DEMOWARE

Deliverable D2.4
Good practice summary for biofilm mitigation in networks

The project “Innovation Demonstration for a Competitive and Innovative European Water Reuse Sector” (DEMOWARE) has received funding from the European Union’s 7th Framework Programme for research, technological development and demonstration, theme ENV.2013.WATER INNO&DEMO-1 (Water innovation demonstration projects) under grant agreement no 619040

Deliverable Title	D2.4 Good practice summary for biofilm mitigation in networks. Performance assessment of CO_2 injection in agricultural irrigation networks and recommendations for biofilm control in urban networks
Related Work Package:	WP2: Process monitoring and performance control
Deliverable lead:	HQuimia/CTM
Author(s):	Ángel Teno (Hidroquimia), Carmen Biel (IRTA), Xavier Martínez (CTM), Montse Calderer (CTM), Irene Jubany (CTM), Carme Bosch (CTM)
Contact for queries	Hidroquimia Tractaments i Química Industrial SLU Orbital 40. Parc Audiovisual de Catalunya Ctra 1274 Km. 1 Ed. Nord L. O22 08225-Terrassa (Barcelona) T +34 93 700 14 81 Fundació CTM Centre Tecnològic, Plaça de la Ciència, 2 08243 Manresa (Barcelona) T +34 93 877 73 73 E irene.jubany@ctm.com.es
Dissemination level:	Public
Due submission date:	31/10/2016 (M34)
Actual submission:	31/12/2016 (M36)
Grant Agreement Number:	619040
Instrument:	FP7-ENV-2013-WATER-INNO-DEMO
Start date of the project:	01.01.2014
Duration of the project:	36 months
Website:	www.demoware.eu
Abstract	This report includes results from the Task 2.4 (subtasks 24.1 and 24.2) developed within the FP7 DEMOWARE project, including two case studies in two different demonstration sites: Torre Marimon and Sabadell, both in Spain.

Table of contents

List of figures

List of tables

Glossary

BES	Biofilm electrochemical signal
BOD	Biological oxygen demand
CFU	Colony-forming units
COD	Chemical oxygen demand
CU	Coefficient of uniformity
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
GW	Groundwater
HPC	Heterotrophic plate count
LRV	Log reduction value
LSI	Langelier Saturation Index
MBR	Membrane Bioreactor
MPN	Most probable number
NGS	Next generation sequencing
n.a.	not analysed
n.d.	not detected
O&M	Operation and maintenance
OTU	Operational taxonomic unit
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
RW	Reclaimed wastewater
SAR	Sodium adsorption ratio
TDS	Total dissolved salts
TIC	Total inorganic carbon
TKN	Total kjeldahl nitrogen
TOC	Total organic carbon
TSS	Total suspended solids
UV	Ultraviolet
VSS	Volatile suspended solids
WW	Wastewater
WWTP	Wastewater treatment plant

Executive Summary

Bacterial biofilms represent a real problem in water distribution facilities, for this reason, proper operation and maintenance is required to control and remove them.

In this sense, the present work evaluates the use of different maintenance strategies to mitigate biofilm in water distribution networks for urban and agricultural applications. The study was performed in two different DEMOWARE demonstration sites, Sabadell and Torre Marimon.

On the one side, in Sabadell demo-site, a pilot membrane bioreactor (MBR) was installed in Riu Sec wastewater treatment plant (WWTP) together with a pilot pipe network, simulating an urban reclaimed wastewater application. The effluent from the pilot MBR was split in 4 different treatment trails: (1) disinfection with sodium hypochlorite, (2) disinfection with sodium hypochlorite plus ultraviolet, (3) disinfection with ultraviolet, and (4) no disinfection treatment. Results indicated that only treatment trails based on the use of sodium hypochlorite could decrease the potential of biofilm growth on pipes. Besides, the use of an electrochemical sensor to predict biofilm growth was investigated. The use of the electrochemical sensor ALVIM, gave promising results under constant flow conditions.

On the other hand, in Torre Marimon demo-site, a pilot irrigation network fed with non-disinfected reclaimed wastewater (RW) from Caldes de Montbui WWTP was installed simulating an agricultural reuse application. Four different effluents were tested for their potential to form biofilms and scaling on pipes and drippers: (1) RW, (2) RW with CO_2, (3) RW with nitric acid, and (4) groundwater from Torre Marimon. Results indicated that the use of reclaimed wastewater led to a better drip irrigation performance, in terms of clogging and drip uniformity, and less scaling formation compared to groundwater. Moreover, treatments with nitric acid and CO2 reduce scaling formation and inactivate microbial pathogens.

1 Introduction

1.1 Fouling formation in reclaimed wastewater distribution and storage facilities

Water distribution facilities require proper operation and maintenance to maintain reliable service to water users. Operation and maintenance (O&M) of water distribution systems include periodic flushing of pipelines to maintain water quality and regular checking of disinfection residuals throughout the system to prevent biofilm formation, besides other activities intended for other purposes.

One of the main problems that O&M face is the formation of fouling in the overall water distribution system, including pipelines, valves, pumps, and storage reservoirs. Fouling refers to the undesirable formation of inorganic and/or organic deposits. Scaling or mineral fouling refers to the deposition of inorganic substances and organic fouling or biofouling to the deposition of organic substances (humic substances, proteins, etc.) with adhesion of micro-organisms. These deposits can induce water quality changes, increase the rate of corrosion at the surface, and increase the fluid frictional resistance. There are several types of fouling and combinations thereof: crystalline or precipitation fouling, corrosion fouling, particulate fouling, chemical reaction fouling, and biological fouling (biofouling).

Fouling formation may be magnified in reclaimed wastewater because the concentrations of dissolved nutrients, salts and residual organic matter usually are higher than in potable water from freshwater resources (Parsons, 2010).

1.2 Water quality management in reclaimed wastewater distribution and storage

Fouling, either from an inorganic or organic nature, is directly related to the quality of feed water. The main water quality parameters that influence the potential of fouling formation are the following (Asano, 2007; Nakayama and Bucks, 1991):

- Physical: temperature and suspended solids.
- Chemical: pH and alkalinity variations, ion composition, total dissolved solids (TDS), reduction of disinfection residuals
- Biological: bacteria, humic substances, algae, nitrogen and phosphorous species, residual dissolved organic carbon (DOC).

An increase of water temperature may occur during storage or pipeline circulation and may accelerate chemical and biological temperature-dependent reactions, for instance, bacterial regrowth in distribution pipelines, algae growth in open reservoirs, salt precipitation or chlorine residual reduction. Suspended solids and particulate matter may narrow flow paths and lead to clogging phenomena.

The formation of scale in water distribution networks is a significant technical, aesthetic and hygienic problem. There are different factors affecting scale formation such as concentration of salts, flow velocity, water temperature and pH of the flowing water. Hydrodynamic parameters such as Reynolds number and shear stress also influence the scale formation. Build-up of scaling in pipes, plumbing fittings and drippers can cause them to clog partially or completely, making them partially or totally dysfunctional (Varaprasad, 2012).

Bacterial growth and regrowth is also a significant water quality problem in most water reuse applications. Bacterial regrowth can occur if the residual disinfectant levels are allowed to fall to low levels in the distribution system. The reduction in disinfectant concentration is caused by the presence of low concentrations of residual organic matter in the reclaimed wastewater, slime build-up on distribution piping, and

exposure to the atmosphere in enclosed and open reservoirs. If bacteria are allowed to grow, the potential for biofilm formation in water distribution systems increases.

Biofilms are composed of bacteria held in a polymeric matrix. The bacteria within these biofilms have a number of effects on the quality of the water. They can be the starting point of proliferation of bigger organisms, affect turbidity, taste, odour and colour of the distributed water, lead to possible health risks and foster corrosion of pipe materials (Hallam et al., 2001). Furthermore, they can cause O&M problems including clogging of sprinklers and drippers in irrigation applications, and an excessive pipeline flushing and storage pond maintenance.

1.3 Options for fouling mitigation in reclaimed wastewater distribution and storage facilities

There are several O&M solutions for the control of bacteria regrowth in distribution systems (Asano et al., 2007):

- Operations management: increase disinfectant residual, decrease residence time, increase turnover in storage facilities, and isolate and disinfect problem area in pipelines.
- Maintenance: conduct periodic unidirectional or zone flushing to remove sediment, scour biofilm from pipe walls with a pig, check presence of sediment in storage facilities.

The most common biofouling control procedure is the use of chlorine as disinfectant. Chlorine oxidizes biofilm polymers causing disruption and partial removal in the shear stress field. Inactivation of a portion of the microbial population also occurs. Altered biofilm ''roughness'' and decreased viable cell numbers will influence "regrowth" rates of the biofilm. Chlorine is also capable of accelerating corrosion processes. Other oxidizing biocidal procedures are: ClO_2, peracetic acid, ozone, hydrogen peroxide, photocatalytic TiO_2 and ultraviolet irradiation (Asano et al 2007).

In contrast, acidification and/or the use of sequestering agents are the most common methods to reduce scale formation. As pH increases the rate of scaling increases, therefore acidification to pH 6.2-6.5 by continuous acid addition helps to reduce mineral deposit to almost completion.

The present report will focus on the formation of biofilms in water distribution systems, mainly those that deliver reclaimed wastewater for urban and agricultural applications, and possible maintenance strategies to mitigate this formation.

2 Objectives

The main objective of this report is to demonstrate the technical feasibility of different mitigation measures to avoid the formation of biofilms and clogging in water distribution systems. The activities involved in this report were developed in the framework of the DEMOWARE project (subtasks 24.1 and 24.2), undertaken at field scale at the following demonstration sites:

- Demonstration study in Sabadell, Spain. Use the effluent of a pilot membrane bioreactor (MBR) for an urban application using two different disinfection strategies for biofilm mitigation: Ultraviolet (UV) and chlorination.
- Demonstration study in Torre Marimon, Spain. Use of secondary treated WW for an agricultural irrigation application using the following clogging mitigation measures: CO_2 and nitric acid in comparison with the use of groundwater.

Figure 1 shows the overall scheme of both demonstration sites with their main features.

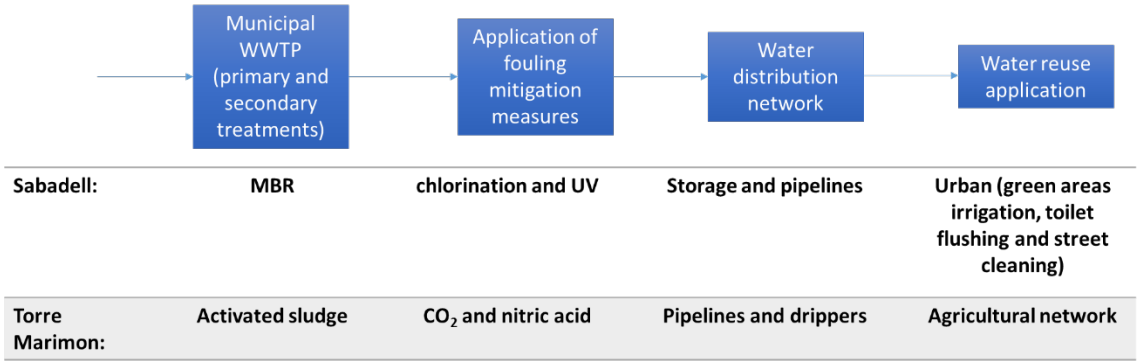

Figure 1 Water distribution networks and main features in the case studies of Sabadell and Torre Marimon

Specifically for the demonstration study in Sabadell, the feasibility of an electrochemical sensor applied as a non-invasive technology to observe biofilm growth has been assessed.

3 Assessment of maintenance strategies in distribution networks for urban application

3.1 Introduction and specific objectives

The main objective of this task is to evaluate the effect of different maintenance strategies on the quality of reclaimed water and its potential to form biofilm in distribution networks for urban application.

All water distribution networks can be affected by the growth of biofilms, this fact could be especially important when using reclaimed water with a certain content of nutrients. Biofilm on pipe walls in water distribution systems are composed of bacteria in a polymeric matrix, which can lead to chlorine demand, coliform growth, pipe corrosion and water taste and odour problems (Hallam, 2001).

In this sense, specific objectives of this task are:

- Study the correlation between reclaimed water quality and biofilm formation.
- Test different disinfection strategies to minimize biofilm formation in pipes.
- Demonstrate the feasibility of an electrochemical sensor as a non-invasive technology to observe biofilm growth and derive appropriate maintenance measures.

The study of maintenance strategies in urban distribution networks was performed in the DEMOWARE demonstration site in Sabadell.

3.2 Site description

Sabadell is a city of 200.000 inhabitants located in the metropolitan area of Barcelona (Catalonia) and crossed by two small rivers (Riu Sec and Riu Ripoll). Drinking water is mainly obtained from the Llobregat River (85%) and from groundwater (15%). Wastewater treatment is performed in two wastewater treatment plants (WWTP), the Riu Sec WWTP and the Riu Ripoll WWTP. CASSA is the company in charge of the whole water management in the city.

Since 2003, Sabadell has promoted the use of non-potable water for uses such as street cleaning or urban irrigation (with groundwater) or re-establishing the ecological flow of Riu Ripoll (with treated water from the Riu Ripoll WWTP). Reclaimed wastewater is currently being used for urban purposes, mainly street cleaning and green areas irrigation and for commercial uses such us toilet flushing in a commercial area. However, an ambitious wastewater reuse program is planned. New uses are being promoted in order to supply golf courses in the region for irrigation. For this purpose, a separate distribution network has already been constructed (25 km of non-potable water network).

In DEMOWARE, the study site is the Riu Sec WWTP, located at the south west of the city. This plant has a primary, secondary and tertiary treatment in order to produce reclaimed water (Figure 2). The treatment system has a design capacity of 2500 m^3/h (21.9 hm^3/yr) and features flat-sheet membrane bioreactors (MBR) followed by disinfection based on UV irradiation and hypochlorite dosing.

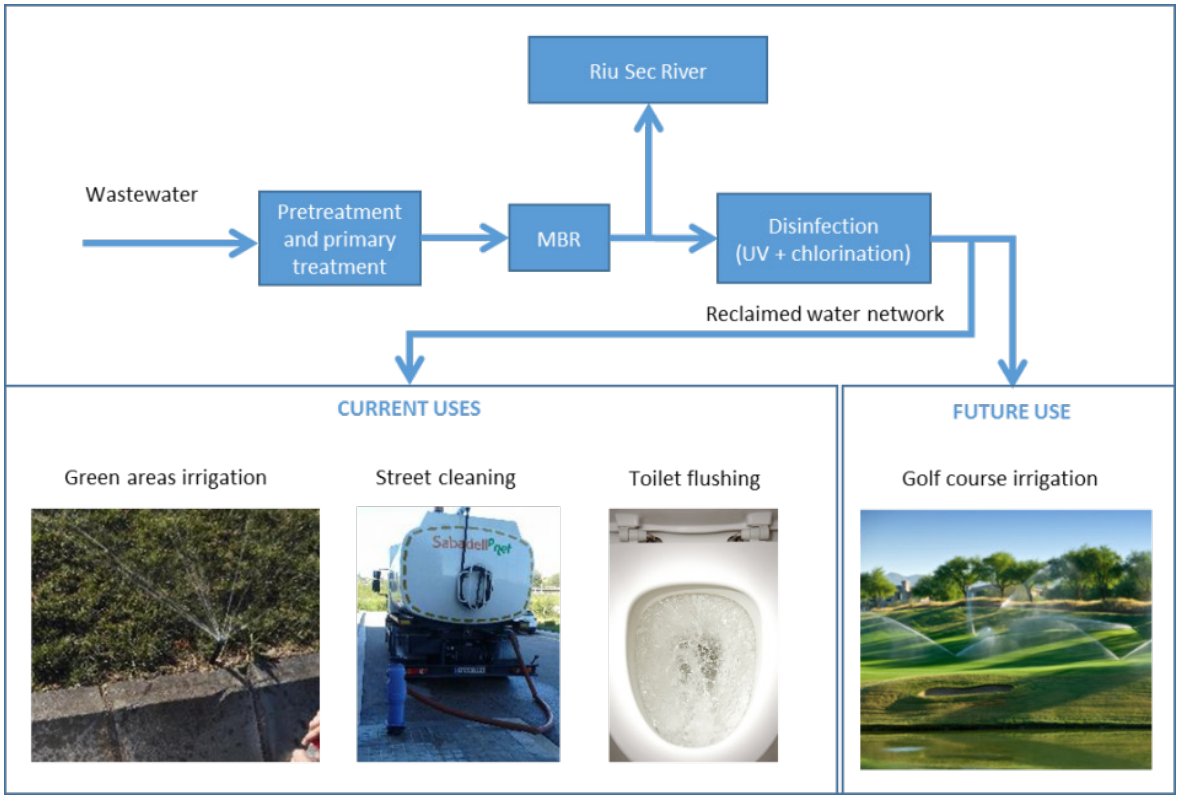

Figure 2 Simplified flow sheet of Riu Sec WWTP and current and future reclaimed water uses

In this task, a pilot plant was installed in Riu Sec WWTP. Effluent of the primary treatment of the WWTP was used as influent of the pilot plant. The pilot plant consisted of a MBR, effluent storage tanks with experimental disinfection treatments and an experimental urban distribution network. Figure 3 shows the location of the pilot plant.

Figure 3 Experimental pilot system site: WWTP Riu Sec (Sabadell)

3.3 Pilot plant

3.3.1 Membrane bioreactor

The pilot MBR plant was designed to treat a flow of 2 m^3 per day. Feed water was taken from the primary reactor of the Riu Sec WWTP and stored in a tank to allow constant flow to the pilot MBR (see Figure 4, feed area).

The pilot MBR consisted of two different chambers. The first one was the anoxic chamber where denitrification took place. This chamber was equipped with a stirrer and a REDOX sensor to monitor the denitrification process. The second one was the aerobic chamber, where the oxygen concentration was maintained between 2 and 4 mg/l for proper organic matter degradation. This chamber was equipped with two independent membrane systems, three blowers and five air diffusers. One of the blowers was used for the aeration of the chamber, while the other two were involved in the membrane cleaning.

Besides, the pilot MBR included a pump involved in the recirculation and purge of the activated sludge. The objective of this pump was to differentiate the cellular retention time from the hydraulic retention time. The purge was done with a three way valve, which was opened in one direction for the recirculation of the sludge and to another when the purge was activated (see Figure 4, MBR system area).

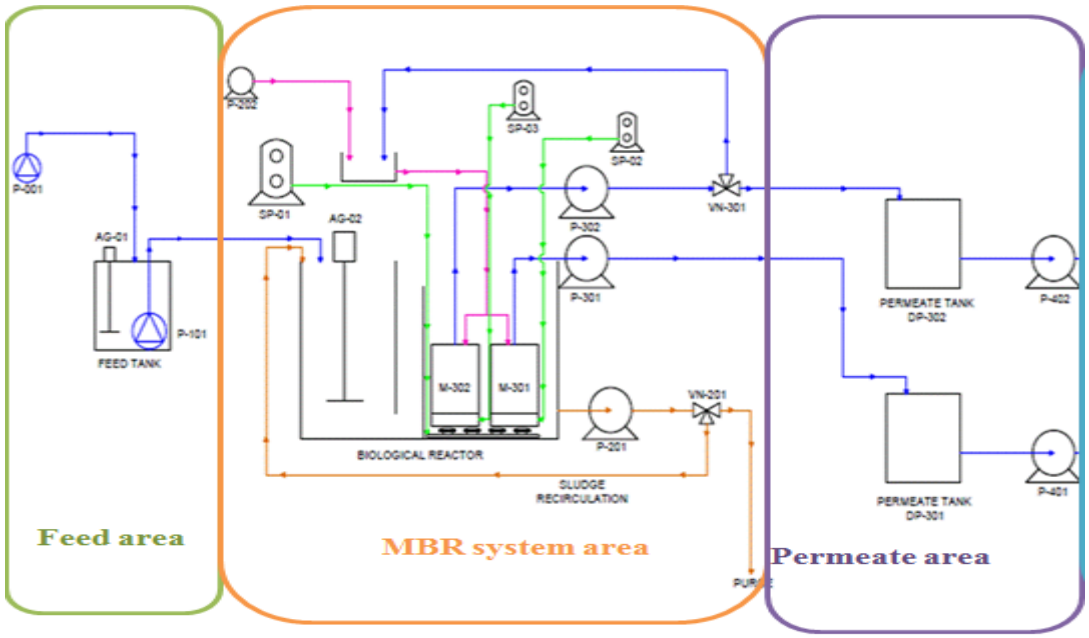

Figure 4 Layout of the pilot MBR installed at Riu Sec wastewater treatment plant

It should be stressed that two independent membrane modules were installed in the aerobic chamber to obtain two different effluents from the MBR and evaluate the effect of membrane integrity on water quality (Deliverable 2.2). Both membrane modules worked continuously during all the whole period of pilot plant operation. The characteristics of the membrane modules are detailed in Table 1.

