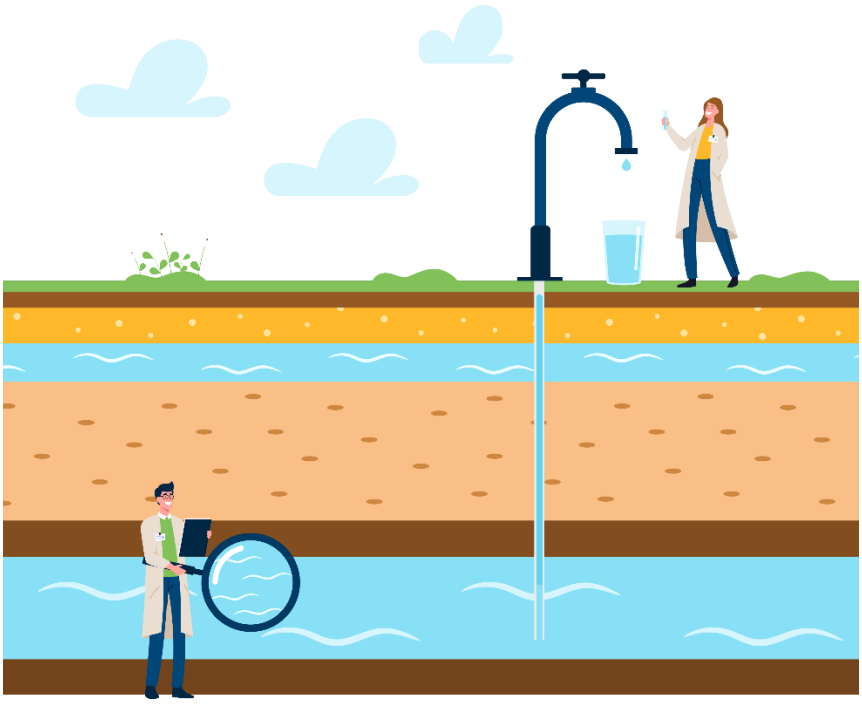
MAR2PROTECT

Report on the preliminary assessment of WP2 technology performances for upscale to TRL 5

University of Bologna, Italy



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PARTNERS AND ASSOCIATED PARTNERS ACRONYMS

ACRONYM	PARTNER
ADTA	ÁGUAS DO TEJO ATLÂNTICO
AQUATEC	AFFILIATED ENTITY OF CETAND. WATER PRIVATE COMPANY (VEOLIA GROUP), SPAIN
CETAND	CENTRO ANDALUZ DE INVESTIGACIONES DEL AGUA
CIIMAR	CENTRO INTERDISCIPLINAR DE INVESTIGAÇÃO MARINHA E AMBIENTAL, PORTUGAL
CT	CITY OF CAPE TOWN
DU	DUNEA DUIN & WATER
FEUGA	FUNDACIÓN EMPRESA UNIVERSIDAD GALLEGA, SPAIN
FHNW	UNIVERSITY OF APPLIED SCIENCES AND ARTS NORTHWESTERN SWITZERLAND
HERA	HOLDING ENERGIA RISORSE AMBIENTE
IHE	IHE DELFT INSTITUTE FOR WATER EDUCATION, THE NETHERLANDS
IT	INSTITUTO DE TELECOMUNICAÇOES, PORTUGAL
ISSBAT	INST. SUPÉR. DES SCIENCES BIOLOGIQUES APPLIQUÉES DE TUNIS
KTU	KAUNAS UNIVERSITY OF TECHNOLOGY, LITHUANIA
NOVA	NOVA ID FCT – ASSOCIAÇÃO PARA A INOVAÇÃO E DESENVOLVIMENTO DA FACULDADE DE CIÊNCIAS E TECNOLOGIA DA UNIVERSIDADE NOVA DE LISBOA, PORTUGAL
SU	STELLENBOSCH UNIVERSITY, SOUTH AFRICA
UNIBO	UNIVERSITY OF BOLOGNA, ITALY

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Abbreviations / Acronyms:

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Executive Summary

The following document is Deliverable 2.4 "*Report of the preliminary assessment of WP2 technology performances for upscale to TRL5*" of the MAR2PROTECT project, funded by the European Union's Horizon Europe research and innovation programme under grant agreement Number 101082048. This document illustrates the results achieved by the WP2 partners during months 1-18 of the MAR2PROTECT project, relatively to the development of technologies for i) the treatment of different water types before their use for managed aquifer recharge, and ii) the FERT-ROOT technology for the reduction of fertilizer leaching into groundwater. Some of the water treatment technologies are based on the use of innovative materials, whose preparation and characterization is reported in Deliverables 2.1 "*Functionalized biomaterials*" and 2.2 "*Report of functionalized biomaterials*". This Deliverable represents the public version of the confidential Deliverable 2.3 "*Preliminary assessment of WP2 technology performances for upscale to TRL 5*".

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Lead Beneficiary	**Deliverable Author (S)**
University of Bologna	Dario Frascari, Elisa Girometti, Giulio Zanaroli, Elena Biagi
Beneficiaries	**Deliverable Co-Author (S)**
NOVA	Ana B. Pereiro, João M. M. Araújo and Srdana Kolakovic
IHE Delft	Sergio Salinas, Vanida Salgado, Vignesh Malaichami, Lynn Tahtouh, Constanza Rosa, Maria Kennedy
DUNEA	Brent Pieterse, Joost Priem, Franca Kramer
FHNW	Ana Dujmovic, Philippe Corvini
CIIMAR	Marisa Almeida
ISSBAT	Atef Jaouani, Hajer Annouri
SU	Christoff Truter, Gideon Wolfaardt, Marielize Botes
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	SEN	Sensitive, limited under the conditions of the Grant Agreement	

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1. INTRODUCTION

Deliverable 2.4 "Report of the preliminary assessment of WP2 technology performances for upscale to TRL 5" of the MAR2PROTECT project illustrates the results achieved by the WP2 partners during months 1-18 of the MAR2PROTECT project, relative to the development of technologies for i) the treatment of different water types before their use for managed aquifer recharge, and ii) the FERT-ROOT technology for the reduction of fertilizer leaching into groundwater. Some of the water treatment technologies are based on the use of innovative materials, whose preparation and characterization are reported in Deliverable 2.2 "Report of *functionalized biomaterials*".

Deliverable 2.4 is articulated into 3 sections, each corresponding to a Task of WP2:

- Removal and biodegradation of micropollutants from **treated wastewater**, corresponding to Task 2.2. This section includes contributions from NOVA, ADTA, UNIBUO, HERA, ISSBAT AND FHNW, relative to a range of technologies: adsorption, including the use of innovative functionalized materials; biodegradation, including the development of innovative reactor types, such as 3D-printed biofilms and granular sludge reactors; phytoremediation by means of constructed wetlands; membrane processes, such as nanofiltration and reverse osmosis.

- Removal and biodegradation of micropollutants from **surface waters**, corresponding to Task 2.3. This section includes contributions from CIIMAR, IHE and DUNEA, relative to a range of technologies: membrane processes, such as ultrafiltration and reverse osmosis; adsorption on activated carbon; in-line coagulation; sand filtration; phytoremediation of coastal saltmarshes through a re-vegetation approach.

- **Reduction of fertilizer leaching into groundwater from agriculture**, corresponding to Task 2.4. This section includes only the innovative FERT-ROOT technology, developed by SU.

For each section and each technology, after a presentation of the experimental methodologies, the main results are presented, followed by a short conclusion relative in particular to a preliminary assessment of the possibility to scale-up each technology in the MAR2PROTECT demo sites. The final assessment of the technologies to be scaled-up in the demo sites will be made in the framework of Del. 5.1 (M24). However, some technologies have already been scaled up during the first reporting period. In particular, the IHE-Dunea technology combination for the treatment of surface water and the SU FERTO-ROOT technology have already been scaled-up in Demo Sites 1 and 5, respectively. Conversely, for other technologies the results obtained during months 1-18 already indicate that the TRL is still too low for a scale-up in the Demo Sites.

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2. REMOVAL AND BIODEGRADATION OF POLLUTANTS FROM TREATED WASTEWATER BEFORE ITS USE FOR MANAGED AQUIFER RECHARGE (TASK 2.2)

2.1 BIODEGARDATION AND REMOVAL PROCESSES WITH INNOVATIVE BIOMATERIALS (NOVA, AdTA)

2.1.1 INTRODUCTION

In the scope of Task 2.2, NOVA is developing the following technologies for wastewater (WW) treatment before its use for MAR:

- TEC 1.1 - Polishing step for the removal of residual contaminants and by-products by adsorption
- TEC 1.2 - Adsorption system to recover greenhouse gases (GHG) released by TEC 3.1
- TEC 2 - Micropollutant concentration using Aqueous biphasic systems (ABS)
- TEC 3.1 - Micropollutants biodegradation by Aerobic granular sludge (AGS)

The flowsheet of the combined process proposed by NOVA is shown in Figure 2.1.1.1.

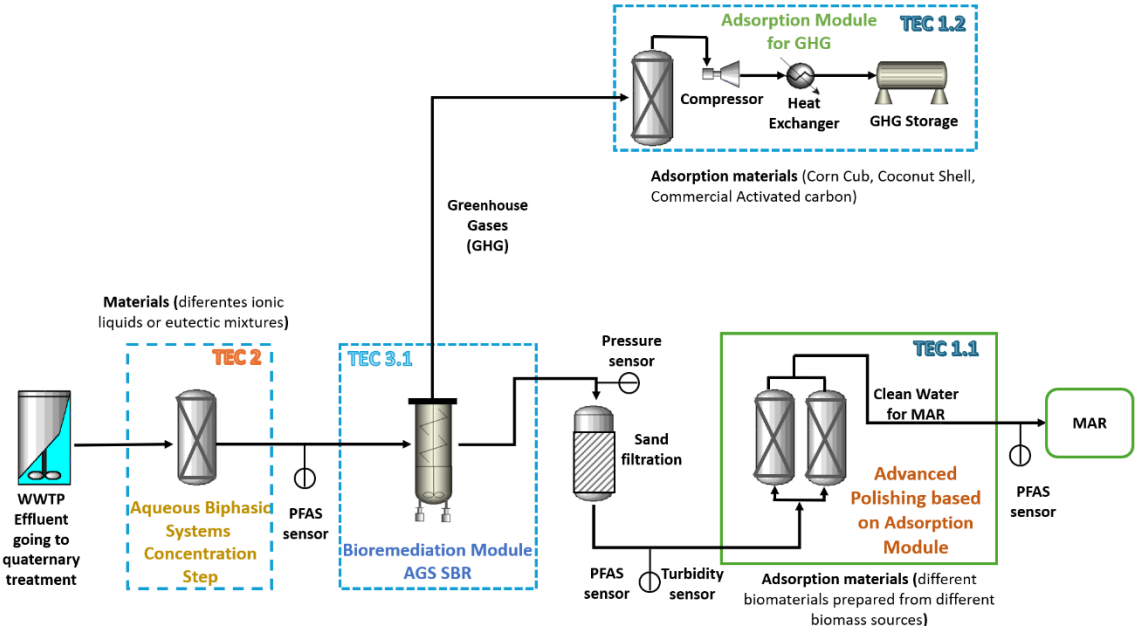

Figure 2.1.1.1 Flowsheet of the technologies developed by NOVA.

The above-mentioned technologies were selected for a potential implementation in Demo Site 3, located in Frielas (Portugal). Based on the initial characterization of the effluent and high persistence of these compounds in the environment, NOVA and AdTA have been focused on the removal and biodegradation of pharmaceuticals and perfluoroalkylated and polyfluoroalkylated substances (PFAS), in particular, diclofenac (DCF), iopromide (IOP), valsartan (VAL), perfluorooctanoic acid (PFOA), perfluorobutane sulfonic acid (PFBS) and perfluoropentanoic acid (PFPeA).

The NOVA activities conducted during the first reporting period (months 1-18) focused on developing innovative (bio)materials, described in Deliverable 2.1, which were

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applied for treatment processes to assess the removal of target pollutants at Demo Site 3. In the scope of Task 2.2, NOVA has conducted the following activities:

- TEC 1.1: a screening was conducted to compare the removal rates of 16 innovative biomaterials synthesised from different biomass sources (Task 2.1) and commercial Norit for the removal of target pollutants (DCF, IOP, VAL, PFOA, PFBS and PFPeA). Finally, the best one was selected to perform kinetic sorption tests, adsorption isotherms and breakthrough assays. The results obtained demonstrate the applicability of this technology for the pilot scale. The optimisation of the process using real samples of wastewater effluent will be performed in the next period.
- TEC 1.2: adsorption of GHGs was tested using 5 biomaterials for the 5 gases with the highest global warming potential (difluoromethane - R-32, pentafluoroethane - R-125, 1,1,1,2-tetrafluoroethane - R-134a, sulphur hexafluoride - SF6 and nitrogen - N2). These GHGs were selected to study the affinity of the innovative biomaterials for fluorinated gases using nitrogen as a reference inert gas. The adsorption of the gases produced in the biodegradation process will be further studied in case the biodegradation process gets scaled up to the pilot plant.
- TEC 3.1: diclofenac biodegradation continuous tests were conducted using AGS and a synthetic mixture containing diclofenac to elucidate the degradation mechanisms and optimise the process. Further tests with a synthetic mixture of three pharmaceuticals will be performed to decide if the technology will be suitable for scaling up to the pilot plant.

As for TEC 2 (Aqueous Biphasic Systems: ABS), the biomaterials will be used as a micropollutant concentration step prior to the biodegradation process. Then, the concentration of these trace pharmaceuticals in the wastewater effluent to be treated will be increased. This preconcentration step can help easier continuous quantification as well as enhance their subsequent biodegradation. NOVA possesses an extensive ABS database (Freire et al. 2012, Shahriari et al. 2013, Carvalho et al., 2024), which will be employed during the second reporting period if the biodegradation process advances to the pilot plant scale. In these aqueous two-phase systems, the biomaterials (ionic liquids or deep eutectic solvents) will be used to produce two immiscible aqueous phases where the micropollutants will be concentrated in one of the phases. This process will be a simple way to concentrate pharmaceuticals using an environmentally friendly and low-cost process.

2.1.2 MICROPOLLUTANT REMOVAL USING ADSORPTION (TEC 1)

22 Ionic Liquids (ILs) from different families (including the 6 fluorinated ILs prepared in Task 2.1) were selected to evaluate their performance in removing PFOA from aqueous solutions. The experimental results showed that phosphonium-based ILs are the most suitable ILs for PFOA removal. The other ILs were not selected and showed three types of behaviour: 1) insoluble in aqueous solutions; 2) saturated signal for quantification; and 3) additive effect in the quantification method.

The ILs selected after the screening were tested subsequently at different incubation times and the results showed that phosphonium-based ILs with short alkyl chains achieved the highest adsorption capacity. This behaviour could be related to better stabilisation over time and more stable aggregation structures. Phosphonium-based ILs with short alkyl chains form more stable aggregates that favour the formation of compact structures.

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A screening was performed to compare the removal rates (%) of 16 biomaterials synthesised from different biomass sources (Task 2.1) and commercial Norit for the removal of DCF, IOP, VAL, PFOA, PFBS and PFPeA from the aqueous solutions, separately. The biomaterials synthesised by NOVA demonstrate a higher capacity for pharmaceuticals and PFAS removal from aqueous solutions than commercial Norit.

According to the experimental results obtained from the screening with the different biomaterials prepared in this project for the removal of PFAS and pharmaceuticals, four biomaterials, two from *Juncus maritimus* (JM), one from coconut shell and *Phragmites australis* (PA), were selected for subsequent adsorption tests on a synthetic matrix of the mixture with the three pharmaceuticals (DCF, VAL and IOP) and a mixture of the three PFAS (PFOA, PFBS PFPeA). The removal capacity of the biomaterials selected was verified.

The results showed that activated carbon based on coconut shell obtained the best performance for all three pharmaceuticals but with similar results to *Juncus maritimus*. The results for the PFAS mixture showed that activated carbon based on *Juncus maritimus* has the best performance for the adsorption of the three acids in a single matrix. Besides, this biomaterial has more affinity towards the long chain (PFOA) than the short chain. Then, *Juncus maritimus* activated carbon was selected for the next experiments.

Kinetic sorption assays were carried out for pharmaceutical and PFAS removal from aqueous solutions with *Juncus Maritimus* activated carbon. The results demonstrated a maximum adsorption capacity higher than 400 $mg \cdot g^{-1}$ for DCF, around 300 $mg \cdot g^{-1}$ for VAL, higher than 350 $mg \cdot g^{-1}$ for IOP, around 890 $mg \cdot g^{-1}$ for PFOA, higher than 700 $mg \cdot g^{-1}$ for PFPeA and around 150 $mg \cdot g^{-1}$ for PFBS. These values indicated the superior adsorption capacity of this biomaterial synthesised from biomass of Demo Site 7 (Lima River estuary): saltmarsh plant *Juncus maritimus (JM)*.

Adsorption isotherm assays were performed for pharmaceutical and PFAS removal from aqueous solutions with *Juncus maritimus* activated carbon. The comparison of the performance of *Juncus maritimus* activated carbon demonstrates that the adsorption capacity of PFAS with short-chain (PFBS and PFPeA) is lower than PFOA and the different pharmaceuticals studied in this demo site.

Real samples of wastewater effluent will be tested, where the influence of other parameters present in the real will be taken into account to evaluate the performance of *Juncus Maritimus* activated carbon. The optimisation of a quantification method using UHPLC MSMS was finalised to measure low concentrations and establish a satisfactory solid phase extraction (SPE) phase for both PFAS and pharmaceuticals.

Adsorption of greenhouse gases (GHGs) was performed with the 2 biomaterials based on coconut shell biomass and the 3 biomaterials based on corn cob biomass for the 5 gases studied in this work (difluoromethane - R-32, pentafluoroethane - R-125, 1,1,1,2-tetrafluoroethane - R-134a, sulphur hexafluoride - SF_6 and nitrogen - N_2). These GHGs were selected to study the affinity of these biomaterials for fluorinated gases with high global warming potential and using nitrogen as our reference inert gas. Afterwards, a comparison was made to evaluate the effectiveness of these biomaterials for GHG adsorption. If this technology is scaled up for the pilot plant, adsorption will be studied with the gases produced in the biodegradation process.

The results show that coconut shell-activated carbon performs better for the adsorption of fluorinated gases. However, corn cob-activated carbon is the most appropriate biomaterial because a high adsorption capacity is obtained at pressures

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close to atmospheric pressure for all gases (0.1 MPa), being the closest to real operating conditions.

2.1.3 MICROPOLLUTANT BIODEGRADATION (TEC 3.1)

A lab-scale sequencing batch reactor (SBR) based on aerobic granular sludge (AGS) technology was set up and operated for the biodegradation of micropollutants. The experimental tests started with synthetic wastewater containing diclofenac.

- *Biomass properties and inventory*

The SBR was operated to favour microorganisms with the ability to form aerobic granules with fast settling. The AGS settling properties gradually improved during the first 2 months of operation and were not affected by a subsequent storage period of 1 month. The main biomass fraction corresponded to granules with 1-0.6 mm.

Gradual diclofenac accumulation in the SBR and the prolonged exposure of AGS to diclofenac concentration values higher than 60 µg/L had a negative impact on the settleability and the structural integrity of the aerobic granules. A shift in the predominant size occurred towards the 0.6-0.2 mm fraction and an increase in bacterial flocs (<0.2 mm) was also observed.

Biomass inventory in the SBR was assessed by total suspended solids (TSS) and volatile suspended solids (VSS) analyses and the results presented a decrease after 5 months of SBR operation, possibly due to the accumulation of diclofenac and granule instability.

Removing diclofenac from the feed resulted in a recovery of the TSS and VSS values. Also, the bacterial floc content decreased and granules ranging from 0.6-0.2 mm became predominant, presenting high settleability.

Extraction and quantification of polyhydroxyalkanoate (PHA) and extracellular polymeric substances (EPS) were performed to assess biopolymer production by AGS. Intracellular PHA content was always detected in very low quantities ($<10^{-4}$ mg/L). On the other hand, biomass exposure to increasing diclofenac concentration values led to increased EPS production as a protection strategy.

