

Bactericidal UVC LEDs for Ultrapure Water Applications

The R&D Notebook 11

Maxime Sorre, Christophe Paragot, Pascal Rajagopalan, Thomas Flint, Laurent Moreau
R&D, Lab Water Solutions, Science & Lab Solutions, Merck, Guyancourt, France

Abstract

Light emitting diode (LED) technologies have boomed during the past years and enabled the design of mercury-free, tailor-made solutions to inactivate and control microorganisms in pure and ultrapure water. This paper investigates the germicidal effect of ultraviolet (UV) beams at 265 nm, the photoluminescence of LEDs, and their integration into pure and ultrapure water producing systems. Log reduction value tests using *Ralstonia pickettii* and UV dose prediction modeling were conducted to assess the efficiency of our newly designed inline reactor and sanitization module. Mixing regime, transmittance, reflectivity and temperature were monitored to reach log reduction values greater than 7.5 and minimum dose of 30 mJ·cm⁻².

Introduction to UV light

What are UV rays?

Ultraviolet (UV) light is electromagnetic radiation with a wavelength of between 100 and 400 nm. UV rays are longer than X-rays but shorter than visible light. UV radiation is subdivided into UVA (315-400 nm), UVB (280-315 nm) and UVC (100-280 nm) according to the International Organization for Standardization.¹ Alternatively, UVC can be defined as between 200 and 280 nm.² Deep UV sources that emit UVC light

are used in a variety of applications such as high-density optical storage, biomedical research, and the treatment and sanitization of air and municipal water supplies.³ Following quantum theory, the energy of a beam is a function of the amount and the energy of emitted photons. The energy of each photon is linked to its wavelength, according to the Planck-Einstein relationship:

$$E = h \frac{c}{\lambda}$$

where E represents the energy (in J), λ the wavelength (in m), c the speed of light in vacuum ($3.00 \cdot 10^8 \text{ m} \cdot \text{s}^{-1}$), and h the Planck constant ($6.63 \times 10^{-34} \text{ J} \cdot \text{s}$).

Light-matter interactions

Because electrons of atoms and covalent bonds are characterized by discrete levels of energy, the amount of energy required for an electron to jump from one level to another must match the absorption of a photon of a specific wavelength. When covalently bonded electrons jump to a higher level of energy, the stability of the molecule is affected, making it available for photochemical reactions. Organic molecules that contain double bonds and benzene rings are particularly susceptible to photochemical reactions under UV radiation.

Fluence & irradiance

Irradiance (in $\text{W}\cdot\text{m}^{-2}$), sometimes designated as intensity or fluence rate, is the amount of electromagnetic radiation power received by a defined surface when directed perpendicular to the surface. In other words, irradiance is the surface density of the radiant power meeting the considered surface. Dose, also known as fluence (in $\text{J}\cdot\text{m}^{-2}$), quantifies the amount of energy transmitted onto a defined surface area and over a defined time span of electromagnetic radiation when directed perpendicular to the surface. Dose and irradiance consequently are connected by the following integral:

$$H_e = \int_0^T E_e(t) dt$$

where H_e is the dose (in $\text{J}\cdot\text{m}^{-2}$), E_e the irradiance (in $\text{W}\cdot\text{m}^{-2}$), t the time variable (in s), and T the exposure duration (in s). Dose and irradiance are noted H index e and E index e (e for "energetic"), respectively, to avoid any confusion with the equivalent quantum-based physics parameters. If irradiance is kept constant throughout the exposure time T , it can be removed from the integral, resulting in:⁴

$$H_e (\text{J}\cdot\text{m}^{-2}) = E_e (\text{W}\cdot\text{m}^{-2}) \times T (\text{s})$$

Dose depends on irradiance, assumed to be of constant power in most cases, and on irradiation time.

Bactericidal effect of UV radiation

Which wavelength inactivates bacteria best?

To disable bacterial replication, DNA and RNA are strategically targeted with the use of radiation.

