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Deliverable Report

D1.4 CECs in product water of v1 prototype

Work package no.: WP1

Lead beneficiary: UNIFE

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1 Introduction to the project SERPIC

The project *Sustainable Electrochemical Reduction of contaminants of emerging concern and Pathogens in WWTP effluent for Irrigation of Crops – SERPIC* will develop an integral technology, based on a multi-barrier approach, to treat the effluents of wastewater treatment plants (WWTPs) to maximise the reduction of contaminants of emerging concern (CECs). The eight partners of the SERPIC consortium are funded by the European Commission and by six national funding agencies from Norway, Germany, Italy, Spain, Portugal and South Africa. The official starting date of the SERPIC project is 1. September 2021. The project has a duration of 36 months and will end next 31 August 2024.

The overall aim of the SERPIC project is to investigate and minimise the spread of CECs and antimicrobial resistant bacteria/antibiotic resistance genes (ARB/ARG) within the water cycle from households and industries to WWTPs effluents, and afterwards via irrigation into the food chain, into soil and groundwater and into river basins, estuaries, coastal areas, and oceans with a focus on additional water sources for food production.

A membrane nanofiltration (NF) technology will be applied to reduce CECs in its permeate stream by at least 90 % while retaining the nutrients. A residual disinfection using chlorine dioxide produced electrochemically will be added to the stream used for crops irrigation (Route A). The CECs in the polluted concentrate (retentate) stream will be reduced by at least 80 % by light driven electro-chemical oxidation. When discharged into the aquatic system (route B), it will contribute to the quality improvement of the surface water body.

A prototype treatment plant will be set-up and evaluated for irrigation in long-term tests with the help of agricultural test pots. A review investigation of CECs spread will be performed at four regional showcases in Europe and Africa. It will include a detailed assessment of the individual situation and surrounding conditions. Transfer concepts will be developed to transfer the results of the treatment technology to other regions, especially in low- and middle-income countries.

2 Report summary

The report contains the main results of the investigation at the first version of the prototype related to the water quality in the two treatment trains, Route A and Route B. The experimental campaign was carried out at the Universidad de Castilla-La Mancha UCLM laboratories as the prototype was assembled and put in exercise there. It was fed with the secondary effluent of the WWTP of Ciudad Real, withdrawn at the beginning of the campaign and stored in an outdoor 10-m^3 tank near the prototype plant. This deliverable reports the results of the analysis of the inlet and the stream after the different treatment steps regarding the conventional chemical and physical parameters regularly monitored as well as the indicator CECs, selected according to the methodology described in deliverable D1.1.

3 Deliverable description as stated in the Project Description

The deliverable reports the main findings and risks connected to the production of the effluent of the 1st version of the prototype adequate for direct reuse for irrigation needs (Route A) and for the release into a surface water body (Route B) by means of SERPIC solution.

4 Experimental setup

The experimental campaign was carried out from August until November 2023 at the v1 prototype plant, build-up from the separate developed components (Deliverable D1.3) and located in the laboratory of the UCLM. Water samples were taken from different sampling points of the v1 prototype plant, see Figure 1, corresponding to the feeding (secondary effluent) and the effluents of the different treatment steps (nanofiltration permeate and nanofiltration concentrate, ozonated effluent, photoreactor effluent). The secondary effluent was collected from the municipal WWTP of Ciudad Real (Spain) on 31st August 2023, and stored in a 10 m^3 - tank near the prototype plant and continuously fed to the prototype for the whole campaign.

Each sample was collected in sterile 1L plastic bottles and subsequently stored at -20 °C until handling and analysis. Organic compounds (diclofenac, iopromide, sulfamethoxazole, and venlafaxine) were extracted from water samples using solid phase extraction (SPE) with polymer sorbent cartridges. Analyses were performed using Liquid chromatography time-of-flight mass spectrometry (LC-MS/TOF). *Escherichia coli* bacterial counts were determined with an indirect impedance method using a Microtrac® 4200 system (SY-LAB). Gene copy numbers of the *sul*1 (ARG) DNA, extracted from the water samples by using the Urine DNA Isolation Kit (Norgen Biotek), was determined by amplification by quantitative real-time Polymerase Chain Reaction (qPCR). Further details are reported in the Deliverable D1.2 *Analytical procedures*.

