

# The purification performance of LaVie system on organic pollutants and microorganism removal



*Stéphanie LEBARILLIER, Lisa FOLI, Anne-Marie FARNET and Pascal WONG-WAH-CHUNG*

## Stable of content

<b>1-Introduction and aims</b> .....	3
<b>2- Material and analytical methods</b> .....	3
<b>2-1- Products and bacterial strains</b> .....	3
<b>2-2- Preparation of the solutions and culture conditions:</b> .....	4
<b>2-3- Purification system LaVie:</b> .....	4
<b>2-4-Analytical methods:</b> .....	5
2-4-1- Measurement of free chlorine and dissolved oxygen: .....	5
2-4-2- Abatement of selected organic compounds.....	5
2-4-3- Enumeration .....	5
<b>3- Results</b> .....	6
<b>3-1- Removal of organic pollutants</b> .....	6
<b>3-2- Removal of microorganisms</b> .....	7
<b>4- Conclusion</b> .....	8
<b>5-References</b> .....	9
<b>6-Appendix</b> .....	10

## 1-Introduction and aims

The quality of tap water is of great concern and LaVie system answers a double issue provides pure, high-quality water, ideal for health and enjoyable to drink, and for people in developing countries, clean drinking water makes a large difference.

To demonstrate the efficiency of LaVie system, the determination of the abatement of organic pollutants is required as well as the reduction in microorganisms. Previous studies focussed on the elimination of a reference compound (diclofenac) and demonstrated that its degradation occurred and is favoured in the presence of free chlorine. However, the studies were realized on a unique compound and at concentrations around mg/L quite far from realistic ones in drinking water sources or drinking water. For instance, some emerging organic compounds (EOCs) such as pesticides (atrazine, metolachlor) and bisphenol A have been detected in ground water at concentration around a few  $\mu\text{g/L}$  (Mukhopadhyay et al., 2022) and other compounds (atrazine, bisphenol A, glyphosate...) in drinking waters at concentration around a few  $\mu\text{g/L}$  to a few ng/L (Owagboriaye et al., 2022; Valbonesi et al., 2021; Khademi et al., 2019).

In this context, this study focused on the behaviour of some EOCs at low concentration during their exposure in LaVie system. Among the organic pollutants, an endocrine disruptor (bisphenol A) and one pesticide (glyphosate) were selected at concentration around 1  $\mu\text{g/L}$ . The abatement measurement will be undertaken on diluted solution of organic pollutants in the presence of free chlorine as it has been previously demonstrated that its presence improved the degradation of other organic pollutants. In addition, the effectiveness of the treatment on microorganisms will be evaluated by monitoring after exposure and 24 hours afterwards (revival potential after repairing its DNA). Several concentrations of microorganisms using two varieties of *Escherichia coli* as an indicator of faecal contamination (potential intraspecific variability in the response to UV in coliforms) will be tested to define more precisely the potential for coliform elimination by this treatment.

## 2- Material and analytical methods

### 2-1- Products and bacterial strains

Bisphenol A (99% purity), atrazine (99.1% purity) and glyphosate (98% purity) were purchased from Sigma Aldrich and Javel water (3.6% active chlorine) was provided by La Croix (Colgate-Palmolive Company).

Two strains of *Escherichia coli* (ATCC 11 303 and ATCC 15 597) were grown on nutrient broth for 3h at 37°C (exponential growth phase) to correlate optical density (OD) with the number of Colony-Forming Units per mL of broth (CFU/mL).

