam(TM), «AquiSense Technologies,» [En línea]. Available: https://aquisense.com/products/research/pearllab-beam/.

[2] K. Sholtes y K. G. Linden, «Pulsed and continuous light UV LED: microbial inactivation, electrical, and time efficiency,» Noviembre 2019. [En línea].

[3] T. Matsumoto, I. Tatsuno y T. Hasegawa, «Instantaneous water purification by deep ultraviolet light in water waveguide: Escherichia coli bacteria disinfection,» Mayo 2019. [En línea].

[4] S. Rattanakul y K. Oguma, «Inactivation kinetics and efficiencies of UV-LEDs against Pseudomonas aeruginosa, Legionella pneumophila, and surrogate microorganisms,» Marzo 2018. [En línea].

[5] K. Song, F. Taghipour y M. Mohseni, «Microorganisms inactivation by continuous and pulsed irradiation of ultraviolet light-emitting diodes (UV-LEDs),» Julio 2018. [En línea].

[6] K. Song, F. Taghipour y M. Mohseni, «Microorganisms inactivation by wavelength combinations of ultraviolet light-emitting diodes (UV-LEDs),» Mayo 2019. [En línea].

[7] S. Vilhunen, H. Särkkä y M. Sillanpää, «Ultraviolet light-emitting diodes in water disinfection,» Junio 2009. [En línea].

[8] A. C. Chevremont, A. M. Farnet, M. Sergent, B. Coulomb y J. L. Boudenne, «Multivariate optimization of fecal bioindicator inactivation by coupling UV-A and UV-C LEDs,» Enero 2012. [En línea].

[9] K. Oguma, R. Kita, H. Sakai, M. Murakami y S. Takizawa, «Application of UV light emitting diodes to batch and flow-through water disinfection systems,» Noviembre 2013. [En línea].

[10] P. O. Nyangaresi, Y. Qin, G. Chen, B. Zhang, Y. Lu y L. Shen, «Effects of single and combined UV-LEDs on inactivation and subsequent reactivation of E. coli in water disinfection,» Diciembre 2018. [En línea].

[11] A. C. Chevremont, A. M. Farnet, B. Coulomb y J. L. Boudenne, «Effect of coupled UV-A and UV-C LEDs on both microbiological and chemical pollution of urban wastewaters,» Junio 2012. [En línea].

[12] K. Y. Nelson, D. W. McMartin, C. K. Yost, K. J. Runtz y T. Ono, «Point-of-use water disinfection using UV light-emitting diodes to reduce bacterial contamination,» Agosto 2013. [En línea].

[13] P. Jarvis, O. Autin, E. H. Goslan y F. Hassard, «Application of ultraviolet light-emitting diodes (UV-LED) to full-scale drinking-water disinfection,» 2019. [En línea]. Available: https://doi.org/10.3390/w11091894.

[14] UV System, «UV System,» [En línea]. Available: https://www.uvsystem.in/what-is-uv/.

[15] Larson Electronics, «Larson Electronics,» [En línea]. Available: https://www.larsonelectronics.com/images/product/LightingFacts/266985.PDF.

[16] Larson Electronics, «Ultraviolet Radiation Safety,» [En línea]. Available: https://www.larsonelectronics.com/images/product/EyeSafety/272823.PDF.

[17] G. A. Mansoori, P. Mohazzabi, P. Mccormack y S. Jabbari, «Nanotechnology in cancer prevention, detection and treatment: bright future lies ahead,» 2007.

[18] P. O. Nyangaresi, Y. Qin, G. Chen, B. Zhang, Y. Lu y L. Shen, «Comparison of the performance of pulsed and continuous UVC-LED irradiation in the inactivation of bacteria,» Junio 2019. [En línea].

[19] G. Q. Li, W. L. Wang, Z. Y. Huo, Y. Lu y H. Y. Hu, «Comparison of UV-LED and low pressure UV for water disinfection: Photoreactivation and dark repair of Escherichia coli,» Diciembre 2017. [En línea].

[20] X. Y. Zou, Y. L. Lin, B. Xu, T. C. Cao, Y. L. Tang, Y. Pan, Z. C. Gao y N. Y. Gao, «Enhanced inactivation of E. coli by pulsed UV-LED irradiation during water disinfection,» Febrero 2019. [En línea].

[21] Typhon Treatment System Ltd., «Typhon Treatment System Ltd.,» [En línea]. Available: https://www.typhontreatment.com/.

[22] S. E. Beck, H. Ryu, L. A. Boczek, J. L. Cashdollar, K. M. Jeanis, J. S. Rosenblum, O. R. Lawal y K. G. Linden, «Evaluating UV-C LED disinfection performance and investigating potential dual-wavelength synergy,» Febrero 2017. [En línea].

[23] Q. Wan, G. Wen, R. Cao, X. Xu, H. Zhao, K. Li, J. Wang y T. Huang, «Comparison of UV-LEDs and LPUV on inactivation and subsequent reactivation of waterborne fungal spores,» Abril 2020. [En línea].