Table 1 Specifications of the membrane modules installed in the pilot-MBR

Manufacturer	Martin Systems, siClaro®
Module	FM611
Membrane area	6.25 m^2
Membrane material	Polyethersulfone
Membrane type	Ultrafiltration
MWCO	150 kDa
Pore size (nominal / maximal)	35 nm / 100 nm
Dimensions (L x W x H)	404 x 291 x 1099 mm
Gaps between membrane sheets	6 mm

The average permeate flow was 100 l/h. Both membrane systems were also provided with a vacuum manometer in order to control the suction pressure of the pump when they were working.

The permeate was stored in two different tanks, one for each filtration line (DP-301 and DP-302) (see Figure 4, permeate area). Figure 5 shows pictures of the different elements of the MBR pilot plant.

Figure 5 Pictures of feed storage tank (left), the MBR aeration tank (middle) and one of the permeate tanks (right)

3.3.2 Disinfection lines

Permeate of the two membrane modules was disinfected differently as to produce four different water qualities which were stored in individual tanks

- UV disinfection + chlorination (tank 501)
- UV disinfection (tank 502)
- Chlorination (tank 503)
- no disinfection (tank 504)

Water from each permeate tank was discharged through a pipe, of which one was equipped with a UV light device for water disinfection. Each line fed two storage tanks, where tentative additional disinfection by chlorination was provided. A schematic of this set-up is illustrated in Figure 7.

Chlorination was done using sodium hypochlorite. The concentration of sodium hypochlorite in the storage tanks was determined as free chlorine by means of a REDOX sensor; the sensor was connected to a

peristaltic pump which added the disinfectant to the water when it was under the desired level of free chlorine. In addition once a week the chlorine level was determined following the method ISO7393-2:1985 which is a photometric method, the sensor was adjusted when necessary.

UV disinfection was performed with a low pressure UV lamp (Sterilux MINI 1000) installed in the permeate line DP301. The system was designed to irradiate the water with a minimum of 25 mW·sec/cm^2 at 98 % transmissivity of water; the flow rate of the pump P-401 (1000 l/h), assures that the time of irradiation of the water is enough, due to the specifications sent by the provider, in order to assure a correct disinfection in the system. However, after some time of operation (see Table 2) it was observed that irradiation was insufficient and UV connection was modified adding a recirculation pump of the permeate to increase water irradiation.

3.3.3 Urban network

After disinfection water was released from the storage tanks (501 to 504) through a 400 m polyethylene pipe of ¾" diameter to simulate a real urban pipe with reclaimed water (pipes 501 to 504). Figure 6 shows pictures of the different elements of the pilot distribution network including storage tanks, 400-m pipes and chlorination system.

Figure 6 **View of 400-m pipeline and storage tanks (left) and detail of chlorination system installed in tanks 501 and 503 (right)**

To summarize, Figure 7 presents a schematic picture of the full pilot plant, including the MBR, the permeate tanks, the storage tanks and pipes.

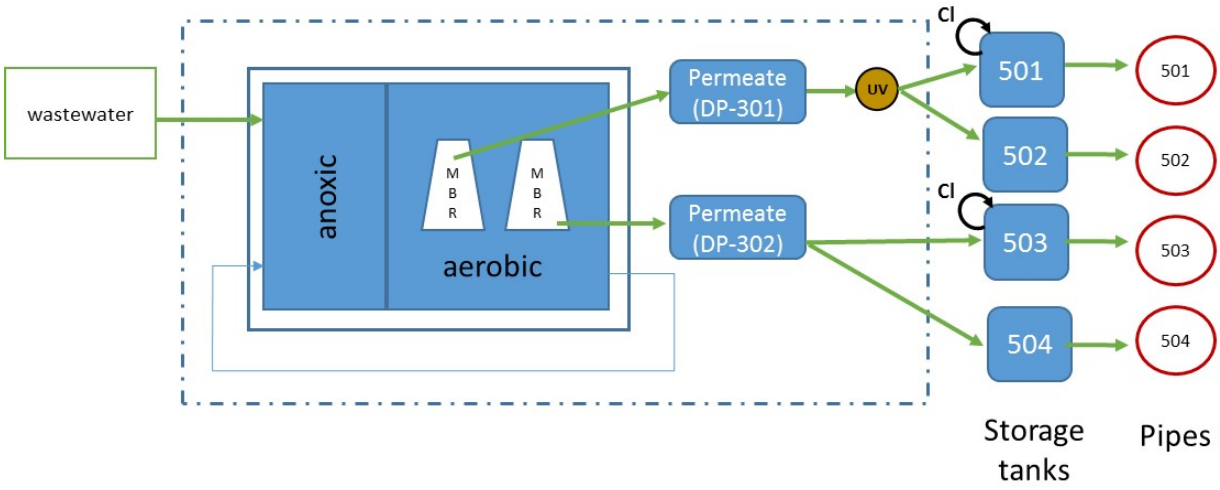

Figure 7 Flow diagram of the whole pilot plant installed in Riu Sec WWTP

The operation of the pilot plant was controlled by SCADA system which logged data of the online sensors and controlled the pumps and valves (see Annex 7.1)

3.4 Pilot plant operation

The pilot plant was operated for 261 days. During this period 2 m^3 of reclaimed water were produced per day (1 m^3 by each membrane module). Each storage tank held 500 l of permeate or disinfected water. The tanks were directly connected to their network pipe so the tank started to fill when the network pipe was full of water. Once all storage tanks were filled, valves at the end of the pipes opened and released the water. The emptying by gravity lasted 4 hours and drained the total storage tank volume (500 liters), in this way the network pipe was always filled with water (stagnant or flowing). In summary the process was 20 hours of filling the tank and 4 hours to emptying by gravity through the network pipe.

Plant operation was divided in two different test periods during which different disinfection strategies were applied, as summarised in Table 2.

Table 2 Characteristics of 1st and 2nd test period

Characteristics	1st test	2nd test
Duration (days)	158	89
Date (from - to)	12/11/2015-18/04/2016	02/05/2016-30/07/2016
Chlorination	0.5 mg/l free chlorine	0.8 mg/l free chlorine
UV	26.56 mW·s/cm^2	110.65 mW·s/cm^2

At the end of 1st test, at day 158 of operation a general disinfection of the whole pipe network was performed, including permeate pipes, storage tanks (501 to 504) and 400-m pipes (501 to 504). Two different disinfection reagents were used sodium hypochlorite and hypochlorous acid for line DP-301 and DP-302, respectively.

The procedure for cleaning and chemical disinfection of the deposits and pipes using sodium hypochlorite and hypochlorous acid was as follows:

- Chlorinate the permeate storage tank with sodium hypochlorite at 52.5 mg /l of free residual chlorine at a temperature not exceeding 30 ° C and a pH of 7-8, the concentration of free chlorine is 37 mg/l if the disinfectant used is hypochlorous acid so the oxidation potential of this acid is much higher. Then turn on the pumps of the circuit ensuring that all the chlorinated water is flowing for all the circuit.
- Control the concentration of free chlorine at the end of the circuit (irrigation pipe). Once the concentration of free chlorine is less than 25 mg/l empty the circuit and start again.
- Once the concentration of free chlorine at the end of the circuit has raised to 25 mg/l maintain 4 hours the chlorinated water in the circuit.
- Neutralize with sodium thiosulfate the amount of free residual chlorine and empty all the circuit.

3.5 Monitoring and analytics

3.5.1 MBR performance

In order to verify the proper operation and degradation performance of the MBR a number of physico-chemical parameters were monitored once per week. Table 3 summarizes the parameters, analytical methods and water samples analysed during the whole pilot plant operation.

Table 3 Physico-chemical analyses of the MBR part of the pilot plant

Parameter	Feed water	Reactor sludge	Permeate line 1	Permeate line 2	Analytical method
pH	X		X	X	ISO10523:2008
Conductivity	X		X	X	UNE EN 27888:1994
Total suspended solids (TSS)	X	X	X	X	UNE EN 872:2006
Volatile suspended solids (VSS)		X			UNE 77032:2015
Chemical oxygen demand (COD)	X		X	X	UNE 77004:2002
Biological oxygen demand (BOD)	X		X	X	UNE 77003-89
Total Kjeldahl nitrogen (TKN)	X		X	X	UNE 25663:1994
Ammonium nitrogen ($N\text{-}NH_4^+$)	X		X	X	UNE ISO 11905-1:1998
Nitrate			X	X	ISO10304-1:2007
Phosphorous	X		X	X	ISO 10304-1:2009

3.5.2 Microbial parameters

3.5.2.1 Analysis of water samples

Table 4 summarizes microbiological parameters and samples analysed during the whole pilot plant operation.

Table 4 Microbiological analyses in water samples

Parameter	Permeate line 1	Permeate line 2	Storage tanks (501-504)	Pipelines (501-504)	Analytical method
Total viable bacteria (Heterotrophs)	X	X	X	X	(Hallam, *et.al*, 2001)
Biofilm				X	(Hallam, et.al, 2001 and Van der Kooij et.al, 2005)
E. coli			X		EN ISO 9308-1:2000
Flow cytometry	X	X	X	X	IRSA method
PMA-qPCR method			X	X	BlueBiolabs method

Samples for analyses of heterotrophic bacteria, biofilm in pipelines, E. coli and flow cytometry were collected at different time intervals during the 1st and the 2nd test. Specifically, 4 sampling campaigns were done in each test. In the 1st test samples were collected at days 14, 33, 84 and 151 of pilot plant operation and in the 2nd test samples were collected at days 0, 49, 63 and 72 of pilot plant operation.

Samples to analyse microbial community through BlueBiolabs method were collected during the 2nd test, at day 63 of operation.

For total heterotrophic bacteria determination 500 ml water samples were collected at the outlet of module 1 (Pipeline 301) and module 2 (Pipeline 302) of the pilot plant, at storage tanks (501-504) and pipelines (501-504). Water samples previously disinfected with sodium hypochlorite were collected in sterile bottles containing thiosulphate (0.5 mL of 1.8% thiosulphate solution) to neutralize any residual chlorine that could be present. Total heterotrophs were analysed by heterotrophic plate count (HPC) using R2A agar and a incubation time of 7 days at 22°C (Hallam, *et.al*, 2001). Each sample was tested in duplicate. Final heterotrophic bacteria concentration was reported as colony-forming units (CFU) per water volume as an averaged value of the duplicate analysis.

Escherichia coli was determined in water samples from the storage tanks 501-504. Water samples previously disinfected with sodium hypochlorite were collected in sterile bottles containing thiosulphate (0.5 mL of 1.8% thiosulphate solution) to neutralize any residual disinfectant. *E. coli* was determined by membrane filtration method according to the EN ISO 9308-1:2000.

For flow cytometry, water samples were fixed in formaldehyde solution (2% final concentration) and kept at 4°C upon the analysis. The aquatic microbial community was characterized by using the Flow Cytometer A50-micro (Apogee Flow System, Hertfordshire, England) equipped with a solid-state laser set at 20 mV and tuned to an excitation wavelength of 488 nm. The volumetric absolute counting was carried out on fixed samples, stained with SYBR Green I (1:10000 dilution; Molecular Probes, Invitrogen) for 10 min in the dark at room temperature. Samples were run at low flow rates to keep the number of events below 1000 events/s. Thresholding was carried out using the green channel. Light scattering signals (i.e., forward and side scatter), green fluorescence (530/30 nm), and red fluorescence (>610 nm) were registered

for the characterization of each single cytometric event. By using fixed gates, single cells (prokaryotes and protozoa) and suspended particles (abiotic debris) were identified according to their green fluorescence signals and side scatter. The instrumental settings were kept the same for all samples in order to achieve comparable data, in accordance with previous published protocols (Gasol and Moran, 2015). The Apogee Histogram Software (v89.0) was used for data handling and visualization.

3.5.2.2 Biofilm analysis

Samples of pipes from the pipe network were collected for biofilm analyses. Pipes were cut with a circular saw and the biofilm was collected by swabbing the entire surface of the pipe (Hallam, *et.al*, 2001 and Van der Kooij et.al, 2005). Swabs were immediately transferred into a volume of 10 ml of ¼ strength Ringer solution. Bacteria extraction was done by vortexing vigorously the swab to release the bacteria into the sterile Ringer solution.

The number of heterotrophic bacteria of the solution was counted using HPC method, as previously described. Final bacterial concentration was reported as colony-forming units (CFU) per unit area of pipe surface, which was determined for each piece of pipe.

3.5.2.3 Biofilm growth potential

In order to collect biofilm samples for analysis by molecular biological methods developed in the DEMOWARE project, glass beads carrier were exposed in the water storage tanks (501 to 504) for two weeks during the 2nd test (from day 49 until day 63 of operation). Likewise, 100 ml of water from pipes were collected and filtered at day 63 of operation. Carriers and the biofilm grown on it and filters were shipped for further processing to partner BluebioLabs in cool (with ice packs) and damp conditions for.

The samples were treated with PMA (final concentration 50 µM) and irradiated with blue light for 15 minutes (performed by Blue Biolabs). The EURx GeneMATRIX Soil DNA Purificatio Kit was used to extract the DNA. The Kit comprises of a mechanical and chemical cell lysis step. By vortexing the glass beads, the attached biofilm on the biofilm carriers was removed in a physiological buffer. 500 µl of the bacteria suspension were used for the following DNA extraction step. For the DNA-Extraction of filtrated samples the filter was put in bead tubes, which were included in the EURx GeneMATRIX Soil DNA Purification Kit. The DNA was eluted in elution buffer and stored at – 20 °C. After that, the DNA samples were amplified by qPCR, using the EURx SG qPCR Master Mix (Roboklon) and the CFX96 Touch Real Time PCR Detection System (BioRad), with species specific primer sets for the following organisms:

- *Legionella sp*.
- *Pseudomonas sp.* 1
- Mycobacteria
- Clostridium
- *Escherichia coli*

In addition a universal bacterial primer set, which has been used to analyze the whole bacterial population, also was used.

3.5.3 Electrochemical sensor for biofilm monitoring

Biofilm formation in pipe networks may pose an operational and health issue in water reclamation systems. Hence, biofilm control and removal is usually done by chemical disinfection. A more closely observation of biofilm growth would offer potential to optimise pipe disinfection protocols by applying the reagents at the moment that biofilm appears.

Several biofilm sensing techniques have been used throughout the years as light scattering, turbidity and electrochemical impedance, but these techniques present different limitations such as the low sensitivity or the fact that they cannot discriminate the biological and inorganic fouling (Pavanello et al., 2011).

Within this work, a study of available sensors in the market to monitor biofilm in water networks was performed to select a suitable device for testing.

3.5.3.1 ALVIM sensor

From sensors found in market (see Annex 7.2), the ALVIM sensor was chosen in this project (Figure 8). Selection of ALVIM sensor was based on the fact that it could detect biofilm in early stages, it work on real-time and it was designed to evaluate the effectiveness of disinfection strategies.

AVLIM sensor bases its work in a cathodic depolarization induced by the biofilm growth. This principle has been studied over the years by different scientific authors, and it was proved that the electrochemical activity of aquatic biofilm is proportional to the surface area covered by bacteria. So measuring the biofilm electrochemical signal (BES) is possible to know the biofilm growth.

The ALVIM sensor is a three electrodes system, in which the zinc counter-electrode plays also the role of pseudo reference. Connected to the zinc and to the stainless steel working electrode where the biofilm growth is evaluated there is an acquisition system, composed of three main parts: the first for substratum conditioning, the second for signal transduction and elaboration, the third for data transmission over local/GSM/GPRS network. The BES (expressed as current density or potential), measured in real time can be registered in several intervals of time and then this data are sent to a data base in order to study the process.

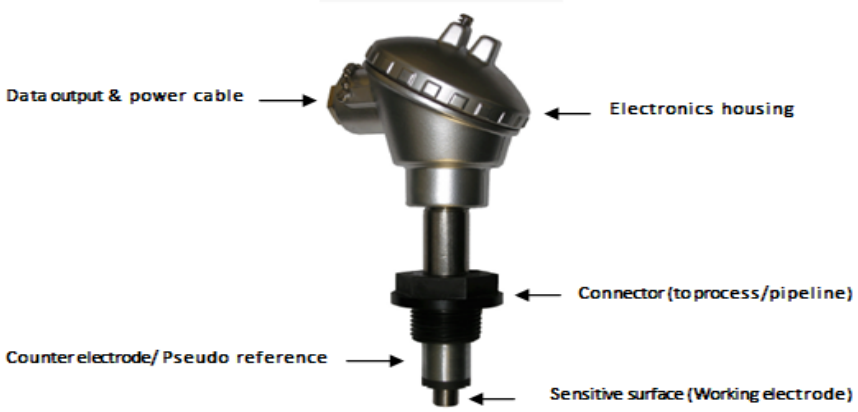

Figure 8 ALVIM probe main parts

As commented before, ALVIM sensor works by basing its measures in the cathodic current density. The variation of this parameter measured at a given time on a stainless steel sample can be expressed by the following equation:

$$i(E,t) = i_1(E) + [i_2(E) - i_1(E)] \times \theta(t)$$

Where E is the potential, t is the time, I (E,t) is the cathodic current density, $i_1(E)$ is the current density measured on the clean fraction of the stainless steel surface and $i_2(E)$ is the one measured on the surface fraction $\theta(t)$ [$0 \leq \Theta \leq 1$] covered by biofilm.

Based in this working principle it is possible to analyse the evolution of the signal during the biofilm growth. Figure 9 shows the evolution of the cathodic curve in the biofilm growth process. Curve 1 shows

the performance at the beginning of the stainless steel in an aerated seawater solution. Curve 4 shows this stainless steel completely covered with biofilm. Curve 2 and 3 show intermediate conditions. This figure shows how the variation of the cathode current is only a function of biofilm growth (Pavanello, et al. 2011).

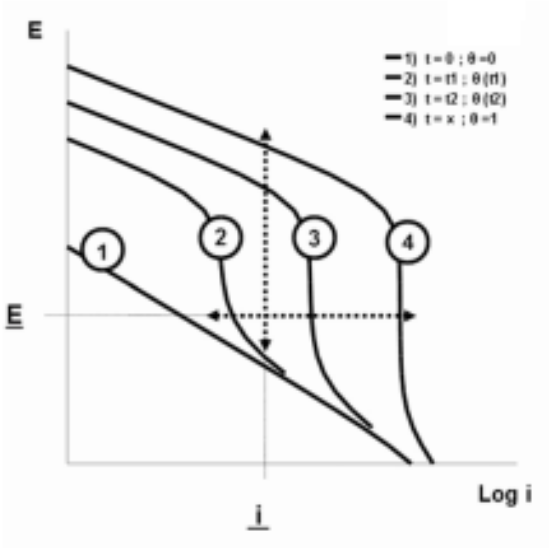

Figure 9 Evolution of the overall cathodic curve during the gradual development of biofilm on a stainless steel surface (Pavanello, et al. 2011)

Figure 10 shows the general tendency of the variation of the potential (E) at a fixed cathodic current (i) during biofilm growth. This figure presents a sigmoid curve which rises rapidly in a relative narrow time reaching a stationary state (Pavanello, et al. 2011).

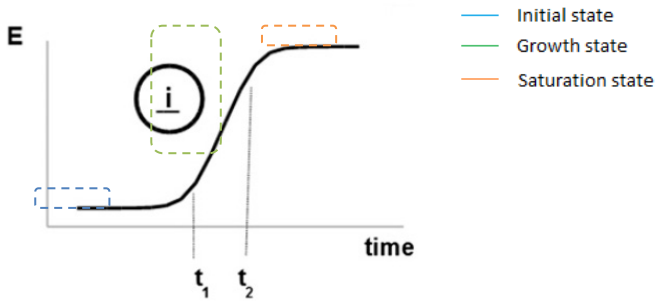

Figure 10 Evolution of the potentials measured at a fixed cathodic current during the biofilm growth (Pavanello, et al. 2011)

3.5.3.2 ALVIM laboratory tests

ALVIM sensor was tested in the laboratory before its implementation in the pilot plant. In particular, two different laboratory experiments were carried out to validate sensor performance and fully characterize its sensibility.

In a first experiment, the sensor was installed in a 3L vessel filled with a secondary effluent (non-disinfected MBR permeate) from Riu Sec WWTP and continuously agitated with a mechanical stirrer. Besides, several polypropylene pipe pieces (2x2 cm) were placed at the bottom of the vessel to allow biofilm growth over it. The experiment was maintained during 45 days at a constant temperature of 35ºC (Figure

11). During all the experiment, the electro-signal BES from ALVIM was automatically registered every 15 minutes and twice a week a pipe piece was removed for biofilm analyses.