Figure 1 shows the absorbance spectra of the four nucleotides in DNA. The absorbance maximum of DNA is assumed to be between 260 and 270 nm.⁴ Generally speaking, the optimal wavelength to disable microorganisms and viruses is around 265 nm.⁴⁻⁶

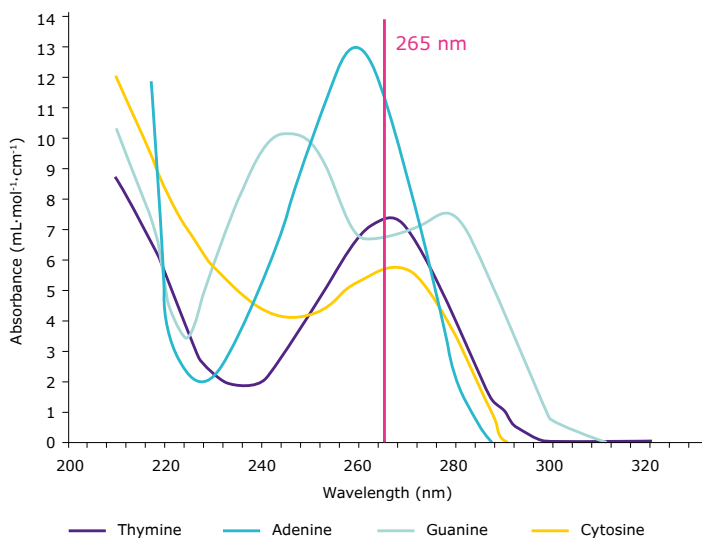


Figure 1. Absorbance curves of adenine, cytosine, guanine and thymine. Adapted from Kowalski 2009.⁸

Other authors state 260 nm as DNA's most absorbed wavelength.⁷ Values may differ depending upon microbial species. For example, 267 nm is cited for *Escherichia coli*,⁴ a ubiquitous microorganism that is often viewed as a standard to assess water quality,⁴ and 260 nm for *Cryptosporidium* parasite.⁵

A wavelength of 265 nm generates several types of lesions in DNA. Two major lesions involve the formation of photo adducts: cyclobutane thymine-thymine dimers and dipyrimidine (thymine-thymine, thymine-cytosine, cytosine-thymine or cytosine-cytosine) 6-4 photoadducts.⁴ For more details on photo adduct formation, please refer to Kano, I., Darboure, D. and Mabic.⁹ Because the nitrogen bases are ubiquitous within DNA, the absorption maximum of DNA does not stray far from 265 nm. However, the required fluence varies considerably between microorganisms due to the varying complexities of cell matrices. At a fixed dose, viruses and spores are the most UVC-resistant microorganisms. This is because access to their DNA and/or RNA is difficult as they are protected by a capsid that vegetative bacteria do not possess.⁹ Other factors, such as cell wall thickness, cell size, the nature of coproducts being synthesized during irradiation, and DNA auto repair abilities, can also play a role.¹⁰ Investigations on more than 50 microorganisms suggest that a UV dose of approximately $10 \text{ mJ}\cdot\text{cm}^{-2}$ is necessary to achieve inactivation amounting to a median log reduction value (LRV) of 3 (i.e., to a 99.9% reduction).⁹

Photoreactivation & excision (dark) repair

Microorganisms, and especially bacteria, are able to self-repair damaged DNA,⁴ thus mitigating the impact of irradiation. The two mechanisms of repair are:

- Excision repair, also known as dark repair
- Photoreactivation, which, contrary to excision repair, requires UVA and visible light,⁴ or even UVB¹¹ as a source of energy

It has been ascertained that photoreactivation is the most prevalent of these two mechanisms, provided the sample is exposed to a sufficient amount of natural light.⁴ This can be explained when considering that 75% of DNA damage occurs through cyclobutane pyrimidine dimer (CPD) formation,¹² in which photoreactivation intervenes. Photoreactivation involves the enzyme, photolyase, which specifically monomerizes CPD lesions.^{4,12} Photolyase is photochemically activated at certain wavelength ranges, which differ slightly depending on the study. The cited ranges lie between 330 and 480 nm,⁴ 310 and 480 nm¹² and 300 and 500 nm.¹¹

Introduction to UVC LED Technology

What is an LED?

An LED (light emitting diode) is a device that uses direct current to emit radiation¹³ of, for example, UV, visible or infrared light. This process of converting electrical energy into light energy is called electroluminescence. The use of LEDs provides numerous advantages^{4,14,15} including:

- A wide range of wavelengths emitted
- No use of mercury
- Compact
- Mechanically solid
- Fast ON/OFF response, as no heating is required
- Energy efficiency, as low voltages are used
- Durability, typically over 10,000 hours at 265 nm¹⁴

LEDs can be easily produced to emit at a certain wavelength spectrum, making them suitable for custom applications with multiple wavelength peaks.¹¹ In the UV range, LEDs differ from low-pressure (LP) mercury lamps as the latter emit discrete monochromatic radiations and the former emit one or several polychromatic peaks centered around the desired wavelength (**Figure 2**).⁶