[24] E. Allahyari, F. Carraturo, A. De Risi, A. Nappo, M. Morelli, A. Cajora y M. Guida, «A sequential utilization of the UV-A (365 nm) fluence rate for disinfection of water, contaminated with Legionella pneumophila and Legionella dumoffii,» Julio 2022. [En línea].

[25] S. Y. Huang, J. C. Lin, X. Q. Huang y W. K. Wang, «Large-area 280 nm LED flexible sterilization light source with improved thermal performance,» Diciembre 2021. [En línea].

[26] S. K. Park, D. M. Jo, M. G. Kang, F. Khan, S. D. Hong, C. Y. Kim, Y. M. Kim y U. C. Ryu, «Bactericidal effect of ultraviolet C light-emitting diodes: Optimization of efficacy toward foodborne pathogens in water,» Septiembre 2021. [En línea].

[27] C. P. Wang y W. C. Lin, «Combination of ultraviolet-C light-emitting diodes and a spiral-channel configuration in a water disinfection reactor,» Agosto 2021. [En línea].

[28] D. K. Kim y D. H. Kang, «Efficacy of light-emitting diodes emitting 395, 405, 415, and 425 nm blue light for bacterial inactivation and the microbicidal mechanism,» Marzo 2021. [En línea].

[29] D. K. Kim y D. H. Kang, «Investigation of a new UVC LEDs array continuous type water disinfection system for inactivating Escherichia coli O157:H7 according to flow rate and electrical energy efficiency analysis,» Enero 2021. [En línea].

[30] W. Kowalski, «Ultraviolet Germicidal Irradiation Handbook,» 2009. [En línea]. Available: https://doi.org/10.1007/978-3-642-01999-9.

[31] ClearWater Industries, Inc., «How Sedimentation Water Treatment Works and How to Make it Efficient,» [En línea]. Available: https://clearwaterind.com/how-sedimentation-water-treatment-works-and-how-to-make-it-efficient/.

[32] Ministerio de Obras Públicas, Transportes y Medio Ambiente, «Real Decreto 509/1996, de 15 de marzo, de desarrollo del Real Decreto-ley 11/1995, de 28 de diciembre, por el que se establecen las normas aplicables al tratamiento de las aguas residuales urbanas.,» Marzo 1996. [En línea]. Available: https://www.boe.es/buscar/act.php?id=BOE-A-1996-7159#:~:text=Ayuda-,Real%20Decreto%20509%2F1996%2C%20de%2015%20de%20marzo%2C%20de,de%2029%2F03%2F1996.

[33] J. Ruza Rodríguez, M. Á. Bordas Martínez, G. Espinosa Expósito y A. Puig Infante , «Manual para la gestión de vertidos.,» [En línea]. Available: https://www.miteco.gob.es/es/agua/publicaciones/Manual_para_la_gestion_de_vertidos_tcm30-137170.pdf.

[34] Ministerio de Obras Públicas, Transportes y Medio Ambiente, «Manual de interpretación y elaboración de informes.,» [En línea].

[35] UE, «Diario Oficial de las Comunidades Europeas,» [En línea]. Available: https://eur-lex.europa.eu/resource.html?uri=cellar:5c835afb-2ec6-4577-bdf8-756d3d694eeb.0008.02/DOC_1&format=PDF.

[36] M. A. Würtele, T. Kolbe, M. Lipsz, A. Külberg, M. Weyers, M. Kneissl y M. Jekel, «Application of GaN-based ultraviolet-C light emitting diodes - UV LEDs - for water disinfection,» Enero 2011. [En línea]. Available: https://doi.org/10.1016/j.watres.2010.11.015.

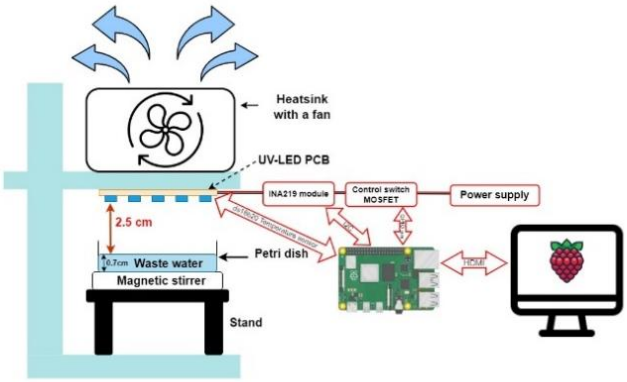
Chapter 7: Resumen de resultados y relación con los objetivos de la tesis

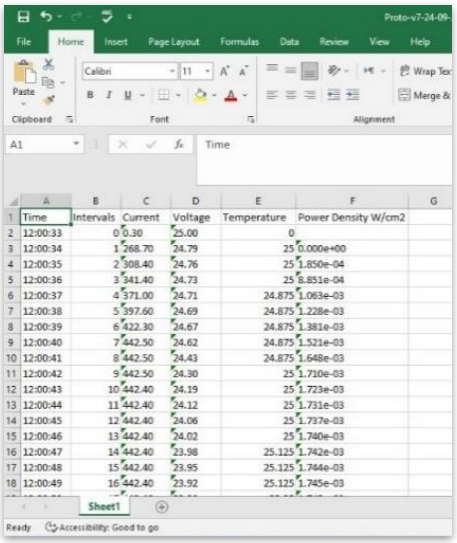
En este capítulo presentamos un resumen de los principales resultados y su relación con los objetivos específicos de la tesis.