- *AGS SBR performance*

The performance of AGS was assessed by the removal of carbon, nitrogen and phosphorus compounds present in the tested wastewater, and by specific diclofenac analysis.

Nitrogen was totally removed in most of the SBR cycles and complete biological phosphorus removal was also generally attained. Diclofenac biodegradation was observed under aerobic conditions, with removal levels up to 30%. However, the accumulation of diclofenac in the SBR and the continuous exposure of AGS to diclofenac concentration values above 30 µg L^{-1} led to removal levels below 10%. After the recovery of the AGS structural stability, diclofenac removal levels were resumed. Biodegradation process optimization based on AGS is currently being pursued and tests with mixtures of relevant micropollutants will be performed.

- *Microbial community characterization*

The abundance of different bacterial genera along the experimental run was analysed for selected samples by high throughput DNA sequencing. Throughout the SBR operation, three main phyla were observed, i.e., *Proteobacteria*, *Bacteroidota*, and *Actinobacteria*. The genera *Propionivibrio* and *Defluviicoccus*, which comprise glycogen-accumulating organisms (GAO), tended to increase in the presence of diclofenac. The genera *Zoogloea*, *Defluviicoccus*, and *Brevundimonas* were found in

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relatively high abundance when AGS was exposed to diclofenac and might be involved in its degradation since these genera have been shown to be involved in the degradation of refractory compounds in AGS systems. The genus *Zoogloea* is also related to the production of EPS. In the absence of diclofenac, the genus *midas_g_23329* tended to increase, but no information regarding its role in AGS has been found in the literature.

2.1.4 CONCLUSIONS AND INDICATIONS FOR SCALE-UP IN THE DEMO SITES

In this work, NOVA has focused on the removal technologies for two groups of emerging pollutants – pharmaceuticals and PFAS.

The adsorption technology using innovative biomaterials prepared by NOVA has presented promising results to remove these persistent pollutants when compared to commercial activated carbon Norit. On the basis of the results obtained during months 1-18, the PFAS and pharmaceuticals adsorption technology is considered suitable for scale-up in demo site 3. Further tests will be conducted in continuous mode with actual wastewater during months 19-24, in order to perform the design of the pilot plant.

Taking into consideration the high PFAS removal achieved in the adsorption tests, and the low biodegradability of these pollutants, as well as the more complicated setup and materials required for PFAS biodegradation tests, PFAS removal was assessed only in the adsorption process. Therefore, the biodegradation process focused only on pharmaceutical removal, testing diclofenac biodegradability using aerobic granular sludge. The results indicated a high persistence of diclofenac, so further tests on the other two target pharmaceuticals need to be completed in order to decide on the applicability of this process for the pilot scale.

NOVA has a large database (Freire et al., 2012; Shahriari et al., 2013; Carvalho et al., 2024) of Aqueous Biphasic Systems (TEC 2) that will be used if the biodegradation technology is selected for the pilot plant. From this ABS database, the selection of the most suitable system (where the ionic liquid or deep eutectic system contains the carbon source to feed the micro-organisms) will be made for the pre-concentration of the micropollutants before entering the bioreactor. Considering the high removal rates of the biomaterials prepared from the different biomass sources, the ABS were discarded for the polishing step.

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2.2 ADSORPTION AND BIODEGRADATION PROCESS (UNIBO, HERA)

2.2.1 INTRODUCTION

UNIBO, in collaboration with HERA, is developing two technologies for WW treatment before its use for MAR:

- Micropollutant adsorption on commercial materials or molecularly imprinted polymers (TEC 1)
- Biodegradation of the desorbed micropollutants in a Membrane Aerated Biofilm Reactor (MABR; TEC 3.2).

The flow-sheet of the combined adsorption/biodegradation process proposed by UNIBO is shown in Figure 2.2.1.1.

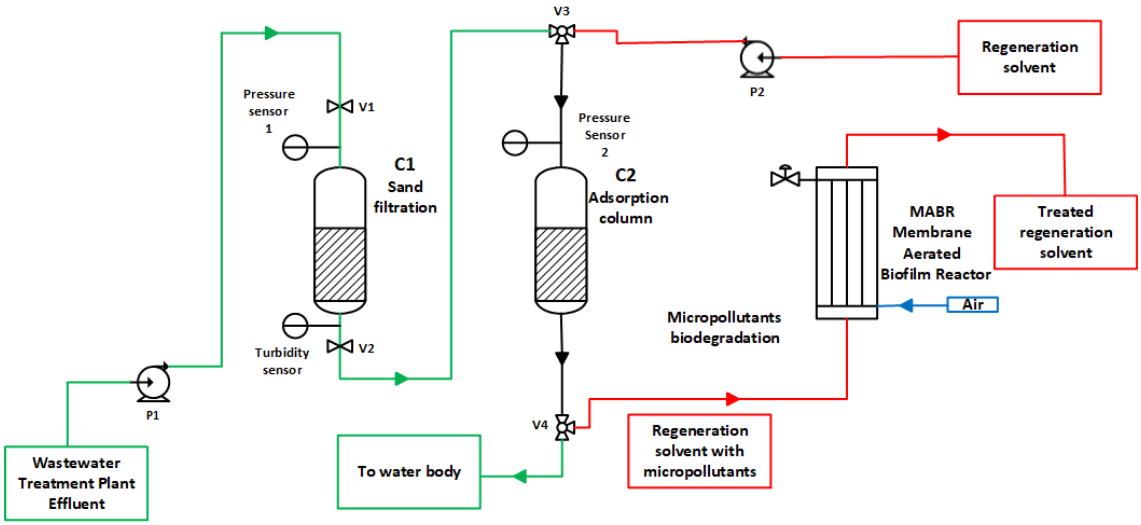

Figure 2.2.1.1 Flow sheet of the pharmaceutical adsorption/biodegradation process proposed by UNIBO.

These technologies are aimed at a potential implementation in Demo Site 4, located in Emilia Romagna (Italy). On the basis of the initial characterization of the effluents of several Wastewater Treatment Plants (WWTPs) located in Demo Site 4. UNIBO and HERA decided to focus on the removal and biodegradation of pharmaceuticals. In particular, the pharmaceuticals detected more frequently in the studied WWTPs, even though at very low concentrations, are ibuprofen, diclofenac, carbamazepine, atenolol and some antibiotics like clarythromicin. During months 1-18, the activities focused on carbamazepine, ibuprofen and diclofenac.

While pharmaceutical adsorption on activated carbons is reported in several studies (Benstoem et al., 2017) and it's the most effective and flexible adsorption solution technology (Gutiérrez et al., 2023), UNIBO focused on testing selective materials like Molecularly Imprinted Polymers (MIPs) in real wastewater treatment plant effluent trying to develop a fixed bed adsorption process with these innovative materials that have the theoretical advantage of being regenerated multiple times (Parlapiano et al., 2021). This aspect is very important as activated carbons have the disadvantage of an expensive and off-site regeneration; in addition, the potential use of MIPs as an alternative to activated carbons can lead to a cost-effective adsorption-desorption process. Moreover, very few studies in the literature tested the adsorption of pharmaceuticals on MIPs in real effluent at low concentrations and in continuous mode.

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As for pharmaceutical biodegradation, it is a promising strategy for the removal of pharmaceuticals. Most research works focused on screening and characterizing in laboratory single microbial strains able to degrade ibuprofen or diclofenac (Ferreira et al., 2023, Jan-Roblero et al., 2023) or in evaluating biodegradation activity in uncharacterized sludges from wastewater-treatment plant (Wu et al., 2023). UNIBO is exploring the biodegradation potential of complex microbial consortia enriched from selected environments, by characterizing the degrading microbial communities (as well as single members) from taxonomical and functional points of view, as well as exploring the communities' features to be exploited in bioprocesses, such as the resistance to different concentrations of solvents utilizable in eluting pharmaceuticals from adsorption columns.

The UNIBO activities conducted during months 1-18 focused in particular on the following aspects:

- Regarding the adsorption process (TEC 1), a wide range of materials were tested by means of batch adsorption tests conducted with single compound solutions of carbamazepine, ibuprofen and diclofenac: activated carbon Norit, XAD16N and MIPs produced with different monomers and cross-linkers; from this initial material screening phase, the best sorbents were selected for each pharmaceutical; this resulted in the selection of commercial materials Norit and XAD16N for all the target pharmaceuticals, and of two MIPs selective towards carbamazepine and diclofenac. As of M18, continuous flow tests with the selected materials are in progress to analyze and compare their performances in fixed bed columns and to find the best operating conditions.
- Regarding the biodegradation process, the UNIBO team focused on enriching microbial communities able to degrade ibuprofen and diclofenac. To date, a microbial mixed aerobic culture able to efficiently degrade ibuprofen (10 mg/L in 10 hours) is available for scale-up and first applications in Membrane Areated Biofilm Reactors, while being characterized under ecological and microbiological point of view (i.e. characterization of the microbial community and functional role of most abundant bacterial species) as well as for its features related to the exploitation in reactors (e.g. resistance to different type and concentration of solvents, ability to cross-degrade other pharmaceuticals). The application of the biodegradation process to the desorbed product obtained from the adsorption process will be performed during months 19-36.

2.2.2 PHARMACEUTICAL ADSORPTION PROCESS

A) EXPERIMENTAL SETUP FOR THE ADSORPTION TESTS

The first part of the UNIBO activity was aimed at developing analytical methods for the analysis of the target pharmaceuticals, which are carbamazepine (CBZ), diclofenac (DCF) and ibuprofen (IBU). UNIBO developed two methods, one using a UPLC-MS instrument and one for an HPLC-DaD instrument.

The procedures for the development and production of Molecularly Imprinted Polymers (MIPs) targeted to the selective adsorption of carbamazepine, ibuprofen and diclofenac are illustrated in deliverable 2.2. In D2.2, 2 different template washing methods are reported: the first one called the Buchner method is referred to in MIP labels as W1; the second one, called the incubation method, is referred to as W2. Each MIP is labelled with the acronym MIP_J_X_Y_Z, where J indicates the target pharmaceutical (CBZ, DCF or IBU), X indicates the cross-linker (B for the bifunctional and T for the trifunctional), Y indicates the monomer (1-11) and Z indicates the template washing method (W1 and W2).

Even though the target pharmaceuticals were detected in the effluents of the studied WWTPs at concentrations varying in the 1-20 μg/L range, they were studied during months 1-18 at higher initial concentrations, in order to simplify and speed-up the

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analytical process, and also to obtain indications relevant also for the application of the adsorption process to industrial wastewaters featuring higher concentration of pharmaceuticals.

The first part of the adsorption tests consisted in batch isotherms, conducted as follows. Single compound solutions were prepared in the concentration range of 500-10000 µg/L for CBZ and IBU and up to 12000 µg/L for DCF, and 50 mL of solutions at increasing concentration were put in contact with 50 mg of the tested sorbents in 125 mL- pyrex bottles. The bottles were put in a rotary shaker for 24 h at T=22°C and a stirring speed of 160 rpm. This part aimed at finding the best sorbent for each pharmaceutical.

During the second part of the work, the adsorption materials that resulted in the best performances in batch conditions were tested by means of continuous flow adsorption tests in a lab-scale experimental setup consisting of 2 tanks, one for the inlet and one for the outlet, a peristaltic pump, a sand column to remove suspended solids and to avoid the adsorption column clogging and the actual adsorption column. The samples were taken at the outlet, filtered with PTFE-0.22 µm filters and analyzed at HPLC-DaD.

B) RESULTS OF THE ADSORPTION TESTS

B1. Carbamazepine

Batch isotherm tests

The first adsorption tests with CBZ were performed in deionized water and the materials that were tested were the commercial Norit GAC and XAD16N, the MIPs with T and different monomers and MIPs with 1 and 2 as monomers and B as cross-linker. Norit GAC outperformed all other sorbents showing an adsorption yield range between 95-100%.

After performing the batch tests in deionized water, isotherm tests were performed with spiked real WWTP effluent to take into account the effects of competitive molecules present in the effluent. The materials that were tested are the 2 commercial materials Norit and XAD16N and different MIPs. The most effective sorbents are the 2 commercial ones as in deionized water and MIP_CBZ_T_1_W2. The best materials performance results are reported in Table 2.2.2.1 in terms of a range of adsorption yield values and sorbed phase concentrations.

Table 2.2.2.1 Carbamazepine's best materials performances results in real effluent.

Material	$C_{L,0}$	$C_{S,eq}$	Adsorption yield
	µg/L	$\mu g_{adsorbate}/g_{adsorbent}$	%
Norit GAC 1240 W	500-10000	500-9500	97-99
XAD16N	500-10000	500-9500	90-99
MIP_ CBZ_T_1_W2	1000-5000	900-4000	86-95

In general, most conducted isotherms present a linear trend. This indicates that even if the tests were conducted at higher CBZ concentrations than those typically found in WWTP effluents, the linear isotherms can reasonably be extrapolated in the low-concentration range typical of WWTP effluents.

Kinetic tests

After the material screening phase, kinetic tests with 2 of the 3 selected materials, that are MIP_CBZ_T_1_W2 and Norit, were performed at a CBZ initial concentration of 2 mg/L, adsorbent material concentration of 1 g/L, T=22°C and different sorbent particle sizes. These tests aimed at analysing the adsorption kinetic rate as a function of particle size and at selecting a reasonable Empty Bed Contact Time (EBCT) for the continuous fixed bed column tests. For MIP_CBZ_T_1_W2, particles sieved in the ranges 0.14-0.355 mm and 0.355-0.71mm were tested, while for Norit, particles sieved

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in the ranges 0.355-0.71mm and 0.71-1.4mm were used. The results showed that MIP_CBZ_T_1_W2 has a faster kinetic rate than Norit: considering the same particle size (0.35<dp<0.71 mm), the CBZ 50% removal is achieved after 10 min for MIP_CBZ_T_1_W2 while after 45 min for Norit even if with Norit a complete removal is achieved while with MIP_CBZ_T_1_W2 a 90% removal is the equilibrium value. Moreover, as expected the adsorption kinetic rate is faster by decreasing the particle size due to the reduction in mass transfer limitation.

Continuous flow adsorption tests

The first continuous flow breakthrough test was started by packing a column with Norit GAC sieved in the range of 0.71-1.4 mm, which is the typical range of full-scale adsorption plants with granular activated carbon. The column that was packed has an inner diameter of 1.3 cm and the packed bed height was 40 cm to obtain a Bed Volume (BV) of about 53 mL. The flowrate was then chosen to obtain an Empty Bed Contact Time (equal to the BV divided by the flow rate) of 23 min which corresponds to a flowrate of 2.3 mL/min. The starting CBZ inlet concentration was 1000 µg/L. This continuous flow test is still in progress and the outlet dimensionless CBZ concentration profile is reported in Figure 2.2.2.1. The latter shows how after about 9000 BVs the normalized outlet carbamazepine concentration is still less than 4% of the inlet concentration coherently with what is found in the literature.

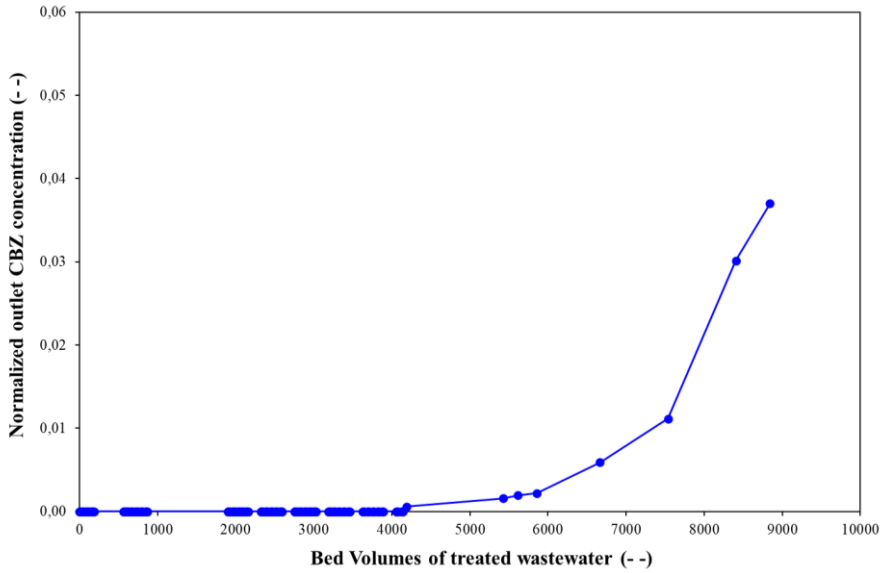

Figure 2.2.2.1 Breakthrough test of carbamazepine adsorption on Norit represented as normalized outlet concentration versus bed volumes of WWTP effluent treated.

After starting the continuous flow test with Norit with operating parameters that are common at full scale, a different approach to reduce the time needed to obtain at least a 20% breakpoint was chosen: the reduction of particle size in the range of 0.35-0.71 mm allows the reduction of the EBCT; this allows to achieve a continuous-mode comparison of the best materials in reasonable times and theoretically without a drastic spreading of the breakthrough curve (Corwin, 2010). For this reason, a packed bed column with an inner diameter of 1 cm and a height of 20 cm was selected, to obtain a BV of about 15 mL. The first material that was tested with these operating parameters was MIP_CBZ_T_1_W2. The selected EBCT was about 3 min, on the basis of the kinetic tests shown above, and the initial CBZ concentration was set to 200 µg/L. The saturation capacity was obtained at about 3000 BVs of effluent treated while a 50% breakpoint was achieved at about 1500 BVs.

B2. Diclofenac

Batch isotherm tests

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In the material screening phase for diclofenac, batch isotherm tests were performed directly with spiked WWTP effluent. The best performing materials are the commercial ones Norit and XAD16N for CBZ and MIP_DCF_T_2_W1. As a consequence of resulting in the best MIP, different washing techniques were studied for MIP_ DCF_T_2_W1: beyond W1 and W2, the other 2 washing techniques were studied are W3 (which consists of using Soxhlet apparatus and just methanol as solvent) and W4 (based on incubation technique with pure methanol at 55°C). The performances resulted quite similar, even though W2 performed slightly better. In Table 2.2.2.2, the best materials performance results are reported in terms of a range of adsorption yield values and sorbed phase concentrations.

Table 2.2.2.2 Diclofenac best materials performances results in real effluent.

Material	$C_{L,0}$	$C_{S,eq}$	Adsorption yield
	$\mu g/L$	$\mu g_{adsorbate}/g_{adsorbent}$	%
Norit GAC 1240 W	500-10000	450-9000	90-100
XAD16N	500-10000	450-7000	73-93
MIP_ DCF_T_2_W1	4000-12000	850-9600	80-90

The DCF continuous flow tests will be conducted during months 19 and 20.

B3. Ibuprofen

Batch isotherm tests

In the material screening phase for IBU, batch isotherm tests were performed directly with spiked WWTP effluent. The results of adsorption batch tests are reported in Table 2.2.2.3 in terms of a range of adsorption yield values and sorbed phase concentration: Norit and XAD16N are the best sorbents for IBU while the tested MIPs performed poorly.

Table 2.2.2.3 Ibuprofen's best materials performances result in real effluent.

Material	$C_{L,0}$	$C_{S,eq}$	Adsorption yield
	$\mu g/L$	$\mu g_{adsorbate}/g_{adsorbent}$	%
Norit GAC 1240 W	500-10000	500-9000	90-100
XAD16N	500-10000	500-7000	70-100

The IBU continuous flow tests will be conducted during months 19 and 20.

2.2.3 PHARMACEUTICAL BIODEGRADATION PROCESS

A) EXPERIMENTAL SETUP FOR THE BIODEGRADATION TESTS

UNIBO activities focused on implementing pharmaceutical degradation in a Membrane Aerated Biofilm Reactor. Over the first 18 months, efforts centred on setting up enrichments of microbial communities capable of degrading target pharmaceuticals (carbamazepine, ibuprofen, diclofenac) under aerobic conditions. Cultures were enriched and monitored for degradation using High-Performance Liquid Chromatography (HPLC). Bacterial strains and microbial consortia potentially degrading IBU were characterized through DNA sequencing.

For IBU, cultures underwent sequential enrichments in a mineral medium supplemented with IBU as a stock solution in methanol to promote solubilization. Starting from these enriched cultures, a parallel selection of bacterial communities able to grow on IBU only (without methanol) was started. An analogous experimental layout was used for DCF.