The method for biofilm analyses was based on the swabbing protocol and HPC method previously described in 3.5.2.2

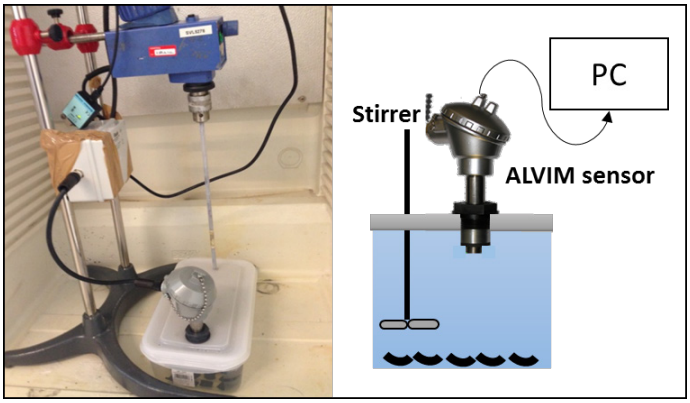

Figure 11 First ALVIM experimental set-up

Since results from the first experiment were not as expected, a second experiment at lab scale was set up. In this second experiment, a tank filled with primary effluent (i.e. effluent after the primary treatment) from Riu Sec WWTP was used to continuously feed the 3L vessel where ALVIM sensor was installed (Figure 12). Therefore, the ALVIM sensor was in contact with a constant water flow that ensures constant oxygen level and simulated real biofilm growth conditions in pipelines. Again, polypropylene pipe pieces (2x2 cm) were placed in the vessel to allow biofilm growth. The experiment was maintained at a constant temperature of 35°C during 72 days. At day 26, 1 g of sodium acetate was added to the primary effluent to promote bacterial and biofilm growth and, at day 70 disinfection of the primary effluent with sodium hypochlorite was applied to study the effect on the sensor signal.

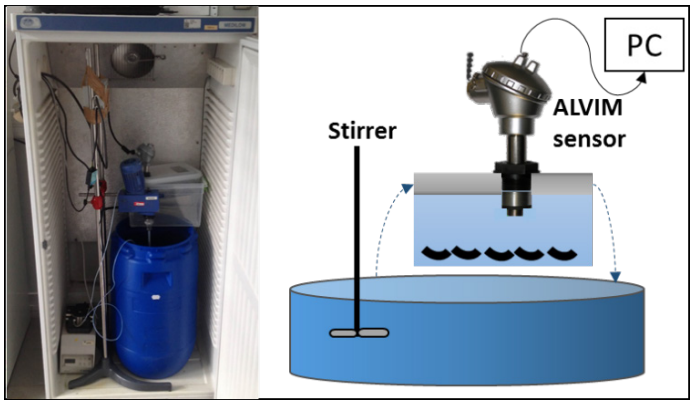

Figure 12 Second ALVIM experimental set-up

3.5.3.3 ALVIM pilot tests

After laboratory tests, the biosensor was installed in Sabadell WWTP under two different operational conditions. First, the sensor was installed in pipe 504 transporting non-disinfected reclaimed wastewater. Second, a portion of the outflow effluent of MBR of the Riu Sec WWTP before disinfection step, was

pumped and channelled throw a PVC pipe (DN75mm) where the ALVIM sensor was installed. In this way, the use of the sensor in two different systems was investigated:

- Discontinuous flow and low water flow for agricultural irrigation systems by discharge from reservoirs direct to drips
- Continuous flow and high water flow for irrigation system by sprinkler.

Table 5 summarizes the operational conditions of both tests.

Table 5 Operational conditions ALVIM test

Characteristics	Discontinuous flow conditions	Continuous flow conditions
Type of water discharge	Discontinuous	Continuous
Location	Pipe 504	Effluent Riu Sec WWTP
Water flow velocity	0.05 m/s*	0.32 m/s

*during time of discharge only

The ALVIM device requires a minimum diameter for installation. For this, a PVC pipe of DN75mm with connection for coupling in the network of experimental pipes was used. The PVC pipe had a length of 2,000 mm with threaded connections ½" at the end of the pipe (Figure 13). The ALVIM sensor was installed on a clamp made of polyethylene. The clamp was a 1 1/2" coupling thread to place sensor inside the tube (Figure 10).

When the sensor test was installed under continuous flow conditions, a self-priming pump was installed at one side of the DN75 pipe and sucted the water from the outflow of the full scale MBR, this water was returned to the WWTP through the other side of the DN75 pipe. The connection of the pump was made by a 1/2 inch so a PVC reduction of the pipe from DN75 to 1/2 inch was needed.

Figure 13 Installation of PVC pipe to the pilot pipe 504 and mounted ALVIM sensor (right)

3.6 Results

3.6.1 MBR pilot plant performance

As previously detailed, the pilot plant was operated for 261 days. During this period 2 m^3 of reclaimed water were produced per day. The pilot plant showed removal rates of about 98 % for TSS, 93 % for COD, 99 % for BOD and 66 % for TKN when averaged over the whole period of plant operation. Nitrification was incomplete with remaining 19 mg/l N-NH_4^+ in the permeate. Due to the lack of phosphorous precipitation in the MBR pilot, no P-removal was observed (Table 6).

Table 6 Average influent and effluent quality of the MBR pilot plant (n=32)*

Parameter	Units	Influent	Effluent (Permeate)
TSS	mg/l	142.6 ± 40.3	2.4 ± 1.9
COD	mg/l	506.2 ± 129.3	37.6 ± 14.5
BOD5	mg/l	382.4 ± 28.5	2.4 ± 2.1
TKN	mg/l	82.2 ± 9.4	27.6 ± 25.3
Ammonium nitrogen (N-NH_4^+)	mg/l	51.2 ± 5.7	19.0 ± 7.4
Nitrate (NO_3^-)	mg/l	n.a.	5.2 ± 0.6
Phosphorous(P_{tot})	mg/l	13.1 ± 2.4	12.1 ± 8.5
pH	-	7.3 ± 0.1	7.3 ± 0.5
Heterotrophic bacteria	CFU/ml	n.a.	$9.4 \pm 9.7 \cdot 10^4$ **

*n: number of samples, ** n=8, n.a.: not analysed

Figure 14 shows the percentage of reduction of TSS between the outlet and inlet of the MBR pilot plant. The removal of TSS was constantly around 98 % in both membrane modules, resulting in less than6 mg/l of TSS, on average 2.4 mg/l of TSS in the permeate during the whole operation period. It should be noticed that a foam episode occurred around the day 50 of operation, directly affecting the removal capacity of the membranes.

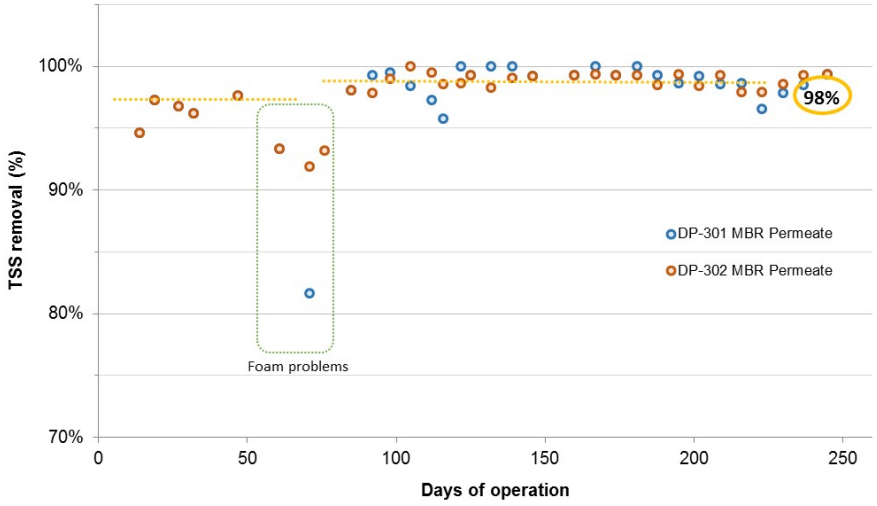

Figure 14 Percentage of TSS removal during MBR pilot plant operation

Removal of COD was around 93% during the whole operation period (Figure 15), however during the foam episode a sharp reduction of COD removal was observed.

Permeated water had an average of 37.6 mg/l of COD during the whole operation period. In the full scale WWTP plant, COD in the permeate is on average 23 mg/l.

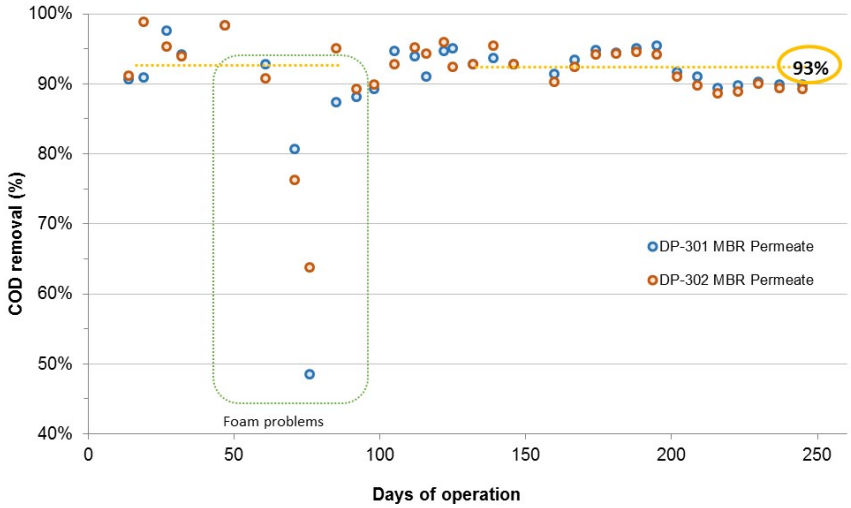

Figure 15 Percentage of COD removal during MBR pilot plant operation

The removal of TKN was initially around 91% but during the foam episode it decreased down to around 50%. After the foam episode, some technical problems with the recirculation pump were detected and TKN removal decreased to levels below 30%. During last 50 days of operation, TKN stabilized around 73% (Figure 16). This results in average in a concentration of TKN in permeate of about 28 mg/l during the whole operation period, whereas in the full scale WWTP concentration of TKN is always below 5 mg/l.

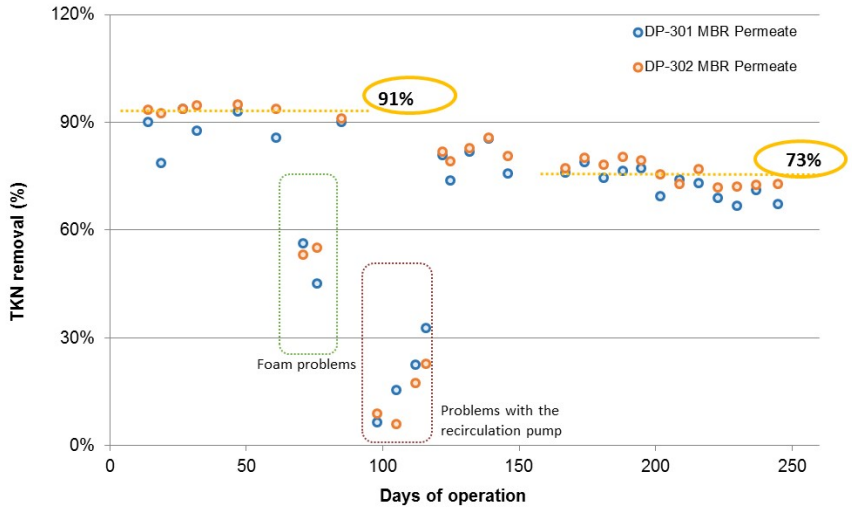

Figure 16 Percentage of TKN removal during MBR pilot plant operation

The MBR pilot plant was not designed for phosphate removal, thus phosphate in effluent directly depended on influent wastewater quality. As indicated in Table 6, average phosphorous concentration in

permeate was 12.1 mg/l, this value is quite high compared to the full scale Riu Sec WWTP which reduces phosphorous and produces a permeate with a concentration below to 1 mg/l. Phosphorous, together with nitrogen and organic matter are nutrients for microorganisms, favouring biofilm formation in reclaimed water networks.

With an average of 9.4 x 104 CFU/ml (n=8) there was quite a high number of heterotrophic bacteria in the effluent of the MBR pilot plant (Table 6). Figure 17 shows results of heterotrophic bacteria in DP-301 and DP-302 permeates over MBR operation time. As it can be observed, slight differences were observed between DP 301 and DP 302 permeate, in average they presented 1.0 ± 7.9·104 (n=8) and 1.2 ± 7.8·104 (n=8), respectively. It should be mentioned that the permeate of the full-scale MBR at Riu Sec WWTP contains around 102 CFU/ml heterotrophic bacteria (result from 1 sample). The performance of the pilot membranes was validated by an integrity test and results showed that both membrane modules were intact.

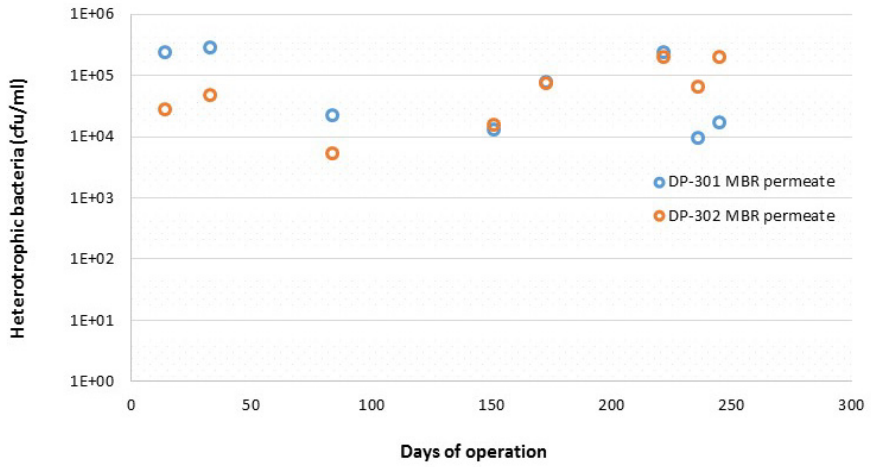

Figure 17 Heterotrophic bacteria in MBR permeate during MBR pilot plant operation

In summary, the permeate quality produced by the two modules of the MBR compared to the full scale Riu Sec WWTP is characterised by:

- High concentration of phosphorous since the pilot plant is not prepared for its removal.
- Variant nitrogen content due to operational problems that affected the biology of the reactor.
- High concentration of heterotrophic bacteria (average 9.4 x 10^4 CFU/ml).

All these characteristics can be linked to a high potential of bacteria and biofilm growth in this specific pipe network. In the next section, the effect of different disinfection strategies to reduce microbial contamination and mitigate biofilm formation is presented.

3.6.2 Effect of disinfection strategies on microbial water quality

As indicated in section 3.3.2, four different treatments were applied to the effluent of the pilot MBR:

- Treatment trail 1: UV disinfection + chlorination (tank 501)
- Treatment trail 2: UV disinfection (tank 502)
- Treatment trail 3: Chlorination (tank 503)
- Treatment trail 4: No disinfection (tank 504)

Their effectiveness in reducing microbial contamination is summarised in the following section.

3.6.2.1 Total viable bacteria (Heterotrophic plate count)

Heterotrophic bacteria in the permeate of the pilot MBR (DP301 and DP302 lines) were between $5.2 \cdot 10^3$ and $2.8 \cdot 10^5$ CFU/ml during the whole operation plant (1st and 2nd tests) (Figure 17).

In both test periods only disinfection with sodium hypochlorite considerably reduced total viable bacteria. However, difference on the sodium hypochlorite concentration used in both tests resulted in difference on the disinfection potential (see 3.6.2.2). Results from the 1st and the 2nd test are depicted in Figure 18 and Figure 19, respectively.

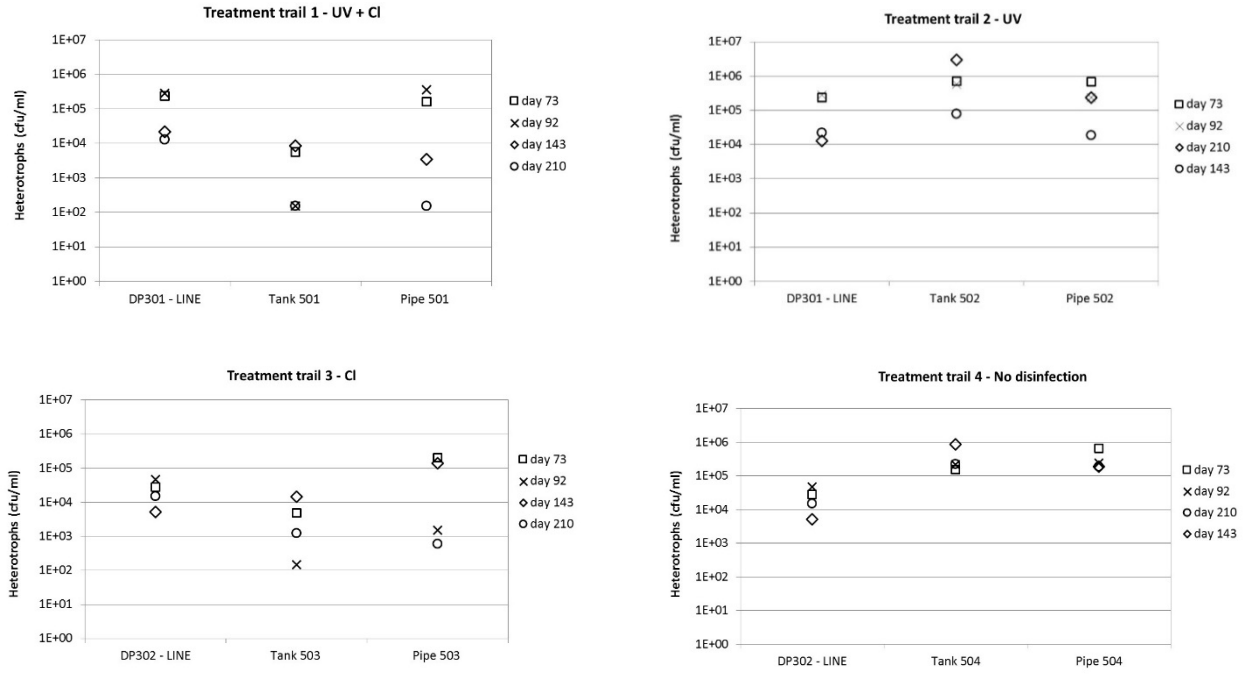

Figure 18 Heterotrophic bacteria along the different treatments trails at different monitoring campaigns in the 1st test

From results shown in Figure 18 and Figure 19, it can be observed that disinfection with sodium hypochlorite was more effective in the 2nd test, when the concentration used of sodium hypochlorite was higher. In the first test, decrease of heterotrophic bacteria was observed in Tanks 501 and 503 only in some sampling campaigns (day 92 and day 210), and usually a regrowth was observed in pipelines 501 and 503.

On the other hand, in the 2nd test, heterotrophic bacteria in tanks and pipelines 501 and 503 was always below the detection limit of 75 CFU/ml. Results from the 1st and 2nd test also indicate that treatment trail 2 with UV was not effective as a disinfection method in this application.