Objetivo 1. Diseñar y fabricar un nuevo prototipo a nivel de laboratorio que desinfecte el agua bruta dentro de una placa de Petri de al menos 60 mm de diámetro, estudiando la caracterización óptica de los LED-UV, el diseño de la placa electrónica y la disposición de los LED-UV, el sistema de refrigeración y la irradiancia UV total.

Este trabajo se llevó a cabo principalmente en el capítulo 3 y la publicación 1 ("Desactivación de cepas ambientales de *Escherichia coli*, *Enterococcus fecales* y *Clostridium perfringens* de un efluente real de aguas residuales mediante LED-UV"), y se completó en el capítulo 4 y la publicación 2 ("Análisis de la ley de reciprocidad de los LED-UV en la desinfección del agua de *Escherichia coli*, *Enterococcus fecales* y *Clostridium perfringens*"). En la primera publicación, se explica el diseño del reactor del experimento, que consiste en una placa de LED-UV unida a un disipador de calor y un ventilador para disipar el calor. Para ajustar la altura sobre la placa de Petri de plástico estéril que contiene el agua bruta que se va a tratar se utiliza un soporte de laboratorio. Las medidas de corriente, voltaje, densidad de potencia óptica y temperatura de placa se registraron manualmente.

En la segunda publicación, además de la experimentación correspondiente al análisis de reciprocidad, se mejoró el sistema de monitorización de parámetros del prototipo. Se utilizó una placa de desarrollo Raspberry-pi-3b con un módulo de corriente-voltaje, densidad de potencia óptica y sensor de temperatura para controlar y supervisar el experimento automáticamente estableciendo la duración del experimento y guardando las mediciones en un archivo Excel (Figura 18).





Time	Intervals	Current	Voltage	Temperature	Power Density W/cm2
12:00:33	0	0.30	25.00	0	
12:00:34	1	268.70	24.79	25	0.000e+00
12:00:35	2	308.40	24.76	25	1.850e-04
12:00:36	3	341.40	24.73	25	8.851e-04
12:00:37	4	371.00	24.71	24.875	1.063e-03
12:00:38	5	397.60	24.69	24.875	1.228e-03
12:00:39	6	422.30	24.67	24.875	1.381e-03
12:00:40	7	442.50	24.62	24.875	1.521e-03
12:00:41	8	442.50	24.43	24.875	1.648e-03
12:00:42	9	442.50	24.30	25	1.710e-03
12:00:43	10	442.40	24.19	25	1.723e-03
12:00:44	11	442.40	24.12	25	1.731e-03
12:00:45	12	442.40	24.06	25	1.737e-03
12:00:46	13	442.40	24.02	25	1.740e-03
12:00:47	14	442.40	23.98	25.125	1.742e-03
12:00:48	15	442.40	23.95	25.125	1.744e-03
12:00:49	16	442.40	23.92	25.125	1.745e-03