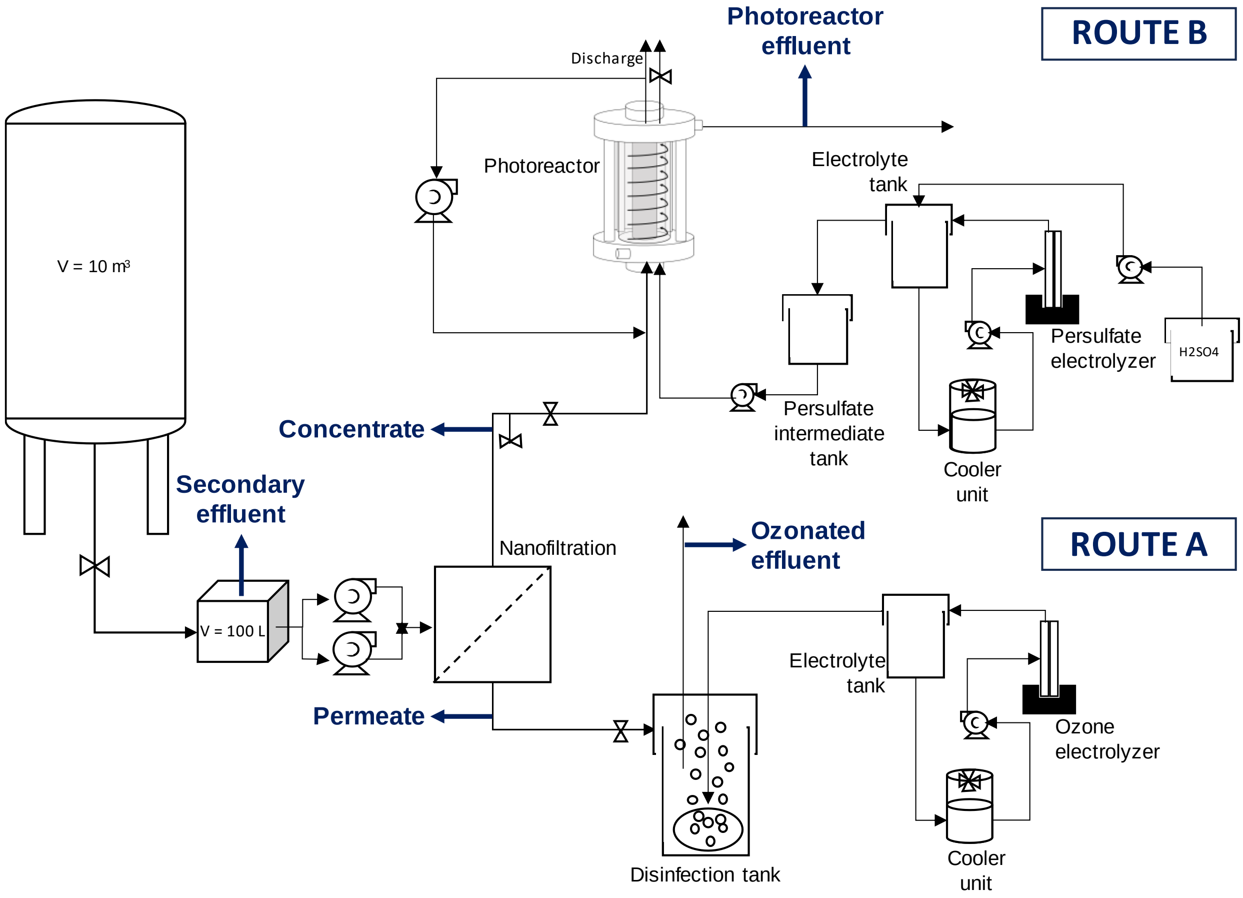


Figure 1: Sampling points in the v1 prototype plant.

Table 1 reports the physico-chemical parameters, the microbial and organic CECs analysed for each sampling point and the number of samples processed for this investigation.

In the case of *E. coli*, the number of samples are higher than for other parameters because it was necessary to carry out additional tests with the different modules of nanofiltration membranes to identify the reasons behind the presence of *E. coli* in the nanofiltration permeate (see below).

Table 1: Number of samples for the different parameters analysed in each sampling point in the v1 prototype plant.