In parallel to aerobic experiments, enrichments have been set up under anaerobic conditions supplementing the cultures with different electron acceptors to promote the onset of different microbial metabolisms.

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B) RESULTS OF THE BIODEGRADATION TESTS

B1. AEROBIC CONDITIONS

Aerobic mixed cultures enriched on CBZ did not achieve detectable biodegradation in 2 months.

Conversely, aerobic mixed cultures enriched on IBU achieved a complete biodegradation in about 3 days. Sequencing of the enriched microbial consortia confirmed that the original microbial community of the sludge used as inoculum evolved into three different microbial consortia with a decreased biodiversity and dominated by different microbial groups, possibly responsible for the degradation. Strains with the ability to degrade IBU were isolated and taxonomically identified.

Selective pressure on these mixed cultures was then increased by eliminating the methanol and enriching cultures able to grow on IBU as the sole carbon source. Three microbial cultures able to grow on IBU only, were obtained and maintained for more than 20 enrichment steps with constant selective pressure. Two out of three cultures degraded IBU at a faster rate, resulting in complete consumption of the chemical within 12 hours. Conversely, one of the cultures exhibited a slower kinetic profile, achieving a full degradation in approximately three days.

Preliminary tests were setup for IBU degradation using methanol, ethanol and acetone as IBU solvent, mimicking a possible situation derived from the elution of the pharmaceutical compounds from the adsorption column, using the culture with the highest degradation rate as inoculum. After a few days of incubation, IBU was degraded in all tested conditions. Degradation kinetics experiments are ongoing in the presence of the different solvents and using different solvent/IBU ratios, aiming at obtaining indications on how and how much the contaminant solutions eluted from the adsorption column will have to be diluted in water to maintain the degradation activity.

Further molecular characterization of the microbial communities enriched at different stages of the maintained selective pressure is ongoing to explore the differences between the initially selected communities, using IBU and methanol as solvent, and the final microbial community able to grow using IBU only as a carbon source.

Glycerol stocks of all IBU degrading cultures are available for subsequent activities and for more studies, which include screening for single strains or combination of strains able to perform degradation, as well as screening of strains able to degrade other pharmaceutical compounds in addition to IBU. Glycerol stocks have been tested for their ability to efficiently grow after freezing and thawing, and it was demonstrated that they maintain the same degradation capabilities as the original culture.

As for DCF, the same experimental set up used for selecting IBU-degrading cultures was applied to enrich cultures degrading DCF starting from aerobic sludges. Degradation activity was observed under aerobic conditions after 2 months of incubation using a methanol stock solution of DCF. After a few enrichment steps, cultures able to degrade part of the DCF in the growth medium were obtained and maintained, even if the amount of DCF degraded after 3 weeks of incubation varied greatly. In all cultures the biomass growth was limited, producing only small flocs at the flask's bottom. Attempts at quantifying the cell concentration were not successful but growth was observed when plating cultures aliquots on rich media, confirming that the cultures were viable. In one of the enrichment steps, there was evidence of biotransformation of the DCF in several other chemicals, accompanied by a colour

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shift in the growth medium from white to bright yellow. However, the subsequent enrichment steps did not show any evidence of DCF degradation nor any colour shift, highlighting the loss of the target phenotype. Attempts at restarting cultures from glycerol stocks resulted in no detected degradation after 4 weeks of incubation. This highlights that species responsible for DCF degradation within the microbial communities could be sensitive to freezing or other environmental variations and easily lost from the pool. Attempts at re-obtaining the target phenotype are still ongoing, starting from other glycerol stocks. Parallelly to these experiments using DCF in methanol stock solution, the inoculation of mineral medium containing DCF only was attempted. In this case, it was not possible to detect any DCF degradation even after 4 weeks of incubation and the biomass growth was negligible.

B2. ANAEROBIC CONDITIONS

Anaerobic mixed cultures enriched on CBZ, IBU or DCF, all supplemented using stock solutions in methanol, did not show significant biodegradation of the target pollutant after more than 2 months, in none of the tested conditions aimed at stimulating different microbial metabolisms. It is important to point out that all anaerobic cultures were confirmed to be alive, as demonstrated by the decreasing concentration of the provided nutrients, as well as by the increasing concentration of carbon dioxide and/or methane in the headspace of anaerobic microcosms.

2.2.4 CONCLUSIONS AND INDICATIONS FOR SCALE UP IN THE DEMO SITES

The UNIBO team worked during months 1-18 on the development of an integrated process of adsorption of pharmaceuticals from WWTP effluents and biodegradation of the desorbed pharmaceuticals. In particular, the activities focused on CBZ, DCF and IBU as target micropollutants.

The adsorption tests, initially conducted in batch mode with actual WWTP effluents, indicate that both activated carbon Norit and resin XAD16N provide interesting adsorption performances towards all the 3 selected pharmaceuticals. In addition, several MIPS were tested, and two MIPs were identified with high adsorption capacity towards CBZ and DCF. The continuous flow tests, conducted so far only with CBZ, and always with actual WWTP effluent, confirm that both Norit and the MIP labelled MIP_CBZ_TRIM_MAA_W2 feature high adsorption performances. The activities planned for months 19-36 include further continuous flow tests conducted with the other target compounds (IBU and DCF), and with mixtures of the different target compounds, at the low concentrations typical of WWTP effluents. In addition, batch and continuous flow tests aimed at the removal of clarythromicin will be conducted.

On the basis of the results obtained during M1-18, the adsorption technology is considered suitable for the upscale to TRL5 in demo site 4. The most suitable adsorption material and the optimal operational conditions will be identified by M24, so as to proceed with the design and construction of the TRL5 pilot plant.

As for the biodegradation process, a microbial consortium capable of biodegrading IBU at a high rate under aerobic conditions was developed, whereas the tests aimed at the biodegradation of CBZ and DCF did not lead to relevant results. Therefore, the activity will proceed with the development of an integrated adsorption/biodegradation process targeted mainly to IBU. To this goal, the IBU degrading culture will be further characterized in terms of degradation rate in the presence of increasing concentrations of different potential desorption solvents (ethanol, methanol, toluene) aiming for the exploitation in a process to treat eluates from the adsorption column. Initial steps in setting up an MABR using the available

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culture to form the biofilm are being taken. To date, the biodegradation process is not yet at a sufficiently high TRL to foresee a scaleup in demo site 4.

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2.3 CONSTRUCTED WETLANDS AND ADSORPTION (ISSBAT)

2.3.1 INTRODUCTION

In the framework of the WP2, ISSBAT is currently developing and testing technologies that will potentially be implemented in Demo Site 2 located in Oued Souhil, Nabeul, Tunisia. These technologies, as presented in Figure 2.3.1.1, will follow a combined technology train including:

- Constructed wetlands (CWs) (TEC4), as tertiary treatment of treated wastewater from SE3 and SE4 WWTPs.
- Low-cost and selective adsorption of selected micropollutants (Diclofenac, Bisphenol A and Ofloxacin) using locally available agricultural residues and treatment of the saturated sorbent with lignolytic fungi (TEC1),
- PV-powered hybrid NF/RO process for salt and micropollutant removal and brine phytoremediation with halophytes combined to bioremediation by bacteria augmented in the halophytes roots (TEC5)

Currently, Non-Point Source pollution has emerged as a critical environmental challenge, which led to a supply of residual pollutants and organic compounds (pharmaceutical compounds and pesticides) into soils and surface waters.
The use of CWs for bioremediation purposes has been gaining considerable attention due to their ability to closely replicate natural and environmentally friendly processes for mitigating a wide range of pollutants. Their successful use is well documented (Ravikumar et al. 2022). However, the performance of CWs for treating organic pollutants, particularly pharmaceutical residues, is still not adequately summarized. The ISSBAT team is developing this technology as a tertiary treatment to improve the quality of treated wastewater used for indirect aquifer recharge. Particular attention is given to the efficiency of constructed wetlands in removing contaminants such as nutrients, pathogens, and residual organic micropollutants. A comparison of the efficiency between different constructed wetland configurations (e.g., vertical flow vs. horizontal flow) and the study of various substrates to enhance the performance of treatment systems will be conducted. Economic evaluation through cost-benefit analysis of constructed wetlands will be performed to assess the feasibility of adopting this technology on a large scale in DS2.

The CW system is followed by and adsorption treatment which is described as the most effective for pharmaceuticals removal. ISSBAT worked on the development of novel cost-effective (bio)materials. Biochar has been shown to be a promising solution due to its ecofriendly and less expensive production compared to the typical activated carbons that require costly and energy consuming activation processes (Puga et al., 2022). In addition, biochar is known for the relatively high porosity and specific surface area, high surface charge and high-water holding capacity (Akintola et al., 2023). In order to remove the target micropollutants (DCF, BPA and OFX), several biomaterials from different available agriculture wastes were tested. A functionalized biochar-Clay composite was prepared to improve the ability of biochar to immobilize the selected micropollutants. Clay minerals have been widely used to develop cost-effective adsorbents for pollutant removal from water, but preparation methods and the clay nature and origin are different and innovative (Han et al., 2019). A further research line is being developed for a low-cost and environmentally friendly bioremediation process to decontaminate the saturated used adsorbents. white rot fungi (WRF) removal mechanisms and degradation pathways remain largely to be elucidated (Asif et al, 2017). Several species of WRF from ISSBAT lab collection are being tested for their

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remediation/degradation applications. ISSBAT is focusing on the biodegradation processes of the selected micropollutants by solid state fermentation (SSF).

Regarding the desalination process which occurs after adsorption, ISSBAT activities aim to compare the desalination efficiency of treated wastewater using reverse osmosis (RO), nanofiltration (NF), and a combination of both technologies. Hybrid RO/NF desalination is still a developing technology and requires further research and development to optimize its performance, energy consumption and cost-effectiveness (Tashtoush et al., 2023). It is a viable option that takes advantage of the strengths of each process and mitigate their weaknesses.

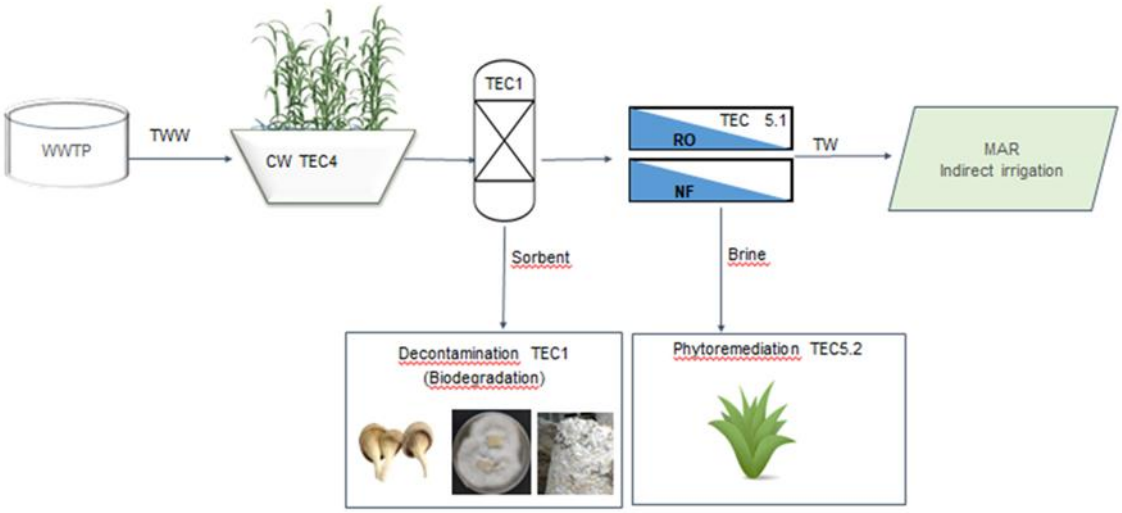

Figure 2.3.1.1 Flowsheet of the technologies developed by ISSBAT.

2.3.2 MICROPOLLUTANT REMOVAL BY MEANS OF CONSTRUCTED WETLANDS (TEC 4.2)

A) EXPERIMENTAL SETUP

During the first reporting period (M1-M18), two sets of lab-scale CW systems have been installed and implemented. The experimentation with the first one failed because of numerous technical issues encountered linked to clogging and plant uprooting and withering. The second lab-scale CW was newly installed in March 2024, replacing the previous one.

Initial lab-scale CWs system

Four lines of lab-scale units of CWs were designed and constructed with polyethylene plastic at ISSBAT. Each line composed of hybrid constructed wetland, consisted of two treatment stages. The first one was a vertical subsurface flow constructed wetland VFCW and the second was a horizontal subsurface flow constructed wetland HFCW (Figure 2.3.2.1). In order to select the most appropriate substrate, several types of media were used. After filling with the appropriate substrates, the constructed wetlands were planted. Before transplanting, the seedlings of macrophytes were washed to eliminate soil and other fines. The experimental units were fed with tap water for 20 days and then by a mix of 50% wastewater and 50% tap water for the following 20 days during the adaptation period. After that, each system was fed with real wastewater. Once stabilized, the system was scheduled to undergo testing for various HRT.

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Figure 2.3.2.1 First lab-scale constructed wetlands.

Second lab-scale CW system

The second lab-scale system consists of four parallel lines, each featuring a hybrid CW system (horizontal and vertical CW). The difference between the lines is based on the substrates used as filter media within the CW units (Figure 2.3.2.2). While gravel is a common substrate, the use of cork in wastewater treatment has recently developed. Preliminary investigations have demonstrated that cork has a promising potential in nitrate removal from agricultural drainage and in reducing some pharmaceutical contaminants. Cork may also be a source of carbon, which is necessary for the growth of hetetrophic bacteria. In this study, cork has been treated differently to obtain three forms of cork. To reduce clogging problems and improve effluent circulation within the system, coarse gravel is used at the inlet and outlet of horizontal CW and at the bottom of vertical CW. The novelty of this study lies in its varied treatment approaches applied to the used substrates.

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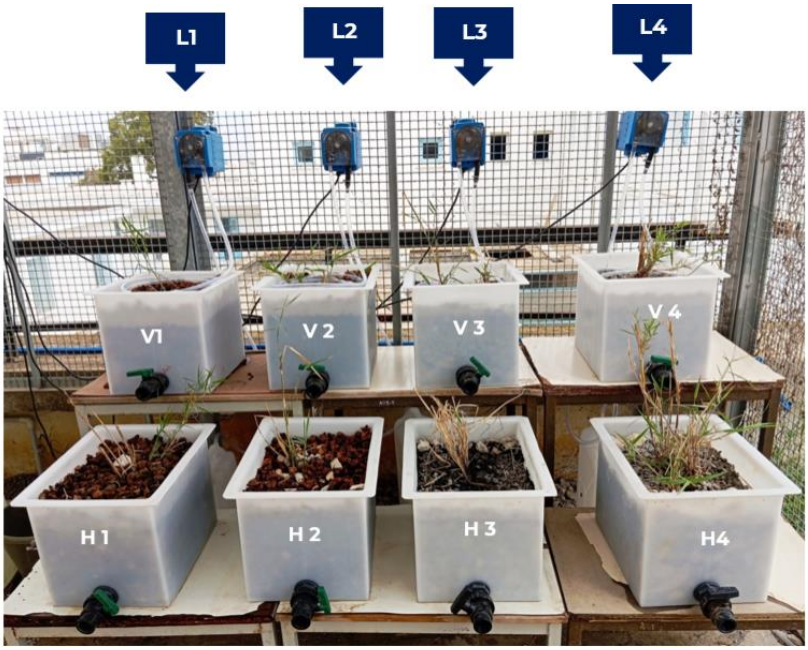

Figure 2.3.2.2 Second lab-scale CW system.

To assess the efficiency of the lab-scale CW system in removing contaminants, various experiments were conducted. These experiments involved adjusting different parameters, notably Hydraulic Retention Time (HRT) and flow rate. Synthetic wastewaters were formulated to accurately replicate the intricate variability observed in real wastewater samples. This tailored formulation will encompass a comprehensive spectrum of components and concentrations commonly encountered in actual wastewater.

During the acclimatization period for about a month, the experimental units were fed with tap water. Plant performance was assessed by physiological and biochemical parameters/indicators, photosynthesis, and membrane stability.

For the performance assessment of the system, a synthetic wastewater was prepared. Water quality monitoring was conducted after various hydraulic retention times in batch mode. Samples were collected from both horizontal and vertical outlets of each basin for analysis.

Physico-chemical analysis for the influent and effluent were performed as follows: Chemical oxygen demand (COD), NH_4-N, and NO_3-N, Electrolytic conductivity (EC) and pH were determined. Total suspended solids (TSS) were determined by standard methods.

B) RESULTS

Initial lab scale CW system

The results of the water analysis performed on the influent and the effluents of the four lines of CWs lab-scale system at HRT=48h showed that the pH remained stable across all treatments, slightly increasing from 7.6 to a range of 7.74-7.91. Total Suspended Solids (TSS) significantly decreased. Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) were substantially reduced, with Line 3 showing the best COD reduction, and both Line 1 and Line 3 achieving the lowest BOD. Nitrate levels dropped from 8 mg/L to as low as 3 mg/L in Line 1, while ammonium was reduced from 6 mg/L to 0.2 mg/L, with Line 2 being the most effective. Total

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Phosphorus (TP) was reduced from 10 mg/L to 0.6 mg/L, with Line 4 performing the best. Notably, total coliform bacteria saw a dramatic reduction in Line 4, highlighting its superior microbial removal efficiency. Overall, Line 4 showed the best overall performance in improving water quality.

Unfortunately, the results obtained were reliable only for the 48-hour HRT. The lab-scale system was recurrently attacked by insects which extensively damaged the plants, and severe clogging of the porous media occurred. Consequently, the first lab scale CWs were abandoned.

Second lab scale CW system

The results of the tested parameters at various retention times obtained with the lab-scale CW system revealed a highly effective treatment process. For Total Suspended Solids (TSS), the lab-scale system demonstrated a robust capability to remove these particles from the influent, with removal efficiency improving over time. The introduction of biochar to the gravel substrate (designated as L3) significantly enhanced the system's ability to filter out TSS, indicating the potential benefits of biochar as a supplementary material.

In terms of Chemical Oxygen Demand (COD), the lab-scale CW system consistently showed high removal efficiency across all tested substrates. The efficiency of COD removal also increased with time, highlighting the system's potential for sustained performance in treating organic pollutants. Similarly, the system effectively reduced Biochemical Oxygen Demand (BOD5) in the wastewater.

The system's performance in removing nitrogen compounds was also noteworthy. It demonstrated substantial effectiveness in the removal of Ammonium Nitrogen (NH4-N), with various configurations contributing to this success. This indicates the system's adaptability and efficiency in nitrification processes. Additionally, the lab-scale CW system exhibits significant proficiency in removing nitrates from the wastewater.

There is a noticeable inactivation of total coliforms across all materials, with L1 displaying the best efficiency of bacterial removal (from 10^5UFC/100mL to 10^3 UFC/100mL).

These results provide an initial insight into the performance of the lab-scale CW system at its early stage of operation. Across various parameters and substrates, differences are observed in the treatment efficiency. For pH, all substrates show a relatively similar range, indicating that the system maintains a neutral to slightly alkaline environment, which is generally favorable for biological treatment processes. Hybrid system combining vertical and horizontal CW seems to be very effective to treat wastewater in terms of TSS, BOD5, NH_4-N, NO_3-N and total coliforms. All tested substrates demonstrate similar efficiency in TSS removal. Gravel amended with biochar revealed the best efficiency removal for COD and BOD_5. Raw Cork and Expanded cork showed better results in removing NH_4-N and nitrates, respectively. Notably, this lab scale system remains in a non-steady state and its optimization is underway.

The ISSBAT workplan for the next period is outlined as follows:

- ✓ Optimization of the lab-scale CW using actual wastewater
- ✓ Microbial community characterization
- ✓ Assessment of the removal of pharmaceutical contaminants

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2.3.3 MICROPOLLUTANT REMOVAL BY MEANS OF ADSORPTION (TEC 1)

A) EXPERIMENTAL SETUP

Experiments were conducted at lab scale in order to design and prepare low-cost innovative highly selective functionalized (bio)materials from agricultural waste. Further tests were aimed at developing a low-cost and environmentally friendly bioremediation process to decontaminate the saturated used adsorbents.