## **2-2- Preparation of the solutions and culture conditions:**

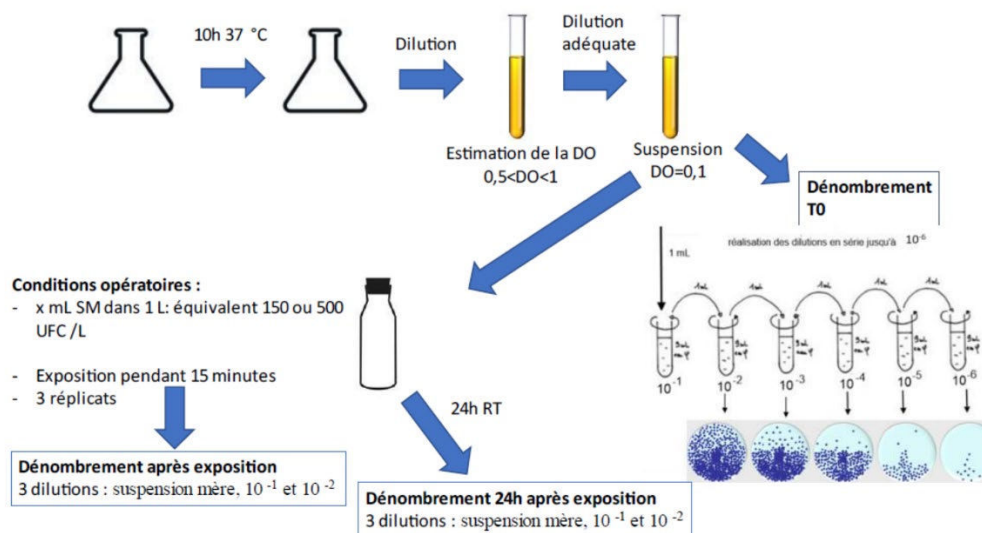
Stock solution of organic pollutants were prepared in tap water before being diluted to a concentration of around 1 µg/L before addition of Javel water at a concentration around 0.1-0.2 mg/L.

To obtain two coliform concentrations of 400 and 600 CFU/L, a culture in a 100 mL Erlenmeyer flask containing 50 mL of nutrient broth was inoculated with 1 mL of a microbial suspension (OD = 0.8) and incubated for 12 hours at 37°C. From this culture, several dilutions were performed to obtain a suspension of OD = 0.1. This suspension, called MS, was enumerated (0.1 mL of dilutions  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$  and  $10^{-7}$  with 3 replicates per dilution) to determine the initial bacterial concentration.

## **2-3- Purification system LaVie:**

The new system is equipped with six 365 Nm LEDs and the reactor is a glass bottle of 1 L. The programmed duration of the exposure is 15 min. The irradiations were realized three times for microorganisms and organic pollutants. The non-exposed sample (t=0) correspond to the solution of organic compounds with the addition of Javel water kept in the dark at room temperature and the exposed sample (t = 15) corresponds to the same solution irradiated during 15 min.

2 mL of the MS suspension was introduced into 1L of sterile distilled water. The so obtained suspension was enumerated without exposition after 15 min to assess the evolution of bacterial concentration (blank). The same UV exposure time was applied on sterile distilled water spiked with bacterial. Between each UV treatment, the bottle was washed with sterile distilled water (500 mL). The suspensions thus treated were counted by spreading on Petri dishes for the mother suspension and the  $10^{-1}$  and  $10^{-2}$  dilutions on PCA agar. To test for possible re-growth, the treated suspensions were then left in the dark at room temperature for 24 h, and coliforms were enumerated for the stock suspension,  $10^{-1}$  and  $10^{-2}$  dilutions (Figure 1). Each manipulation was performed in duplicate.



**Figure 1 :** Experimental protocol to test the effect of the SOLABLE disinfection system on a microbial culture of *Escherichia coli* taking into account the revivification processes.

## 2-4-Analytical methods:

### 2-4-1- Measurement of free chlorine and dissolved oxygen:

To estimate the concentration of free chlorine, AQUALYTIC AL450 photometer was used based on the previous methodology described in the previous report.

### 2-4-2- Abatement of selected organic compounds

To follow the concentration of organic compounds, different analytical methods were used as described in the following table depending on the product.

Compound	Analytical method	LOQ (µg/L)	Uncertainty (%)
Glyphosate	LC-MS-MS after derivatisation	0.020	25
Bisphenol A	ELL, derivatization and GC-MS-MS	0.020	25

**Table 1:** Analytical methods, limits of quantification (LOQ) and uncertainty for organic compounds analysis

### 2-4-3- Enumeration

The enumeration was undertaken on PCA (Plate Count Agar, BioMérieux) agar plates.