Parameters		**Secondary effluent**	**Permeate**	**Concentrate**	**Ozonated effluent**	**Photoreactor effluent**
Physico-chemical parameters	pH, conductivity, chloride, bromide, nitrate, phosphate, sulphate, sodium, ammonium, potassium, calcium, magnesium	12	12	12	12	9
	TOC	8	8	8	6	9
Organic CEC	Diclofenac (DIC), iopromide (IOP), sulfamethoxazole (SMX), venlafaxine (VNLX)	9	5	5	1	3
Microbial CEC	*E. coli*	16	15	16	9	-
	Total coliforms	2	2	2	2	9
	*Sul*1	10	7	7	4	3

As reported in **Table 2**, the pH of the secondary effluent increased from 7.54 (August 2023) to 8-9 (end of November 2023), probably because of the formation of algae in the tank where the secondary effluent was stored. These conditions could explain the fact that *E. coli* was not present in the secondary effluent at the end of November during the last days of investigation (Table 2 shows minimum *E.coli* concentration equal to 0). For this reason, in order to evaluate the disinfection capacity of the technology, it was decided to perform analysis for total coliforms (2 samples were collected and analysed on November 28th and 29th 2023). They were analysed using the same analytical methods of *E. coli* with a different substrate (Coliform Chromogenic Agar, CCA).

This also explains why total coliform result at a lower concentration than *E. coli*.

Table 2: Physical-chemical parameters, microbial and organic CECs concentrations in the secondary effluent, collected from the municipal WWTP of Ciudad Real (Spain), and fed the prototype plant. The experimental campaign was carried out from August until November 2023. The last column reports the limit of detection (LOD) for some parameters (useful in the following).

Parameters	**min**	**max**	**mean**	**LOD**
pH	7.54	9.09	8.40	
Conductivity, µS/ cm	1309	1571	1370	
TOC, mg C/L	7.6	8.2	7.9	
Chloride, mg/L	151	162	155	
Bromide, mg/L	<0.5	<0.5	<0.5	0.5
Nitrate, mg/L	9.24	27.2	18.6	
Phosphate, mg/L	< 0.4	2.33	1.40	0.4
Sulfate, mg/L	155	284	184	
Sodium, mg/L	97.4	123	103	
Ammonium, mg/L	<0.1	0.43	0.28	0.1
Potassium, mg/L	27	36	29	
Calcium, mg/L	72	129	106	
Magnesium, mg/L	46	59	52	
VNLX, µg/L	0.64	0.83	0.73	0.08
DIC, µg/L	0.91	1.25	1.02	0.09
SMX, µg/L	0.29	0.44	0.34	0.04
IOP, µg/L	1.54	2.35	1.96	0.28
E.coli, CFU/ 100 mL	<1	2.8×10^3	3.1×10^2	1
*sul*1, nº Copies/ ml	24	260	85	1
Total coliforms, CFU/ 100 ml	158	178	168	1

The membrane photoreactor was tested at the end of November with different concentrations of electrogenerated persulphate (0.44 mM, 0.99 mM and 1.60 mM). The photo-oxidation tests were carried with a feed flow rate (QF) of 21 L/h and a recirculation flow rate (QR) of 91 L/h, corresponding to a residence time of 2.7 minutes. Three samples were then taken under steady-state conditions.

5 Results

5.1 Route A

In Route A, the nanofiltration unit reduces the concentration of TOC and organic CECs, with an average removal efficiency higher than 80 % as shown in Table 3. Regarding microbial contamination, *E. coli* and *sul*1 gene were analysed for most of the sampling days and their average removal was 25 % and 38 %, respectively. During two days no removal of *E. coli* was observed which led to additional investigations as described below. The achieved removal of total coliforms (sampling days 28 and 29 November 2023) was on average 91 %.

Table 3: Removal efficiencies achieved in nanofiltration unit (Route A) for TOC, organic and microbial CECs.

Sampling day	**TOC** η**, %**	**VNLX,** η**, %**	**DIC,** η**, %**	**SMX,** η**, %**	**IOP,** η**, %**	***E.coli*** η**, %**	***sul*1** η**, %**	**Total coliforms** η**, %**
31/08/23	80.3	82.1	90.8	83.3	84.8	No removal	69.6	
05/09/23	80.4	79.5	91.7	79.5	> 99	42.1	22.0	
07/09/23	81.5	81.0	90.3	87.0	84.5	No removal	52.6	
08/09/23						36.2	35.7	
12/09/23						98.61	10.6	
14/09/23	82.5							
16/10/23	81.6							
09/11/23	82.6							
28/11/23	80.1	82.5	90.6	87.0	84.5		3.31	88.8
29/11/23	82.1	79.4	89.7	87.0	84.6		70.9	92.4
min	80.1	79.4	89.7	79.5	84.5	No removal	3.3	88.8
max	82.6	82.5	91.7	87.0	> 99	98.61	70.9	92.4
average	81.4	80.9	90.6	84.8	87.7	24.53*	37.8	90.6

* The average value for the removal of *E. coli* also takes into account the days in which there was a release in the permeate.