Preparation and functionalization of (bio)materials for adsorption

The preparation and functionalization of (bio)materials for adsorption of the selected micropollutants (Diclofenac, Bisphenol A and Ofloxacin) was carried out. Several biomaterials were tested. Based on availability of materials and higher recovery rates after pyrolysis, ISSBAT proceeded with 10 biomaterials from 2 biomass sources.

The raw material of each substrate was washed to remove residual oils and phenolic compounds and then dried at ambient air. The obtained material was grinded and sieved. The biochar preparation was performed by pyrolysis in a muffle furnace without gas supply and then, cooled down to room temperature. Prior to use, biochars were ground and sieved to give a regular particle size.

The biochar functionalization was carried out to improve biochar adsorption performance via the introduction of surface functional groups. Several solvents were tested. Surface functionalization with clayey material was also realized. The prepared adsorbent was subsequently used in adsorption batch tests.

A first set of adsorption tests was performed batchwise to investigate the performance of each adsorbent and the effect of the various operating conditions such as pH, temperature, and adsorbent initial concentration.

An amount of the adsorbent was added to the solution containing Diclofenac (DCF), Ofloxacin (OFX) or Bisphenol A (BPA). They were stirred in a set of agitated flasks. Samples were taken every 15 min. Samples were collected continuously, immediately centrifuged, and then analysed using a UV-Visible spectrophotometer. A λ max of 275 nm, 274 nm and 275 nm corresponding to the maximum absorption of DCF, BPA and OFX, respectively, was determined after scanning the wavelengths in the domain (200 <λ< 400 nm). All the experiments were conducted with the same initial micropollutant (DCF, OFX, BPA) concentration and the same quantity of adsorbent.

The percentage of micropollutants removal was calculated following the equation (1):

$$Removal\,\% = \frac{(C0-Cf)}{C0} \cdot 100 \qquad (1)$$

where C_0 and C_f (mg/L) represent, respectively, the initial and final concentration between 2 points.

Based on these preliminary adsorption tests, the most promising biomaterials were characterized. The FTIR (Fourier Transform Infrared) analysis was performed using a FTIR spectrometer (Perkin Elmer/Frontier IR/FIR) to identify the different functional groups of the adsorbents. X-ray diffraction analysis (XRD) using a Bruker AXS/D8-ADVANCE powder diffractometer was used to determine the atomic and molecular structure of the materials. The surface morphology was studied using Thermoscientific Q 250 Scanning Electron Microscopy coupled to Energy Dispersive Spectrometry (EDS), providing detailed insights into the morphology, surface structure, and elemental composition of the different adsorbents. Brunauer–

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Emmett–Teller (BET) was used to determine the specific surface area and pore size distribution.

An optimisation step of the different operational conditions and adsorption parameters was performed, as presented in Deliverable 2.2.

Bio-adsorbents decontamination using solid state fermentation by ligninolytic fungi

A further research line was aimed at developing a low-cost and environmentally friendly bioremediation process to decontaminate the saturated used adsorbents. Several species of white rot fungi (WRF) from ISSBAT lab collection were tested based on their growth rate and known enzymatic pool. 3 white-rot fungi were selected to be used for tolerance tests.

The tolerance test was conducted by tracking the fungal mycelium mat diameters in solid malt dextrose agar (MDA) media, at the micropollutants concentration range of 0.1 to 100 mg/L.

The plates were inoculated with ± 4 mm of fungal mycelium disk and incubated at 30 ± 1.5°C for 9 days. The micropollutants' fungal tolerance index test was run in duplicate and calculated by evaluating the fungal mycelial mat according to the following Equation (2).

$$FTR\,\% = \frac{DFT}{DFC} \cdot 100 \qquad (2)$$

With:

FTR (%) is the fungal tolerance; DFT (cm) is the diameter of fungal growth in the test plate exposed to each micropollutant and DFC (cm) represents the diameter of fungal growth in the control plate. Fungal growth diameters were evaluated by measuring the expanding colonies.

B) RESULTS

Preparation and functionalization of (bio)materials for adsorption

Preliminary adsorption tests:

The obtained adsorption results for the selected micropollutants (DCF, BPA and OFX) using the different adsorbents from the first biomaterial show that the highest adsorbed amounts of DCF, OFX and BPA were obtained with functionalized Biochar-clay composite (65%, 64 % and 50 %, respectively). The adsorption reached equilibrium in about 90 min, 180 min and 240 min for DCF, BPA and OFX, respectively.

Adsorption capacity for DCF and BPA showed a rapid increase in adsorption amount during the first 15 min. This fast adsorption capacity at the initial stage by biochar-clay composite is probably due to the high availability of adsorption sites. However, lower removal efficiencies were obtained for Ofloxacin.

Concerning the second tested biomaterial, best results were also obtained with biochar-clay composite (68% of DCF and 62 % of BPA). The OFX removal rate was found to be under 30% and must be further validated.

Overall, the best results were obtained with biochar and biochar-clay composite for the 2 biomaterials. These 4 conditions were used for the optimization step.

The optimisation of pH, temperature and adsorbent dosage was performed for these 4 biomaterials (details are reported in Deliverable 2.2). Based on the final results, pH, temperature, micropollutant concentration, adsorbent quantity and the equilibrium time were fixed to be tested during the next period on real treated wastewater. Overall

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results allowed to select the functionalized biochar-clay composite as the best material.

Bio-adsorbents decontamination using solid state fermentation by ligninolytic fungi

Tolerance tests were conducted for a period of 9 days. BPA and OFX were found to be tolerated by the three tested fungal strains up to the concentration of 100 mg/L of each target micropollutant. The addition of DCF, BPA and OFX delayed the growth of the mycelium compared to the control during the first days, but the fungi ultimately reached the same size. The tolerance index against the 3 micropollutants was relatively high and varied from 65 to 80% for the three fungi.

Fungal Tolerance assays to DCF, OFX and BPA in liquid media are currently proceeding. The experiment is conducted in aerated batch flasks, using a mixture of micropollutants in liquid media. The fungal enzymatic production is also being monitored for lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac).

The fungi's efficiency in removing selected organic micropollutants from the saturated sorbents using solid state fermentation (SSF) will be assessed during the second reporting period. The operating conditions will be optimized (Temperature, pH, co-substrate, C/N ratio, etc...). Extraction techniques of the micropollutants will be developed, and high-performance liquid chromatography (HPLC) will be employed to accurately quantify the removal rate of the pollutants. If the adsorbent's properties remain unchanged after fungal treatment, it can be effectively reused. If not, the primary objective remains the decontamination of the substrate.

2.3.4 DESALINATION OF TREATED WASTEWATER

A) EXPERIMENTAL SETUP

The desalination system shown in Figure 2.3.4.1, is made up of three parts: pre-treatment, desalination, and renewable energy production. The pre-treatment stage is composed of an activated carbon column and micro-filters. The desalination stage includes two spiral wound membrane modules, each housing a nanofiltration (NF) and reverse osmosis (RO) membrane housed within reinforced fiberglass pressure vessels.

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Figure 2.3.4.1 Desalination pilot and PV Solar panels at ISSBAT Facilities.

Filtration tests were conducted under crossflow configuration conditions, maintaining constant operating pressures and an ambient temperature of 23 °C. The feed water, drawn from a 500 L tank via a vertical multistage centrifugal pump, was directed to either NF or RO membranes, depending on single or hybrid configurations. Flow sensors, connected to microprocessors for data logging, were utilized to measure the concentrate and permeate flow rates. Pressure adjustments were achieved through a control valve during the experimental procedures. Pressure gauges were employed to measure the pressures at the feed inlet, permeate outlet, and retentate outlet of the membrane module.

The desalination process yields a salt-depleted permeate stream and a salt-concentrated retentate stream. The conductivity of NF and/or RO permeate and retentate were monitored using online sensors.

The technical specifications for each component of the desalination pilot are shown in Table 2.3.4.1.

Table 2.3.4.1 Detailed technical data for the PV-fed NF/RO system.

Item	Specifications
PV Module	Number of modules: 16 Total panels area: 34 m^2 Total power output: 7280 Wp (455 Wp/Each)
Filmtec Membranes NF 90 and BW30	Membrane type: Thin-Film Composite Membrane material: PA (polyamide) Element configuration: Spiral-Wound Number of NF and RO modules: 1/each
HHP	Diaphragm pump 24 VDC, 1.0 A Open flow = 89 L/h Number of HHP: 2
Feed pump	Output power: 60 W

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	Operating flow: 35 L/min Centrifugal type
Pre-treatment module	Filter 20 µm Activated carbon column: 25 L, manual regeneration Dosing pump: anti scaling, 5 L/h at 5 bar Filter 05µm Filter 01µm
Built-in controls	TDS meter Permeate flow meter Three pressure controllers (raw water, NF membrane and RO membrane) ON/OFF switch Screen control
Built-out controls (online)	NF and RO permeate TDS meter NF and RO permeate /reject flowmeter Feed pressure sensor Data acquisition system

The desalination facility, powered by solar energy with a capacity of 7.3 kilowatts peak (kWp), is linked to the power grid. This system is composed of sixteen mono-crystalline silicon solar panels and a solar inverter that connects to the grid. It also includes metering equipment and a distribution network for managing the power supply. The electricity produced by the solar panels is estimated to cover the energetic needs of the desalination process.

The salinity of WWTP effluents in Oued Souhil demo site used for irrigation/aquifer recharge varies between 2.6 and 5 g/l. Accordingly, two sets of experiments were conducted using two synthetic brackish waters BW1 and BW2 that correspond respectively to the two levels (min and max) described for the real brackish water for the main salts.

Desalination experiments using NF and RO modules were conducted with varying pressures. For each salinity (2.6 and 5 g/L) the working pressure is listed below:

- NF: 6, 10 and 13 bars
- RO: 10, 20 and 30 bars
- NF-C-RO: NF+RO desalination process operated in series. The concentrate “C” corresponding to the NF retentate obtained at 13 bars is fed to the RO stage. Subsequent RO experiments were conducted at 3 different pressures: 10. 20 and 30 bars.

After desalination, the brine – i.e. refers to the intensely concentrated saltwater solution - was further used for irrigation experiments of *Aloe vera*: a medicinal plant with significant salt tolerance property. The brine produced by the different desalination experiments ranged between 3.19 g/L and 14.46 g/L.

The pots were cultured in natural light. After being transplanted, 0.5 L of each type of water was applied once a week during 60 days. Since this experiment is currently in progress, the results will be presented in the next reporting period.

B) RESULTS

A campaign of desalination runs was conducted in the pilot plant installed in ISSBAT, where various parameters were continuously tracked: pressure, salt rejection, permeate water flux, rejection and conductivity.

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Two sets of experiments were conducted using two synthetic brackish waters, BW1 and BW2 of initial salinity of 2.6 g/L and 5 g/L. The experimental protocol includes NF single stage, RO single stage and NF-C-RO two-stage.

The performance of NF, RO and NF-C-RO membranes were investigated with respect of flux, TDS rejection and recovery rate of the product water (permeate) from brackish water. For a given feed water, the permeation fluxes of water through all the membranes increased linearly with the applied pressure.

Permeate flux.

For a given feed water, the permeation fluxes of water through all the membranes increased linearly with the operating applied pressure. The difference between the transmembrane pressure and the osmotic pressure of the solutions on both sides of the membrane serves as the driving force for water permeation. With an increase in the operating pressure, the driving force for permeation increases and so does the permeation flux. While water production could be increased by increasing the operating pressure without increasing the membrane area, excessive pressure will not only consume more energy but also reduce the service life of the membrane module. Thus, the desalination processes are usually operated at low pressures to reduce the operating costs associated with power consumption and maintenance and to extend the membrane life, as long as the water production and product water purity meet the specific needs.

At a given operating pressure, the permeation flux decreased with an increase in the salt concentration in the feed water. This variation of flux with concentration is attributed to the increase in osmotic pressure with increase in feed concentration which causes a reduction in the driving force for the permeation of solvent across the membrane and a subsequent decrease I the permeate flux.

Water recovery

The water recovery for both NF and RO increase with an increase in operating pressure. The water recovery increases because higher pressure allows enhanced flow of water through the membrane.

The water recovery was observed lowest at higher feed concentration. Lower water recovery is because of higher salt concentration, causing the negative effect of concentration polarization.

Salt rejection

The salt rejection of NF and RO membrane did not show any significant change with pressure at different feed concentrations. Single NF, single RO and NF-C-RO hybrid configuration revealed a high salt rejection.

Specific energy consumption (SEC)

For all tested initial salt concentrations, the SEC of NF and RO membranes decreased with increasing operating pressure. This is because the permeate flow rate and the recovery increase with applied pressure. For both NF and RO membranes, SEC increased with concentration ranging from 2.6 g/L to 5 g/L. This trend was observed because the minimum value of required energy linearly increases as a function of the solution concentration.

Comparison between NF and RO membranes

The performance of NF and RO membranes were investigated with respect to recovery rate, rejection and SEC of the produced water (permeate) from brackish water. The operational pressure for the two membranes was adjusted to 13 bar for NF and 30 bar for RO.

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The lowest water recovery rate in a single stage was found with RO membrane at 30 bar. Also, RO membrane revealed a high salt rejection and SEC, while the highest recovery rate and the lowest SEC are observed for NF membrane. As a higher feed water recovery would result in smaller installation size of the membrane unit as well lower installation and operating costs, several attempts have been made to enhance water recovery of membrane process. So, NF stage when used as a softener before RO may allow the increase of RO membrane performance. Moreover, an increase in recovery by combination of NF and RO may result in a reduction in specific energy consumption (SEC), and hence for the diminution of the overall system cost.

The lowest energy consumption was observed for the NF-C-RO hybrid configuration for all range of feed concentration. It is noteworthy that the use of NF membrane prior to RO membrane reduces the energy consumption.

The photovoltaic system comprises 16 modules with a total panel area of 34 m^2. The energy generated by photovoltaic panels showed variation in accordance with variations in the environmental conditions (insolation and ambient temperature). Records indicate that the energy produced ranged from 30 kWh/day in January 2024 to 82 kWh/day in July 2023. It is noteworthy that for the NF-C-RO system, the required energy is approximately of 35 and 84 kWh/day, for initial salinity of 2.6 and 5 g/l, respectively. Therefore, photovoltaic panels must be sized appropriately based on the scale of the desalination plant and the specific technology adopted (whether NF, RO, or NF-C-RO) to ensure that energy demands are fully met.

In general, NF seems to be efficient in the tested salinity range. Indeed, NF permeate water flux is higher than RO permeate water flux at the same concentration of feed water. Moreover, excessive pressure consumption needed for RO not only escalates energy usage but also shortens the service life of the membrane module. Therefore, desalination at lower pressures allows the mitigation of operational expenses related to power consumption and maintenance and to prolong membrane longevity. This is particularly the case of NF compared to RO since the water production and product water quality align with the requirements. All these factors are playing in favour of using NF instead of RO to decrease the salinity of treated wastewater. However, if initial salinity is below 3 g/L it is not recommended to use membrane desalination processes. Reverse osmosis (RO) is a well-established and extensively employed technology ideal for the large-scale desalination of brackish water exhibiting elevated salinity levels (exceeding 5000 mg/L) as well as seawater (Honarparvar et al., 2021).

Phytodesalination using halophytes is proposed as an alternative for brine management. *Aloe vera*, stands out as a significant medicinal plant with diverse uses ranging from the pharmaceutical industry to the cosmetic sector, offering substantial economic potential. Phytodesalination experiments with brine, is currently running for a whole period of 60-day. At the end of the experiments, the following parameters will be measured:

- Determination of leaves dry matter and water content
- Determination of mineral content in leaves and plant
- Quantitative analysis of chlorophyll content in leaves
- Assessment of the fraction of salinity load provided through irrigation actually uptaken by the Aloe biomass

Based on the forthcoming results, a comprehensive evaluation will be conducted to determine the feasibility and relevance of implementing phytodesalination technology in Demo Site 2.

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2.3.5 CONCLUSIONS AND INDICATIONS FOR SCALE UP IN THE DEMO SITES

In conclusion, the initial performance evaluation of the lab-scale CW system shows promising results for treating wastewater to enhance water quality. Hybrid system combining vertical and horizontal CW seems to be very effective to treat wastewater in terms of TSS, BOD_5, NH_4-N, NO_3-N and total coliforms. All tested substrates demonstrate similar efficiency in TSS removal. An optimization of the lab-scale system is underway. This technology will be further tested and optimized using real wastewater, for an efficient and sustainable wastewater treatment solutions in Demo Site 2. On the basis of the preliminary results, the CW technology is considered suitable for scale-up in Demo Site 2.

As for adsorption, results highlight the efficacy of biochar and functionalized biochar-clay composite, in adsorbing DCF, OFX, and BPA from water. Optimal conditions were identified through pH, temperature, and adsorbent dosage optimization. Interestingly, tolerance tests indicated that certain fungal strains can tolerate the target pollutants at concentrations up to 100 mg/L. Ongoing research includes fungal tolerance assays in liquid media and monitoring of pollutant removal alongside enzymatic activities.

In the coming months, experiments in continuous mode using real wastewater will be conducted. Regarding the treatment of adsorbents saturated with micropollutants using WRF, preliminary findings are promising. However, validation of the performance of lignolytic fungi under non-sterile real conditions is necessary. We anticipate that by M22, a decision will be made regarding the feasibility of applying this technology in Demo Site 2.

The experimental results have demonstrated that the recovery rates were higher in the NF-C-RO hybrid configuration compared to single NF and RO systems operated under the same conditions. The NF-C-RO configuration exhibited salt rejection rates that were superior to the individual NF system and comparable to the individual RO system. Moreover, the NF-C-RO setup reported lower specific energy consumption (SEC) compared to RO. Based on these findings, the NF-C-RO hybrid system emerges as a more technologically attractive option, offering significant improvements in recovery rate, energy efficiency, and total dissolved solids (TDS) reduction. This makes the NF-C-RO system a promising solution for enhancing the efficiency and sustainability of desalination processes in wastewater treatment for aquifer recharge applications.

Besides, it is evident that the desalination processes are composed of multifaceted components including technical performances, energy consumption, and associated costs. So, it is necessary to optimize these factors for the NF-RO combinations before implementing and larger scale.

In Demo Site 2 treated wastewater is utilized for both irrigation and indirectly for aquifer recharge. Currently, there are no specific regulations governing the quality of water used for aquifer recharge. Thus, we envisage to consider the standards established for the reuse of treated wastewater. This also applies to salinity levels, meaning that the salinity limits for aquifer recharge are the same as those for irrigation. High salinity water, with EC above 3.0 dS/m and Total Dissolved Solids above 2000 mg/L, significantly impacts most crops and necessitates desalination or the use of highly salt-tolerant crops. Managing irrigation water salinity is essential for sustainable agriculture, and adjustments must be made based on crop tolerance and local soil and climate conditions (Hanson et al., 1999).

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In addition to desalination, another viable strategy for managing irrigation water with relatively high salinity is to dilute it with water of lower salinity. By blending saline water with fresh or less saline water, the overall salinity can be reduced to acceptable levels for agricultural use. This approach is particularly advantageous in Demo Site 2 where desalination is cost-prohibitive and logistically challenging. This method not only helps in conserving freshwater resources but also reduces the need for extensive desalination processes, thereby saving energy and costs. However, careful monitoring and management are required to ensure consistent water quality and to prevent long-term soil salinization issues.

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2.4 BIODEGRADATION BY 3D PRINTED BIOFILMS (FHNW)

2.4.1 INTRODUCTION

FHNW is developing and applying technology TEC 3.3 - Biodegradation by 3D printed biofilms, for the ad. In recent years, this technology has seen developments in a broad range of applications, including biomedicine, bioremediation and biomaterial engineering (Lazarus et. al., 2023; Liu et al., 2022).