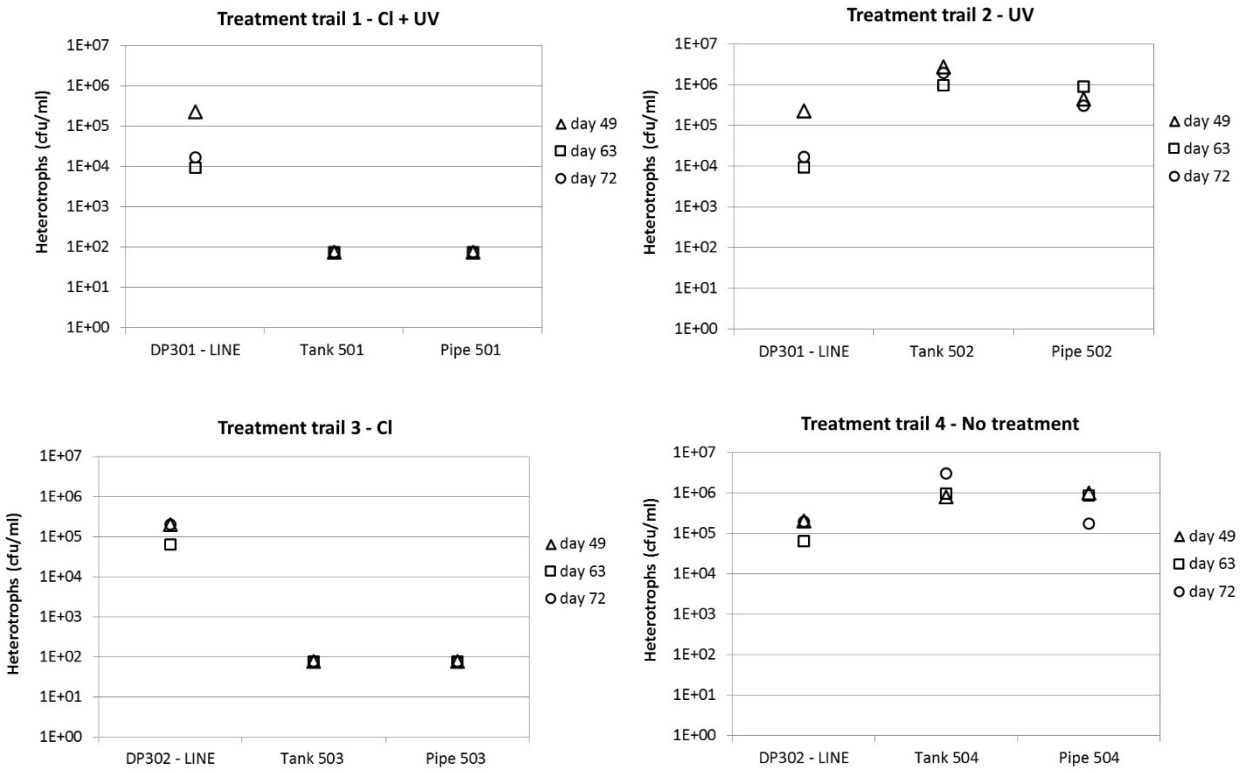

Figure 19 Heterotrophic bacteria along the different treatments trails at different monitoring campaigns in the 2nd test

The effect of disinfection during both tests was further evaluated through calculating the heterotrophic bacteria log reduction value (LRV) by the disinfection step as follows:

$$LRV = LOG10 \cdot \left(\frac{Heterotrophs\ in\ permeate}{Heterotrophs\ in\ storage\ tank}\right) \qquad (1)$$

Besides, the possible regrowth of heterotrophs in pipe network was further evaluated as follows:

$$Regrowth = LOG10 \cdot \left(\frac{Heterotrophs\ in\ pipe}{Heterotrophs\ in\ storage\ tank}\right) \qquad (2)$$

Results are presented in Table 7. As it can be observed, LRV clearly indicates that only in treatment trails using chlorination (1 and 3) an effective disinfection was achieved (LRV positive). A clear difference is observed between test 1 and 2. In test 1, using a lower chlorination dose, LRV obtained in treatment trails 1 and 3 are lower (1.8 and 1.0, respectively) than in test 2, where chlorination dose was increased (2.6 and 3.3, respectively). Regarding the use of ultraviolet together with chlorination (treatment trail 1) compared to the use of chlorination alone (treatment trail 3) it should be mentioned that similar results have been observed in both tests 1 and 2, indicating that probably the additional use of UV does not improve disinfection efficiency. On the other hand, when using ultraviolet alone (treatment trail 2), an increase of microorganisms is observed in the storage tank 502 in both test 1 and 2 (LRV negative). Finally, growth of microorganisms is also observed in Tank 504, where no disinfection treatment is applied.

Concerning regrowth, as it can be observed in Table 7, it should be mentioned that it was not important in any of the treatment trails from both tests, 1st and 2nd, regrowth values are in general around 0.

Table 7 Average LRV and regrowth in the 1st and 2nd test for the different disinfection strategies (n=4)

Treatment trail	Test 1		Test 2	
	LRV disinfection	Regrowth	LRV disinfection	Regrowth
1. UV + Cl	1.8 ± 1.2	1.5 ± 1.9	2.6 ± 0.7	n.d.
2. UV	-0.9 ± 1.0	-0.5 ± 0.4	-1.7 ± 0.5	-0.5 ± 0.4
3. Cl	1.0 ± 1.2	0.8 ± 0.8	3.3 ± 0.3	n.d.
4. No disinfection	-1.2 ± 0.7	0.0 ± 0.5	-1.0 ± 0.3	-0.4 ± 0.7

n.d.: not determined since heterotrophic concentration determined in pipes and tanks is below the detection limit

3.6.2.2 Effect of disinfectant dose

The effect of the different disinfectant doses applied in the two test periods is illustrated in Figure 20 which shows the concentration of heterotrophic bacteria in tanks. It was found that:

- chlorinated streams in the storage tanks showed lower heterotrophic bacteria than UV treated water differing by two orders of magnitude (some hundreds to ten thousands compared to hundreds of thousands to millions per ml)
- an increase of free chlorine in the storage tank from 0.5 ppm to 0.8 ppm resulted in HPC being approx. 1.5 orders of magnitudes lower (5.2 x 10^3 CFU/ml compared to less than 75 CFU/ml)
- with a more than 4-fold increase of the UV dose (from approx.. 25 to 110 mWs/cm2) the level of total counts was almost unchanged ($1.1 \cdot 10^6$ CFU /ml compared to $1.9 \cdot 1010^6$ CFU /ml)
- there were no distinct differences between tank 501 (UV+Cl) and 503 (Cl), indicating that the use of ultraviolet combined with hypochlorite did not increase the disinfection potential.

This finding is also reflected in the log reduction achieved by the different disinfection strategies in the two test periods (see Table 7). It should be mentioned that as the samples were only taken from the storage tank the findings do not necessarily correctly assess the effectiveness of the UV dose which might well result in inactivation of bacteria. However, due to lack of any residual disinfection potential the storage tank potentially acted like a "fermenter" where surviving organisms could grow due to presence of nutrients and organic matter made more easily available by UV radiation. Therefore, it can be stated that the use of UV is not appropriate for this application, or at least, it is not appropriate when initial effluent presents high concentration of bacteria and chemical characteristics that can promote regrowth (i.e. nutrients, organic matter, etc.).

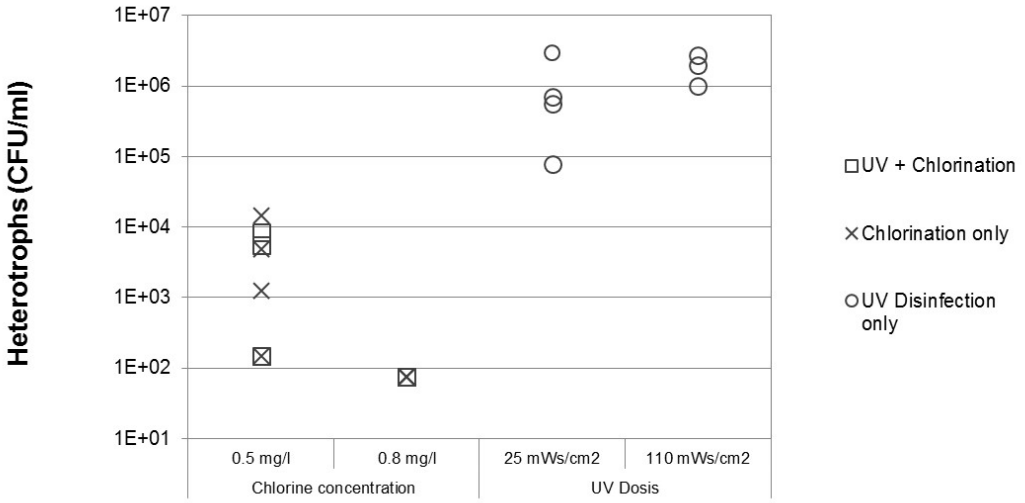

Figure 20 HPC in storage tanks holding differently disinfected water

3.6.2.3 Flow cytometry

Flow cytometry results were compared with the results of total viable bacteria obtained. Figure 21 shows flow cytometry results compared to heterotrophic bacteria results in the 1st test. As it can be observed, flow cytometry analyses gave consistent results with heterotrophic bacteria concentration, obtaining nearly a lineal correlation. However in the 2nd test this lineal correlation was not obtained (data not shown). No obvious reason for this mismatch was found, however, samples from the 2nd test were more murky than samples from the 1st test and the flow cytometry analyses turn out to be more complicated. This could be an explanation for difference observed between 1st and 2nd test but anyway, more research on this aspect should be done in order to validate the use of flow cytometry analyses for determining microbial population in reclaimed wastewater disinfected.

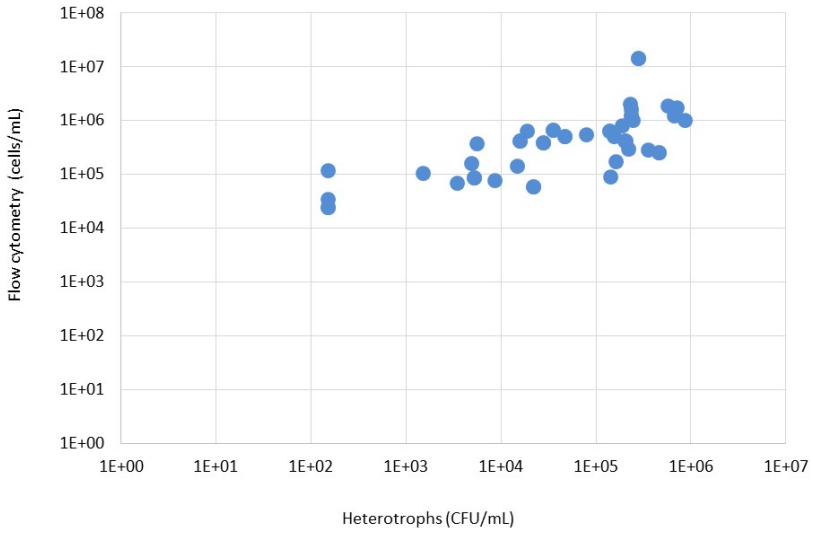

Figure 21 Flow cytometry results versus heterotrophic bacteria on the 1st test

3.6.2.4 Indicator organisms

3.6.2.4.1 E. coli

Figure 22 shows results of *E. coli* in storage tanks during the 1st and 2nd test of plant operation. As it can be observed tanks disinfected with chlorine (tank 501 and 503) present in general a smaller concentration than the other tanks. In fact, in all the sampling campaigns from the 1st and 2nd test E. coli was below the detection limit of 1 CFU/100ml in tanks 501 and 503, except in day 84 from 1st test where 15 CFU/100ml were detected in Tank 501.

Results of E.coli in tank 502 and 504 are more scattered, especially during the 2nd test, but in most of sampling campaigns present values higher than the detection limit. Average concentrations of E. coli in Tank 502 are 32±21 (n=4) and 950±974 (n=4) in test 1 and 2, respectively, and in Tank 504 25±40 (n=4) and 280±546 (n=4) in test 1 and 2, respectively.

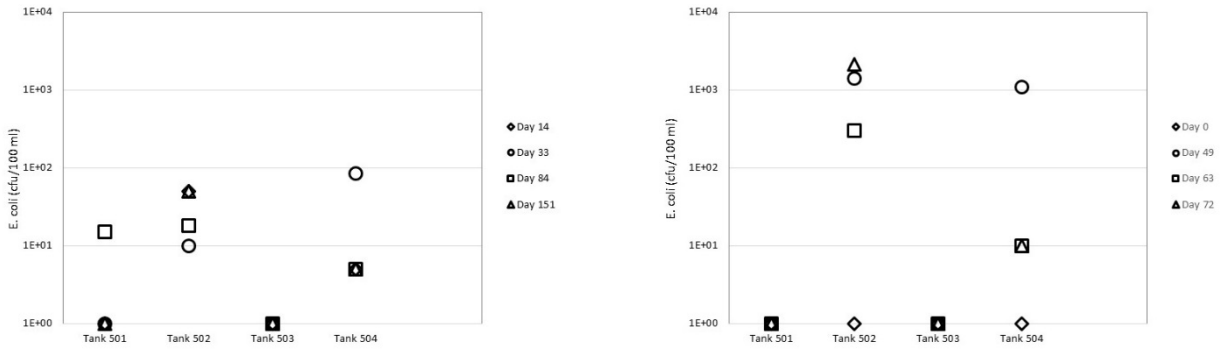

Figure 22 ***E. coli*** **in storage tanks at different monitoring campaigns in the 1st (left) and 2nd test (right) (LD: 1 CFU/100ml)**

3.6.2.4.2 Pathogens and other indicators

Figure 23 shows results of microbial characterization by PMA-qPCR in water in pipes at day 63 of the 2nd test. It should be mentioned that only one sample of water was collected for each pipeline, therefore these results pretend to be a first indication of the microbial population found in water from pipes but not many conclusions can be determined from the results.

In terms of total bacteria (universal primer set) no big differences were detected between pipes 501, 502 and 503 (approx. 300 DNA copies/µl). However, a somewhat higher concentration is observed in pipe 504 with no disinfection (1000 DNA copies/µl). It must be stressed that these results are not in line with the results of total viable bacteria, which clearly indicate differences in function of the disinfection strategy.

It is interesting to observe that in those pipes with chlorinated reclaimed water (501 and 503) *Pseudomonas sp.* present a higher concentration than in other pipes (502 and 504), probably due to chlorination has caused a change on the microbial population, favouring *Pseudomonas*, which seem to have a bigger resistance to chlorination. With many universal 16s rDNA primer sets it has to be taken into account that, while a high percentage of the known bacterial sequences can be amplified, certain species are not amplified with the same efficiency. In addition the qPCR method used in this study was no multiplex PCR and the DNA amplification for each primer set was performed in separate reactions. These two factors could explain why the results of the *Pseudomonas sp.* 1 primer set slightly exceeded those of the universal primer set in Pipe 502.

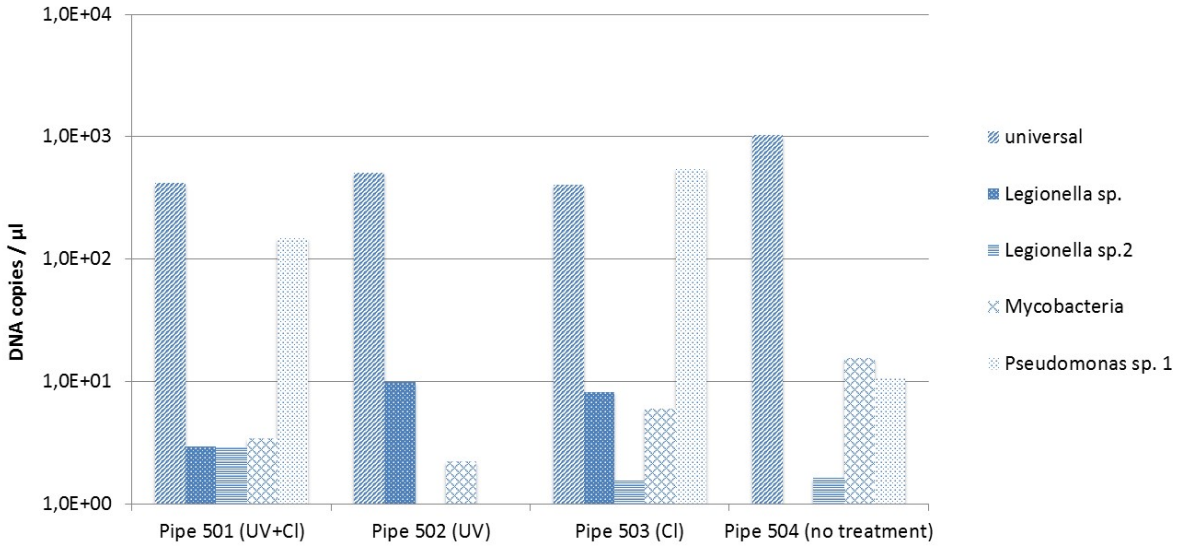

Figure 23 Detection (by qPCR methods) of different microorganisms in water from pipes

3.6.3 Effect of disinfection treatment on biofilm growth

Just like water quality, also biofilm growth was influenced by the disinfection treatment applied. Figure 24 shows biofilm growth in the different pipes (501 to 504) over time during the second testing period with day 0 representing the day of intensive cleaning. As it can be observed, biofilm was not detected in pipes with chlorinated reclaimed wastewater. In pipes 502 and 504 biofilm detected at day 49 remained stable until the end of the experiment at day 72. Therefore, main growth took place before 49 days of plant operation.

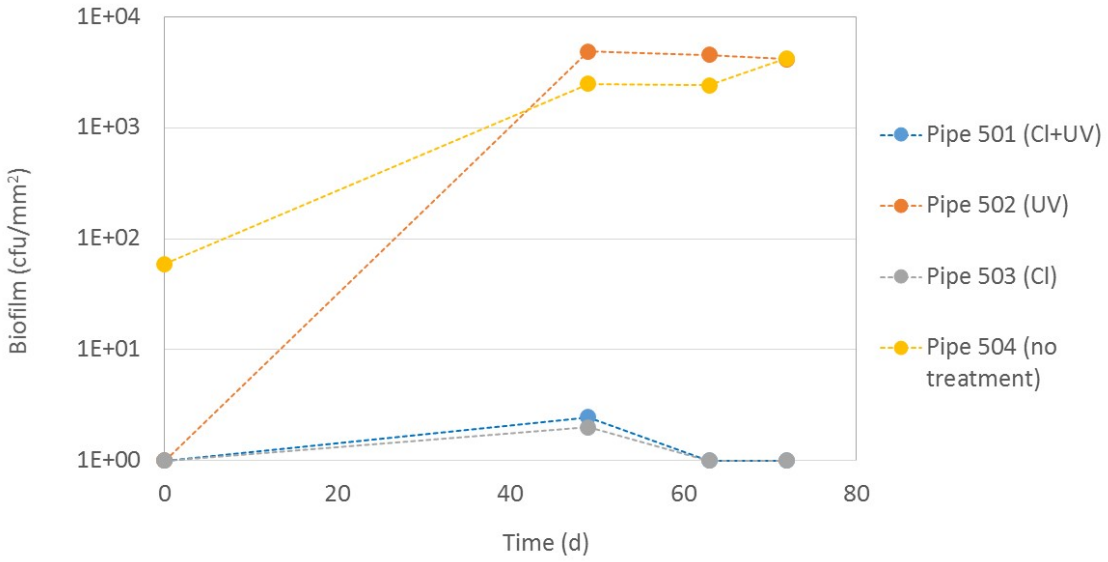

Figure 24 Biofilm growth over time in pipes. x-axis representing days passed since intensive cleaning

Once again, results show that the use of UV is not recommendable for this application, since it has not potential to avoid biofilm growth after its application. Anyway, it should be mentioned that the characteristics of the implemented pilot plant could favour bacterial regrowth in terms of chemical and biological characteristics of the effluent and the presence of storage tanks before pipelines.

In pipes receiving chlorinated water (501 and 503) no or minor biofilm growth was observed. The residual chlorine concentration found in these pipes was on average 0.05 mg/l and 0.22 mg/l during test period 1 and 2 respectively (0.5 mg/l and 0.8 mg/l free chlorine respectively).

Figure 25 presents results from biofilm analyses by qPCR in tanks. As previously commented, biofilm analysed refers to the biofilm grown in tanks during 2 weeks (from day 49 till day 63 of the 2nd test). Again results refer to only one sample, thus they are a first indication of the microorganisms forming biofilm in each tank, more samples would be needed to obtain conclusions.

As it can be observed in Figure 25, in terms of total bacteria (universal primer set), higher concentrations are detected in tank 502 disinfected with UV and in tank 504 where no treatment is applied. These results are in line with the results of heterotrophic bacteria previously commented (Figure 20). Among the specific species analysed, *Pseudomonas sp.* 1 is the one with higher counts, not only in the chlorinated tanks but also in the ultraviolet tank.