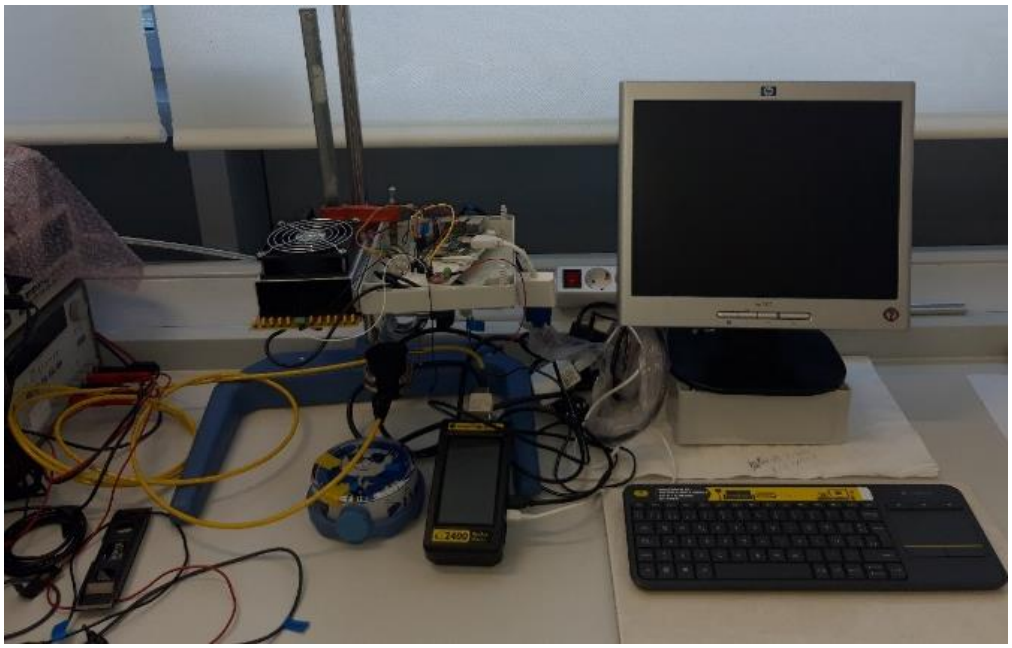


Figura 18 – Montaje del experimento y mediciones guardadas

Objetivo 2. Determinar la longitud de onda del LED-UV óptima para la inactivación de cada uno de los microorganismos estudiados (*Escherichia coli*, *Enterococos fecales* y *Clostridium perfringens*) utilizando cepas ambientales reales procedentes del efluente de la depuradora municipal de Linares.

Este trabajo se realizó en la publicación 1. Aquí se utilizaron los nuevos prototipos de fabricación para tratar aguas residuales procedentes del efluente de la depuradora de Linares a tres longitudes de onda diferentes: 265 nm, 275 nm y 310 nm. Tal y como se observó en los experimentos, cuanto menor es la longitud de onda, más rápido es el proceso de desinfección. En general, *Escherichia coli*, *Enterococos fecales* y *Clostridium perfringens* son sensibles a las longitudes de onda de 265 nm y 275 nm, vs. la longitud de onda de 310 nm que requiere dosis UV más altas y por tanto más tiempo para desinfectar las distintas bacterias. Además, se observó que algunos microorganismos parecían ser más sensibles a determinadas longitudes de onda que a otras, por ejemplo, la longitud de onda de 275 nm fue ligeramente más eficaz para desactivar *Escherichia coli* y Enterococos fecales, pero *Clostridium perfringens* se desactivó en mayor medida con la longitud de onda de 265 nm que con la de 275 nm, como se muestra en los gráficos.

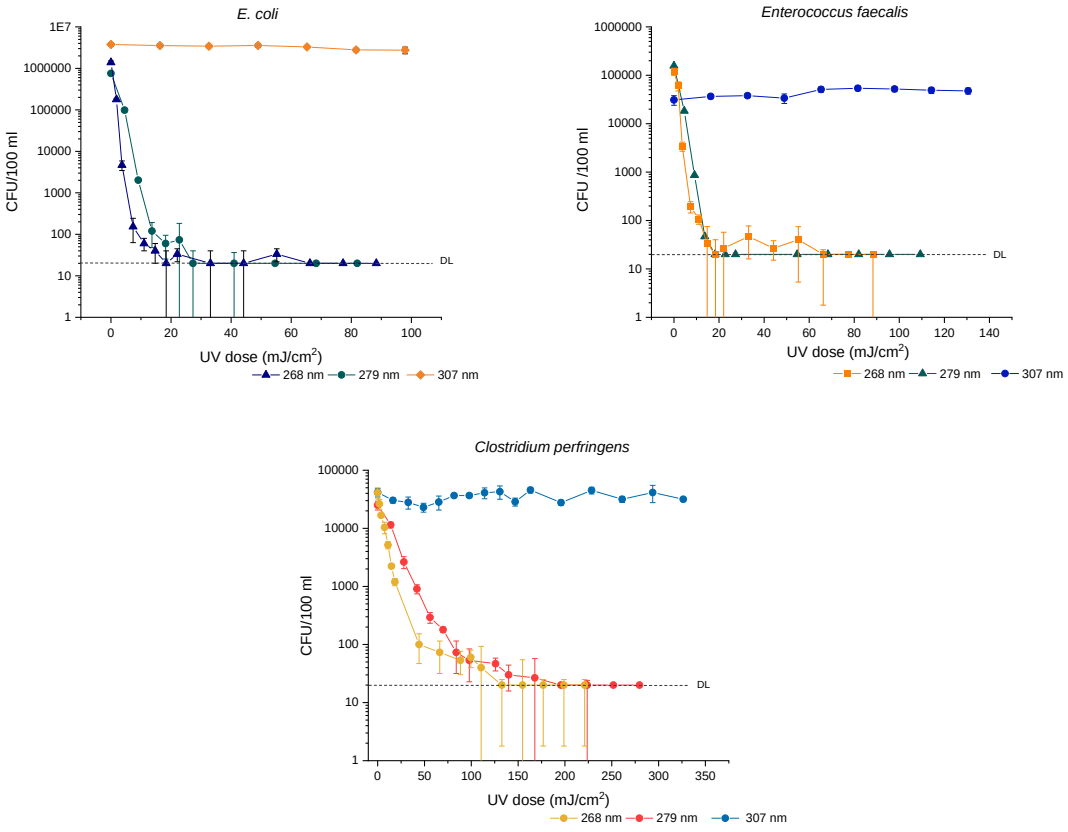


Figura 19 – Dosis UV vs CFU/100 ml. Gráfica para E. col, Enterococcus faecalis y Clostridium perfringens de la publicación [1]

Objetivo 3. Determinar la dosis UV para la inactivación de cada microorganismo y la longitud de onda (tiempo de exposición e irradiancia UV).