Due to the unexpected low removal of *E. coli,* as well as no removal cases, additional tests were performed with different membrane modules equipped in the prototype in order to inspect them. Results for *E. coli* concentrations are reported in Table 4. The influent was analysed at the beginning of the two tests and after 90 and 75 min for the two modules to compare the concentrations. The permeate and the concentrate were instead sampled and analysed more frequently (every 15 or 30 min) to evaluate if there was a decline in the removal performance. The inspected modules showed that the performance was not as expected (i.e., total removal of *E. coli* due to size exclusion was not achieved). The modules were thus sent to the membrane manufacturer for a more accurate inspection and clarifications. Tests carried out by him revealed that the membranes were defective and the lower removal is due to this fact.

Table 4: *E. coli* concentrations in the two additional tests performed on the different modules of nanofiltration membranes to identify the reasons of the presence of *E. coli* in the nanofiltration permeate.

***E. coli*, CFU/100 mL**				
Module, time		**Secondary effluent**	**Concentrate**	**Permeate**
Module 1	0 min	8.8×10^3		
	15 min		2.4×10^4	3.3×10^2
	30 min		3.4×10^4	2.5×10^2
	60 min		2.1×10^4	5.0×10^2
	90 min	7.7×10^3	1.9×10^4	5.5×10^2
Module 2	0 min	7.8×10^3		
	15 min		1.4×10^4	1.5×10^2
	30 min		1.4×10^4	2.2×10^2
	60 min		1.4×10^4	2.6×10^2
	75 min	6.9×10^3	1.5×10^4	1.7×10^2

The following step, a disinfection by using electrogenerated ozone, completely removed total coliforms, diclofenac, sulfamethoxazole and iopromide (average removal equal to 58.9 %, Table 5) and reduced the concentrations of venlafaxine (average removal equal to 44.9 %,

Table 5), *E. coli* and *sul*1 (average removal equal to 97.1 % for both, Table 5). As reported in Table 5, TOC was not substantially decreased after ozonation (average removal is 1.1 %), but its inlet concentration was always very low as it was highly removed in the previous nanofiltration unit: average removal of 80 % leading to an average concentration in the permeate of 1.6 mg/L).

Table 5: Removal efficiencies achieved in the disinfection step (ozonation, Route A) for TOC, organic and microbial CECs.

Sampling day	TOC η, %	VNLX η, %	DIC η, %	SMX η, %	IOP η, %	*E. coli* η, %	*sul*1 η, %	Total coliforms η, %
31/08/23	5.20					99.6		
05/09/23	1.30					95.0		
07/09/23	No removal					97.1		
08/09/23						96.5		
09/11/23	0.73							
28/11/23	0.40	20.2	51.1	52.4	58.2		94.3	95.0
29/11/23	No removal	69.7	52.1	53.5	59.7		100	91.67
min	No removal	20.2	51.1	52.4	58.2	95.0	94.3	
max	5.2	69.7	52.1	53.5	59.7	99.6	100	
average	1.9	44.9	51.6	52.9	58.9	97.1	97.1	93.3

The analysis of the performance of the treatments is completed with Table 6 which reports the concentrations of total suspended solids (TSS), BOD_5 and turbidity in the Route A effluent. These are further parameters for which the European Regulation 2020/741 defining minimum requirement for water reuse set quality standards (last row of Table 6).

Table 6: Concentrations of the parameters for whom the European Regulation defined quality standards.