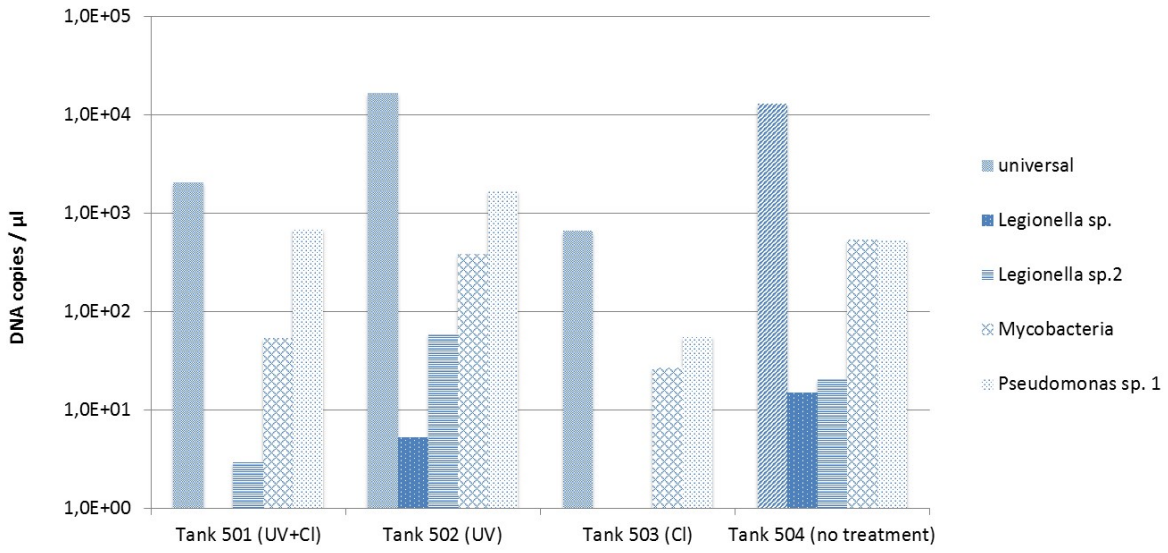

Figure 25 Detection (by qPCR methods) of different microorganisms in biofilms grown on carriers exposed in reclaimed water tanks

3.6.3.1 Network maintenance strategies

As previous commented, between 1st and 2nd test of pilot plant operation a general disinfection of the irrigation system with sodium hypochlorite and hypochlorous acid was done. Line 1, corresponding to pipes 501 and 502 was disinfected with sodium hypochlorite whereas, line 2, corresponding to pipes 503 and 504 was disinfected with hypochlorous acid. Figure 26 shows biofilm in pipes before and after the general disinfection. As it can be observed, both strategies of disinfection were able to eliminate the biofilm formed in pipes during 1st test.

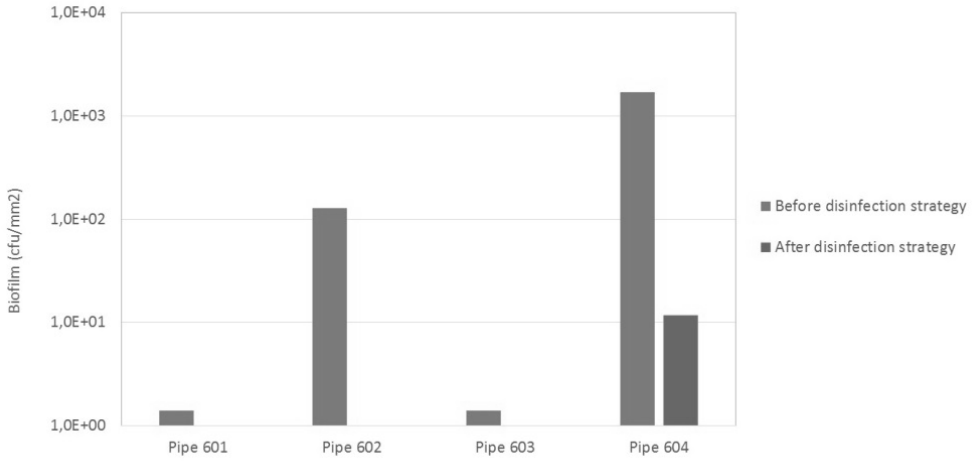

Figure 26 Biofilm in pipes before and after the disinfection

3.6.4 Electrochemical sensor for biofilm monitoring

3.6.4.1 Laboratory tests

Results from the first experiment at laboratory, where ALVIM was installed in a 3L vessel with secondary effluent from Riu Sec WWTP, show a matching trend between BES signal and biofilm growth in polypropylene pieces. However, it should be mentioned that the fact that water was stagnant produced low oxygen conditions in water that caused BES low levels (Figure 27). According to ALVIM's manufacturer BES baseline should be around 600-700 mV and an increase of at least 150 mV should be detected with biofilm growth.

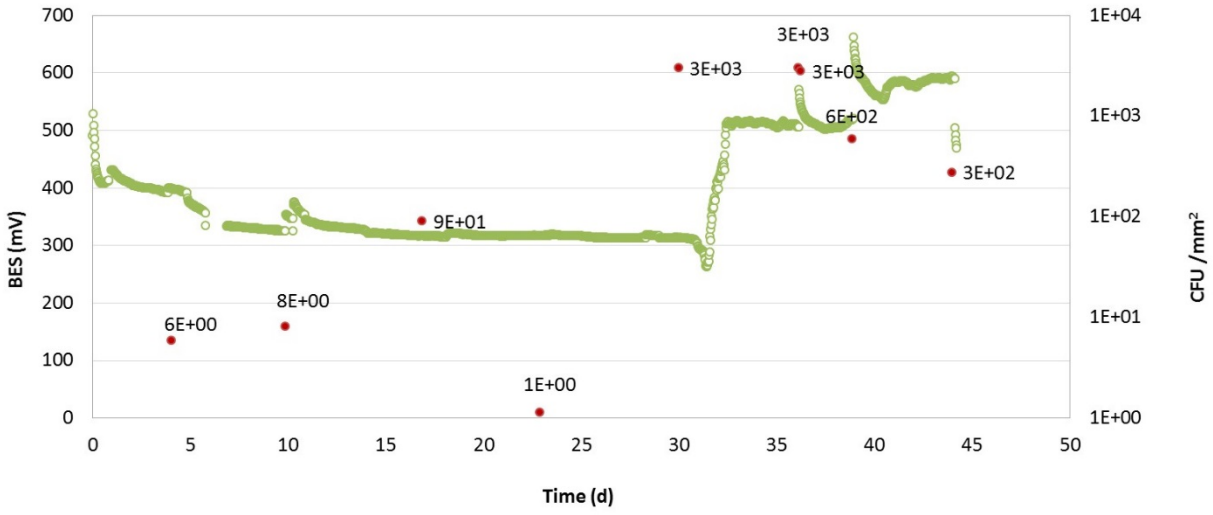

Figure 27 Results from ALVIM experimental set-up with un-renewed water

In view of these results, it was decided to set-up a second experiment at lab scale where ALVIM was installed in a vessel with a continuous flow of primary effluent from Riu Sec WWTP. In this case, primary effluent was used instead of secondary effluent in order to promote biofilm growth.

Results from this second experiment showed correlation between BES and biofilm growth in polypropylene pieces (Figure 28). During 56 days BES signal remained quite stable around 600-700 mV and biofilm in pipe pieces, measured as heterotrophic bacteria, was around 10^3 CFU/mm^2. After day 56, BES increased and exceeded the threshold of 750 mV indicated by the ALVIM manufacturer as an indicator of biofilm growth. At this moment, biofilm in pipe pieces also showed an increase up to 10^5 CFU/mm^2. Finally, it should be mentioned that the application of sodium hypochlorite resulted in a decrease of BES and biofilm in pipe pieces.

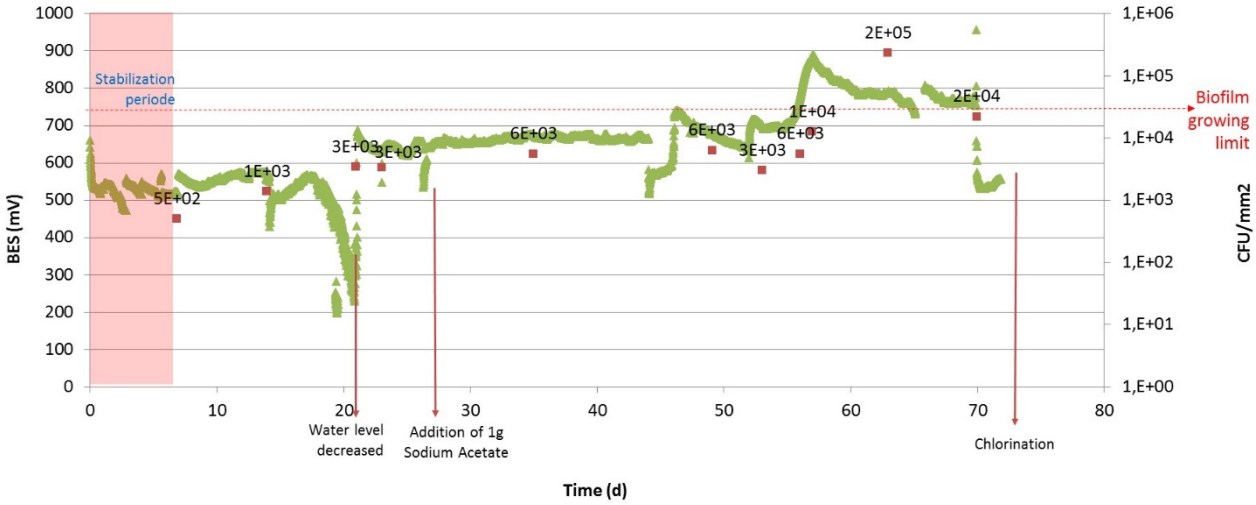

Figure 28 Results of ALVIM testing in lab. Experiments conducted with continuously renewed water

3.6.4.2 Pilot tests

Figure 29 shows the ALVIM signal obtained when installed under intermittent flow conditions in pipe 504 from day 0 to day 37 of the 2nd test. It should be mentioned that under this condition ALVIM was installed in contact with water with high concentration of nutrients and bacteria (see Table 6) thus, with a high potential to form biofilm.

As it can be observed in Figure 29Figure 29 ALVIM signal under intermittent flow conditions when installed in the experimental distribution network (pipe 504), a steady signal could not be achieved during all this period, probably due to the fact that the intermittent flow conditions resulted in a decrease of dissolved oxygen in water pipes which caused a daily drop in the ALVIM signal. As previously indicated, in view of these results and according to the indications of the manufacturer, the ALVIM was installed in constant flow conditions at the effluent of the full scale WWTP.

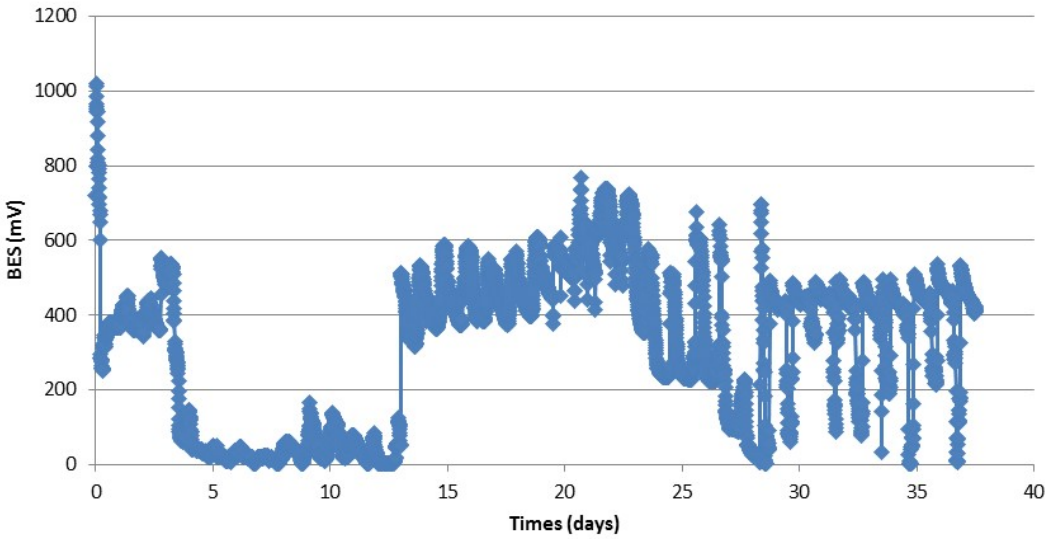

Figure 29 ALVIM signal under intermittent flow conditions when installed in the experimental distribution network (pipe 504)

Figure 30 shows the results of the electrochemical sensor when installed in the full scale Riu Sec WWTP. It should be mentioned that in this case the ALVIM was installed in contact with permeated water from the full scale plant, which contained lower concentrations of nutrients and viable bacteria. In average 23 mg/l COD, 5 mg/l TKN, <1 mg/l P and 10^2 CFU/ml.

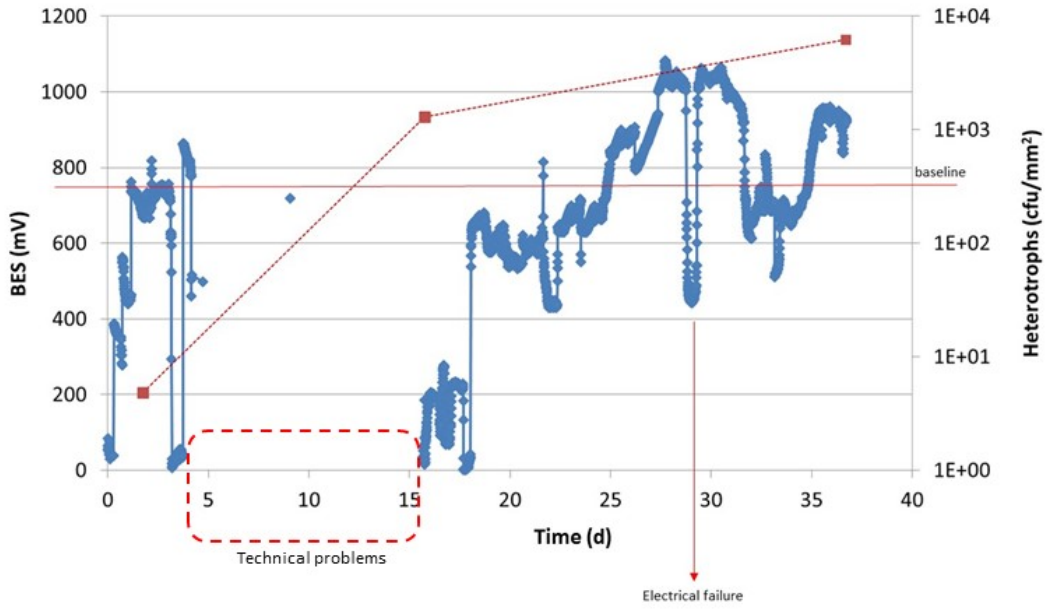

Figure 30 ALVIM signal under continuous flow conditions

As it can be observed in Figure 30, under these conditions it was also difficult to maintain a steady signal. However, signal drops were not so often and a baseline between 600 and 750 mV can be established during the first 25 days of the test. At day 25 a rapidly increased of the signal was observed, exceeding

the baseline of 750 mV. From this moment until the end of the test, signal remained in general higher than 750 mV, however, it should be mentioned that the signal was not completely stable.

The graphic also shows concentration of heterotrophs in pipe analysed through the swabbing method. These results indicate that biofilm growth took place during the whole testing period, from less than 10 to $6 \cdot 10^3$ CFU/mm^2 in 36 days.

The mismatch between ALVIM sensor and biofilm growth in pipes can be due to the fact of using different pipe section, fact that is related to a different water flow velocity and thus can influence to biofilm growth.

3.7 Conclusions

In this work the effect of 4 different maintenance strategies (UV + chlorination, UV, chlorination and no treatment) on the quality of reclaimed water obtained from a pilot MBR and its potential to mitigate biofilm formation in distribution networks for urban application was investigated.

The pilot MBR, installed in Riu Sec WWTP, was operated for 261 day. Reclaimed water obtained during the whole operation period, was characterized by a high concentration of phosphorous, nitrogen and viable bacteria compared to reclaimed water obtained in the full scale plant from Riu Sec. These characteristics might be linked to a high potential of bacterial growth and biofilm formation.

Main results from this task demonstrate that chlorination is the only disinfection strategy from the ones studied able to reduce bacteria in storage tanks and biofilm growth in pipes. Free chlorine concentration was linked to the disinfection potential, the use of the lowest concentration of 0.5 mg/l of free chlorine resulted in a LRV of 1.8 and 1 in treatment trail 1 and 3, respectively, whereas the use of the highest concentration of 0.8 mg/l of free chlorine resulted in a LRV of 2.6 and 3.0 in treatment trail 1 and 3, respectively.

The strategy based on UV (treatment trail 2) was not able to avoid regrowth of bacteria in storage tanks and biofilm formation in pipes at both doses tested 26.56 and 110.6 mW·s/cm^2. Therefore this disinfection strategy alone is not recommended for urban application, especially when initial water presents a high concentration of bacteria and a chemical characteristics (nutrient concentration etc.) that can promote biofilm growth.

The use of the electrochemical sensor ALVIM to predict biofilm growth was tested under laboratory and field scale conditions. Results at laboratory scale indicated that the sensor could correctly predict biofilm formation in pipes. However, at field scale, it was difficult to maintain a steady signal due to operational and/or environmental factors. Two different conditions were tested: intermittent and constant flow conditions. Under intermittent flow conditions ALVIM was not able to maintain a steady signal, probably due to the decrease of dissolved oxygen in water. Under constant flow conditions, ALVIM could maintain a more stable signal compared to intermittent flow conditions, but not conclusive results could be obtained.

Finally, the effectiveness of disinfection with sodium hypochlorite and hypochlorous acid (52.5 and 37.0 mg/l, respectively) on biofilm removal were tested. Results indicate that both strategies could remove biofilm attached in pipe networks.

4 Assessment of fouling control in irrigation distribution system with mineral acid and CO_2 injection

4.1 Introduction and specific objectives

Drip irrigation has been considered a very appropriate application use for reclaimed wastewater because there is no contact with plants and staff, does not form aerosols, and there is less percolation (Capra and Scicolone, 1998, 2004, 2005). In drip irrigation, emitter uniformity and flow rates are very important to ensure the correct water doses application. For this reason, fouling formation control is very important. Follow-up of individual drippers discharge should be performed in the field in order to evaluate irrigation performance, and for irrigation with reclaimed wastewater this assessment is even more needed.

Emitter clogging in irrigation is directly related to the quality of the irrigation water. There exist four interlinked mechanisms of clogging in drip irrigation (Bucks *et al.,* 1979; Nakayama and Bucks, 1981; Nakayama *et al.,* 2007):

1) particulate matter clogging narrow flow paths due to the presence of suspended solids in feed water;
2) scaling, due to formation/chemical precipitation of soluble salts at a concentration above the saturation product (e.g. carbonate, phosphate, sulphate);
3) adsorption, due to hydrophobic interaction of soluble or colloidal organic macromolecules (e.g. humic substances, soluble microbial products, cell debris) and
4) biological, due to biofilm formation and algal growth. The more acute form of fouling is the *in situ* formation of particulate material by supersaturation (scaling), hydrophobic interaction (adsorption) and biofouling (biofilm).

Water quality parameters that have high influence in dripper/emitter clogging are the following: total suspended solids (TSS), total dissolved solids (TDS), pH, bacterial counts and ion composition (Nakayama and Bucks, 1981, 1991; Capra and Scicolone, 1998; Nakayama *et al.* 2007). Fouling formation may be boosted in water reuse application because reclaimed wastewater probably contains higher concentrations of macro and micronutrients, dissolved salts and residual DOC than freshwater resources.

Levels of calcium, magnesium, and sulphate can be highly variable according to the source of the freshwater, the sewage origin (domestic, industrial or agricultural) and the treatment applied. The presence of nutrients and residual carbon may affect malodour generation and algal growth during storage and distribution as well as biofilm formation.

Table 8 **Main mechanisms of clogging of a drip irrigation system, denoting factor/consequence relationship**
Source Asano et al 2007; Bucks et al 1979; Nakayama and Bucks 1991; Nakayama et al 2007

Suspended material (particles and colloids)	Chemical precipitate (scaling)	Organic adhesion (adsorption)	Biological precipitate (biofouling)
Inorganic particles	Salts	Coating	Biofilms
Sand	Ca/Mg and carbonates	Soluble microbial products (SMP)	Bacteria
Clay	Ca/Mg and bicarbonates	Natural organic matter (NOM)	Slime
Silt	Ca/Mg/Fe and phosphates	Phenols	Suspended biomass
Organic particles	Ca/Fe and sulphate	Tannins	Algae

Suspended material (particles and colloids)	Chemical precipitate (scaling)	Organic adhesion (adsorption)	Biological precipitate (biofouling)
Phytoplankton/algae	Ca and silicates	Sequestering	Sloughing biomass
Zooplankton	Hydroxides	Fe	Microbial deposit
Biosolids		Mn	S, Fe, Mn oxidation
Planktonic bacteria			

The most prevalent reactions leading to precipitate formation involve soluble cationic species such as Ca^{+2}, Mg^{+2} and Fe^{+2} and soluble anionic species such as carbonate/bicarbonate, phosphate, sulphate and to minor extent silicate. The scale forming reactions depends on pH, temperature, supersaturation concentration, contact time, common ion effect, presence of particulates, evaporation rate, flow rate and irrigation regime. Once precipitates are formed, they do not redissolved under natural conditions, and require acid treatment.

Solubility of most calcium and magnesium salts decreases with an increase in temperature (Eaton et al 1995). Therefore, evaporation in between irrigation periods along with elevated temperatures (45ºC and above) developed in the irrigation lines (most are black to suppress phototrophic activity) lower the equilibrium solubility and promote precipitation.