Este trabajo se realizó en la publicación 1 y en la publicación 2. La dosis UV utilizada para inactivar los microorganismos se ve afectada por diversos factores, como la turbidez del agua residual utilizada, la longitud de onda utilizada en el método de desinfección, la concentración inicial del microorganismo y la profundidad (altura de la capa de agua) del depósito de aguas residuales durante el experimento (que en nuestro caso es una placa Petri de 60 mm de diámetro).

Por ejemplo, en la publicación 1, la población inicial y el nivel de turbidez de *Escherichia coli* utilizando longitudes de onda de 265 nm, 279 nm y 310 nm fueron de 1,4 x 10^6 UFC/100 ml (12,4 NTU), 7,6 x 10^5 UFC/100 ml (6,3 NTU) y 3,8 x 10^6 UFC/100 ml (8. 9 NTU) respectivamente, y las dosis UV de inactivación en estos casos fueron de 18,4 mJ/cm^2 de dosis UV (XX irradiancia y 50 segundos) para 265 nm, 27,3 mJ/cm^2 de dosis UV (X irradiancia y 60 segundos) para 275 nm y 97.9mJ/cm^2 después de 180 segundos para 310 nm. Las longitudes de onda de 265 nm y 279 nm tuvieron un efecto similar en la desactivación de *Escherichia coli*; sin embargo, parece que la longitud de onda de 265 nm presenta mayor inactivación, especialmente considerando la mayor turbidez del agua durante este experimento vs. la turbidez del experimento de 275 nm (12,4 NTU con 265 nm frente a 6,3 NTU con 275 nm).

Del mismo modo, en la publicación 2, la concentración inicial de *Escherichia coli* variaba entre 4,4 x 10^5 UFC/100 ml y 4,6 x 10^5 UFC/100 ml con un nivel de turbidez de 8 NTU para 50mW potencia óptica UV-LED. La dosis UV necesaria para desactivar *Escherichia coli* fue de 58 mJ/cm^2 utilizando los LED UV de 50 mW y longitud de onda de 265 nm. En el caso de los LED-UV de 275 nm y 50 mW, para un nivel de turbidez de 8 NTU la dosis UV necesaria fue de 13 mJ/cm^2.

En el caso de *Enterococos fecales*, en los experimentos de la publicación 1, con LEDs-UV de baja potencia (en torno a 2 mW), para la longitud de onda de 265 nm la población inicial fue de 1,2 x 10^4 UFC/100 ml con un nivel de turbidez de 12,4 NTU, y se alcanzó la línea de detección después de 50 segundos utilizando 18 mJ/cm^2 de dosis UV. Bajo la longitud de onda de 275 nm, la población inicial fue de 1,6 x 10^5 UFC/100 ml y la turbidez de 6,3 NTU. Se necesitaron 40 segundos y 18,2 mJ/cm^2 de dosis UV para alcanzar la línea de detección. Por último, para la longitud de onda de 310 nm, la población inicial fue de 3,1 x 10^4 UFC/100 ml y la turbidez de 5,4 NTU. En este caso no se alcanzó inactivación total y la concentración final del microorganismo tras el proceso de desinfección fue de 4,8 x 10^4 UFC/100 ml, lo que indica que en este caso no hubo desactivación del microorganismo.

En la publicación 2, utilizando ya LEDs-UV de mayor potencia (50 mW), la concentración inicial de *Enterococos fecales* osciló entre 5,7 x 10^3 UFC/100 ml y 6,9 x 10^4 UFC/100 ml (con una turbidez de 8 NTU) utilizando 1.6mW y 50mW con UV-LED 265 nm. La dosis de

UV fue ligeramente similar, 31 mJ/cm^2 de dosis de UV y 34 mJ/cm^2 de dosis de UV para los LED de 265 nm de potencia baja y media, respectivamente. Para la longitud de onda de 275 nm, 17 mJ/cm^2 de dosis UV (40 segundos) y 13 mJ/cm^2 de dosis UV (11 segundos) para los LED UV de potencia baja y media respectivamente.

La inactivación total de *Clostridium perfringens* en la publicación 1 utilizando LEDs-UV de 265 nm y 2 mW de potencia se alcanzó tras 360 segundos, con una dosis UV equivalente de 133 mJ/cm^2. La concentración inicial fue de 4,1 x 10^4 UFC/100ml y la turbidez de 7,3 NTU. En el caso de LEDs-UV de 275 nm la concentración inicial fue de 2,5 x 10^4 UFC/100 ml y la turbidez de 4,8 NTU. En esta ocasión, se alcanzó la línea de detección tras 420 segundos (dosis UV equivalente de 196 mJ/cm^2). Por último, bajo irradiación a 310 nm y con 4,2 x 10^4 UFC/100 ml de concentración inicial y turbidez de 9,5 NTU, tras 600 segundos (10 minutos) (dosis UV equivalente de 326 mJ/cm^2) la concentración final fue de 3,2 x 10^4 UFC/100 ml, alcanzando solamente un 31% de inactivación.