This novel application of forming synthetic 3D-printed bacterial biofilms to target micropollutant biodegradation is expected to outperform microbial cultures inoculated in the treated water. Compared to standard batch cultures in optimised liquid media, the 3D-printed biofilms are expected to apply directly to the polluted water, avoiding the need for media exchange. Immobilisation of bacterial cells in the bioink facilitates control of spatiotemporal distribution and enhances the density of available biomass in the contaminated media while providing longer survivability and reusability compared to in-situ enzyme applications. (Mehrotra et. al., 2021)

Selected bacterial strains or consortia, capable of degrading the targeted pollutants, are incorporated into a hydrogel, which is formed through additive 3D bioprinting into a defined shape. The defined spatial organisation of different bacterial degraders could facilitate the stepwise degradation of otherwise hard to eliminate pollutants or improve the collective degradation of multiple combined pollutants. The ink for the 3D bioprints is based on specific nutrients, boosting growth and or degradation of the selected bacteria and thereby providing a richer environment than the treated water bodies. This approach tries to provide the required habitat for a multi-species biofilm, facilitating the interactions of different species in targeting different chemical compounds and allowing the co-existence in a complex polluted system, which has not yet been studied in dept and plays a key role in the successful application in the field of bioremediation (Lazarus et. al., 2023; Liu et al., 2022).

This technology could potentially be applied to wastewater treatment plant effluents in Demo Site 4 (Emilia Romagna, Italy). Therefore, FHNW activities focused on the biodegradation of pharmaceuticals found at Demo Site 4. The bacterial selection was thus performed on atenolol, diclofenac, ibuprofen and paracetamol, while further results suggested focusing mainly on ibuprofen.

Within the first reporting period (M1-18), different approaches have been implemented to select possible bacterial degraders for the targeted pollutants, as summarised in Figure 2.4.2.1 in section 2.4.2 A). The main source of bacterial inoculation was the in-house wastewater treatment plant at FHNW, as well as the membrane bioreactors operated by the FHNW team in the framework of a different project. Through multiple generations of aerobic batch cultures on increasing concentrations of the targeted single pollutant, a set of single-strain degraders for paracetamol and atenolol was found. As a common degradation pathway for paracetamol involves the intermediate 4-aminophenol, which itself can be toxic to certain bacteria, 3D bioprinting of a combination of strains could overcome this challenge. By employing two different bacterial strains targeting paracetamol and 4-aminophenol respectively, the accumulation of the intermediate could be avoided and thereby the degradation accelerated. While for ibuprofen, only consortia were found to be able to successfully deplete the contaminant. Further isolation experiments are being conducted.

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As a crucial first step in diclofenac degradation could include reductive dichlorination, enrichment cultures were set-up in an anaerobic environment; nevertheless, no degradation could be observed.

Furthermore, the suitability of the used 3D bioink composition and its effect on bacterial growth were tested for paracetamol strains and ibuprofen consortia. The latest finding on the ibuprofen degradation assay with the consortia suggests the need for a drastic change in bioink composition.

Finally, the degradation of ibuprofen was tested in a set of batch cultures, containing 3D bioprints, abiotic 3D prints or bulk inoculation of the bacterial consortia respectively. Further degradation assays are planned for months 19-36, in order to better assess the relative degradation capacity, fine-tune the bioink composition and test combinations of different degraders.

2.4.2 BIODEGRADATION BY 3D PRINTED BIOFILMS (TEC 3.3)

A) EXPERIMENTAL SETUP

The approach followed at FHNW for TEC 3.3, depicted in Figure 2.4.2.1, starts with the cultivation of a variety of microorganisms isolated from several polluted sites and incorporation into the medium-based bioink. Through 3D bioprinting, biocatalysts from selected bacterial or fungal strains are immobilised in a defined structure, which is then incubated in polluted waters, allowing the stepwise degradation of emerging and classic organic pollutants.

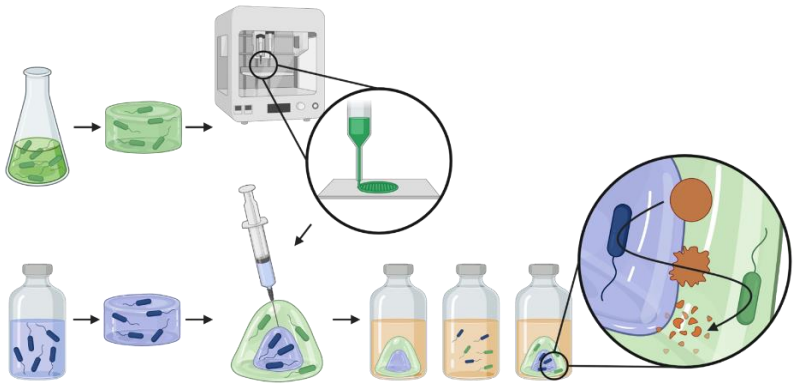

Figure 2.4.2.1 Schematic overview of 3D bioprinting approach for bioremediation. The steps include the cultivation of a variety of microorganisms isolated from several polluted sites and incorporation into a hydrogel-based bioink. Immobilisation of biocatalysts from sele selected bacterial or fungal strains by 3D bioprinting. Incubation of prints in polluted water and Stepwise degradation of emerging and classic organic pollutants.

The pharmaceuticals targeted by FHNW were atenolol, ibuprofen and paracetamol from Demo Site 4, as well as diclofenac from both Demo Site 2 and 4. Where no bacterial isolate known to degrade the target pollutant was present, multiple generations of aerobic or anaerobic enrichment cultures were performed. All bacterial cultures were grown and preselected using standard microbiological methods.

The 3D bioink formulation was based on Brunner Mineral Medium, supplemented with synthetic sewage as recommended by the Organisation for Economic Co-operation and Development [OECD, 2012] (BMM). If necessary, further adjustments to nutrient composition were specifically targeted to the used bacterial strain, by growing in a BioLog Nutrient plate, as well as tested bacterial performance. Methacrylated alginate, ι-Carrageenan and Carboxymethylcellulose were added

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subsequently, by rough stirring and heating to 80°C once the previous component was completely dissolved.

3DDiscoveryTM Evolution Bio-printer from RegenHU was used both with a pressure-based (pressure: 50 – 500 kPa, feed rate: 15 – 35 mm/s, layer thickness: 150 – 350 µm) and mechanically driven printhead (flow rate: 10 – 30 µL/s, feed rate: 20 – 40 mm/s, layer thickness: 200 – 400 µm).

The bacterial culture was concentrated and a volume corresponding to a theoretical OD600 of 0.5 within the desired volume of bioink was manually mixed into the ink. For each mL of bioink 5 µL of eosin Y (0.5 % in 1-vinyl-pyrrilidone) and 50 µL of triethanol amine (5.0 M) in HEPES buffer (0.1 M, pH = 7.4) were mixed in manually, before wrapping all containers in aluminium foil and storing in the dark.

The finished 3D bioprints were photo-crosslinked under green light, before incubating in the corresponding sample matrix or culture medium.

For degradation assays, as shown in Figure 2.4.2.2, both the 3D prints containing bacteria and abiotic 3D prints were incubated in 30 mL WWTP effluent from Demo Site 4 in Emilia Romagna and spiked with 5-10 mg/L of the targeted contaminant. In the initial months, when no samples from Demo Site 4 were available yet, artificial ground water was used instead. The composition of the artificial groundwater was based on Evian mineral water and adjusted according to Table 2.4.2.1 to better fit local ground water at Muttenz (CH). Additionally, a set of flasks with artificial ground water or WWTP effluent was inoculated with the corresponding amount of bacteria, without the presence of additional supplemented nutrients in the form of the bioink for comparison and evaluation of degradation efficiency. As positive control, a set of flasks with artificial wastewater was spiked with the same amount of pollutant and inoculated with the corresponding amount of bacteria, again with no bioink present.

All flasks were incubated at 25°C, 120 rpm and the degradation of ibuprofen was monitored through HPLC analysis. Each run was set over a gradient ranging from 20% - 95% MeOH and concentration was determined through peak area.

Moreover, after the first and second weeks of incubation, a piece of the 3D bioprint or bulk liquid was used as inoculum in artificial wastewater and 50 gm/L ibuprofen. This survival assay should evaluate whether the cells of the sample survive and thus grow, as well as whether they preserve their ibuprofen degradation ability.

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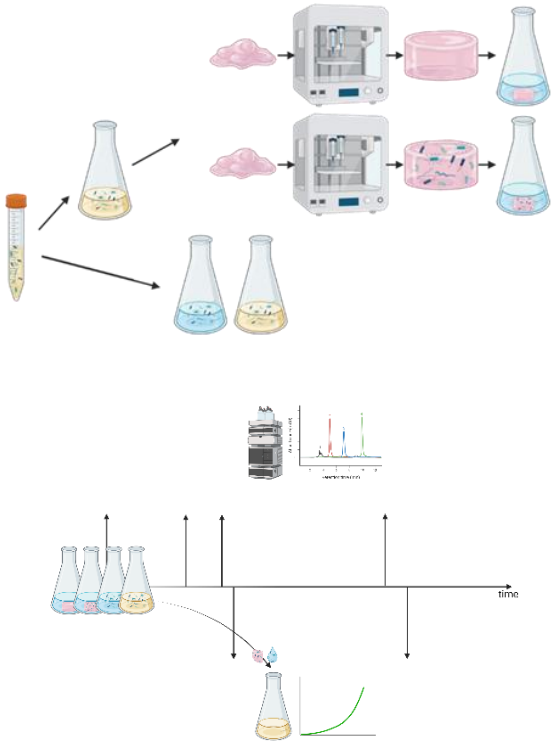

Figure 2.4.2.2 Experimental approach to assess biodegradation of ibuprofen with the use of 3D printed biofilms. The selected bacterial consortium was grown in nutrient-rich conditions (simulated wastewater from BMM and OECD), incorporated into a 3D-bioprint and followed by incubation in contaminated water (simulated ground or Demo site effluent water supplemented mg(L of ibuprofen. In parallel, an abiotic 3D-print was prepared under the same conditions, as well as a bulk inoculum of the equivalent amount of bacteria without any bioink in both rich and poor nutrient conditions. Over time, each flask was sampled for HPLC analysis and inoculation of survival assay.

Table 2.4.2.1 Composition of artificial groundwater.

Compound	HCO_3	Mg	Ca	Na	K	SO_4	NO_3	Cl	pH
(mg/L)	360	26	103	10.5	2	27.1	16.1	12	7.2

B) RESULTS

The search for suitable microbial strains or communities yielded multiple single-strain degraders for paracetamol and atenolol, both present at Demo Site 4. While the strongest degraders for paracetamol were able to deplete up to 500 mg / L of paracetamol within 3 days, the highest atenolol degradation by single strains was found to be about 25% degradation within 3 weeks.

A growth assay of all paracetamol single strain degraders in the presence of different concentrations of 4-aminophenol showed that the growth of the strain *Raoultella planitcola* was opposite proportional to the applied concentration of 4-aminophenol. *Microbacterium sp. strain* BR1 was selected as the main 4-aminophenol degrader.

The only overlapping contaminant of demo sites 2 & 4, diclofenac, did not yield any suitable biodegrades, as no significant decrease in diclofenac concentration could be observed.

The enrichment of ibuprofen in aerobic cultures yielded two different microbial consortia, named MBR2Ibu and MBR5Ibu, able to completely degrade ibuprofen in optimal conditions within 3 days. All isolates successfully extracted from the consortium were not able to perform any ibuprofen degradation on their own.

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The results of the ibuprofen degradation assay are reported in Figure 2.4.2.3, showing the samples inoculated with the MBR5Ibu consortium in different shades of brown. The positive control in rich medium (light brown) shows as expected the fastest degradation rates, while both the consortium in simulated ground water (blue) and the 3D bioprint in simulated ground water (brown) take a longer time to initiate degradation, also showing a high variability until the starting time between different replicates. The abiotic 3D print in simulated ground water (shown in dark blue) does not decrease in ibuprofen concentration over time.

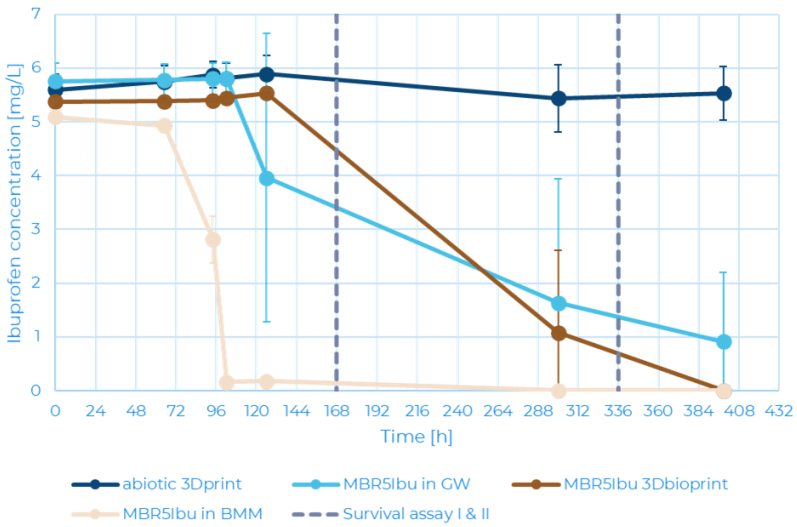

Figure 2.4.2.3 Ibuprofen degradation assay with 3D printed biofilms. Positive control of MBR5Ibu inoculated in artificial wastewater is shown in light brown. 3D prints with or without the addition of MBR5Ibu bacteria incubated in artificial ground water are shown in brown and in dark blue. The corresponding amount of bacteria in artificial groundwater shown in blue. All samples were spiked with 5 to 6 mg/L of ibuprofen.

Although the survival assay did show growth for all printed samples, indicating contamination of the bioink, the ibuprofen degradation ability was only preserved in the samples actually inoculated with our consortium.

As the 3D prints in simulated ground water showed much shorter stability than previously experienced, it was assumed that certain bacteria in the consortia might be degrading one of the crucial components of the bioink. Indeed, lower degradation rates of ibuprofen were shown, when the consortium was incubated with the amount of MASA corresponding to a 4 mL bioprint. The same experiment yielded faster ibuprofen degradation for the MBR2Ibu consortium (50 mg/L in 60h), as well as less influence on the presence of MASA, which is why it was employed in the new round of 3D bioprinting and degradation assay in the matrix from Demo Site 4 shown in Figure 2.4.2.4.

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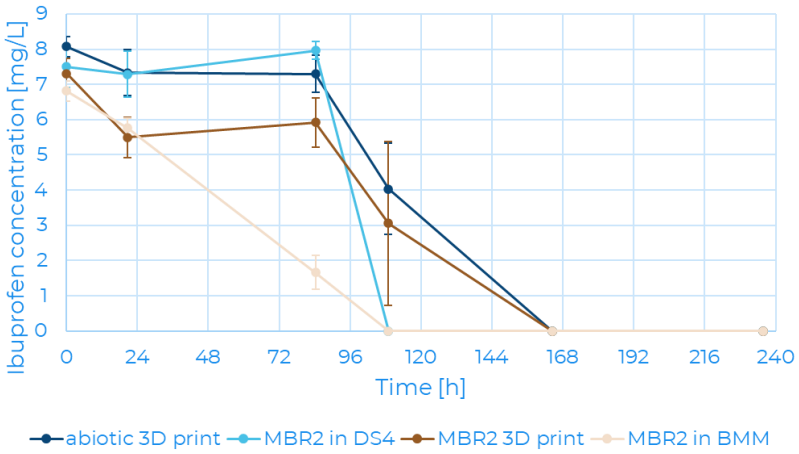

Figure 2.4.2.4 Ibuprofen degradation assay of MBR2Ibu cultures and 3D bioprints in Demo Site 4 water. The 3D-printed biofilm of the MBR2Ibu consortium is shown in brown, while the abiotic 3D print is shown in dark blue. The corresponding amount of bacteria without any bioink shown in blue. Positive control inoculated from the corresponding amount of MBR2IBu bacteria in artificial wastewater shown in light brown. All simples were spiked with 7-8 mg/L of ibuprofen.

As expected, the positive control (light brown) in the complete BMM was able to deplete ibuprofen the fastest. However, the empty 3D print (dark blue) in sample water from Demo Site 4 showed a significant ibuprofen degradation until complete depletion after 1 week, suggesting the presence of a competing bacterial consortium. The inoculated 3D bioprint (brown) showed initial degradation similar to the positive control, indicating that the boost of essential nutrients inside the bioink would be beneficial to the inoculated bacteria. However, as the degradation rate dropped to match the empty print, competition between organisms from the wastewater sample was assumed.

Furthermore, it is assumed that the bacteria present in the Demo Site 4 matrix were further destabilising the 3D prints, leading to a lifetime of only a few days, instead of the expected weeks.

2.4.3 CONCLUSIONS AND INDICATIONS FOR SCALE UP IN THE DEMO SITES

As ibuprofen was shown to be a relevant micropollutant of Demo Site 4 samples, the initial search for degraders was focused on ibuprofen. Both consortia MBR2Ibu and MBR5Ibu showed promising degradation rates, with 100% ibuprofen removal within 4-5 days of incubation in optimal conditions. The 3D bioprints of both consortia were able to remove 100% of ibuprofen within 1-2 weeks. As the additional nutrients of the 3D bioink might provide an easier accessible nutrient source than ibuprofen, it is crucial to find the best composition in order to optimise the degradation rates.

As the last results indicate a competing bacterial consortium capable of ibuprofen degradation within the sample matrix from Demo Site 4, more research needs to be conducted in order to investigate the interaction between the 3D bioprints and the indigenous consortium of the target effluent. In order to apply the 3D bioprints with consortia MBR2Ibu or MBR5Ibu for a downstream polishing step of the pharmaceutical biodegradation for the effluent of Demo Site 4, an additional pre-treatment and removal of local bacteria would be required, highly increasing the cost of the technology. Alternatively, the bioink composition could be further modified to increase the crosslinking rate and possible lifetime of the 3D prints despite the presence of possible degraders in the targeted Demo Site.

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As 3D printed bioinks represent a highly innovative technology at the early stage of development, at the moment the TRL appears to be too low for scale-up of this technology in the MAR2PROTECT demo sites.

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3. REMOVAL AND BIODEGRADATION OF POLLUTANTS FROM SURFACE WASTEWATER BEFORE ITS USE FOR MANAGED AQUIFER RECHARGE (TASK 2.3)

3.1 PHYTO- & BIO-REMEDIATION OF ESTUARINE SALTMARSHES (CIIMAR)

3.1.1 INTRODUCTION

In general, the first step in contaminants removal from water is their retention in sediments, either through adsorption or precipitation. But then strategies should be established to remove/eliminate those contaminants from sediments to prevent their increasing environmental toxicity as well as to maintain this removal in the long term, preventing contaminants from spreading in the area and from reaching coastal areas and/or eventually contaminating underlying aquifers. Phytoremediation by estuarine saltmarshes (that combines the role of saltmarsh plants and their associated rhizosphere microbial communities) is a promising technology to degrade and uptake micropollutants, ultimately protecting estuaries and related aquifers. The technology chosen for Lima river estuary (Demo site 7) is, therefore, an integrated phyto-/bio-remediation approach for the removal of micropollutants from estuaries, based on re-vegetation of impacted areas and non-vegetated areas. Saltmarshes can be protected and restored using nature-based solutions, such as saltmarsh restoration, saltmarsh creation, and saltmarsh management becoming itself a nature-based solution to prevent, protect and recover impacted estuarine areas. However, the implementation in estuaries is hampered by the adequate selection of salt marsh plants as well as the understanding of the physicochemical conditions of the site for effective removal of both organic and inorganic micropollutants. The latter can influence phytoremediation success, such as sediment characteristics (namely organic matter content and grain size), the contaminant characteristics and the simultaneous presence of both inorganic, such as metals, and organic contaminants, such as pharmaceuticals.

So, laboratory-scale experiments were carried out to evaluate the phytoremediation potential of two saltmarsh plants commonly present in estuarine areas including the Lima estuary, *Juncus maritimus* and *Phragmites australis*, to remove both metals and pharmaceuticals. The selection of the target compounds to be tested in lab scale experiment, two metals, Cu and Cd., and two pharmaceuticals, venlafaxine and ketoprofen, was based on a recent monitoring survey of the Lima river estuary. These metals, one a micronutrient (Cu) and another a toxic element with no known biological function (Cd) were detected recently in Lima river estuarine water (unpublished results) as well as in saltmarsh plants and sediments of the area (Cunha et al 2024). The pharmaceutical compounds were also recently detected in estuarine water and/or sediments (unpublished results) and are relevant types of pharmaceutical families, commonly used in human medicine and commonly detected in aquatic environments.