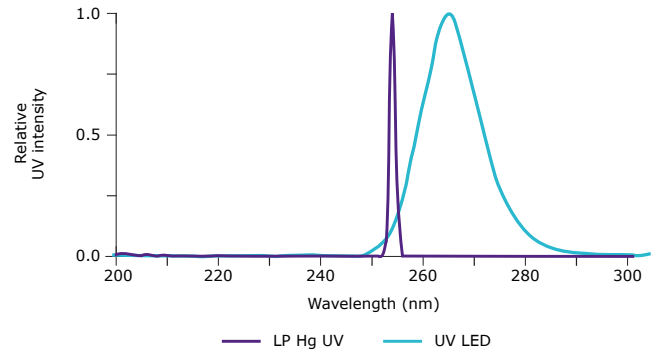


Figure 2. Relative intensity vs. wavelength of a low-pressure mercury (LP Hg) UV lamp and a UV LED. Adapted from Fujioka et al. 2020.¹⁶

An LED is composed of a small chip that incorporates a semiconductor crystal protected by a resin (e.g., epoxy resin when emitting in the visible spectrum) or by a lens (for UV applications). The small size of LEDs (about 1 mm²)^{15,17} increases the design possibilities when inventing new custom applications. Although LED technologies are booming, they are still at an infant stage of their development³ and challenges remain. For instance, for a given irradiance, the shorter the wavelength, the more expensive and the less energy-efficient an LED will be.¹¹

Electroluminescence within an LED is summarized in **Figures 3** and **4**. The crystal within the LED is basically composed of n-type and p-type layers of doped materials with, respectively, electron-donor and electron-acceptor elements. Because electrons repel each other in the n-type material, the electrons reorganize themselves at the p-n junction, the border between these materials. Applying current reorganizes electrons and positive holes in the matrix, which generates energy in the form of light of a wavelength determined by their respective energy.

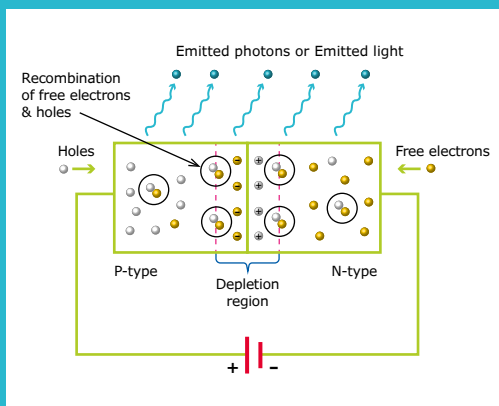


Figure 3. Summary of photon generation of LEDs. Adapted from www.physics-and-radio-electronics.com/electronic-devices-and-circuits/semiconductor-diodes/lightemittingdiodeledconstructionworking.html.

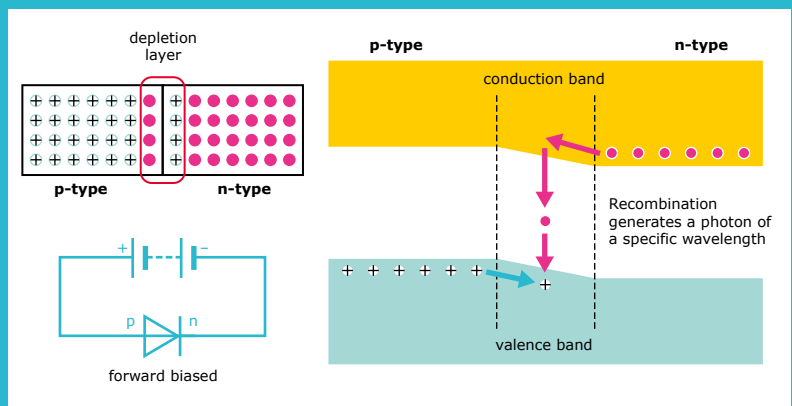


Figure 4. Generation of a photon following electron and hole recombination in a forward bias diode. Adapted from www.youtube.com/watch?v=32vMzGTCzPU.

Group 13 element nitrides for deep UV emission

The brightness and wavelength of the emitted light depend on the material used for producing the LED and on the applied direct current flowing through the LED. The efficiency in generating light increases with increasing current but decreases with increasing temperature. The bandgap in the depletion layer between the conduction band and the valence band must correspond to the required wavelength, in this case 265 nm.