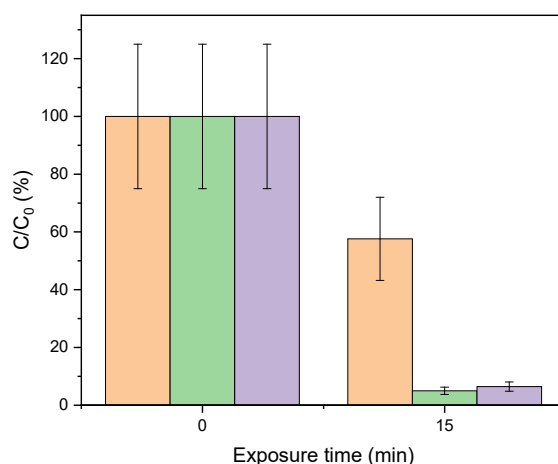
### 3- Results

#### 3-1- Removal of organic pollutants

The removal of the selected organic pollutants was estimated after addition of Javel water and exposure. The results are presented on the following figure and in appendix. On the figure, the initial and final concentration after exposure is indicated in percentage and the errors bars correspond to the uncertainties of the analytical method.

For bisphenol A (BPA) at an initial concentration of around 1 µg/L, its total elimination was observed in both sample (t = 0 and 15) with final concentration of bisphenol A under the quantification limit (see appendix Table 1S). Such behaviour can be explained by the presence of hypochlorite ions (coming from Javel water) in the solution which can induce bisphenol A degradation. This reaction has been previously observed by Yamamoto and Yasuhara (2002) who demonstrated that this phenomenon is improved with high ratio of chlorine versus BPA. In our conditions, assuming that this ratio is between 120 to 160, the total disappearance of BPA occurs in around 15 min.

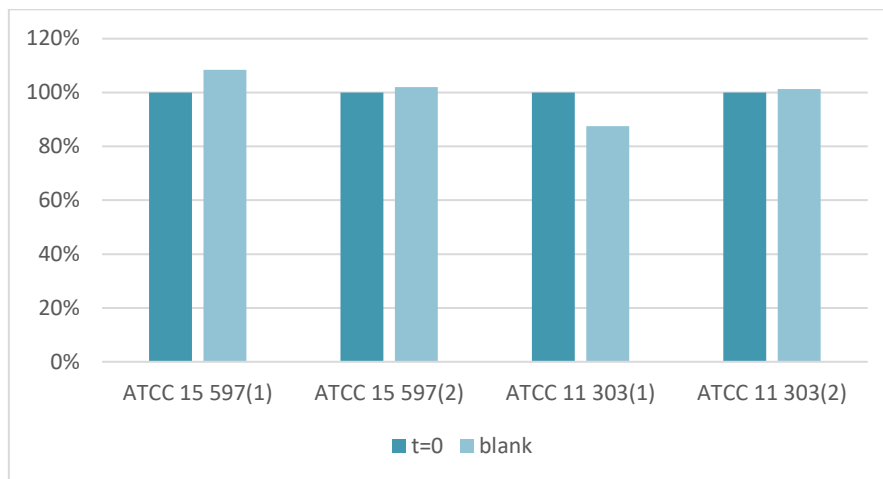
For glyphosate (GLY), the results presented in Figure 2 show that GLY elimination is systematically and significantly observed after 15 min of exposure; the removal is in the range 42 to 95%. The difference in the abatement could probably be explained by slight variation in initial concentration of free chlorine and GLY (see Table 1S).



**Figure 2:** Degradation percentage for glyphosate after irradiation in LaVie system

### 3-2- Removal of microorganisms

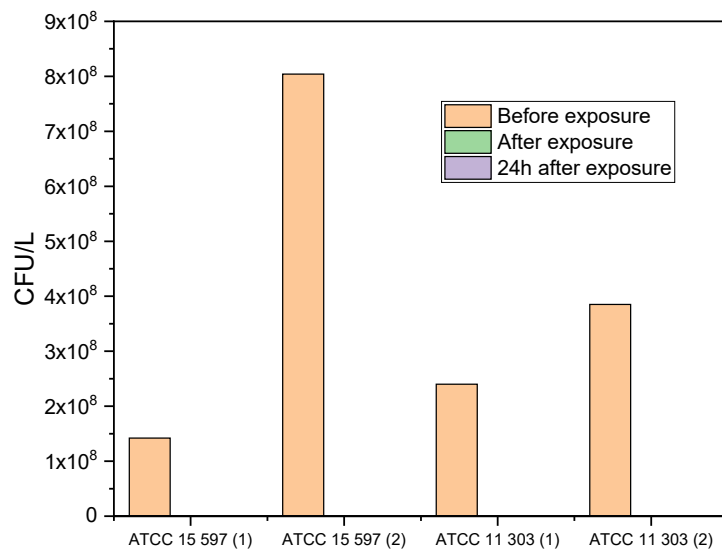
Experiments without exposure in LaVie system and in the absence of chlorine ions were carried out for both *Escherichia coli* strains (ATCC 15 597 and ATCC 11 303) with initial concentration in the range  $1.4$  to  $8.0 \times 10^8$  CFU/L (Table 2S). The results presented in figure 3 show that the dilution of mother solution SM in sterile distilled water did not induce a significant abatement of bacterial colony after 15 min within the experimental errors. EC concentrations determined during this set of experiments are given in Table S3.