En la publicación 2, cuando trabajamos ya con LEDs-UV de mayor potencia, la concentración inicial para *Clostridium perfringens* osciló entre 3,2 x 10^4 UFC/100 ml y 4,9 x 10^4 UFC/100 ml (4.9 NTU) utilizando 1.6mW y 50mW con UV-LED 265 nm, con una turbidez de 4,9 NTU. Para los LED de 265 nm, la inactivación se alcanzó aplicando dosis UV de 100 mJ/cm^2 (240 segundos) y dosis UV de 124 mJ/cm^2 (70 segundos) para las potencias baja (2 mW) y media (50 mW), respectivamente. En el caso de la longitud de onda de 275 nm, el nivel de turbidez fue de 10 NTU, y se requirieron dosis UV de 159 mJ/cm^2 (360 segundos) para los LED UV de 1,6 mW y la misma dosis UV para los LED UV de 50 mW (221 segundos).

Objetivo 4. Analizar las pérdidas de transmitancia de la luz UV de los LED a través del agua para determinar la profundidad máxima del agua del reactor y evaluar además el efecto de la turbidez.

Este trabajo se realizó en la publicación 2, probando cómo se atenuaba la irradiancia UV de LEDs-UV de dos longitudes de onda diferentes, 265 nm y 275 nm, con dos potencias ópticas diferentes, 1,6 mW y 50 mW utilizando agua sin turbidez y con turbidez. Se iluminaba un reactor de agua con cada placa de LEDs-UV de distinta longitud de onda y distinta potencia óptica y se medía la irradiancia a distintas distancias (profundidades) de la capa inicial de agua (Figure 20).

Los resultados demuestran que tanto la turbidez como la altura de la capa de agua (profundidad) afectan al proceso de desinfección: las pérdidas de irradiancia UV aumentan por la alta turbidez y la profundidad del recipiente que contiene las aguas residuales. Se observó una pérdida máxima de irradiancia UV del 45,6 % utilizando los LED UV de 275 nm y 50 mW con un nivel de turbidez de 0 NTU a 2 cm de profundidad, y del 73,7 % utilizando los mismos LED UV con un nivel de turbidez de 8 NTU a la misma profundidad.

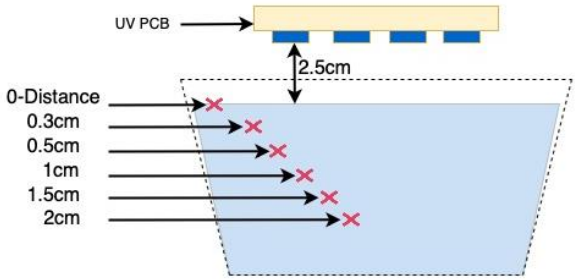


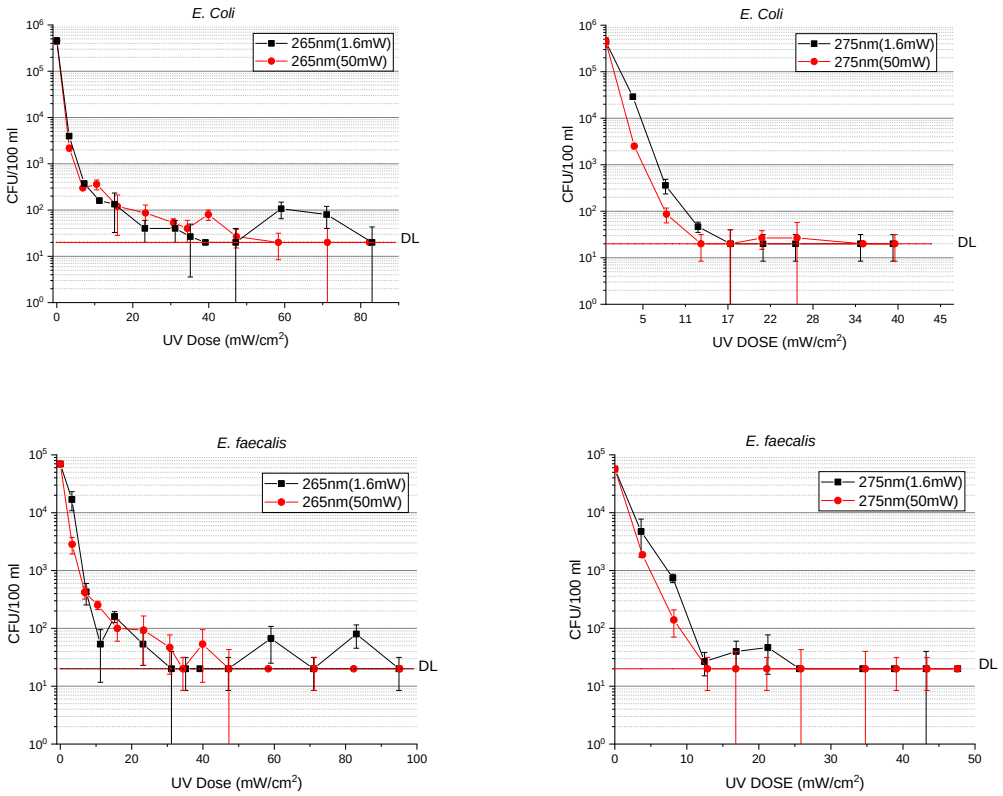
Figure 20 – Ilustración para la medición de la potencia óptica de los LED UV y la profundidad del agua.