Active-region materials are sandwiched between n-type and p-type semiconductors to confine the depletion layer. The following kinds of active-region materials may be used:

- An electron blocking layer (EBL) that is rich in electrons, near the p-type layers, which prevents reverse leakage current
- Multiple quantum well (QW/MQW) layers that alternate with quantum barrier layers to trap electrons and holes, fostering their recombination

In this context, the chemical composition to create deep UV source semiconductors include Group 13 element (B, Al, Ga, In...) nitrides, particularly aluminum and gallium nitride ($\text{Al}_x\text{Ga}_{(1-x)}\text{N}$). The discovery of $\text{Al}_x\text{Ga}_{(1-x)}\text{N}$ to generate UVC granted I. Akasaki, H. Amano and S. Nakamura the Nobel Prize in Physics.¹⁸

Several parameters determine the energetic performance of commercial UV LEDs:

- **Wall-plug efficiency, or radiant efficiency** (no unit): Ratio of emitted irradiance outside the LED on inlet electrical power.⁴
- **External quantum efficiency (EQE)** (no unit): Ratio between photon flux emitted from the LED and the electrical current (electrons flow) that goes through the device. Photon flux can be calculated by dividing irradiance by the energy per photon.
- **Internal quantum efficiency (IQE)** (no unit): Ratio between the flux of photons produced within the active region and the flow of electrons that goes through the device. Not all the photons produced within the active region are emitted to the outside. Similar to EQE, the photon flux of the active region is obtained by dividing the optical power that the active region emits by the energy per photon.

Process engineering parameters of UV irradiation

Reactor hydrodynamics and mixing regime

A UVC LED can either be incorporated into a static environment reactor or an inline dynamic reactor,¹⁹ where a reactor is defined here as a container in which substances undergo UV irradiation. In both cases, the LED must be integrated into the reactor, whose performance is linked to how well the hydrodynamic and radiative patterns interact with each other.¹⁹ As fluence, rather than irradiance⁶, is the key parameter to enhance bacterial inactivation efficiency at 265 nm, it is crucial to optimize the residence time distribution (RTD). The aim is to homogenize as much as possible the fluence directed at all locations of the reactor and to prevent shortcuts with high velocity, which are detrimental to the overall LED reactor performance of inactivating microorganisms. To model hydrodynamics within chemical processes, real reactors, which are non-ideal, are usually interpreted as constructions based on two ideal reactors of an opposite behavior: the continuous stirred-tank reactor (CSTR) and the plug-flow reactor (PFR). A CSTR reactor homogeneously mixes all microorganisms in suspension within the whole volume whereas a PFR is characterized by heterogeneous mixing along the flow direction of the microorganisms, therefore following a gradient of concentration. For a UVC process, a real reactor that approaches the behavior of a CSTR is advantaged: even if irradiance is heterogeneously distributed, its mixing regime eventually makes all microorganisms undergo the same accumulated fluence, on average.¹³ Other investigations confirm the relevance of a regime close to CSTR.²⁰ In all cases, this is crucial to master the process design, for instance by baffling the pathway through the reactor to enhance performance.¹³

Transmittance

UV radiation, throughout its path, is absorbed by the molecules it crosses and by the media it passes through. For example, water transmittance is affected by the presence of contaminants, such as organics, colloids or dissolved gases, and by the water itself.²¹ In tap, pure and ultrapure water, UVC energy quickly declines as it advances from the source, and its penetration is strongly impacted by contaminants. Common organic contaminants, such as humic and fulvic acids, are particularly impactful UV light scavengers. This means that water treated by UVC for bactericidal control should already be refined by upstream purification technologies, such as reverse osmosis (RO) and electrodeionization (EDI). Furthermore, in addition to the continuous loss of light energy along its path through water, a part of the light energy is also lost by diffraction through the air-quartz and quartz-water diopters it passes through.

Reflectivity

A fraction of light is reflected by the reactor walls, which directly impacts LRV.²¹ The impact of reflectivity is greater at low flows and high irradiance²¹ and the closer a mixing regime is to the model CSTR.¹³ Moreover, the reflectiveness of a reactor depends on its shape and geometry, on the distance between the UVC source and the target location,¹⁹ and to a lesser extent, on the rugosity of the wall.²¹ Our investigation led us to conclude that polytetrafluoroethylene (PTFE) is the best reflective material available on the market, given its reflectivity of 95% at 265 nm.²²

UVC LED in Milli-Q® Water Purification Systems

Development of our inline bactericidal reactors

What were our expectations?

Table 1 summarizes some of our considerations and the choices we made when designing our inline bactericidal reactor.

Table 1. Parameters considered for bactericidal efficiency and our design approaches.