Proper management of emitter clogging includes: filtration to remove particulate material, using biocides to restrict microbes, reduce nutrient content to avoid biofouling, acidification and/or sequestering agents to reduce scale formation and flushing to empty the irrigation lines.

The specific objectives of the present demonstration study were, firstly, to compare the potential of fouling formation of reclaimed wastewater in irrigation networks in contrast to groundwater, and secondly, compare the standard maintenance cleaning of drippers and pipes with mineral acids with a novel system based on the application of CO_2 in reclaimed wastewater. The initial hypothesis was that reclaimed wastewater, susceptible to have higher content on nutrients compared to conventional freshwater sources, would favour the formation of biofilms in irrigation networks. To avoid biofilm formation and scaling, different mitigation alternatives can be applied. CO_2 injection acidifies water and increases pressure thus, decreases the appearance of inorganic scaling and biofilms in the irrigation networks. In the present study, several parameters were followed to assess fouling formation in irrigation networks: uniformity in drippers water flows, pressure variation along the pipe, and bacterial counts and biofilm characterisation by means of qPCR in the drippers outflow waters and biofilm inside the dripper. Fouling was characterised in irrigation networks using reclaimed wastewater (RW) and groundwater (GW).

4.2 Set-up and methods

4.2.1 Site and treatments description

Two types of water were tested in this activity: groundwater (GW) and reclaimed wastewater (RW). GW was pumped from a well and stored in a 2.000 m^3 reservoir. RW was non-disinfected secondary treated wastewater from Caldes de Montbui WWTP. WWTP from Caldes de Montbui (Barcelona Province, Catalonia, Spain) collects the WW effluents from the municipality, mainly including domestic WW but also few industrial effluents. The WWTP has a design capacity of 30,000 population equivalent and to treat a hydraulic load of 6.000 m^3/d. The water treatment in WWTP Caldes de Montbui includes a physicochemical primary treatment and biological and settling secondary treatments. This effluent was periodically delivered by tankers and stored in a reservoir (27 m^3) without disinfection.

Two different treatments were used as measure for clogging reduction and applied in RW. The pH of the RW was lowered by chemical addition via two options as follows:

- pH adjustment by nitric acid: nitric acid diluted on the water through a venturi system (Dosatron International SAS, France). The nitric acid, with a richness of 60% and a density of 1.38 g/mL, was diluted in a 50 litres tank with a concentration of 11.5 g nitric acid/litre. Then, from that tank was injected through a dosatron (Compact D07RE125) with a 1.25% dilution into the irrigation system. The final acid nitric dose was 0.142 g/L/min.
- pH adjustment by CO_2 injection: CO_2 gas (99.9%) was injected from a gas bottle into the 300L storage tank with a pressure of 3.5 bars until a pH of 6.5 was achieved.

Summing up, four water treatments (RW, RW+HNO_3 , RW+CO_2, GW), and 2 drippers types have been tested (see 4.2.2.1 and Figure 31).

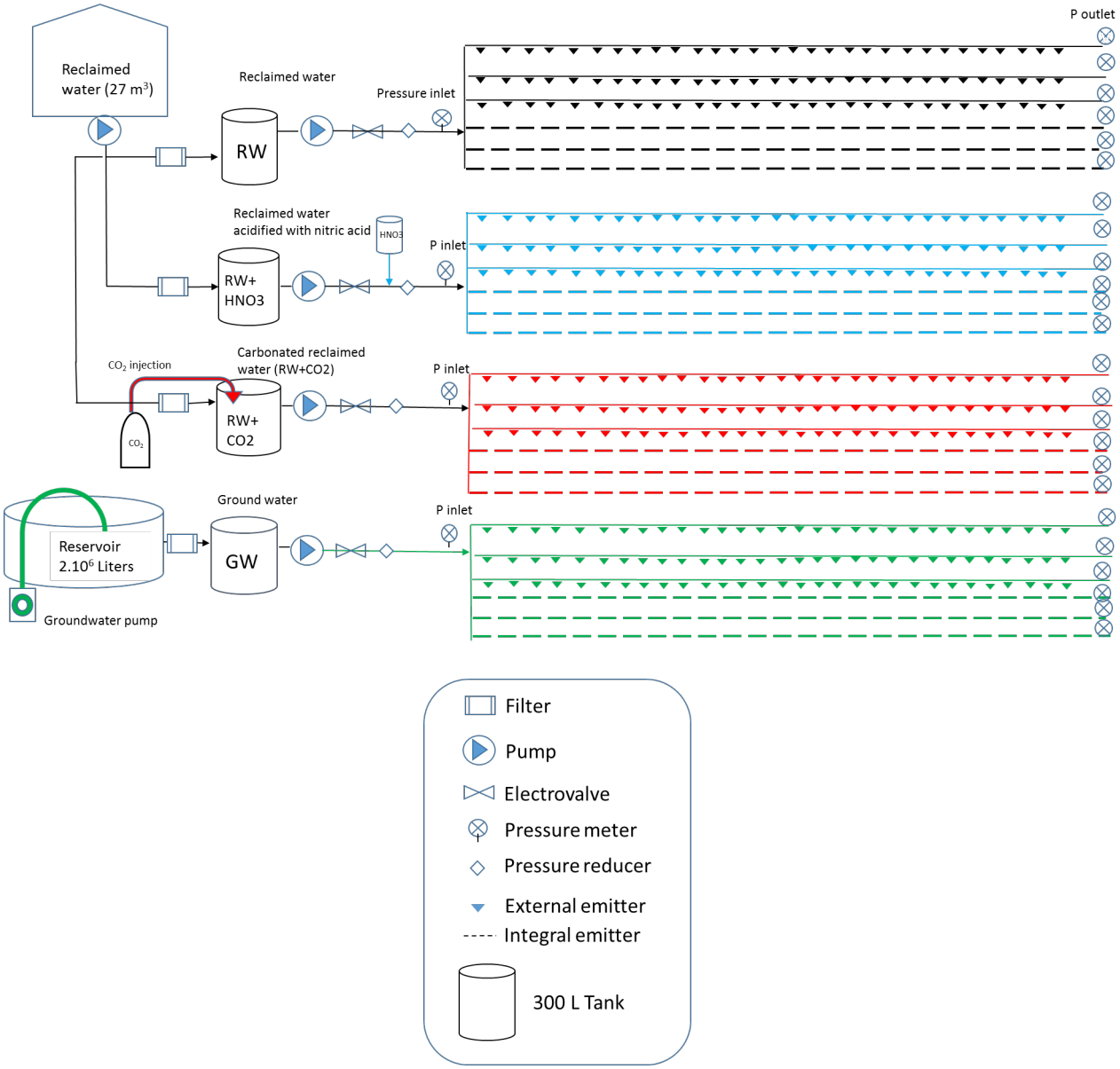

Figure 31 Scheme of irrigation network and different water qualities
(RW, black; RW+HNO_3, blue; RW+CO_2, red; GW, green) and dripper types (external, line with inverted triangles; integral, dashed line)

Figure 32 View of the irrigation network

4.2.2 Experimental irrigation scheme

4.2.2.1 Layout and operation

The irrigation network consisted of six polyethylene pipes per treatment (Figure 31). The pipes were of 16 mm diameter and 15 m length, and closed at the end. The number of drippers per line was 30. All pipes were fed with pre-filtered water that had gone through a plastic screen filter (Technoplastic ¾) of 120 µm mesh before the water tank in each water treatment to remove suspended solids (Figure 33).

Figure 33 Filter distribution installation (left) and filter detail (right)

The layout of the irrigation scheme is illustrated in Figure 31 and a general view of the experimental study can be seen in Figure 32.

4.2.2.2 Types of drippers

For each water quality, two types of drippers were installed (Figure 34). They were both pressure-compensating and anti-drain drippers:

- external dripper PCJ Junior™, Netafim, Isreal), and
- integral dripper (Uniram RC, Netafim, Israel)

with a nominal flow rate of 2 $L \cdot h^{-1}$ at the working pressure between 1.4-1.8 kg/m^2. In our case we regulated the pressure to 2 kg/m^2 with a pressure regulator (model ARBM). We measured some variation of inlet pressure between 1.8 and 2.1 kg/m^2 and mean flow rate of 2.4 $L \cdot h^{-1}$.

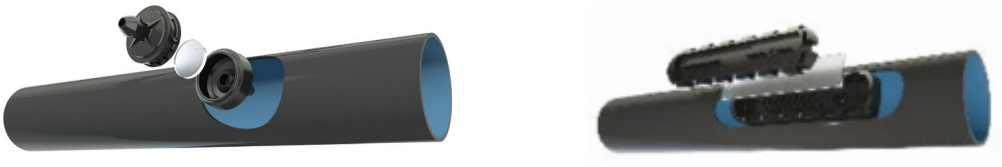

Figure 34 Illustration of different dripper types used in the experimental plot.
External Pressure compensated dripper, model PCJ Junior Netafim Israel (left); Integral dripper, model Uniram RC Netafim, Israel (right). Source: www.netafim.com

4.2.2.3 Mode of operation

The irrigation network was operated intermittently from September 2014 to September 2016, according to the following schedule:

- Irrigation water was applied for 10 min, 2 times a day at 9:00 and 16:00 h.
- Water was stagnant from 9:10 to 16:00 h and from 16:10 to 9:00 h of the following day.
- As an example, the daily water flow, taking into account the water flow measured under these conditions was: 0.040 $L \cdot min^{-1}$ x 20 $min \cdot d^{-1}$ = 0.8 $L \cdot day^{-1} \cdot dripper^{-1}$. These are representative irrigation dosages for spring.
- In April 2016, the irrigation schedule was changed: 5 min and 4 times a day in order to increase the water movement into the irrigation pipes.

4.2.3 Monitoring and sampling

4.2.3.1 Physico-chemical parameters in water

The pH value and the conductivity of the irrigation waters were measured weekly in water samples taken from the pipes/drippers using a pH meter (pH25, Crison, Spain,) and a conductimeter (CR35, Crison, Spain), respectively. A complete analysis including the following parameters: macro and micronutrients, TOC, TIC, BOD_5, turbidity, and TSS was performed periodically, in both RW and GW, following analytical standard methods for water quality.

4.2.3.2 Dripper flow rate and pressure difference

The dripper flow rate was determined monthly. Three out of 30 drippers per line were tested. They were located in the beginning, the middle, and the end of each pipe. The water of each dripper was collected for 2 min and measured volumetrically. The flow rate is given as ml/min. To assess the emission uniformity of the drippers, coefficient of uniformity (CU) of the flow rate was calculated as follows (Wilcox and Swailes, 1947):

$$CU = 100*(1-(\text{Standard deviation}/\text{average flow rate}))$$

Once a month, the working pressure in each pipe was measured with a fixed pressure gauge in the inlet of the pipe and a portable device at its end (Figure 35). The pressure at inlet is controlled by the pressure regulator. The pressure difference was calculated as follows:

$$\text{Pressure difference} = (1-((P_{inlet}-P_{end})/P_{inlet}))*100$$

Figure 35 Inlet fixed pressure gauge, water meter and pipes (left) and the portable pressure gauge at the end of the pipe (right)

4.2.3.3 Microbial analysis

4.2.3.3.1 Analysis of biofilm attached to pipe walls

Bacterial counts in biofilms attached to pipe walls from the irrigation network were quantified at 245 and 287 days of system working (May and June 2015). Pipe removable sections of approximately 20 cm long for each different water treatment were sampled in sterile sampling flasks and transported to the laboratory in insulated cold boxes to examine and characterize biofilm growth.

In order to quantify the spatial variability of biofilm attached to the surface of tubing material, three different subsections were cut for each different water treatment for biofilm analysis. The method used for determining the density of aerobic and facultative anaerobic heterotrophic bacteria was spread plate technique as mentioned in section 3.5.2.2 (Hallam *et al.*, 2001; Boe-Hansen *et al.*, 2003).

During the sampling, each pipe section was cut with a circular saw and biofilm was collected by swabbing the entire surface of the pipe subsection (Hallam, *et al.*, 2001; Van der Kooij *et al.*, 2001). Swabs were immediately transferred into a volume of 10 ml of ¼ strength Ringer solution. Bacteria extraction was done vortexing vigorously the swab to release the bacteria into the sterile Ringer solution. Tenfold to 10.000 fold dilutions were prepared and 0.1 ml were pipetted into sterile petri plates by duplicate. The number of heterotrophic bacteria of the solution was counted using spread plate technique and enumeration according to ISO 8199:2005. Final bacterial concentration was reported as colony forming units (CFU) per unit area of pipe surface (mm^2), which was determined individually for each pipe subsection.

4.2.3.3.2 Culture-dependent and independent methods for microbial assessment of biofilm inside the drippers

In order to obtain the biofilm inside the dripper, drippers with visible biofilm were incubated with 0.025 mmol/L tetrasodium pyrophosphate solution, shaken for 1h and subsequently sonicated (180s, 40 W output, Branson) to detach and disaggregate cells (Velji and Albright, 1986) for obtaining the biofilm suspensions. The sampling for this purpose was performed at days 596 and 688 after the start of the assay.

To determine total aerobic heterotrophic microbial populations, miniaturized most probable number (MPN) was carried out in microtiter plates (8 replicates per dilution) from ten-fold dilutions conducted from biofilm resuspensions on tetrasodium pyrophosphate solution as described before. R2 broth was utilized as rich liquid growth medium.

Also, molecular biology techniques (RNA/DNA-assisted) were applied to compare the microbial structure and diversity of biofilms with that of water samples. Total genomic DNA (see 4.2.3.3.3) was extracted from pellets obtained after a centrifugation step (1mL of biofilm suspensions centrifuged at 14000 g for 5 min). Moreover, directly from scrapped biofilm dripper surface, RNA and DNA were extracted simultaneously the by PowerMicrobiome RNA extraction kit (MoBio) following the manufacturer's instructions. In order to stabilize the RNA extracts, they were retrotranscribed to cDNA. Then, DNA, cDNA and RNA extracts were kept frozen at -80ºC until subsequent analyses. Microbial assessment was carried out by qPCR and high-throughput sequencing techniques (NGS) by means MiSEq (Illumina) sequencing platform. rRNA gene libraries targeting eubacterial region V1-V3 of 16S rRNA and fungal region ITS1-5,8S-ITS2, were sequenced at Molecular Research DNA.

Downstream MiSeq data analysis was carried out by using QIIME software version 1.8.0. The obtained DNA reads were compiled in FASTq files for further bioinformatic processing. Trimming of the 16S rRNA barcoded sequences into libraries was carried out using QIIME software version 1.8.0 (Caporaso et al., 2010). Quality filtering of the reads was performed at Q25, prior to the grouping into Operational Taxonomic Units (OTUs) at a 97% sequence homology cutoff. The following steps were performed using QIIME: Denoising using Denoiser (Reeder and Knight, 2010). Reference sequences for each OTU (OTU picking up) were obtained via the first method of UCLUST algorithm (Edgar, R.C., 2010). For sequence alignment was used PyNAST (Caporaso et al., 2010b) and Chimera detection was used ChimeraSlayer (Haas et al., 2011). Reference sequences for each OTUs were then taxonomically classified by using BLASTn against GreenGenes and RDP Bayesian Classifier database and compiled into each taxonomic level (DeSantis, Hugenholtz et al. 2006).

Data from MiSEq will be submitted in the meantime to the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) for further scientific publication at SCI indexed journal.

4.2.3.3.3 Analysis of total heterotrophic population in drippers water outflow

The same day of the dripper sampling (day of operation 596 and 688), water from RW and GW treatments at the entrance of the system and water outflow of the drippers were sampled. Water was collected from tanks and drippers by three independent replicates in sterile falcons at 4 °C (at the same of dripper biofilms, see 4.2.3.3.2). Liquid samples were immediately filtered in sterile conditions by Swinnex® Filter Holders (Millipore) using 0.22 µm pore diameter membranes of cellulose acetate (Whatman®). Filtrates were kept frozen at -20ºC until DNA extraction which were performed by using a bead-beating protocol (PowerSoil™ DNA Isolation Kit, MoBio Laboratories, Inc., Carlsbad, USA), according to the instructions of the manufacturer. To determine total aerobic heterotrophic microbial populations, miniaturized most probably number was carried out in microtiter plates (8 replicates per dilution) from ten-fold dilutions conducted from water samples. R2 broth was utilized as rich liquid growth medium.

4.2.3.4 Determination of fouling composition

The fouling composition in integral drippers that had been using RW and GW during a period of approximately 2 years was determined at the end of the demonstration study. Fouling (inorganic precipitates and biofilm) from drippers was extracted in 30 ml of an acid solution (0.05 M HCl) by ultrasonication during 20 min. The extract was analysed for cations and anions by ionic chromatography (ICS-2100, Dionex, Thermo Fisher Scientific, Sunnyvale, US) and for TOC (multi N/C 3100, Analytik Jena AG, Jena, Germany). New drippers were also extracted as field blanks. Results were corrected by the blanks and expressed as mass of the fouling constituent per dripper. The amount of carbonates which are lost during the acid extraction was estimated to balance the ionic composition.

4.3 Results

4.3.1 Water quality

As expected, RW treated with CO_2 or nitric acid showed lower pH than non-amended RW (Figure 36), around 6.5 and 8.0 for treated and non-treated RW, respectively. This slightly-acid pH, in addition to helping to the mitigation of fouling formation, favours plant nutrient uptake due to the higher solubility and availability of the micro and macronutrients. Electrical conductivity was similar between treated and non-treated RW. The pH of RW increased during storage in the tank, in some cases of 0.5 to 1 units (Figure 36). In average, the pH was 8.0 and electrical conductivity was 1.4 $dS \cdot m^{-1}$ in RW. Those values should be taken into account for crops fertirrigation because it can affect the availability of some nutrients and in some salt sensitive species can affect its growth and production.

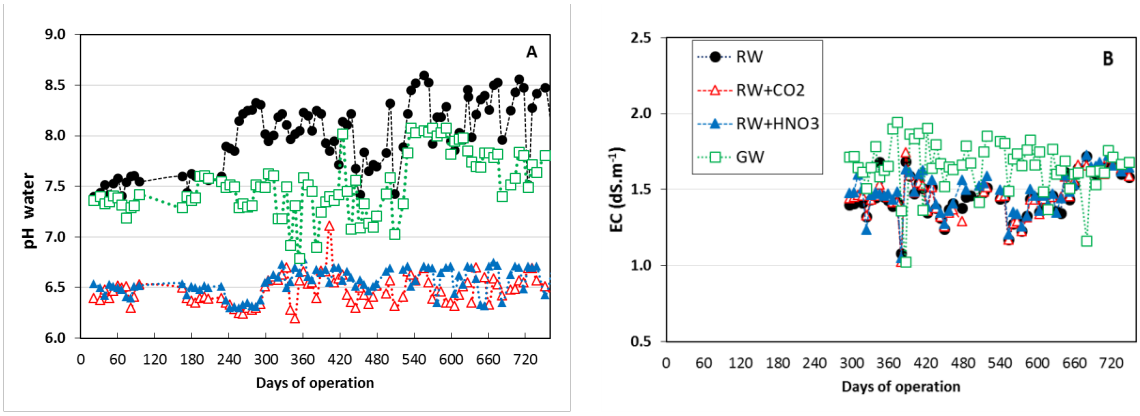

Figure 36 pH and electrical conductivity evolution in reclaimed wastewater (RW), reclaimed wastewater treated with CO_2 (RW+CO_2), reclaimed wastewater treated with nitric acid (RW+HNO_3), and groundwater (GW).

Little variation on macronutrients concentrations have been observed in RW throughout the duration of the demonstration experiment (Figure 37). Secondary nutrients as sodium, sulphate and chloride showed high values, 174 $mg \cdot L^{-1}$, 79 $mg \cdot L^{-1}$, and 269 $mg \cdot L^{-1}$, respectively (Table 9). These values were in the normal range in water for irrigation use (Ayers and Westcot, 1994). However, RW presented a SAR (sodium adsorption ratio, the sodium to square root of calcium + magnesium) of 4.8 that can restrict its use depending on soil texture because can cause soil salinization. Within the micronutrients, RW had boron (0.3 ppm), but the levels were under the limit of 2 ppm, the threshold limit recommended in irrigation waters (Ayers and Westcot, 1994).

As expected, the chemical composition of RW treated with CO_2 and nitric acid showed only differences in bicarbonate and nitrate concentrations compared to non-treated RW. RW+CO_2 had 26% higher content of bicarbonates and RW+HNO_3 had a five times higher content of nitrates, compared to non-treated RW (data not shown).