	Perdida de irradiancia UV (%) con 0 NTU				Perdida de irradiancia UV (%) con 8 NTU			
Profundidad (cm)	265 nm 2.5 mW	265 nm 50 mW	275 nm 1.6 mW	275 nm 50 mW	265 nm 2.5 mW	265 nm 50 mW	275 nm 1.6 mW	275 nm 50 mW
0.3	11.6	9	8.7	8.7	10.5	16.6	16.8	20.5
0.5	16	13.7	13.1	15.9	19.6	26.8	20	29.6
1.0	26.4	24.9	23.6	27.9	41.4	45	41.3	49.9
1.5	36.2	34.8	32.5	37.3	57.1	62.5	57.5	63.3
2.0	44.5	41.9	40.2	**45.6**	69.9	73.3	68.5	**73.7**

Tabla 1 – Porcentaje de pérdidas debidas a la profundidad del agua y al nivel de turbidez.

Objetivo 5. Verificar la ley de reciprocidad para la desinfección del agua mediante LED-UV. Se utilizan LED-UV de alta y baja potencia para tratar las aguas residuales con la misma dosis de UV. Se analizará el contenido microbiológico (*Escherichia coli*, *Enterococos fecales* y *Clostridium perfringens*) para observar si con la misma dosis UV pero diferente irradiancia inicial la inactivación de las bacterias es la misma.

En la publicación 2 se examinó la ley de reciprocidad para la desinfección de aguas residuales utilizando dos longitudes de onda UV (265 nm y 275 nm) con dos potencias ópticas diferentes (1,6 mW y 50 mW) para desinfectar tres microorganismos (*Escherichia coli*, *Enterococos fecales* y *Clostridium perfringens*). Se observaron índices de inactivación similares para las dos potencias ópticas, con la excepción de los LED UV de 275 nm, que mostraron valores ligeramente superiores para los LED de 50 mW en el caso de *Escherichia coli* y *Enterococos fecales*. Se puede concluir que sí se cumple la ley de reciprocidad pero en el caso de 275 nm se podría investigar más a fondo si existe alguna diferencia al repetir los experimentos de manera exhaustiva.



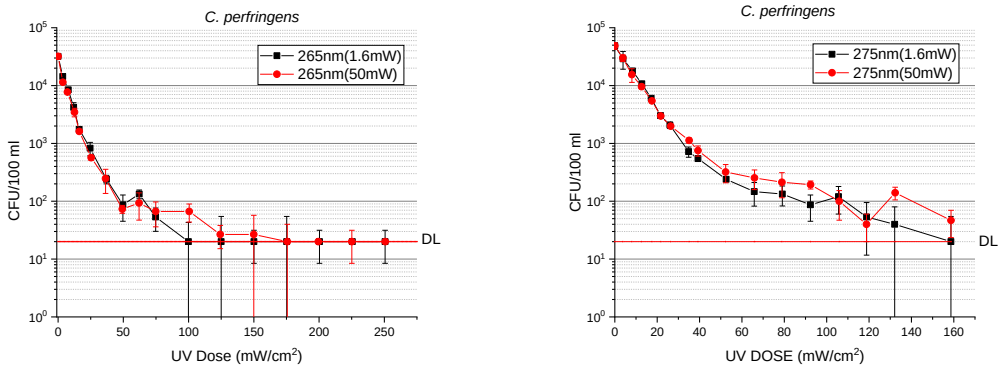


Figura 21 – Dosis UV frente a UFC/100ml para E. coli utilizando 265nm (izq.) y 275 nm (drcha.) con diferentes potencias ópticas (1,6mW y 50mW). De arriba abajo E. coli, E. faecalis y C. perfringens.

Chapter 8: Conclusión

El capítulo de conclusiones de esta tesis presenta los resultados de mi investigación sobre el uso de LEDs ultravioleta como método alternativo para la desinfección de aguas residuales. La investigación demuestra que los LEDs ultravioleta son una alternativa viable a las lámparas ultravioleta tradicionales, ya que ofrecen una serie de ventajas como ser menos frágiles, no contener sustancias tóxicas y estar disponibles en una variedad de longitudes de onda. Además, los LEDs ultravioleta requieren un voltaje más bajo para funcionar en comparación con las lámparas UV de mercurio convencionales, lo que facilita su uso en modo pulsante, que tiene el potencial de reducir costes. Por otra parte, la literatura científica está demostrando que los LEDs ultravioleta tienen el potencial de ser más eficientes y eficaces en el futuro, y que los costes van disminuyendo progresivamente.