Previous work carried out by the team at lab scale, both in elutriate solution (simplified medium, simulating the estuarine interstitial water to which plants are exposed) and in mesocosm conditions simulating estuarine conditions (non-vegetated and vegetated sediments subject to dry and flooded periods with brackish water, simulating daily tidal variations) showed that both *J. maritimus* and *P. australis* had

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potential to remove Cd and Cu present in the aquatic environment, either by accumulating metals in plants tissues or retaining metals in the sediment surrounding plants belowground structures (the rhizosphere) (Fraga et al. 2022, Silva et al. 2014, Oliveira et al. 2014,). Elutriate solution experiments have shown also that *Phragmites australis* can contribute to the removal of pharmaceuticals, namely paroxetine and bezafibrate, from the aqueous medium (Dias et al. 2020). Moreover, lab experiments in elutriate solution have shown that Cu phytoremediation by saltmarsh plants (*Halimiones portulacoides* and *P. australis*) can be affected by the presence of different organic contaminants, including pesticides, hydrocarbons and pharmaceuticals (Almeida et al. 2009, Fraga et al. 2022) and that pharmaceutical removal can be affected by Cu presence (Dias et al. 2020). However, that influence depended on the organic contaminant characteristics. So, one should evaluate the phytoremediation potential for the selected target compounds, as information is still missing, namely when they are simultaneously present in the medium.

Experiments carried out so far were done in simplified conditions, elutriate solution and elutriate solution + sediment. The obtained results will allow the selection of the most suitable saltmarsh plant for contaminant removal and to understand which contaminant combinations might affect the phytoremediation process. The following experiments will be done in mesocosm conditions simulating estuarine conditions (adapting from previous experiments (Silva et al. 2014, Oliveira et al. 2014)) to evaluate phytoremediation potential during re-vegetation approaches for an integrated phyto-/bio-remediation approach for removal of micropollutants from estuaries.

3.1.2 PHYTO- & BIO-REMEDIATION OF ESTUARINE SALTMARSHES COMBINED TO ADSORPTION (TEC 1, 4.1)

A) EXPERIMENTAL SETUP

Experiments were done by exposing plants for 7 days to elutriate solution (obtained by mixing estuarine water with vegetated sediment (collected in the estuarine area just before the experiment) as described in Dias et al. 2020) or to elutriate solution + sediment to have a more realistic medium and simulate interactions among estuarine water, sediments and salt marsh plants. The elutriate solutions were initially doped with the selected contaminants. Samples of elutriate solution, sediment (when present) and plant tissues were collected at the beginning and end of the experimental period for contaminants concentration determinations and removal percentages assessment (considering initial and final levels).

Several sets of experiments were carried out (Figure 3.1.2.1). For venlafaxine, due to technical constraints associated with the measurement of the compound, the experiments were only done with *P. australis* in the absence or presence of Cu.

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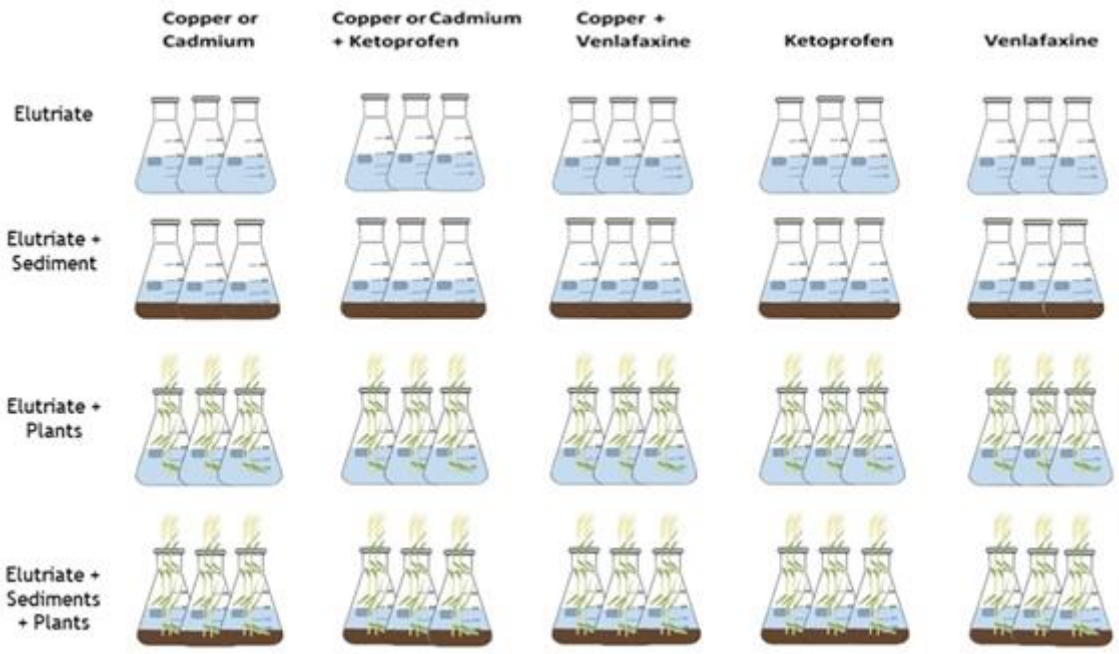

Figure 3.1.2.1 Elutriate experiments set up.

Metals were determined by atomic absorption spectrometry with flame atomization (AAnalyst 200, Perkin Elmer) in solutions (directly in elutriate solution and after acidic high-pressure microwave digestion of sediments and plant tissues) as described in Fraga et al. (2022). Pharmaceutical compounds were determined in elutriate solutions by high-performance liquid chromatography with diode ray detection (Hitachi LaChrom Elite HPLC System) after solid phase extraction, using a previously optimised methodology (unpublished results). Pharmaceuticals were not determined in plant tissues and sediment due to a lack of validated analytical methodology, which will be optimised for future experiments. In all experiments, chlorophyll levels in plant leaves were measured spectrophotometrically (as in Silva et al. 2014) as indicators of possible plant damage or stress.

B) RESULTS

For *P. australis* chlorophyll levels showed that in general there was some impact of the experimental conditions (chlorophyll levels decreasing up to ca, 20 % (depending on the experimental set) during the experimental period), but in general no significant effect of the contaminants added to the medium was observed. For *J. maritimus* however, there was a higher (ca. 3-fold) decrease of chlorophyll levels when exposed to the metal alone.

Results show that there is no clear contribution of the plant for the removal of the venlafaxine compound, as similar removal from elutriate solution occurred also in the absence of the plant, probably due to bounding to colloid matter still present in the solution. Colloids may present an enhanced sorption affinity for some organic compounds (Yan et al., 2015). The presence of Cu increased venlafaxine removal from the solution, but again with no clear contribution of the plant.

For ketoprofen, in the experiments with *P. australis*, in the absence of sediment, no significant removal of ketoprofen from the elutriate solution was observed, indicating that this plant has no clear contribution to the removal of this compound. No metal effect was observed. As so, tests were made for another salt marsh plant, *J. maritimus*. For this, no removal of ketoprofen from the elutriate solution was observed, either in

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the presence or absence of Cd or Cu, indicating that this plant also has no clear contribution to the removal of this compound.

In the presence of sediment, venlafaxine removal was almost total, independent of the presence of the plant or Cu. The compound was probably retained in the sediment, as this is a hydrophobic compound. Compounds with log K_{OW} > 3, as is the case of venlafaxine tend to be tightly bound to soil organic matter (Carvalho et al. 2012), which is significant in vegetated sediment.

For ketoprofen, in the presence of sediment, results were contradictory, with no removal or significant removal, indicating that experiments must be repeated. Results are in according with the fact that ketoprofen has been detected mostly in estuarine waters and not in estuarine sediments, with the opposite being observed for venlafaxine (unpublished results).

Regarding metal removal from elutriate solution, results show in general a role of both plants, for both Cd and Cu, in general, with no pharmaceutical influence. This removal is confirmed by metal accumulation in plant tissues, mainly in belowground tissues. In fact, Cu levels in *P. australis* plant roots increased up to 750 times. However, no metal translocation was observed, with shoot levels remaining identical after the exposure. For Cd, levels increased more than 1000 times in plant roots, again with no significant metal translocation to aboveground tissues. No apparent toxicity signs were observed despite the high metal accumulation probably due to its compartmentalisation in root cells. Metal accumulation was lower in the presence of sediment, as observed before, due to metal retention and lower bioavailability. The presence of the organic contaminants (venlafaxine or ketoprofen) decreased Cu accumulation by half. For Cd, a decrease was also observed (ca. 20 %) but only in the absence of sediment.

In *J. maritimus* Cu levels in plant belowground structures (roots and rhizomes) increased almost 130 times, whereas in plant shoots only a 4 times increase was observed. For Cd, increases were even higher, of 160 and 10 times in belowground structures and shoots, respectively. The presence of ketoprofen, in general, did not affect metal levels in solutions but influenced metal accumulation in plant belowground tissues, decreasing Cu levels by 30 % and increasing Cd levels in ca. 20 %. For shoots, no influence of ketoprofen on metal levels was observed, despite the fact that metal impact was reduced as indicated by chlorophyll levels.

Again, sediment had a significant role in metal removal, mainly due to metal adsorption as observed previously (Fraga et al. 2022), with sediment levels increasing significantly. This adsorption capacity is according to the very low levels of metals being detected in Lima estuarine water (unpublished results), showing metals are retained in sediment. Therefore, phytoremediation strategies should be tested and investigated to remove/eliminate those contaminants from sediments. Current results show that even in the presence of sediment both plants can accumulate metals. Moreover, previous work has shown that vegetated sediment has a higher metal removal capacity than non-vegetated sediment. So, plants' presence in estuarine sediment is advantageous.

Results also show that the simultaneous presence of metals and pharmaceuticals might alter metals removal, mostly decreasing it, a fact that should be taken into consideration when considering phytoremediation strategies for the estuarine area.

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3.1.3 CONCLUSIONS AND INDICATIONS FOR SCALE UP IN THE DEMO SITES

The obtained results show the relevant role of plants in metal removal, which can be impacted by the presence of organic contaminants. Sediment retention is another relevant metal removal process, that reduces metal uptake by plants, probably due to a lower metal bioavailability. On the other hand, for pharmaceutical compounds, plants do not have an active role, pharmaceutical adsorption to sediments is probably the main initial removal process of the compounds from the aquatic environment.

New experiments in mesocosms scale simulating the estuarine environment are still needed to fully understand the process underpinning metal and pharmaceutical removal, including when they are simultaneously present, and most importantly, to develop strategies that can enhance this removal. Despite the fact that contaminants can be removed from the aquatic environment by adsorption into sediments, strategies should be established to remove/eliminate those contaminants from sediments to prevent their increasing environmental toxicity as well as to maintain this removal in the long term. For metals, this can be achieved by phytoremediation, with saltmarsh plants, such as *P. australis* and *J. maritimus* showing potential to accumulate them. For pharmaceuticals, their removal from sediments will occur probably by microbial degradation. Nevertheless, plants can also have a key role here as previous studies have shown that microbial communities in vegetated sediments are more efficient.

Obtained results so far, indicate that re-vegetation of estuarine areas could be a suitable approach to remove contaminants being introduced in the estuarine aquatic environment. This removal will contribute to the good quality of surface waters, preventing contaminants from spreading through the estuarine area, from reaching coastal areas and eventually from reaching underlying aquifers, thus protecting groundwater.

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3.2 TREATMENT OF LAKE WATER BY MEANS OF RAPID SAND FILTRATION AND ULTRAFILTRATION (IHE, DU)

3.2.1 INTRODUCTION

The WP2 activities conducted by IHE and DU in Demo Site 1 (DS1) are aimed at developing a suitable treatment for the surface water of Lake Valkenburg, so, as to produce water suitable for infiltration in dunes and/or for drinking water production. The treatment process implemented by IHE and DU is based on combinations of the following technologies:

- Inline coagulation
- Full coagulation/flocculation
- Dissolved air flotation (DAF)
- Rapid sand filtration (RSF)
- Ultrafiltration (UF)
- Reverse osmosis (RO)
- Granular activated carbon (GAC) filtration (in the future)

These technologies have been organized into two alternative treatment lines, illustrated in Figure 3.2.1.1. Inline coagulation is not included in this Figure, as it has been dismissed based on preliminary results. The general goal of the proposed treatment trains is to remove the following surface water pollutants: natural organic matter, suspended solids, and fouling-related parameters. The research activity was also aimed at implementing pilot scale monitoring parameters, such as the Bacterial Growth Potential (BGP) and the Modified Fouling Index (MFI-0.45 and MFI-UF), aimed at monitoring the potential of clogging/fouling of the membrane processes (UF, RO), with the ultimate goal to identify operational condition characterized by a minimization of the energy consumption and operational costs.

Given the previous expertise of IHE and DU in surface water treatment, the above-listed technologies have been scaled up at a pilot scale since the beginning of MAR2PROTECT, in a pilot plant articulated in 2 treatment lines, with a total capacity of 16 m3/h combined capacity. GAC filtration/adsorption will be added in the upcoming months. As for RO, due to operational challenges, a very limited amount of data was collected on the quality of the RO effluent during the first reporting period. It is also possible that the RO will be moved to a different treatment location (to be confirmed by Dunea).

A pilot plant (16 m3/h) with two pre-treatment lines prior to reverse osmosis (RO) was constructed. Initially, the first pre-treatment line comprised inline coagulation followed by rapid sand filtration (anthracite and sand) and the second line included inline coagulation followed by ultrafiltration UF (7.2 m3/h). The coagulant used is ferric chloride (FeCl3). However, Lake Valkenburg has a high concentration of natural organic matter (NOM) and DOC ranges from 9-14 mg/L, depending on the season. The elevated levels of carbon and other nutrients are anticipated to be potential contributors to biofouling within the RO membrane system, given their propensity to serve as a substrate for bacterial proliferation and biofilm formation (van der Kooij, 1992, Jung et al., 2006, Zularisam et al., 2006, Chen et al., 2007, Ghernaout, 2014). At present, inline coagulation did not reduce the DOC concentration sufficiently (removal was < 15% with inline coagulation and RSF) and hence, it has been replaced by full-scale coagulation (with coagulant doses between 10-30 mg/L Fe3+) followed by dissolved air flotation (DAF), which feed either rapid sand filtration (RSF) or ultrafiltration.

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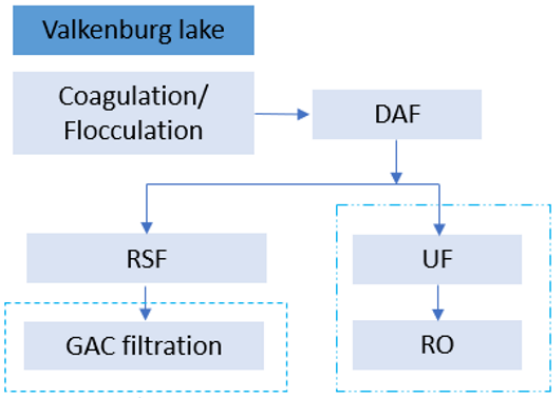

Figure 3.2.1.1 Schematic of treatment lines at DS1. NB. Coagulation/flocculation followed by dissolved air flotation was introduced in early 2024 to replace inline coagulation.

Coagulation is frequently applied to remove DOC/NOM. The coagulation efficiency for NOM removal will depend on pH, coagulant dose and (rapid and slow) mixing conditions, temperature, NOM properties, the presence of divalent cations and destabilizing anions and alkalinity (Duan and Gregory, 2003, Matilainen et al., 2010). Coagulation is used as a pre-treatment and is usually followed by sedimentation or dissolved air flotation and filtration (RSF or UF).

For waters with elevated NOM levels (TOC > 8 mg/L) (Anderson et al., 2023) as is the case with Lake Valkenburg, coagulation/flocculation followed by sedimentation or DAF is used as a pre-treatment (Thompson, 2001) before filtration. It should be noted that NOM forms low-density flocs with very low settling velocities which need high settling time. Hence, when coagulation/flocculation/sedimentation is compared with coagulation/flocculation/DAF the former can treat water with low-density particles (Edzwald, 2006). Hög et al. (2015) reported the removal of 34% DOC treating river water (TOC: 6.2-10.1 mg/L) using FeCl3 (10 mg Fe3+/L with 0.1 mg/L polyacrylamide as flocculant aid) and air flotation as a pre-treatment before ceramic membrane filtration. Moreover, coagulation/flocculation/DAF can produce water with low turbidity (<1 NTU), and reduces DOC and pathogens compared to sedimentation (Ladouceur et al., 2023).

In order to ensure optimal and sustainable operation of infiltration wells and RO systems, high-quality feed water is required (Turbidity <1 NTU, TSS <0.1mg/L, DOC <2 mg/L, AOC < 10 µg ac-C/L, MFI-0.45 < 3-5 s/L2) (van der Hoek et al., 2000, Vrouwenvelder et al., 2008, Stuyfzand and Osma, 2019, Pérez-Paricio and Carrera, 2020). These targets must be achieved consistently during pre-treatment. Hence, continuous assessment and monitoring of particulate/colloidal matter as well as nutrients (carbon and phosphate) is required to optimize the pre-treatment processes (coagulant dosing, mixing, filtration rate, etc) and guarantee good quality feedwater for RO and infiltration extending the lifespan of the membranes and reduce operational costs of the RO system and infiltration wells.

3.2.2 TREATMENT OF LAKE WATER BY MEANS OF RAPID SAND FILTRATION AND ULTRAFILTRATION

A) Description of the pilot installation

A.1 Rapid sand filters

An overview of the pilot plant installation focusing on the rapid sand filter treatment line is presented in Figure 3.2.2.1.

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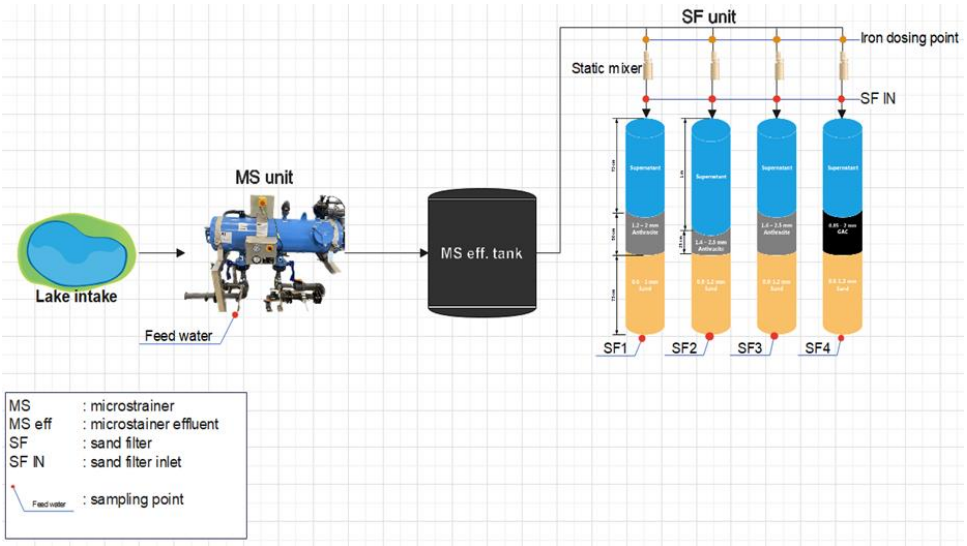

Figure 3.2.2.1 Pilot plant schematic – Rapid sand filters treatment line.

The rapid sand filter unit, presented in Figure 3.2.2.1, consists of four filter columns of 40 cm diameter each with,

- a supernatant total height of 2 m; to provide a minimum of 2 mwc head-loss at the bottom of the filter, and
- a dual media filter with sand as the bottom layer and anthracite or granular activated carbon (GAC) as the top layer. Moreover, the SF2 media filter height is 100 cm whereas it is 125 cm for SF1, SF3, and SF4.