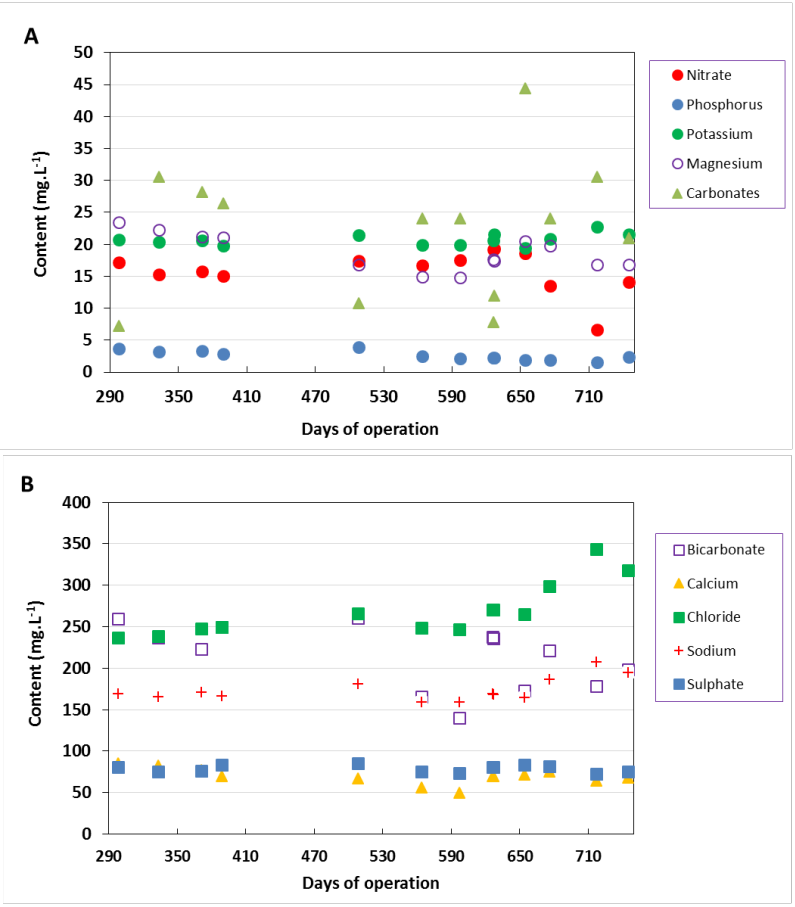

Figure 37 Macronutrients content evolution in RW

As expected, the chemical composition of RW treated with CO_2 and nitric acid showed only differences in bicarbonate and nitrate concentrations compared to non-treated RW. RW+CO_2 had 26% higher content of bicarbonates and RW+HNO_3 had a five times higher content of nitrates, compared to non-treated RW (data not shown).

GW showed different values in nitrate concentration, phosphorous and sulphates in comparison to RW. Nitrate concentration and sulphate were 24 and 2 times higher in GW than in RW, respectively. Phosphorous showed lower concentration in GW (-98%) (Table 9). Non-treated RW was characterized by rather low content of biodegradable carbon and suspended solids, low to moderate turbidity, slightly high in ions, mainly sodium and chloride. GW had even lower content of biodegradable carbon, suspended solids and turbidity. However, this GW had two main considerations: high content of nitrates that have to be accounted for the fertilization dosage in case of an irrigation reuse and high content of scale-forming ions. In this regards, the Langelier Saturation Index for GW indicated that this water was supersaturated with respect to calcium carbonate and inorganic scaling probably occur. In contrast, RW had a lower LSI although was still supersaturated in CaCO3 but scale forming was less likely than in GW.

Table 9 **Physicochemical water quality of the secondary WWTP effluent from WWTP Caldes de Montbui (RW) *versus* groundwater (GW) (2015-2016)**

Parameter	Units	RW *average ± deviation ¦ n*		GW *average ± deviation ¦ n*	
TSS	mg/L	3.6 ± 4.8	7	<2	2
Turbidity	NTU	3.4 ± 3.6	8	1.2	2
TOC	mgC/L	5.7 ± 0.6	5	1.6	2
BOD_5	mgO_2/l	7.2 ± 7.3	3	2.0	2
pH	upH	8.0 ± 0.02	16	7.7 ± 0.24	6
Conductivity	mS/cm	1.4 ± 0.04	16	1.6 ± 0.15	6
TIC	mgC/L	47 ± 2	3	54	2
Chloride	mg/L	269 ± 10	16	129 ± 12	6
Sulphate	mg/L	79 ± 1.2	16	123 ± 16	6
Bromide	mg/L	0.5 ± 0.1	16	0.4	6
Nitrate	mg/L	16 ± 0.9	16	388 ± 46	6
Nitrite	mg/L	0.8 ± 1.3	1	<0.2	1
Phosphorous	mg/L	2.6 ± 0.2	16	0.1 ± 0.0	6
Ca^{2+}	mg/L	70 ± 2.7	16	180 ± 14	6
Mg^{+}	mg/L	18.7 ± 0.8	16	52.6 ± 4.3	6
Na^{+}	mg/L	174 ± 4.0	16	39.0 ± 3.3	6
K^{+}	mg/L	20.7 ± 0.3	16	5.5 ± 0.7	6
NH_4^{+}	mg/L	0.5 ± 0.8	16	<0.2	1
Boron	mg/L	0.3 ± 0.9	16	<0.25	6
SAR		4.82 ± 0.14	13	0.67 ± 0.06	5
Langelier Saturation Index, LSI (20°C)	-	0.065		0.31	

The microbiological composition of RW and GW is shown in Table 10. The heterotrophic bacteria counts in RW were 4.5 times higher than in GW, when determined by plating and quantifies as colony forming units (CFU). Samples of both types of water analysed by MPN determination showed no differences. The high number of bacteria present in the GW can be explained for the outdoor location of the reservoir.

Table 10 **Microbiological characterization of the secondary WWTP effluent from WWTP Caldes de Montbui (RW) *versus* groundwater (GW) (2015-2016)**

Parameter	Units	RW *average ± deviation ¦ n*		GW *average ± deviation ¦n*	
Total heterotrophic bacterial count	$CFU{\cdot}mL^{-1}$	1.67E+05 ± 1.39E+05	8	3.0E+04 ± 2.86E+04	3
Total heterotrophic bacterial count	$MPN{\cdot}mL^{-1}$	1.77E+05 ± 1.35E+05	2	3.17E+05 ± 2.57E+05	2
Clostridium perfringens	$CFU{\cdot}mL^{-1}$	n.d.		n.a.	
E. Coli	$CFU/100mL^{-1}$	<1		n.a.	
Protozoa (*Giardia spp.* and *Cryptosporidium*)	oocysts/L	<8		n.a.	
Taenia spp.	$egg{\cdot}10\ L^{-1}$	< 1		n.a.	
Helminth eggs	$egg{\cdot}10\ L^{-1}$	< 1		n.a.	

4.3.2 Irrigation system performance

4.3.2.1 Pipe pressure difference

In an irrigation network there are pressure differences between the inlet and the end of the pipe due to the roughness of the material, friction and the dripper configuration. Variations on pressure are indicative of possible dripper clogging. The difference between the inlet pressure and the pressure at the end of each line was measured in triplicate and the average is shown in Figure 38. Till January 2016 (operation day 490) values were lower than 100% that means that the inlet pressure was higher than end point. From beginning of 2016 (operation day 498), the GW drippers showed values higher than 100%, meaning that the pressure at the end was higher due to drippers clogging. For the RW line there were non-explainable values around 60% during winter 2015, but no negative differences throughout the duration of the study. This implies that drippers were not clogged. In acidified RW, the relative pressure difference showed small differences between RW+CO_2 and RW+HNO_3 but always lower than 100%, in average 91% and 92% for RW+CO_2 and RW+HNO_3, respectively.

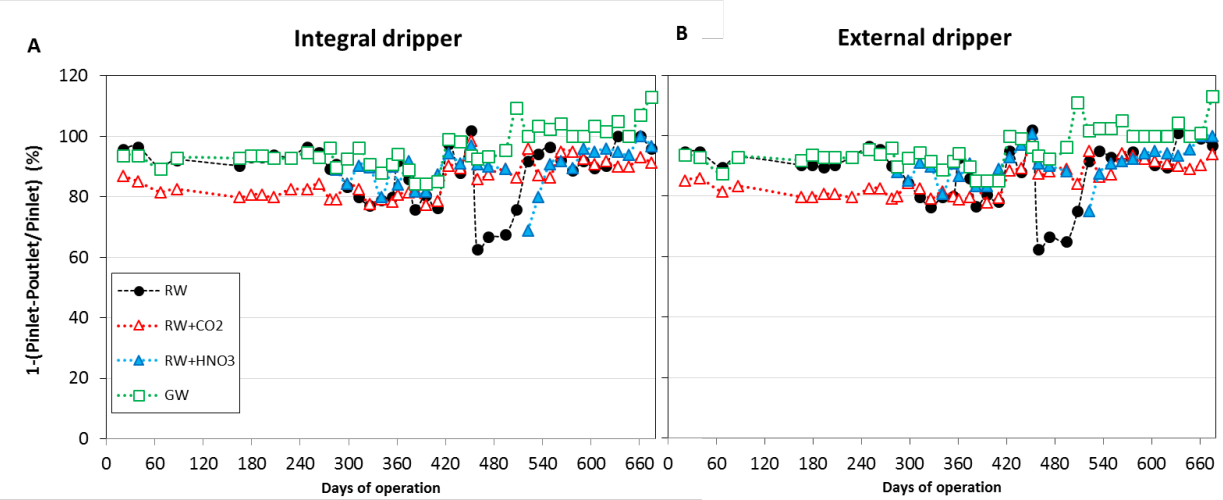

Figure 38 Pressure difference in integral (A) and external (B) drippers
Relative pressure difference between inlet and end point in the irrigation pipe respect to the inlet (in %). The values are the average of three pipes per treatment.

4.3.2.2 Dripper flow

The flow rate of the emitters showed punctual/sporadic differences mainly between GW and the other treatments (Figure 39). The main reason is that the GW drippers showed more clogging. At the end of the experimental study, 28 % and 14 % of the external and integral drippers, respectively, were clogged. This caused higher water flow in the remaining functional drippers. This confirms the finding explained afterwards that fouling was quantitatively more important in GW pipes compared to both treated and non-treated RW pipes. An increase of water flows in GW and RW was observed during autumn 2015, more or less after 6 months of functioning. In winter-spring months during 2016, RW-external drippers showed low values, but in the following summer they recovered the same values of the others drippers. The same pattern was observed for the coefficient of uniformity (CU) of the water flows (Figure 40), the GW drippers got sporadically lower uniformity after 12 months of functioning.

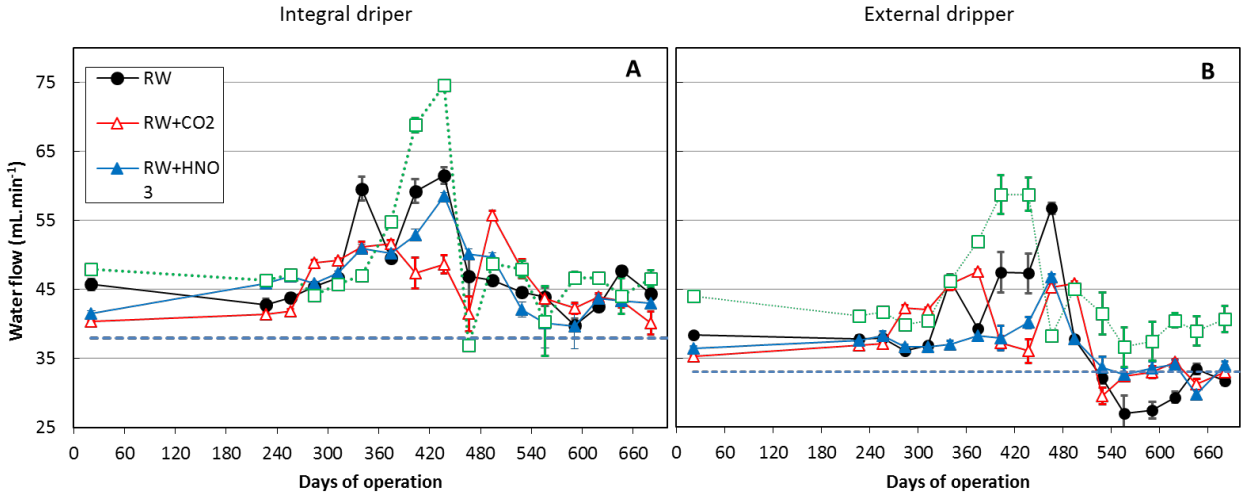

Figure 39 Drippers water flow rate evolution in integral (A) and external drippers (B).
Symbols represent the average of 9 measures and bars are standard error. Dotted blue line represents nominal flow rate of each dripper.

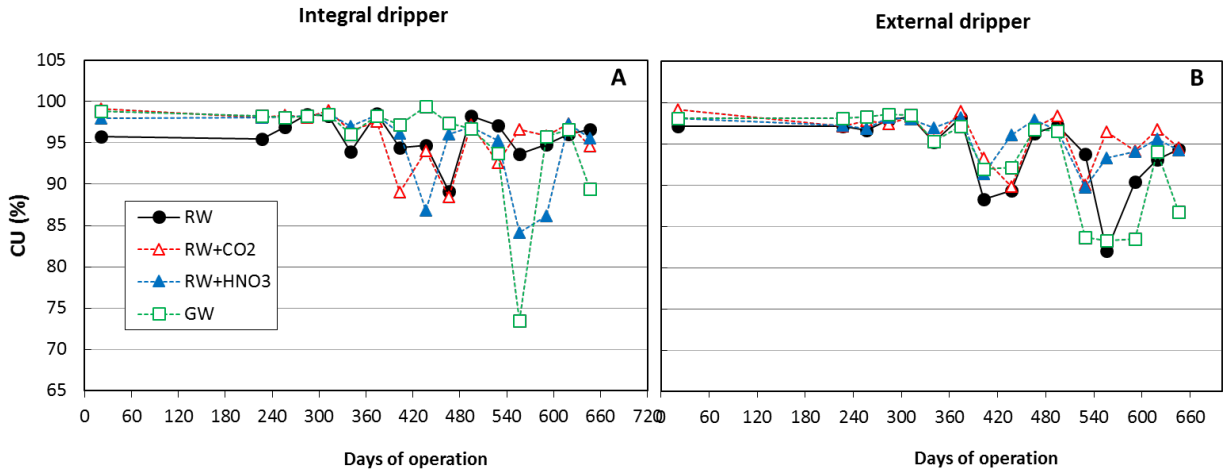

Figure 40 Coefficient of uniformity of the flow rate evolution in integral drippers (A) and external dripper (B).

4.3.3 Biofouling

4.3.3.1 Biofilm attached to pipe walls

After approximately 9-10 months of irrigation performance (May-June 2015, 247 and 287 days of operation), we analysed the biofilm attached to the pipe walls, including the drippers, as is explained in section 4.2.3.3.1. In May-June 2015, the bacterial count in the pipe walls was 66 CFU·mm^{-2} in average and showed slight differences between treatments. A *priori* it seems that integral drippers presented more biofilm than external drippers, even though differences were not significant. No differences between water treatments were observed probably because the irrigation network had been working for nine months and there was probably no time for biofilm formation.

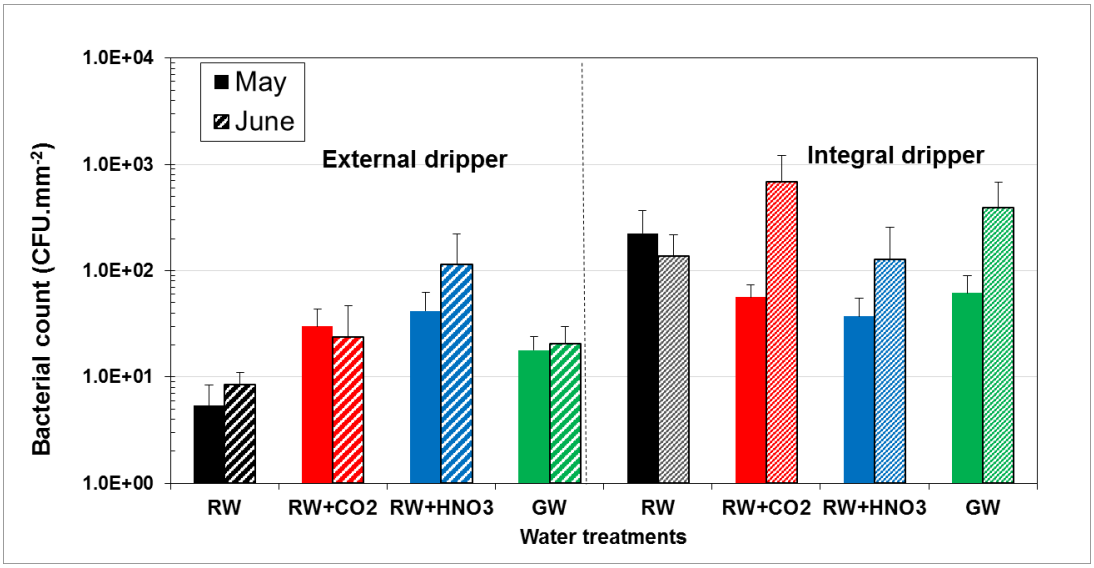

Figure 41 Bacterial count (CFU·mm^{-2}) in the biofilm formed in the pipe walls in May and June 2015
The values are the average of 3 replications in May and 2 replicates in June.

4.3.3.2 Biofilm inside the drippers

Towards the end of the experimental study (April-July 2016, 596 and 688 days of operation), biofilm inside the drippers was analysed as explained in section 4.2.3.3.2. The 16SrRNA gene copies are an indicator of total eubacteria biomass, whereas bacterial counts expressed as MPN or CFU indicate only active bacteria able to grow in a standard culture media.

Microbial biomass, expressed as 16SrRNA gene copies, showed lower values in GW and RW+HNO_3 compared to RW and RW+CO_2 (Figure 42). However, in April 2016, RW+HNO_3 had 70% less bacterial counts than RW, and RW+CO_2 had 119% more than RW (Figure 42). In July 2016, the behaviour was different: all treated RW showed more biofilm than RW, 21 and 89% in RW+CO_2 and RW+HNO_3, respectively. The GW drippers showed similar values between April and July 2016, almost the lowest in both cases. The relationship between 16SrRNA gene copies and MPN revealed a seasonal effect, there are different communities in RW and GW. To sum-up, no conclusive results were obtained to assess biofilm formation by means of bacterial counts, by the different water sources and treatments. It might be that biofouling due to bacteria formation has not been enough developed in order to observe significant differences between treatments.

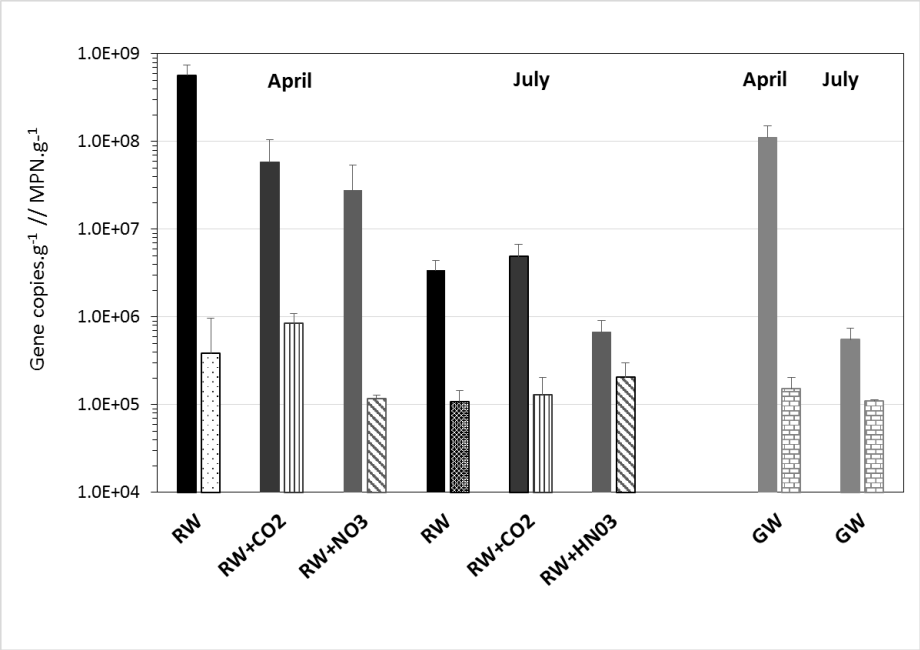

Figure 42 **Microbial biomass (filled bars) and bacterial count (pattern bars) in the biofilm attached at drippers in April and July 2016.**
Microbial biomass expressed as 16S rRNA gene coy number per dripper mass and bacterial count expressed in MPN units per gram of dripper. The values are pooled from 3 drippers.