Parameter	Design approach
Transmittance of quartz	Highly UVC transparent quartz
Transmittance of water	UV treatment to take place downstream the removal of most organics and ions
Reflectivity of reactor inner walls	Highly reflective material to be selected (needed especially for low flow rates)
Flow	High impact on reactor sizing specifications
Mixing regime	CSTR preferred over PFR
Temperature of LED	LED cooling system to be taken into account
Intensity of input LED power	Generally higher (but on the downside, this increases LED temperature)

We decided to develop a PTFE reactor internally for cost and properties reasons. Its design would attempt to maximally address the key considerations described in **Table 1**.

Bacteria that were identified upstream the UVC reactor stage include:

- Some rod-shaped Gram-negative, oxidase-positive bacteria
- Ralstonia pickettii*
- Pseudomonas aeruginosa*
- Sphingomonas paucimobilis*
- Brevundimonas*

As *R. pickettii* is one of the most robust germs able to proliferate in pure and ultrapure water,^{23,24} all challenge tests to measure the bactericidal efficiency of UVC LED emitting at 265 nm were performed on this species. We found that the minimum required UV dose necessary is 22 mJ·cm⁻² to inactivate *R. pickettii*.²⁵ According to the American National Standard, the minimum required dose of a mercury lamp emitting at 254 nm is 16 mJ·cm⁻², if the irradiator is fitted with a calibrated intensity meter, and 30 mJ·cm⁻² if no calibrated meter controls the irradiator.²⁶

Flow schematic: Integration of an LED inline reactor

[Milli-Q® IQ 7003/05/10/15 tap to pure to ultrapure water systems](#) are composed of a water purification unit connected to a tank for Type 2 pure water storage. Water purification takes place in two main steps:

1. Purification to create Type 2 water: Within the water purification unit, tap water is initially purified through the IPAK Gard® pretreatment pack, which contains a pleated filter and a carbon block to efficiently remove particles, colloids and free chlorine. Advanced RO then removes 95 to 99% of contaminants, including ions, particles, bacteria and large organics (>200 kDa), while ensuring a constant product flow rate and constant water quality. RO water then enters the Elix® EDI module to remove remaining ions, where ion-exchange resins are continuously regenerated by an electrical field. The purified water then passes through a UVC LED reactor emitting at 265 nm where bacteria are further eliminated, resulting in Type 2 pure water that is stored in the storage tank.

2. Polishing to create Type 1 water: Type 2 pure water stored in the tank is then further purified into Type 1 ultrapure water. This is achieved by ion exchange combined with activated carbon adsorption, followed by photo-oxidation of organic compounds using a vacuum UV mercury-free lamp, such as an excimer lamp.