Sampling day	TSS mg/L	Turbidity NTU	BOD_5 mg/L	*E. coli* CFU/100 mL
31/08/23	<1	0.30	<5	13
05/09/23	<1	0.28	<5	11
07/09/23		0.25		10
08/09/23		0.32		1
12/09/2023		0.20		< 1
14/09/2023		0.31		
26/09/2023		0.33		
16/10/2023		0.24		
09/11/2023		0.19		< 1
11/11/2023		0.20		< 1
28/11/23	<1	0.27	<5	< 1
29/11/23	<1	0.26		< 1
EU standard	≤ 10	≤ 5	≤ 10	≤ 10

5.2 Route B

In Route B, nanofiltration concentrate is fed to the membrane photoreactor. Due to the absence of *E. coli* in the secondary effluent during the sampling campaign on the membrane photoreactor, total coliforms were investigated. The results are summarized in Table 7. The photoreactor achieved the complete removal for diclofenac, sulfamethoxazole, iopromide and total coliforms regardless the applied persulfate dose. On the other side, the removal of TOC, *sul*1 and venlafaxine is influenced by the dose of persulfate applied.

Table 7: Removal efficiencies achieved in membrane photoreactor (Route B) for TOC, organic and microbial CECs at different electrogenerated persulfate concentrations (0.44 mM, 0.99 mM and 1.60 mM).

Day	Persulphate, mM	Sample no.	TOC η, %	VNLX, η, %	DIC, η, %	SMX, η, %	IOP, η, %	*sul*1 η, %	Total coliforms η, %
29/11/23	0.44	1	29.1						99.60
29/11/23	0.44	2	26.8	56.0	97.3	96.3	96.4	68.8	99.60
29/11/23	0.44	3	27.6						99.60
28/11/23	0.99	1	38.4						99.60
28/11/23	0.99	2	35.7	64.3	97.4	96.1	96.3	99.5	99.60
28/11/23	0.99	3	39.7						99.60
28/11/23	1.6	1	42.8						99.60
28/11/23	1.6	2	42.0	70.3	97.4	96.1	96.3	99.5	99.60
28/11/23	1.6	3	41.0						99.60

5.3 Conclusion

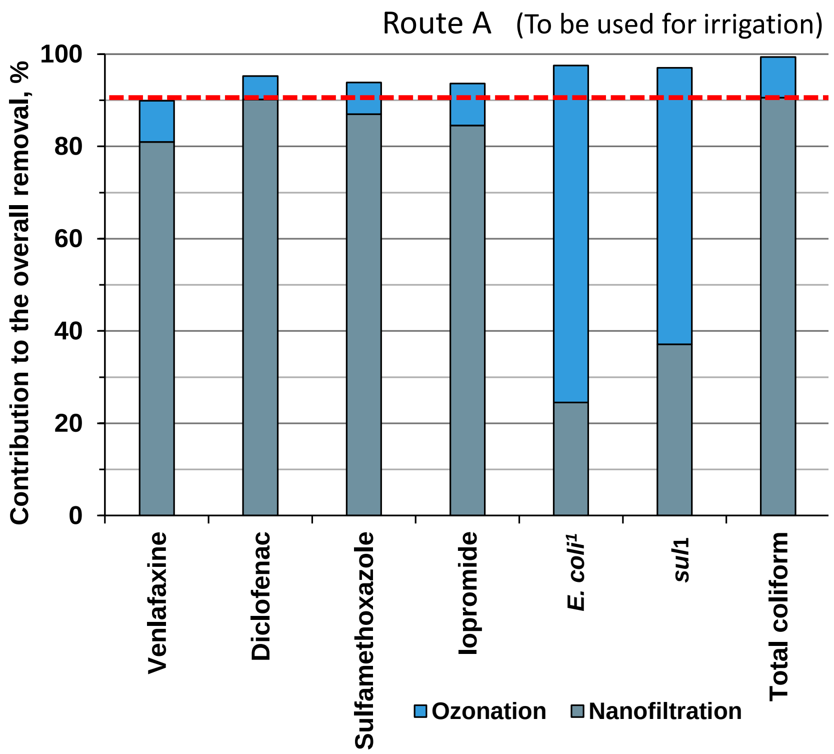
The goals of SERPIC project are the removal of the target CECs by 90 % in Route A, complying with targets set by the EU regulation on minimum requirements for water reuse for agricultural irrigation. In Route B, the removal targets are:

- ARB, ARGs, analgesic drugs: > 99 %
- pharmaceuticals: > 90 %
- antiretrovirals: > 80 %
- anticonvulsants: > 80 %
- preservatives in personal care products: > 99 %
- illicit drugs: > 90 %
- industrial micropollutants: > 80 %

Considering the overall removal shown in Figures 2 and 3, the goals are achieved for most of the analysed compounds. In Route A, average removal for all organic and microbial CECs are higher than 90%. In Route B: diclofenac (pharmaceutical), sulfamethoxazole (pharmaceutical), iopromide (industrial micropollutants) and total coliform have an average removal equal to 100 %. The removal of venlafaxine in Route B is less than the limit of 90 % set for pharmaceuticals for all persulphate dose applied (from 56.0 % for 0.44 mM to 70.3 % for 1.6 mM). In addition, *sul*1 achieved the threshold of 99% for ARGs using persulphate concentrations of 0.99 mM and 1.6 mM.