A solution of ferric chloride FeCl3 is used as a coagulant with a 40 % purity for iron; the concentration of iron in the solution is 200 g Fe3+/L. The solution is stored in a dosing cabinet and is pumped to the sand filters unit. To set the pump flow, the following should be taken into consideration: flow rate of sand filters, coagulation dose desired, and concentration of iron in the dosing solution. The table below presents the pump flow values for the variation of filtration rate and coagulation dose.

For RSF, the head-loss breakthrough which triggers the BW happens at an earlier stage than the turbidity breakthrough to avoid compromising the water turbidity produced by the RSF unit. However, the DP condition set for automatic BW at the pilot does not prevent the RSFs from reaching a turbidity breakthrough. The BW stages and duration are as follows:

- 5 to 10 mins to drain the filters,
- 10 mins for air BW, and
- Around 8 mins for water BW.

A.2 Ultrafiltration

The Lake Valkenburg pilot also comprises 2 Inge's UF modules with inline coagulation, as a major pre-treatment unit prior to the RO feed. The capacity of the ultrafiltration process line is 7.2 m3/h.

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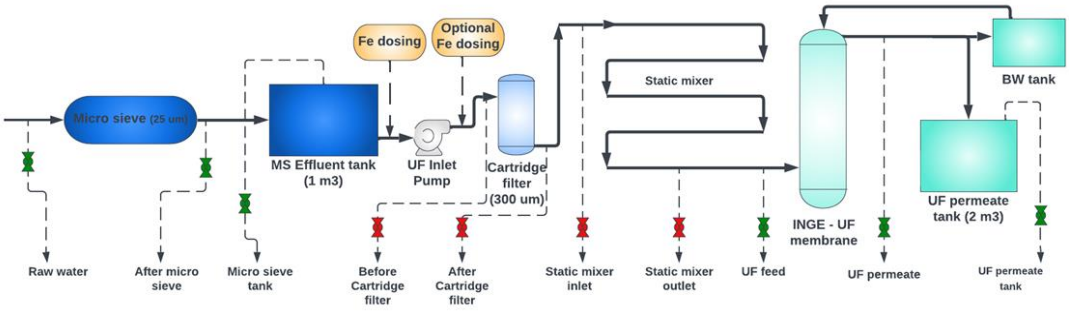

Figure 3.2.2.2 UF process scheme and sampling points for monitoring of water quality.

The UF module mounted in the skid is a patented Multibore® membrane technology, which is an in-house development of the manufacturers Inge GmbH, Greifenberg, Bavaria Germany. These Multibore® membranes have 7 capillaries of the same diameter in a single fiber. Inge boasts that the Multibore® membranes were only made in a single step with only one material- PES (polyethersulphone), which apparently does not peel away unlike the conventional composite membranes and proves to be advantageous in terms of membrane integrity. These are operated in dead-end mode and backwashed at periodic intervals. The membrane module at the pilot is of 0.9 mm diameter of the capillaries. These membranes are integrated into encased pressure vessels, resulting in an array called the inge® dizzer® module (Inge, 2016).

Table 3.2.2.1 Lake Valkenburg pilot's UF module.

Manufacturer: Inge; Model: dizzer® modules with Multibore® 0.9	
Temperature	0 - 40 ^{o}C
Surface area	60 m^2
Filtration flux	60 - 180 (L/m^2/h)
Backwash flux	230- 300 (L/m^2/h)
Operation	Inside out, dead end
Pore size	0.02 µm
MWCO	100 kDa
Recovery	90 - 98 %
CEB frequency	Every 30 – 32 sets
Filtration pressure (TMP)	0.1 - 1.5 bar
backwash pressure (TMP)	0.3 - 3.0 bar

Standard inge® modules are operated in two cycles. Filtration bottom followed by backwash bottom and filtration top followed by backwash top. The filtration lasts for 30 minutes and the hydraulic backwash lasts for 3 minutes (inclusive of valve positioning and forward flushes). In total, an Operation Cycle (OC) requires, 33 minutes to complete. Meanwhile, the coagulant was dosed inline prior to the UF feed pump, to improve the mixing efficiency and homogenization prior to feeding the UF.

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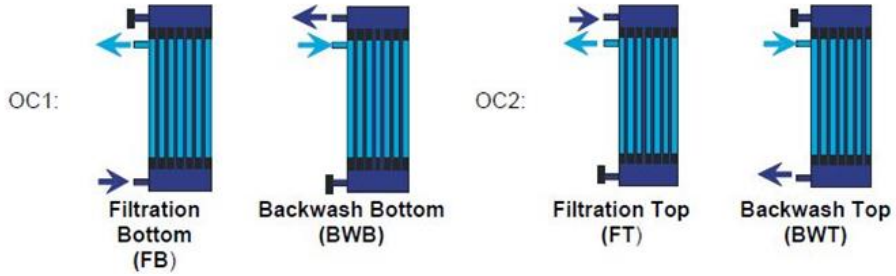

Figure 3.2.2.3 Illustration of operation cycles (Inge, 2016)

3.2.2.1 Experimental methods applied onsite

3.2.2.1.1 Bacterial growth potential (BGP) ATP-based method

This method measured the ATP from bacteria present in the water to monitor bacterial growth. To evaluate the BGP with an initial cell count, samples are pasteurized at 70˚C for 30 min and cooled down, before inoculation, in a water bath. A natural bacterial consortium from lake water collected for each experiment is added as an inoculum to all the pasteurized samples and artificial lake waters. The concentration of the inoculum is ~10x103 ICC/mL (measured with flow cytometry) as suggested in previous studies (Hammes and Egli, 2005). After inoculation, samples are incubated in the dark for 7 days (until decay is observed) at 30˚C and Total and Free ATP are measured daily (Figure 3.2.2.4).

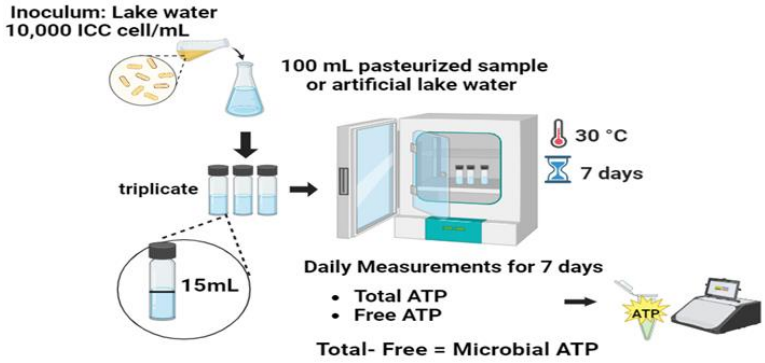

Figure 3.2.2.4 BGP procedure.

It should be considered that the current BGP method was developed for seawater (where the higher concentration of salts reduces the luminesce signal). In this study, BGP measurements will be performed on lake water with low levels of salinity (EC 820 µS/cm – 1003 µS/cm) and higher concentrations of organic matter (DOC in seawater 1-2 mg/L, while DOC in lake water 9-14 mg/L) and total cells compared to seawater (North sea water 1.2×106 cells/mL (Abushaban, 2019), while Valkenburg lake water average value ranges from 4.1×106 cells/mL, with a maximum value of 15×106 cells/mL).

3.2.2.1.2Modified fouling index 0.45

It considers that pore blocking occurs in three subsequent mechanisms, first, followed by cake filtration and finally, cake blocking, or enhanced compression occurs. On the contrary to the silt density index, MFI-0.45 can be used to test the particulate fouling potential of ultrafiltration permeate (Salinas-Rodríguez 2021). The recommended MFI-0.45 value to prevent fouling in RO membranes is <1 s/L2 (van der Hoek et al., 2000, Vrouwenvelder et al., 2008).

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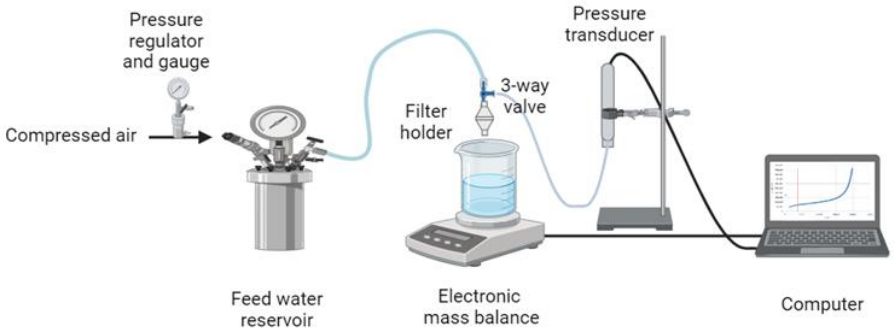

Figure 3.2.2.5 Schematic of laboratory apparatus used for MFI-0.45 measurement.

3.2.2.1.3 Modified fouling index ultrafiltration

In practice, it was observed that particles <0.45 µm were responsible for fouling. In RO systems the main particulate fouling mechanism is cake/gel filtration. Therefore MFI-UF at constant flux (operation mode in RO) was developed using hollow fibre PAN 13 kDa membrane and modified using 25 mm PES UF membrane (Boerlage et al., 2003). MFI-UF depends on pore size, the smaller the pore size, the higher the MFI-UF value and during this research 10 kDa membranes will be used during the measurements.

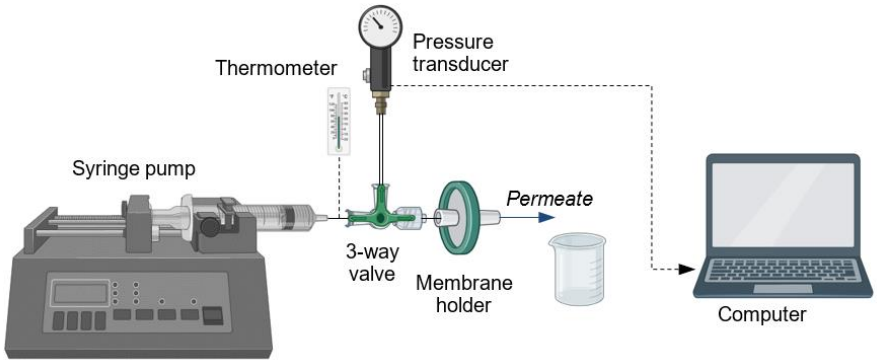

Figure 3.2.2.6 Schematic of laboratory apparatus used for MFI-UF measurement.

B) RESULTS

In the following figures, the average results of the various monitoring campaigns in the reporting period are presented for the target parameters, namely:

- Natural organic matter characterization: liquid chromatography coupled with organic carbon detection (LC-OCD)
- Biological fouling: assimilable organic carbon (AOC) and bacterial growth potential (BGP)
- Phosphate (PO4-P)
- Physical parameters: suspended solids, turbidity
- Particulate and colloidal fouling: modified fouling index (MFI-0.45) and MFI-UF.

It is important to mention that the dissolved air flotation unit was introduced in early 2024 to replace the inline coagulation process due to its limited effect on performance and water quality produced at the pilot.

Remark: In some of the results, UF permeate and RO feed concentration values are reported. In between these two sampling points, there is a buffer tank that may influence the water quality over time.

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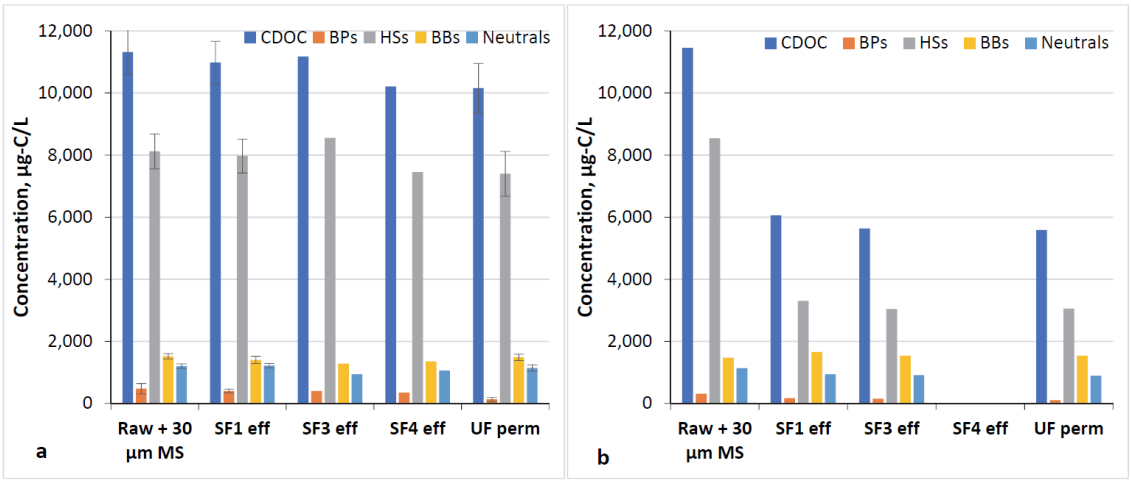

Figure 3.2.2.7 Fractions of natural organic matter, measured by LC-OCD a) Period 2022-2023 without DAF, b) Period 2024 where DAF was introduced before RSFs and UF.

There is a significant effect on the removal of natural organic matter by full coagulation/flocculation followed by dissolved air flotation versus inline coagulation. In addition, no significant difference was observed between RSF and UF processes. DOC was removed 20% by UF while 16% by the RSF treatment line; this value increased to about 50% when full coag/floc/flotation started to work in 2024. The biopolymers fraction of NOM is about 70% removed by UF while 30-50% by the RSF treatment line. Humic substances were removed about 20% by inline coagulation followed by UF or RSF but the performance increased to about 65% when full coagulation/flocc/flotation was installed.

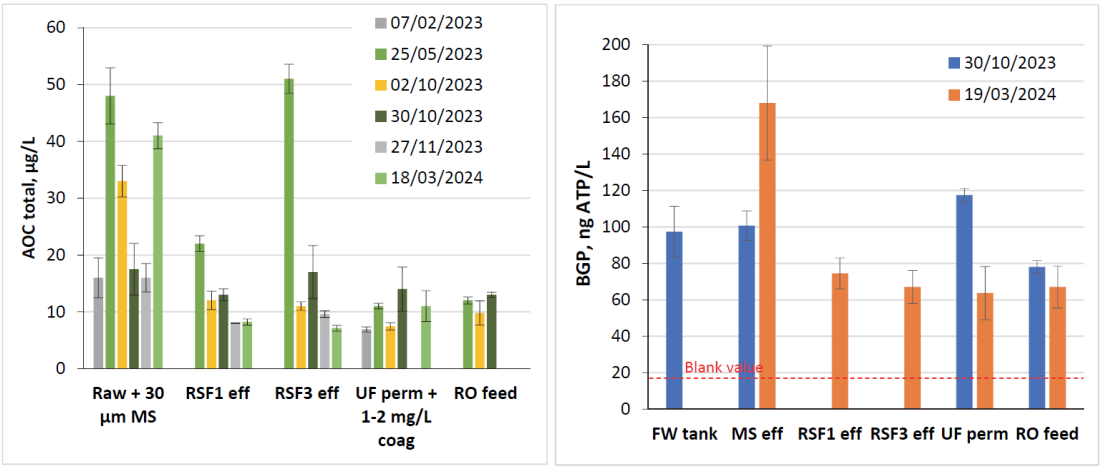

Figure 3.2.2.8 Assimilable organic carbon (AOC) (left) and preliminary bacterial growth potential test (right) concentrations along the treatment lines.

The AOC guideline value for drinking water is 10 µg-C/L. UF permeate and RSF effluent values were close to this guideline value showing a good performance by both treatment lines.

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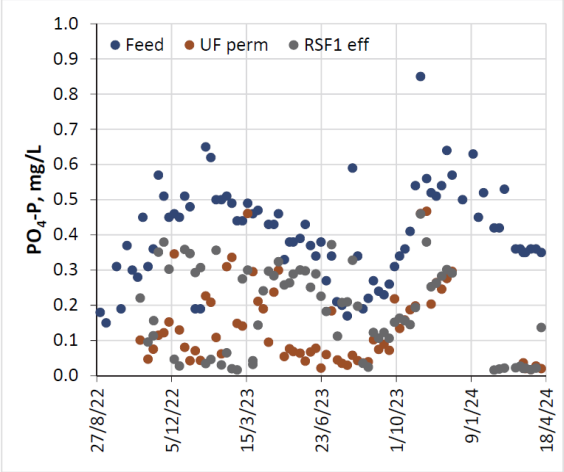

Figure 3.2.2.9 Monitoring of orthophosphate at the pilot plant. In 2024 the DAF system was introduced.

Phosphate was removed by the two treatment lines with a significant performance, in particular after the introduction of DAF in 2024 achieving 92-94% PO4-P removal in 2024.

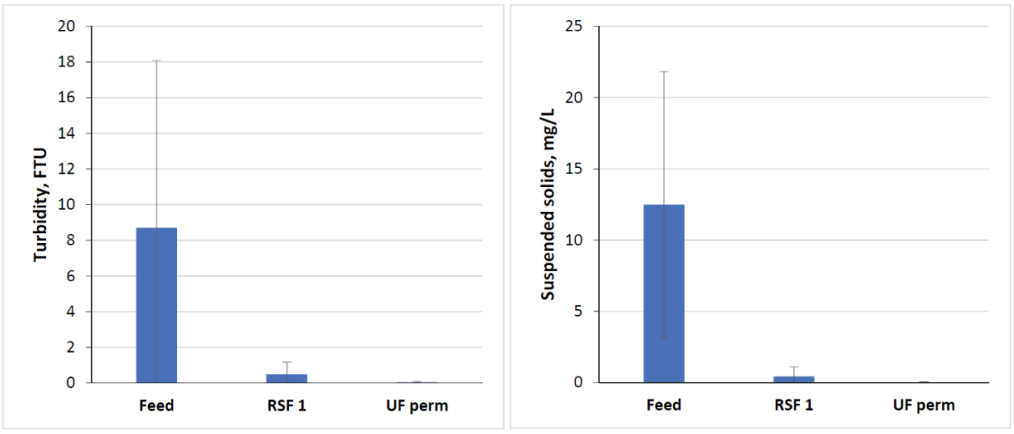

Figure 3.2.2.10 Average turbidity (left) and suspended solids (right) concentration to date.

UF is a robust barrier for turbidity and suspended solids removal despite the large variation in the lake water over time. Also, RSF was effective, although not at the same level as the UF.

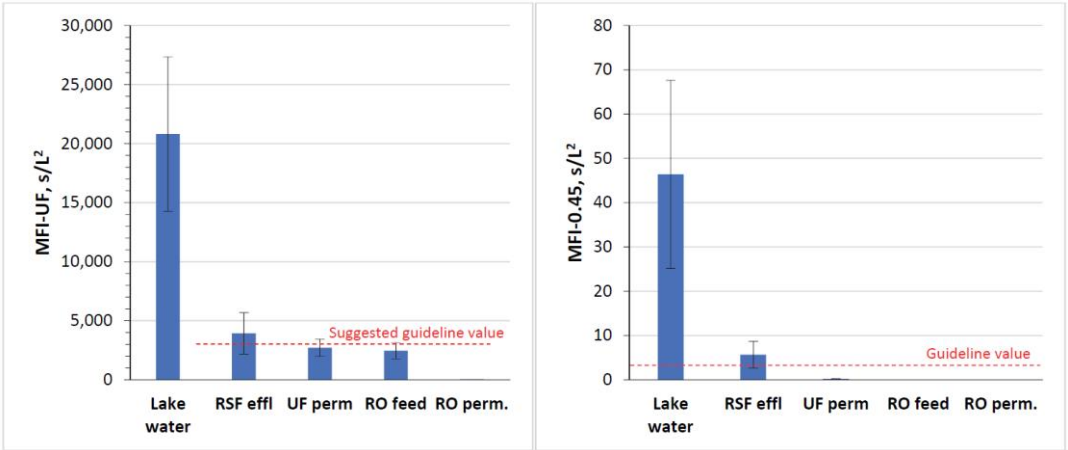

Figure 3.2.2.11 Monitoring of MFI-UF and MFI-0.45 at the pilot installation.

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The removal of particulate and colloidal fouling monitored by MFI-0.45 and MFI-UF, respectively, showed very good performance for the UF system, producing values lower than the guidelines for RO feedwater.