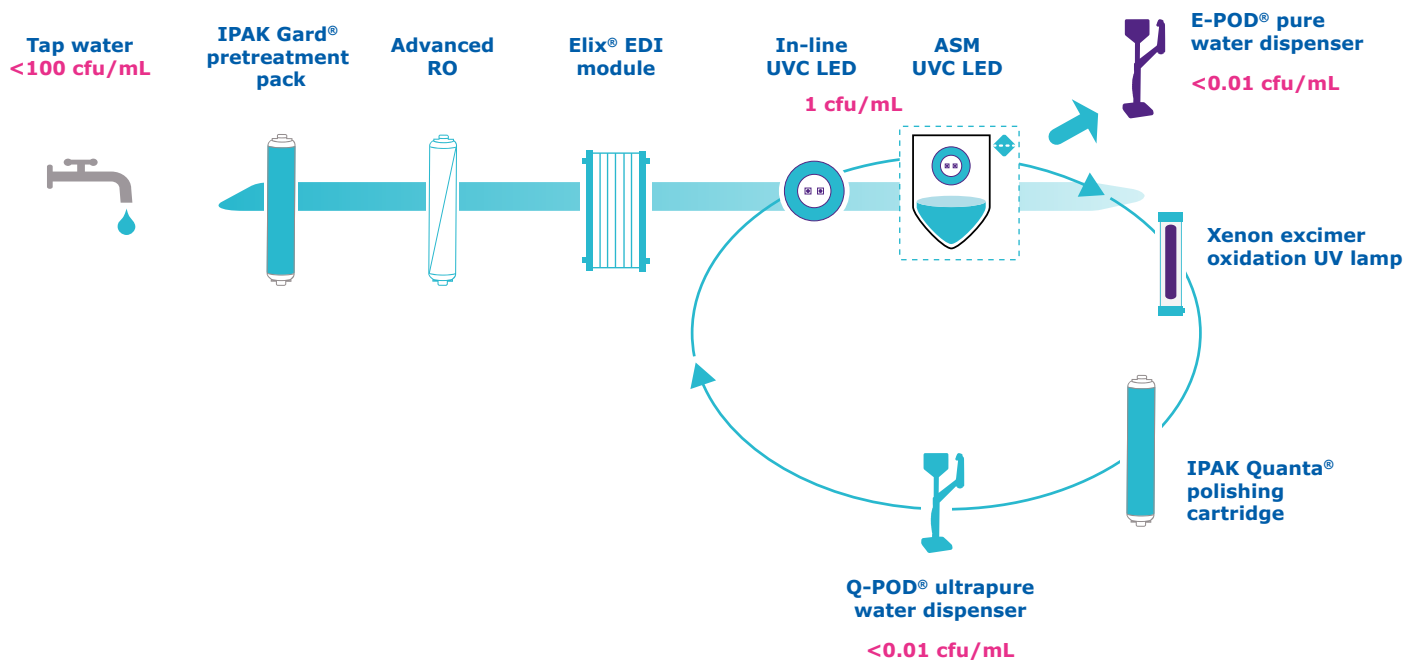


Figure 5. Flow schematic of Milli-Q® IQ 7003/05/10/15 water purification systems showing water flow, UVC LED locations, and bacterial concentrations. For both pure and ultrapure water, bacteria concentrations were $<0.01\text{ cfu/mL}$ ($<10\text{ cfu/L}$) with a Millipak® Gold filter when installed and used in a laminar flow hood. ASM, automatic sanitization module; EDI, electrodeionization; RO, reverse osmosis.

Before distribution of Type 2 water or polishing of Type 1 water, stored tank water is protected by an UVC LED automatic sanitization module (ASM) and a vent filter. **Figure 5** shows a flow schematic of a Milli-Q® IQ 7003/05/10/15 water purification system that includes the location of both LED chips within the process as well as the typical microbiological concentration throughout the purification journey.

Performance assessments

The efficiency of our solution (**Figure 5**) was assessed by multi-physical thermal, optical, fluid dynamic and particulate tracing simulations. Theoretical studies were compared with experimental results. Extensive research work and design development were required to arrive at the optimal final design.

Log reduction value (LRV)

All experimental tests were conducted with ultrapure water samples spiked with *R. pickettii* at 10^5 cfu/mL . Several reactor designs were tested (**Figure 6**). The final reactor version reached $\text{LRV} >7.5$, a performance similar to that of a traditional mercury lamp.

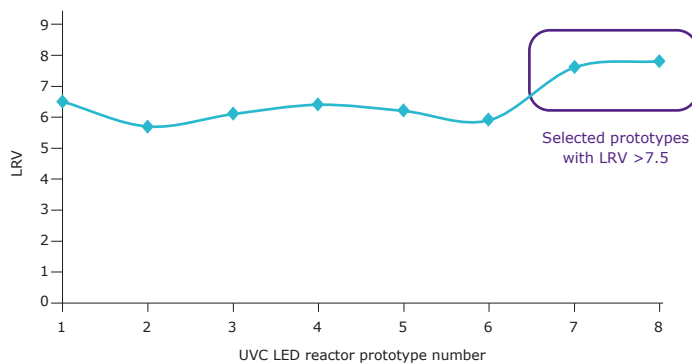


Figure 6. Log reduction value (LRV) for various reactor designs at maximal flow rate (55 L/h) with an *R. pickettii* concentration of 10^5 cfu/mL .²⁵

Computational fluid dynamics (CFD) and UV intensity modeling for UV dose determination

We used a multi-physics simulation to combine Eulerian-model particulates tracing of residence time (**Figure 7A**) and velocity fields (**Figure 7B**) with a static UV fluence rate model (**Figure 7C**). From these, we modeled UV dose distribution inside the reactor (**Figure 7D**). CFD modeling through Eulerian particle tracing enables a certain notion of fluence to be considered separately: the UV dose simulated in CFD modeling is not the locally delivered fluence received at one particular location of the reactor over a defined time frame, but rather the accumulated fluence defined as the infinite sum of all fluences undergone by the Eulerian fluid particle all along its journey in the reactor over a defined time frame.¹³