**Figure 3:** Percentage of bacterial colony in sterile distilled water at initial time (t=0) and after 15 min (blank): first (1) and second (2) test

Samples that received UV treatment in LaVie system had no *E. coli* present following the treatment whatever the strain (ATCC 15 597 and ATCC 11 303) and the initial concentration (Table 4S). Thus, after 15 minutes of UV exposure in the system, no bacterial colony ATCC 15 597 was observed after 24 h of incubation at  $37^\circ\text{C}$  on PCA agar for the concentrations tested, i.e.,  $1.42 \cdot 10^8$  CFU/mL or  $8.04 \cdot 10^8$  CFU/mL inside the device. This corresponds to a reduction in *E. coli* in the range  $8.16\text{-log}_{10}$  to  $8.91\text{-log}_{10}$ . For ATCC 11 303, the reduction is in the range  $8.38\text{-log}_{10}$  to  $8.54\text{-log}_{10}$ .

Moreover, 24 hours after irradiation, no bacterial colony was observed after spreading on Petri dishes and incubation according to the protocol indicated in Figure 1, thus revealing the absence of DNA repair by the exposed bacterial cells (dark repair phenomenon). The results are also presented in Table 5S.



**Figure 4:** Measured *E. coli* concentration before and after 15 min exposure and 24h after exposure

#### 4- Conclusion

The efficiency of the UV LED treatment to eliminate coliforms has been confirmed on two different *Escherichia coli* strains with an average of 8.50- $\log_{10}$  reduction and without any re-growth process (AND repair). The removal of high concentrations of coliforms tested can be explained by the particularly long exposure time (15 minutes). It should also be noted that the coliform species used in these experiments, *Escherichia coli*, is a mobile bacterium (*Peritrich ciliature*) which favors the homogeneity of the UV exposure. Indeed, during the irradiation, no stirring of the microbial suspension is performed in the device. Thus, in the case of non-mobile microorganisms, a sedimentation is likely to take place, limiting the effects of the irradiation, the microorganisms being found at the bottom of the bottle after a certain time. This is the case for example of fecal streptococci or spores of sulfite-reducing anaerobes which are also indicators of fecal contamination (recent and old respectively).

Moreover, the efficiency of this UV LED treatment on organic compounds in the presence of hypochlorite revealed the significant elimination of glyphosate and bisphenol A and in a lesser extent atrazine one.

To give even more weight to these results, it would be useful to test i) coliform strains known to be more resistant to UV (DNA repair system) and readily available commercially; ii) strains of fecal streptococci that have different bacterial walls (Gram-positive microorganism) and are immobile.

Moreover, for organic compounds, even if some compounds seem to be removed in the LaVie system, additional experiments are needed considering irradiation time and source, effect of hypochlorite concentration (higher than 0.1-0.2 mg/L) and probably other organic or metallic pollutants.

## 5-References

Mukhopadhyay et al., Emerging organic contaminants in global community drinking water sources and supply: A review of occurrence, processes and remediation, *Journal of Environmental Chemical Engineering*, 10, Issue 3, 2022, 107560.

Valbonesi et al., Contaminants of emerging concern in drinking water: Quality assessment by combining chemical and biological analysis, *Science of The Total Environment*, Volume 758, 1 March 2021, 143624

Owagboriaye et al., Outcome of the first survey of atrazine in drinking water from Ijebu-North, South-West, Nigeria: Human health risk and neurotoxicological implications, *Toxicology Reports*, 9, 2022, 1347-1356.