En la publicación 1, los resultados experimentales revelan la eficacia del uso de LED UV de baja irradiancia a varias longitudes de onda (268 nm, 279 nm y 307 nm) en la inactivación de diversas cepas de bacterias presentes en efluentes reales de aguas residuales. Los resultados indican que la longitud de onda de 268 nm fue la más eficaz y rápida en términos de inactivación bacteriana, seguida de la de 279 nm. Sin embargo, la tasa de inactivación fue significativamente inferior cuando se utilizó una longitud de onda de 307 nm. Por ello, no se recomienda el uso de esta última longitud de onda (307 nm) en la desinfección de aguas residuales con LED UV debido a su elevado consumo de energía en comparación con las otras dos longitudes de onda.

Por otra parte, los datos obtenidos de los experimentos también demuestran la capacidad de los LEDs UV para inactivar tres cepas bacterianas diferentes (*Escherichia coli*, *Enterococos fecales*, *Clostridium perfringens*) de forma eficiente y con tiempos de exposición cortos, del orden de minutos. También se observó que la dosis de UV necesaria y el tiempo de exposición correspondiente variaban entre las distintas cepas bacterianas. En concreto, *Escherichia coli* y *Enterococos fecales* se inactivaron con una dosis UV mínima (18,4 mJ/cm^2) y un tiempo de exposición correspondiente de tan sólo 1 minuto. En cambio, *C. perfringens* necesitó dosis UV más elevadas y tiempos de exposición más largos, de hasta 6 minutos. Aunque todavía es posible desinfectar esta cepa bacteriana, es necesario seguir investigando el uso de LED UV de mayor potencia que puedan reducir el tiempo de exposición y facilitar así la ampliación de pequeños reactores prototipo a reactores de tamaño completo que puedan implantarse en instalaciones de tratamiento de aguas residuales.

En la publicación 2, se ha comprobado que la ley de reciprocidad es válida para la desinfección LED-UV utilizando LEDs con longitudes de onda de 265 nm y 275 nm y dos potencias ópticas diferentes de 1,6-2,5 mW y 50 mW. Esto se determinó mediante pruebas con tres microorganismos presentes en el efluente de la planta de aguas residuales de Linares: *Escherichia coli*, *Enterococos fecales* y *Clostridium perfringens*. Los

índices de inactivación fueron similares para ambas potencias ópticas, con la excepción de los LED de 275 nm que mostraron resultados ligeramente mejores en el case de los LEDs de potencia de 50 mW para *Escherichia coli* y *Enterococos fecales*. Sin embargo, es necesario seguir investigando para confirmar estos resultados y tener en cuenta el impacto de la turbidez y la transmitancia UV en los resultados, ya que a 2 cm de profundidad del agua se produce una disminución significativa de la transmitancia.

Trabajo futuro:

En el futuro, está previsto ampliar el alcance del proyecto:

- Aumentando los volúmenes de aguas residuales en los experimentos de desinfección. Este aumento requerirá el desarrollo de nuevas placas de circuito impreso para cubrir el recipiente que contiene las aguas residuales y un sistema de gestión de la temperatura para acomodar las nuevas placas de circuito impreso. Además, se diseñará un nuevo sistema electrónico para controlar y supervisar el nuevo reactor para una desinfección eficiente y eficaz.

- Probar la eficacia de la desinfección UV-LED en una gama más amplia de microorganismos, incluidas la *Salmonella* y la *Legionella*, que son objeto específico de la normativa española en lo que respecta a su presencia en los sistemas de tratamiento de aguas residuales. Ello permitiría comprender mejor las capacidades de la desinfección LED-UV en este contexto y contribuiría a garantizar el cumplimiento de la normativa pertinente (Real Decreto 1620/2007, de 7 de diciembre).

- Otra línea futura sería investigar el uso de LEDs-UV más potentes, como los que tienen una potencia de salida de 200 mW, ya que no han sido ampliamente examinados en el campo de la desinfección de aguas residuales.

- Además, en el campo de la desinfección de aguas residuales mediante LEDs-UV, hay casos en los que las bacterias reaparecen después del tratamiento, por lo que ese podría profundizar en las causas subyacentes de la reactivación de las bacterias para entender cómo mitigar este fenómeno y desarrollar estrategias para eliminarlo.

- Otra área de interés sería implantar el uso de la modulación de anchura pulsada (PWM) como alternativa al modo continuo. La PWM consiste en suministrar a los LEDs-UV señales de tensión pulsadas a una frecuencia y un ciclo de trabajo específicos. Empleando esta técnica, se determinará la eficacia en la desactivación de microorganismos y también se reducirá el consumo de energía durante el proceso de desinfección.

- Por último, se podría diseñar y desarrollar un reactor portátil que utilizase los LEDs-UV más eficaces y se alimente mediante un sistema de paneles solares, lo que lo podría ser apto para su uso en zonas remotas donde el acceso a la electricidad pueda ser limitado.