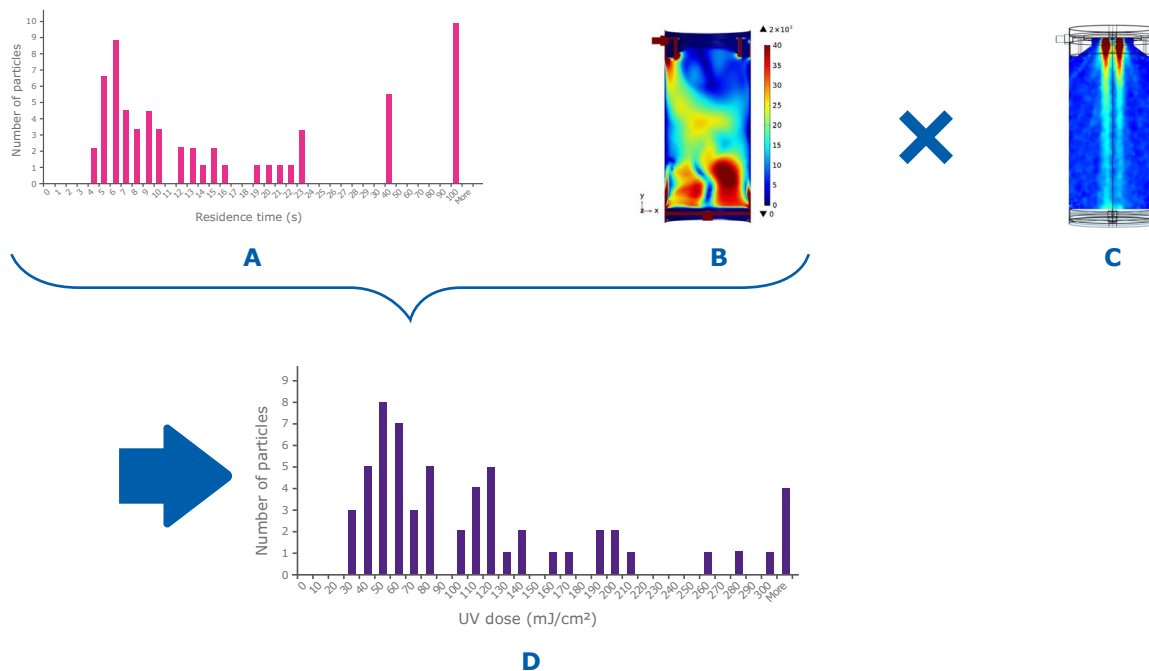


Figure 7. Multi-physics simulations. (A) Residence time (particulate number vs. time in s); (B) Surface velocity magnitude ($\text{mm}\cdot\text{s}^{-1}$); (C) UV intensity (arbitrary units); (D) Accumulated UV dose ($\text{mJ}\cdot\text{cm}^{-2}$).²⁵

Although our simulations did not show a typical normalized Gaussian pattern, all UV fluence values of the particulates were greater than or equal to $30 \text{ mJ}\cdot\text{cm}^{-2}$. The combination of all these tests enabled us to modulate the current values (in mA) depending upon the operating mode of the system: recirculation only, pure water production only, and recirculation and production happening at the same time. Increasing the fluence rates at the locations where water velocity is higher is a strategy known as fluence-rate hydrodynamics conformity.²⁰ For this, LEDs offer the flexibility to modulate the optical power and thus to adjust to a flow rate and a mixing regime that vary with time. In other words, it becomes possible to enslave inlet power on flowmetering.¹³ This feature could enhance the process efficiency whilst saving energy and LED lifetime.

Temperature management

Excess heat negatively impacts the light output and lifetime of UVC LEDs. Heat generation is caused directly by power dissipation (W), which can be easily estimated through multiplying forward voltage (V) and forward current (A). To increase LED lifetime, we selected the UVC LED with the best operating time and optimized the thermal management of the printed circuit board assembly (PCBA) through water cooling. Aluminum and copper increased the thermal transport of LED, PCBA and water throughout the process, helping to successfully develop a design that meets our thermal requirements.

Development of our tank Automatic Sanitization Module (ASM)

Microorganisms exposed to UV irradiation are not actually killed, but rather inactivated through dimer formation on their DNA. As photoreactivation and dark repair allow bacteria to recover from these detrimental DNA modifications, the quality of stagnant purified water inevitably degrades with storage time. In other words, water quality within a storage tank is not static and inalterable. In order to maintain a constant and high degree of purity, a protective environment and technology must be applied. With carefully selected raw materials, an optimized air-vent filter and a bactericidal UV lamp, it is possible to maintain high-quality purified water during storage.²⁷ In this context, LEDs have been investigated as an alternative to traditional mercury-based ASMs.

Our ASM solution

As beam intensity varies depending upon the angle, we placed the LED assembly of our ASM at the top of the tank, thus irradiating stored pure water from above with minimum blind spots (Figure 8). From this position, beams spread in a dome-shaped irradiation pattern into the bulk of the tank, homogeneously inactivating bacteria to prevent their regrowth. This is in contrast to a low pressure lamp that emits parallelly,²⁸ which prevents radiation from efficiently reaching the bottom of some storage tanks.