Khademi et al., Direct detection of glyphosate in drinking water using corona-discharge ion mobility spectrometry: A theoretical and experimental study, *International Journal of Mass Spectrometry*, 442, 2019, 29-34

Yamamoto T. and Yasuhara A., Chlorination of bisphenol A in aqueous media: formation of chlorinated bisphenol A congeners and degradation, *Chemosphere*, 46, 2002, 1215-1223

## 6-Appendix

**Table 1S:** Organic pollutants and free chlorine concentration in LaVie system

Compound	Sample	Free chlorine (mg/L)	Concentration (µg/L)
Bisphenol A	T0	0.12	< 0,02
	T15		
	T0	0.14	
	T15		
	T0	0.16	
	T15		
Glyphosate	T0	0.06	0.92
	T15		0.53
	T0	0.08	0.48
	T15		0.024
	T0	0.16	0.62
	T15		0.04

**Table 2S:** EC ATCC 15 597 and ATCC 11 303 concentrations in SM and spiked sterile distilled water

EC ATCC 15 597 (1) concentration					MS (CFU/mL)	LaVie system (CFU/L)
Dilution	10 <sup>-7</sup>	10 <sup>-6</sup>	10 <sup>-5</sup>	10 <sup>-4</sup>	71×10 <sup>6</sup>	1.42×10 <sup>8</sup>
CFU/0.1 mL	0	6	83	> 300		
EC ATCC 15 597 (2) concentration					MS (CFU/mL)	LaVie system (CFU/L)
Dilution	10 <sup>-7</sup>	10 <sup>-6</sup>	10 <sup>-5</sup>	10 <sup>-4</sup>	268×10 <sup>6</sup>	8.04×10 <sup>8</sup>
CFU/0.1 mL	5	35	186	> 300		
EC ATCC 11 303 (1) concentration					MS (CFU/mL)	LaVie system (CFU/L)
Dilution	10 <sup>-7</sup>	10 <sup>-6</sup>	10 <sup>-5</sup>	10 <sup>-4</sup>	120.5×10 <sup>6</sup>	2.40×10 <sup>8</sup>
CFU/0.1 mL	2	12	121	> 300		
EC ATCC 11 303 (2) concentration					MS (CFU/mL)	LaVie system (CFU/L)
Dilution	10 <sup>-7</sup>	10 <sup>-6</sup>	10 <sup>-5</sup>	10 <sup>-4</sup>	128.5×10 <sup>6</sup>	3.85×10 <sup>8</sup>
CFU/0.1 mL	1	16	97	> 300		

**Table 3S:** EC ATCC 15 597 and ATCC 11 303 concentrations in spiked sterile distilled water at initial time (t = 0) and after 15 min (blank).

EC strain	EC concentration (10 <sup>8</sup> CFU/mL)			
	t = 0	t = 15 min	t = 0	t = 15 min
ATCC 15 597	1.42	1.54	8.04	8.20
ATCC 11 303	2.40	2.10	3.85	3.90

**Table 4S:** EC ATCC 15 597 and ATCC 11 303 concentrations in spiked sterile distilled water after 15 min of exposure in the absence of free chlorine (left) and after 24h in the dark at room temperature (right).

ATCC 15 597 (1) after 15 min exposure				ATCC 15 597 (1) 24h after 15 min exposure			
Dilution	SM	10 <sup>-1</sup>	10 <sup>-2</sup>	Dilution	SM	10 <sup>-1</sup>	10 <sup>-2</sup>
CFU/0.1 mL	0	0	0	CFU/0.1 mL	0	0	0
ATCC 15 597 (2) after 15 min exposure				ATCC 15 597 (2) 24h after 15 min exposure			
Dilution	SM	10 <sup>-1</sup>	10 <sup>-2</sup>	Dilution	SM	10 <sup>-1</sup>	10 <sup>-2</sup>
CFU/0.1 mL	0	0	0	CFU/0.1 mL	0	0	0
ATCC 11 330 (1) after 15 min exposure				ATCC 11 330 (1) 24h after 15 min exposure			
Dilution	SM	10 <sup>-1</sup>	10 <sup>-2</sup>	Dilution	SM	10 <sup>-1</sup>	10 <sup>-2</sup>
CFU/0.1 mL	0	0	0	CFU/0.1 mL	0	0	0
ATCC 11 330 (2) after 15 min exposure				ATCC 11 330 (2) 24h after 15 min exposure			
Dilution	SM	10 <sup>-1</sup>	10 <sup>-2</sup>	Dilution	SM	10 <sup>-1</sup>	10 <sup>-2</sup>
CFU/0.1 mL	0	0	0	CFU/0.1 mL	0	0	0