Furthermore, the ozone disinfection provided complete removal of microbiological contamination demonstrating the added value of additional disinfection step in the treatment train, especially when unrestricted reuse of reclaimed water for crops irrigation is considered.

Figures 2 and 3 report the same goals of 90 % in Route A and > 99 % in Route B for total coliform, even if they are not ARB. These target values were kept as specific targets were not established for indicator microorganisms not included among the ARB.



[1]*E. coli* < 10 MPN/100 mL

Figure 2: Overall removal achieved in Route A. The contribution to the overall removal of nanofiltration and ozonation steps is reported. The red dotted lines are the removal goals set in the SERPIC project.

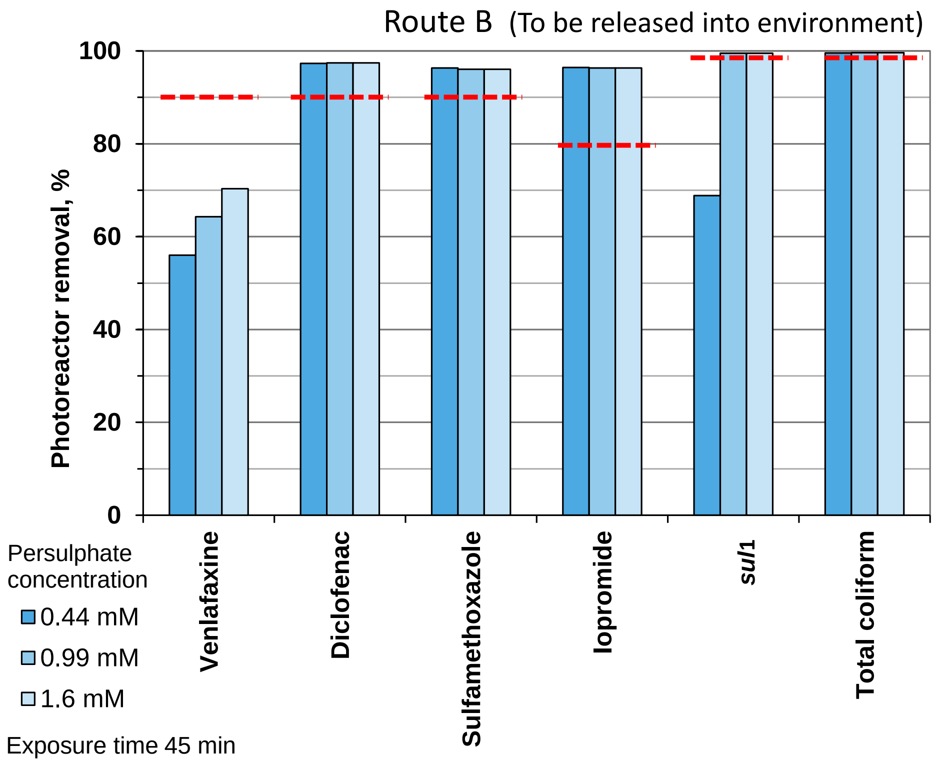


Figure 3: Overall removal achieved in Route B. The removal is a function of the persulphate concentration (dose) and the treatment lasted 45 min. The red dotted lines are the removal goals set in the SERPIC project.

According to the results obtained, several additional tests will be made within the re-engineering activities, trying to clarify several aspects, mainly the effect of the treatment on the disinfection.

6 Publications and other dissemination activities

A leaflet for the stakeholders was prepared with average removal achieved for the selected CECs.

A Factsheet of the project is under preparation in collaboration with DECHEMA and will be available online.

An online workshop will take place 28th of August 2024 from 14:00-15:00 CET within the event World Water Week which will be held 25.-29.8.2024 in Stockholm.

A scientific paper is under preparation with the results of the investigation.