As a cooling strategy, we applied an air-based solution with a fan, as water is unable to contact the quartz lens at the top of a storage tank that is not full. The LED chip is equipped with the same printed circuit board assembly (PCBA) and the same 265 nm wavelength as for the inline reactor described above.

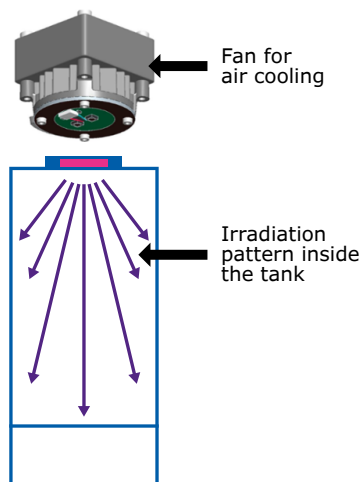


Figure 8. Crosswise view of our automatic sanitization module (ASM).

Performance assessment

To evaluate our ASM, we used a 100-liter tank filled with water spiked with *R. pickettii* at 10^5 cfu/mL. After applying continuous illumination, we obtained a sanitization performance similar to a traditional mercury lamp, with a final microbial concentration less than 1 cfu/mL (Figure 9).

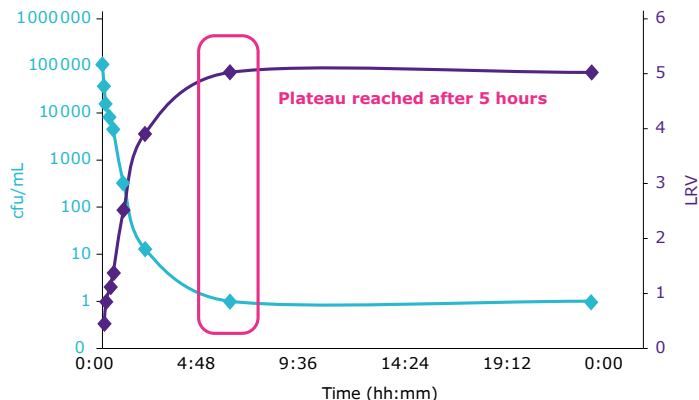


Figure 9. Reduction of bacterial count in *R. pickettii*-spiked water in a 100-L tank exposed to our UVC LED ASM over time (cfu/mL in cyan, LRV in purple).²⁵

As Figure 9 shows, a plateau is reached after approximately 5 hours of irradiation by our UVC LED ASM. It is therefore more efficient to turn on the ASM for 5 consecutive hours per day rather than splitting emission into several periods. An optimal energy-efficient strategy is to apply this continuous illumination when water is most likely to be stagnant, for instance during the night. We consequently set the ASM to be switched on for 300 minutes every day, starting at 2 am (default software setting). In day-to-day usage, we confirmed the ASM's ability to reduce bacterial contamination to <1 cfu/mL after 5 hours of continuous irradiation. This result was in accordance with our expectations and similar to a UV mercury lamp's performance.²⁵

The ASM is primarily used to maintain water quality within the tank. It is not meant to remove bacteria that have managed to settle on the wall, forming a biofilm. Although reducing biofilm by UV irradiation is possible, it requires higher fluence and irradiance. We determined that an important diminution of bacteria concentration from biofilm samples is possible through a continuous 7-day illumination (via a sanitization boost procedure offered in our Milli-Q® Services portfolio). However, sanitization boosting should remain occasional as it could prematurely exhaust the ASM. Fortunately, the daily 5-hour sanitization cycle is efficient enough under most circumstances to prevent any renewed biofilm growth. The key is regularity. The ASM could be characterized by the famous expression "An ounce of prevention is worth a pound of cure".

Conclusion

Theory, method and experiments helped us to develop an optimally designed, mercury-free UVC LED inline reactor and a UVC LED tank sanitization module to be embedded in Milli-Q® water purification systems, such as in Milli-Q® IQ and IX series systems. Although we detailed how the ASM and the bactericidal reactor work individually, it is important to emphasize that UVC LEDs perform optimally when integrated into a complete water purification chain (Figure 5). While other steps of the process, such as RO and EDI, are required upstream to remove contaminants

and improve UV transmittance of water, both 265 nm UVC technologies described here are dedicated to microbiological contamination control. This combination of purification technologies and especially, the sequence of the purification chain, are critical to achieve consistently high-quality ultrapure water. Indeed, reversing the order of RO, EDI and UV irradiation steps would jeopardize the quality of final ultrapure water being produced. As UVC LED chips continue to evolve, we will work to continuously innovate for the benefit of our future generations of ultrapure water systems.

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