3.2.3 CONCLUSIONS AND INDICATIONS FOR SCALE UP IN THE DEMO SITES

Technologies implemented at DS1 were already scaled up at the TRL5 pilot scale. The focus so far has been on monitoring the performance of the treatment lines under changing water quality due to seasonal variations. The goal is to assess the performance of the treatment units in producing water quality for shallow infiltration in dunes. Thanks to the implementation of full-scale coagulation and flocculation followed by dissolved air flotation (DAF), both treatment lines showed promising results in terms of removal of assimilable organic carbon, phosphate and suspended solids. Ultrafiltration resulted in a robust barrier of particulate and colloidal matter, producing better water quality than the rapid sand filters. The following Table 3.2.3.1 summarizes the average removal obtained by the two treatment lines.

Table 3.2.3.1 Summary of average values and removal rates for various parameters monitored at the pilot installation.

				Removal	
Parameter	**Feed**	**UF perm**	**RSF eff**	**UF perm**	**RSF eff**
Dissolved organic carbon, µg/L	11,313	9,013	9,455	20.3	16.4
Biopolymers, µg/L	477.3	125.3	323.0	73.8	32.3
Humics, µg/L	8,119	6,311	6,683	22.3	17.7
Dissolved organic carbon[1], µg/L	11,450	5,589	5,846	51.2	48.9
Biopolymers[1], µg/L	308.0	106.0	160.5	65.6	47.9
Humics[1], µg/L	8,536	3,055	3,170	64.2	62.9
Turbidity, FTU	8.7	0.0	0.5	99.5	94.4
Susp. Solids, mg/L	12.5	0.0	0.4	99.9	96.6
PO_4-P, mg/L	0.40	0.15	0.21	62.3	48.6
PO_4-P[1], mg/L	0.40	0.02	0.03	94.0	92.2
MFI-0.45, s/L^2	46.4	0.2	5.7	99.5	87.8
MFI-UF, s/L^2	20,809	2,716	3,937	86.9	81.1

[1]Values for after the introduction of Coag+Floc+DAF. NB: In the table, the comma is used as a thousand separator, and the dot is used as a decimal indicator.

RO membranes reduced ~96% the electrical conductivity in the feed water from ~94 mS/m to about 4.4 mS/m. The average value for the RO concentrate was about 300 mS/m.

The results of the MFI-0.45 for both treatment lines suggest that the produced water quality is of sufficient quality for infiltration (MFI-0.45 < 3 s/L^2). UF produces consistently values less than 1 s/L^2 while RSF produces values around 6 s/L^2 which is expected to decrease under 3 s/L^2 once GAC filtration is introduced. There is no guideline value for MFI-UF, but when comparing the measured values with water produced by existing facilities treating water for deep well infiltration, we can observe that the water produced by the UF is at the same level.

In the coming months, granular activated carbon will be added after the rapid sand filters. This addition is expected to determine a significant improvement in the overall water quality produced by the RSF treatment line. The final choice between the two

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alternative treatment lines will be made at the end of the second reporting period, on the basis of the effluent quality obtained in the RFS line after the addition of GAC adsorption, and in the UF/RO line, once more consistent data on the RO effluent will be available.

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4. MITIGATION OF AGRICULTURAL N/P LEACHING TO GROUNDWATER THROUGH THE FERT-ROOT TECHNOLOGY (SU, TASK 2.4)

4.1.1 INTRODUCTION

SU is developing and evaluating the FERT-ROOT technology (TEC 6) for the mitigation of agricultural leaching of nitrate and phosphate to groundwater. FERT-ROOT applies the HumeFlo photocatalytic oxidation device in combination with biostimulants to stimulate an active and diverse microbiome in the root zone, enhance nutrient uptake and ultimately reduce leaching of the applied fertilizers. Although oxidation technology for irrigation systems is widely used for preventing scaling and biological fouling, the HumeFlo system is novel in its approach to utilise photocatalytic oxidation to address soil structure and composition challenges. HumeFlo is operated with the organic sequestering agent MacroPlex. Applying a combination of two unique soil biostimulants (organic carbon and nitrogen sources) further expands the potential of the FERT-ROOT approach to mitigate nitrate and phosphate leaching of agricultural origin.

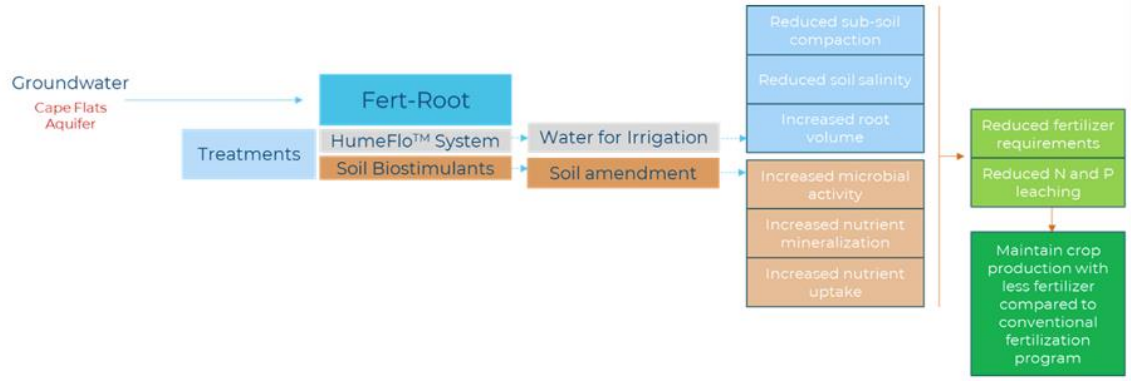

Figure 4.1.1.1 Flow chart of the proposed implementation of the FERT-ROOT technologies for the mitigation of agricultural nitrate and phosphate leaching.

A) EXPERIMENTAL SETUP

A1) Implementation of the integrated FERT-ROOT system in a vegetable farm in the Philippi Horticultural Area

Three independent field trials were performed to assess the performance of FERT-ROOT and the associated potential to reduce nutrient leeching into groundwater. The primary assessment was performed on a commercial vegetable farm in the Phillipi Horticultural Area, Cape Town, utilizing the irrigation infrastructure already in place. The site was fitted with a HumeFlo for in-line treatment of irrigation water, whereas FERT-ROOT biostimulants were applied weekly by a tractor. The soil's physical, chemical and biological parameters were characterised by collecting data at various sampling locations per experimental block. The endpoints assessed included horizontal and vertical soil compaction, bulk density, chemical composition, and biological composition.

The second field trial was performed on a strawberry farm. The goal was to assess the impact of the two soil biostimulants that form part of the FERT-ROOT technologies. The HumeFlo system was excluded from the trial because the scale of irrigation activities was too small for such a device, built for the treatment of large volumes of water. The experiment included a control section, treated with the conventional

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commercial program for fertiliser application, and a soil biostimulant section, where the 2 soil biostimulants were added to the conventional program. All products were injected weekly into the fertigation line. Soil samples were collected from the topsoil to assess soil biological activity. Additionally, soil electrical conductivity was measured to determine the efficiency of the soil biostimulants to immobilise nutrients in the root zone. Nitrate concentrations in the leaf petiole sap were also measured, as well as the final fruit weight for both the treated section and the control section.

A3) Application of the HumeFlo / MacroPlex technology in a citrus field trial

The HumeFlo system was installed at a commercial citrus farm. The area treated by the HumeFlo system in combination with MacroPlex was 28 ha, with a control section of 1.2 ha. MacroPlex was administered at a concentration of 4 ppm, and calibrated to the electrical conductivity of the irrigation water at the trial site. The trial extended over a period of 3 months, from August to November 2023. Soil profile pits were dug to assess root development and determine root volume. Sub-soil compaction was further determined. Soil samples were collected for chemical characterization. Water samples representing pre- and post- HumeFlo + MacroPlex treatment were also collected. Only the HumeFlo system of the FERT-ROOT technologies was evaluated in this trial. The soil biostimulants were not implemented to assess the effect of only the HumeFlo system on soil structure.

A4) Application of the FERT-ROOT soil biostimulants in laboratory-scale soil columns

Laboratory scale experiments were set up to assess the effectiveness of the soil biostimulants to immobilise nutrients with the use of soil columns. The columns were filled with sandy soil collected from the Phillipi Horticultural Area FERT-ROOT site and flushed with 5 L of reverse osmosis water to remove unbound nutrients. The trial included a control group in which a fertiliser mixture containing nitrate and phosphate was administered, and a treatment group, where the same mixture was added together with the FERT-ROOT biostimulants. Leachates from all columns were collected and submitted for analysis to determine whether nutrient leaching was impacted by the FERT-ROOT biostimulants. Only the FERT-ROOT biostimulants were evaluated in the column test, as the HumeFlo system requires large volumes of water to reach operational concentrations of the produced free radicals. Additionally, the soil column tests cannot be conducted on the HumeFlo system, as the preparation of the columns disturbs the soil structure, making soil compaction evaluation unreliable.

B) RESULTS

B1) Implementation of the integrated FERT-ROOT system in a vegetable farm in the Philippi Horticultural Area

Unfortunately, results obtained from the Philippi FERT-ROOT site are not sufficient for reporting, due to practical challenges encountered during the test, including theft and vandalism. In particular, pipes were broken and the valves of all the irrigation lines coupled to the FERT-ROOT experimentation were removed to harvest copper parts for re-selling. This incident resulted in the loss of months of data and the need for a complete restart of trials. The consequent additional demands of repeated trials have created a negative sentiment with the farmer and he is no longer willing to allow future experimentation on his land. Therefore, two additional experimental sites that also fall within the Cape Flats Aquifer catchment area have been identified for future experiments. These sites are in safer and more controlled areas, where theft and vandalism would be less likely. These possible sites also pose the advantage that drip irrigation is used and installed in such a way that the soil biostimulants could be

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administered with minimal effort to the commercial farmer who would incorporate the technologies in his conventional crop production programs.

B2) Application of the FERT-ROOT soil biostimulants in a strawberry field trial

The results obtained from a trial evaluating the FERT-ROOT soil biostimulants in strawberry fields show promise in terms of stimulating soil biology and immobilising nutrients within the root zone. The average soil CO_2 levels of biostimulant-treated plots increased from 5.3 ppm to 7.2 ppm after a 12-week treatment, compared to a change of only 0.2 ppm in the control field. The data indicate increased biological activity in response to biostimulant treatment. Soil electrical conductivity changes suggest improved nutrient immobilization in the root zone, whereas, leaf petiole sap NO_3 levels showed an increase in nitrate uptake in the biostimulant-treated field relative to the control. As the final indicator of biostimulant effectiveness, fruit weight was significantly higher in the treated section than in the control. The increased nutrient immobilisation in the root zone, as well as the increased uptake of nitrate and the higher fruit yield, show the potential of the soil biostimulants to ultimately reduce leaching of nutrients without compromising crop yield. Additionally, the data suggests the technology could potentially reduce the initial fertilizer inputs, without compromising final crop yield.

B3) Application of the HumeFlo / MacroPlex technology in a citrus field trial

The HumeFlo oxidation system drives the breakdown of carbonates to free-bound cations such as calcium, potassium and magnesium, where calcium is known to enhance aggregate formation. With more free cations in the irrigation water, it allows for the formation of aggregates, the process where negatively charged soil particles are clumped together with positively charged cations by the electrostatic forces, increasing pore size between soil particles and ultimately alleviating sub-soil compaction. In the citrus trial, the HumeFlo treated water contained higher concentrations of calcium, potassium and magnesium, and decreased carbonate levels, indicating effective oxidation. Sub-soil compaction was completely alleviated three months after the HumeFlo treatment. With the alleviation of sub-soil compaction, the root volume per 100 m of citrus row was calculated to have increased from 7.07 m^3 to 28.26 m^3. Conversely, the control section received untreated irrigation water and showed no improvement in subsoil compaction and root development. The significant increase in root volume obtained in the HumeFlo-treated trees indicates a high potential for increased nutrient uptake and ultimately less nitrate and phosphate leaching.

B4) Application of the FERT-ROOT soil biostimulants in laboratory-scale soil columns

The column tests were initiated after the Phillipi trial was terminated due to theft, in order to continue testing on the effectiveness of FERT-ROOT in the sandy soils of the region. Final results are not available yet and trials are ongoing. The technique still requires further optimisation for successful evaluation of the soil biostimulants' ability to immobilise nutrients.

3. CONCLUSIONS AND INDICATIONS FOR SCALE UP IN THE DEMO SITES

Based on the results obtained from the field trials, the FERT-ROOT technologies show promise for the mitigation of the leaching of agriculturally applied nitrate and phosphate to groundwater sources. The reduced soil compaction and better soil structure resulting from FERT-ROOT implementation promote better root development, enabling the extraction of more nutrients from the soil and leaving

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fewer nutrients to leach into groundwater. Additionally, the complexation or chelation of nutrients with the organic molecules induced by MacroPlex and the supply of soil biostimulants immobilise nutrients within the root zone, allowing for higher nutrient uptake by the better-developed roots, ultimately mitigating fertiliser leaching. The principle on which the FERT-ROOT technologies work does not accommodate well to small-scale assessment, especially the HumeFlo system, as it requires a minimum flow rate of irrigation water of 30 m^3/h. Small-scale pot trials are also unfeasible to assess the HumeFlo system as the preparation of soil/sand for pot trials disrupts soil structure and would not be an accurate representation of how the technology influences soil characteristics in the field. Therefore, the MAR2PROTECT FERT-ROOT tests were conducted mainly at the field level.

An important lesson learned from the SU field trials is that the weather conditions highly influence the successful implementation of both FERT-ROOT technologies, as wind speed at the time of irrigation and soil biostimulant application influences the delivery success rate of both the treated irrigation water and the soil biostimulants. As a result of the theft and vandalism that occurred in the Phillipi farm where the complete FERT-ROOT system (HumeFlo/MacroPlex + FERT-ROOT biostimulants) was tested at field level, two additional sites for field testing were identified, and the corresponding results will be presented in month 36. In this Deliverable, only the results relative to the field testing of the components of FERT-ROOT taken separately were presented (HumeFlo/MacroPlex in one field trial, FERT-ROOT biostimulants in another).

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5. CONCLUSIONS

The MAR2PROTECT partners conducted during months 1-18 an intense research activity in the framework of WP2, leading to the following preliminary conclusions.

A) Removal and biodegradation of (micro)pollutants from treated wastewater before their use for managed aquifer recharge.

Adsorption, tested in wastewater samples from Demo Sites 2 (Oued Souhil), 3 (Frielas) and 4 (Emilia Romagna), proved to be a very effective process for the removal of a large spectrum of PFAS and pharmaceuticals. In addition to commercial materials, such as activated carbon and polymeric resins, a wide range of innovative materials produced by MAR2PROTECT partners was tested: Molecularly Imprinted Polymers (MIPs), biochars obtained from agricultural residues, adsorbents obtained from agricultural biomasses and ionic liquids. So far, the best results were obtained with biochar derived from palm leaves (Demo Site 2), a sorbent derived from *Juncus Maritimus* (Demo Site 3) and activated carbon (Demo Site 4). An innovative approach to regenerate biomass-based saturated sorbents based on the use of lignin-lytic fungi is under development in Demo Site 2 with preliminary positive results. Thus, adsorption is considered suitable for scale-up in these 3 Demo Sites. The final choice of the materials to be implemented in each Demo Site will be made during months 19-30, on the basis of further tests conducted in continuous flow mode with real WWTP effluents.

Biodegradation was tested in wastewater samples from Demo Sites 3 (Frielas) and 4 (Emilia Romagna), targeting different pharmaceutical compounds: ibuprofen, diclofenac and carbamazepine. Different bioreactor types were tested:

- anaerobic-aerobic sequencing batch reactor: only limited biodegradation of diclofenac was attained;
- batch tests preliminary to the development of a membrane-aerated biofilm reactor: a consortium capable of rapidly degrading up to 10 mg/L of ibuprofen was developed;
- 3D printed biofilms: a consortium capable of rapidly degrading up to 6 mg/L of ibuprofen was developed, but the results are similar to those of the non-3D printed indigenous consortium of Demo Site 4.

On the basis of these results, there is a preliminary indication that these approaches for pharmaceutical biodegradation are not yet ready for scale-up in an operational environment. However, this point will be further assessed in month 24 on the basis of the latest research results.

Hybrid Constructed wetlands were tested on synthetic wastewater mimicking the actual WW of Demo Site 2. Preliminary results indicate the attainment of high removals of BOD, COD, suspended solids, ammonium, nitrate and total coliforms. This technology appears to be suitable for scale-up in Demo Site 2.

The implementation of **nanofiltration and reverse osmosis** for the desalination of brackish wastewater is under development for a potential implementation in Demo Site 2. The pilot plant is powered by a photovoltaic system. Preliminary results indicate salt rejections in the 92-95% for NF, and 97-99% for RO. Energy consumption is in the 1.4-4 kWh/m^3 range, depending mainly on water salinity. The 34 m^2 photovoltaic system resulted to be sufficient to provide in all seasons the energy required by the desalination process in the case of WW with a salinity of 2.6 g/L. Conversely, the energy required by the desalination of water with 5 g/L of salts was completely supplied by

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the PV panels only during the summer. The development of an innovative approach for brine phytodesalination through the irrigation of halophytes is in progress.

B) Removal and biodegradation of (micro)pollutants from surface waters before their use for managed aquifer recharge.

IHE and DU implemented two technology trains for the treatment of lake water in Demo Site 1 before its use for MAR: **a) coagulation/flocculation + dissolved air flotation + sand filtration (+ adsorption on activated carbon, *ongoing*); b) coagulation/flocculation + dissolved air flotation + ultrafiltration + reverse osmosis**. Both treatment trains were rapidly scaled up at pilot scale (16 m3/d combined capacity) during months 1-18. Both treatment lines showed promising results in terms of removal of assimilable organic carbon, phosphate and suspended solids. The UF/RO treatment allowed to attain a higher removal of suspended solids and particulate and colloidal matter. The final choice between the two alternative treatment lines will be made on the basis of i) the produced water quality for infiltration and ii) the performance of the two treatment lines in terms of reliable, and robust operation.

CIIMAR is developing in Demo Site 7 an innovative **phytoremediation/revegetation** approach for the decontamination of surface water and sediments in a saltmarsh. Two types of saltmarsh plants were tested, J. maritimus and P. australis. The lab-scale tests indicate that the integrated action of plant uptake, adsorption on sediments and biodegradation by the rhizosphere microbial community is very effective in the removal of both pharmaceuticals (ketoprofen and venlafaxine) and heavy metals (Cu and Cd), with marked accumulation of Cu and Cd in the tissues of both plants. The results obtained during months 1-18 indicate that this technology is suitable for scale-up in Demo Site 7.

C) Reduction of fertilizer leaching into groundwater: the FERT-ROOT technology

Unfortunately, the main field test for the assessment of the integrated FERT-ROOT technology (HumeFlo / MacroPlex irrigation water treatment system + The FERT-ROOT soil biostimulants AminoFix-3000 and Nitro-15) did not lead to any result, due to theft and vandalism that occurred in the selected farm. However, the field assessment of the components of FERT-ROOT taken separately yielded very positive results: the application of HumeFlo/MacroPlex in a citrus field test led to a marked increase of root volume and of the crop capacity to uptake cations and anions, and the application of the FERT-ROOT biostimulants in a strawberry field test led to a strong accumulation of nitrate in leaves and a marked increase in crop yield. In addition, laboratory column tests for the assessment of the FERT-ROOT biostimulants and a new field test for the assessment of the integrated FERT-ROOT technology (HumeFlo / MacroPlex irrigation water treatment system + The FERT-ROOT soil biostimulants AminoFix-3000 and Nitro-15) were set up. The results will be presented at the end of the second reporting period.

In relation to the upcoming scale-up of technologies in the Demo Sites, the results presented in this Deliverable show that in Demo Sites 1 and 5 the technologies were already scaled up in operational environments with encouraging results. In addition, technologies that are almost ready for field implementation in Demo Sites 2, 3, 4 and 7 have already been developed and identified: adsorption, constructed wetlands, desalination, and phytoremediation/re-vegetation. As for Demo Site 6, dedicated to the field assessment of managed aquifer recharge by Cetaqua, no implementation of water treatment technologies before water use for MAR is planned, according to the project's Grant Agreement.

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