In April 2016, fungal population, accounted by copies of the gene ITS1, was 57 % and 87 % lower in RW+CO_2 and RW+HNO_3, respectively, compared to non-treated RW. In July 2016, these percentages were 14 % and 39 % lower in treated-RW than in untreated-RW (Figure 43). Fungal community in GW was 80% lower respect to RW in April and there were no differences in July.

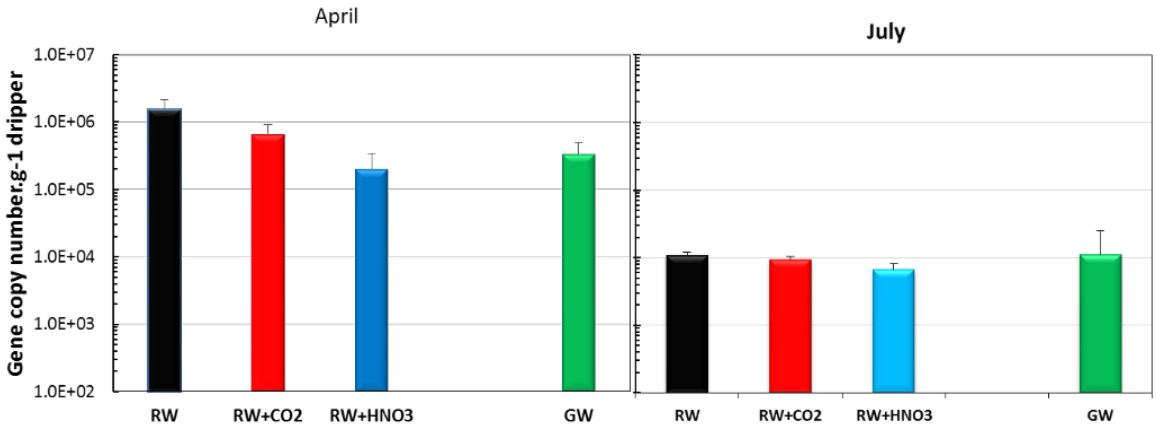

Figure 43 **Quantitative abundance of fungal populations in the dripper biofilm determined by quantifying ITS1 gene copies by qPCR.**

Interestingly, 16S rRNA/ITIS1 rRNA-qPCR assessment revealed that in July 2016, biofilms contained less microbial biomass abundance (100-500 fold lower in bacterial and 50-100 lower in fungal populations) compared with biofilms in April 2016. The high water temperatures reached inside the black dripper and the pipes in summer (above 30 °C) in daily hours may cause a higher cell lysis and death in summer.

4.3.3.3 Bacterial counts in dippers inflow and outflow waters

In this section, we compared the microbiological population in the initial water tank and the water getting out the dripper. In order to know the total bacterial population present at water samples, qPCR and MPN was carried out in tanks and in the drippers outflow waters of the different treatments. In April 2016, all the outflow waters with treated-RW had higher bacterial biomass content than RW from the tank (Figure 44). In July 2016, the bacterial biomass was lower in the RW drippers outflow water and higher in RW+CO_2. In April in GW the results were similar between gene copies and MPN, being these lower in the dripper than in the tank. In July gene copies results were lower.

The results showed similar values of bacterial counts between RW and GW tanks (Figure 44). Between treatments, there are different patterns in April and July 2016. In April, the dripper outflow with RW showed an increase of 480 % compared to the RW-tank and higher values respect to RW+CO_2 (+92 %) and RW+HNO_3 (+71 %) (Figure 44). In July, RW+HNO_3 showed lower values compared to the other treatments (a decrease between -319% and -485% compared to RW and RW+CO_2). In GW samples, the water getting out the dripper showed less bacterial populations than the water in the tank, values showed a decrease of 59 % and 63 % in April and July 2016, respectively. The relationship between the MPN and 16S rRNA gene copies results showed that all gene copies values are higher than MPN values, it does mean that there are bacteria dead, but there are bacteria non cultivable but viable or quiescent present in all the treatments that can be active in the future (Figure 44).

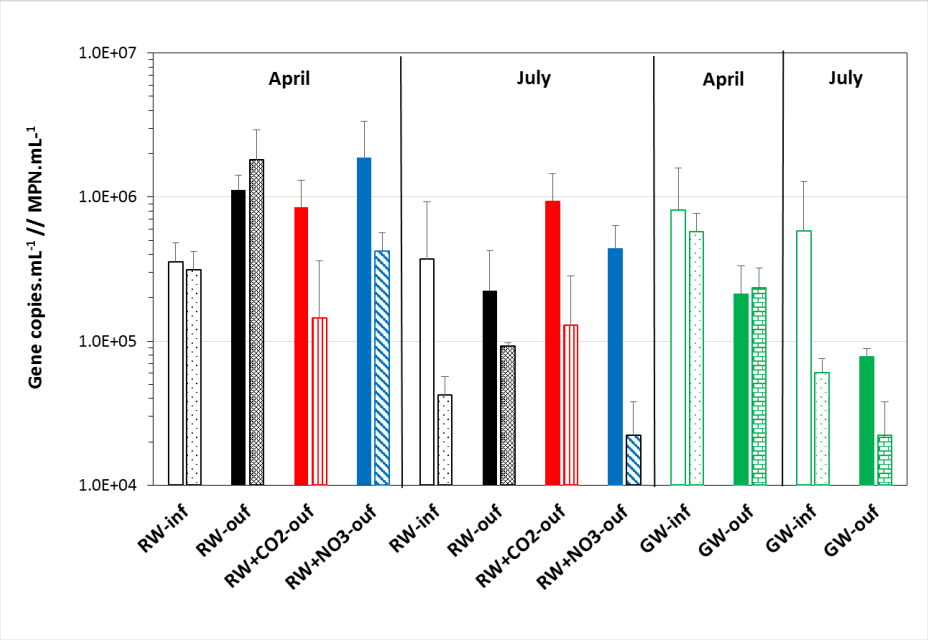

Figure 44 Microbial biomass (as gene copies - filled bars) and bacterial counts (as MPN - pattern bars) in the tank of reclaimed wastewater (RW-inf) and groundwater (GW-inf) and in the water getting out the dripper (ouf) of the different treatments in April and July 2016.
Microbial biomass expressed as 16S rRNA gene-based qPCR and bacterial count expressed as MPN

Regarding fungal population, the water outflow the dripper in April 2016 showed more fungal biomass than the inflow water (Figure 45).

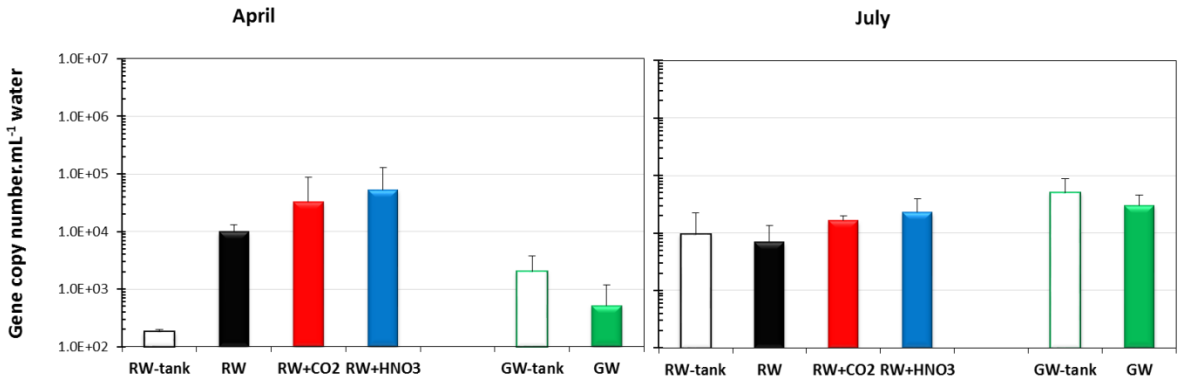

Figure 45 Quantitative abundance of fungal populations in water samples (inflow and outflow irrigation water) determined by quantifying ITS1 gene copies by qPCR

Although microbial biofilms (eubacteria and fungi) in drippers were highly reduced in July 2016 compared to April 2016, water outflow from drippers remained enriched with bacterial and fungal communities.

Deciphering total and active microbial communities (eubacteria and fungi) in different irrigation water and dripper biofilms, by applying 16SrRNA/ITS rRNA-based High-Throughput Sequencing (MiSEq platform) in spring (April 2016) and summer (July 2016), by means of NGS technology (MiSeq) combined with DNA/RNA-based assessment, allow us to distinguish and identify those most present/active microbial groups (eubacteria and fungi) in the biofilm, including plant and human pathogenic microorganisms. In addition, NGS assessment revealed the effect of the type of water source (GW *versus* RW), and the effect of the water treatments (CO_2 and HNO_3) on the effluent water and dripper biofilm. Also, the effect of biofilm diversity structure and active biomass on the final effluent from drippers has been assessed.

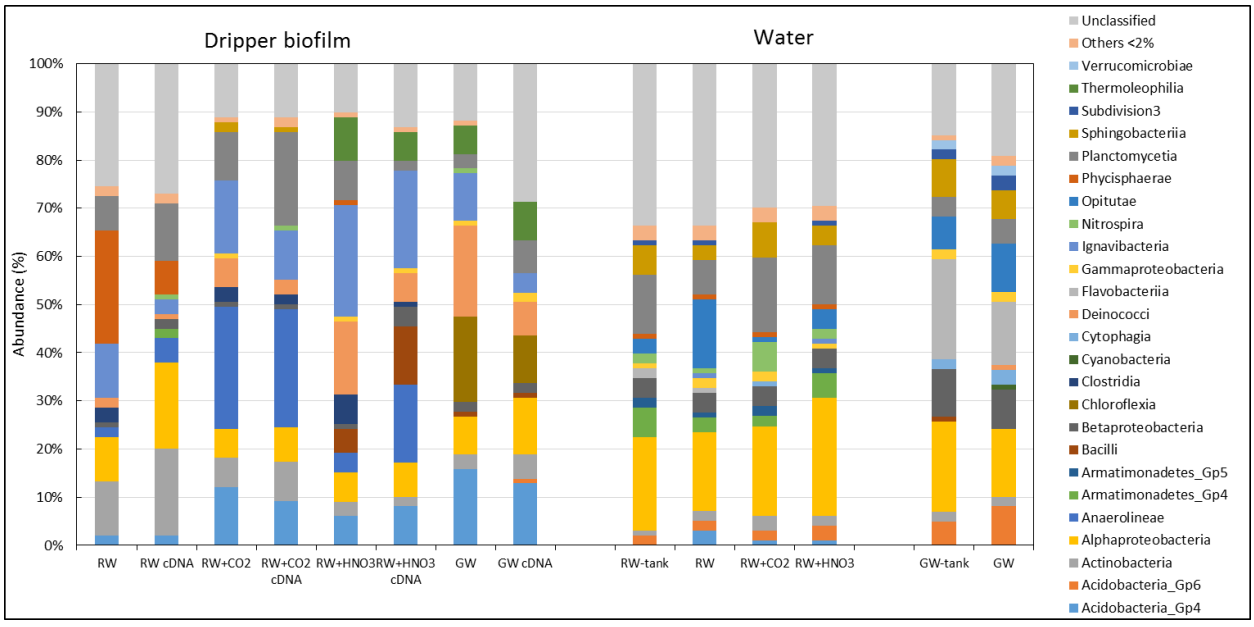

Figure 46 Eubacterial community structure at class level assessed by MiSEq sequencing

i) in biofilm samples from drippers a simultaneous DNA- and RNA based (cDNA) was conducted to distinguish present and active microbial populations; ii) in water tanks of reclaimed water (RW-tank) and GW (GW-tank) and the water outflow the drippers in all the treatments in July 2016. Values are the average of two independent DNA/cDNA extracts.

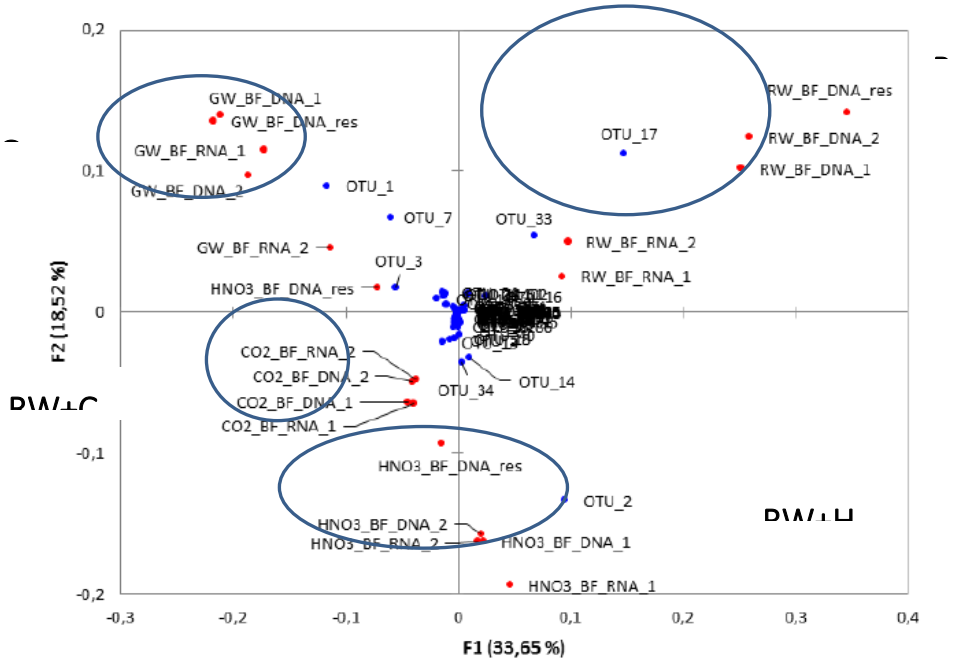

Figure 47 Contribution biplot of correspondence analysis (CA) conducted from relative abundances distribution of main OTUs (>1%)

From eubacterial biofilms grown in drippers in different kinds of water (GW, RW, and treated RW with HNO_3 and CO_2)

Miseq sequencing of eubacterial community (16S rRNA based) revealed that microbial community stablished on the dripper biofilms was clearly different and specialized compared to microbial communities found in water (both GW and RW) (Figure 46). In addition, both the water source (GW *versus* RW) and the treatment applied to RW (HNO_3 and CO_2), conditioned the microbial community enriched on dripper biofilms (Figure 47). However, shared OTUs belonging to known thermophilic bacterial classes such as *Ignavibacteria* and *Deinococci* were highly enriched on the biofilms (accounting for 15-30% of the total and active microbial community) regardless of RW treatment and water source.

Regarding the abundance and distribution of potential fungal pathogens in water and biofilms, *Cladosporium spp.* related OTUs were the more abundant in the untreated RW released from drippers in July 2016 (Figure 48). Interestingly, the relative abundance of *Cladosporium spp.* in dripper water dramatically decreased when treated RW was utilized confirming certain inhibitory effect of CO_2 and HNO_3, probably water pH which was below 6.8 (untreated GW and RW pH was 7.6 and 8.1, respectively), creating local acidic regions in drying exogenous surfaces upon dripper material. It is noteworthy that *Cladosporium spp.* were hardly detected inside the drippers on the biofilms, confirming that *Cladosporium spp.* were only able to grow on the external part of dripper with optimal oxygen availability. Moreover, it was also enriched in GW and dripper GW probably by the availability of nutrients-N such as nitrates, which were more abundant in GW than in RW (see Table 9).

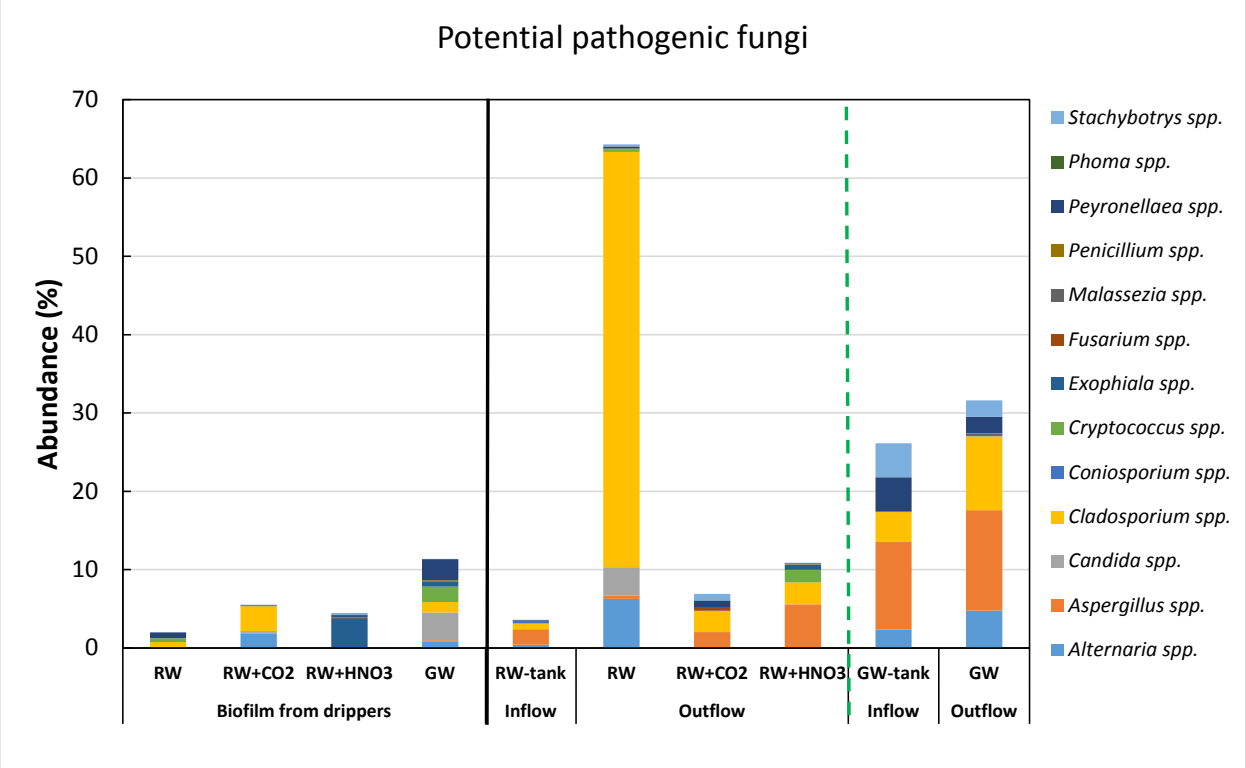

Figure 48 **Relative abundance of potential pathogenic fungi in biofilms and water samples determined by means of MiSEq sequencing of massive libraries of ITS1 rRNA region in July 2016.**

Additionally, known pathogenic human bacteria such as *Legionella* and *Mycobacterium* were found by MiSEq sequencing. Their absolute abundance was determined by combining the abundance of total bacteria (16SrRNA-based qPCR) and the relative abundance found of these genera by NGS.

Data revealed that both genera were less abundant in GW (10^1-10^2 and 10^2-10^4 16S gene-based copies·mL^{-1} of *Legionella* and *Mycobacterium,* respectively) compared with RW (10^2-10^3 and 10^4 16S gene-based copies·mL^{-1} of *Legionella* and *Mycobacterium,* respectively) (Figure 49).

Interestingly, both genera were not enriched on dripper biofilms. In fact, *Mycobacterium* accounted below of 0.5% of relative abundance in RW-based biofilms and 0.1-0.7% in GW-based biofilms, being 40-200 fold lower relative abundance than observed in RW (1-3% of relative abundance) and similar to that observed in GW (0.03-1.4%) (Figure 50). As observed in case of *Mycobacterium*, *Legionella* relative abundance in biofilms was quite low accounting for 0.02-0.06% of total population (Figure 50).

Total specific populations of *Legionella* in dripper biofilms utilizing groundwater achieved $10^1 \cdot g^{-1}$. However, when reclaimed water was utilized, *Legionella* populations were 10-50 fold higher accounting for 10^3 16S gene based copies·g^{-1}, both in untreated and CO_2-treated reclaimed water, and 10^2 16S gene-based copies·g^{-1} in HNO_3-treated RW. *Mycobacterium* populations in GW-based biofilms accounted for 10^3 16S gene base copies·g^{-1} and interestingly in HNO_3-RW, being 10 fold higher in untreated RW and CO_2-RW which were close to 10^4 16S gene-based·g^{-1} (Figure 50). Globally cDNA assessment in biofilms revealed that *Mycobacterium* and *Legionella* were less metabolically active (6-80 times lower in 16S based-cDNA copies) in treated-RW than in untreated RW, being HNO_3 the more effective treatment against *Legionella* as was previously described for DNA-based copies as well.

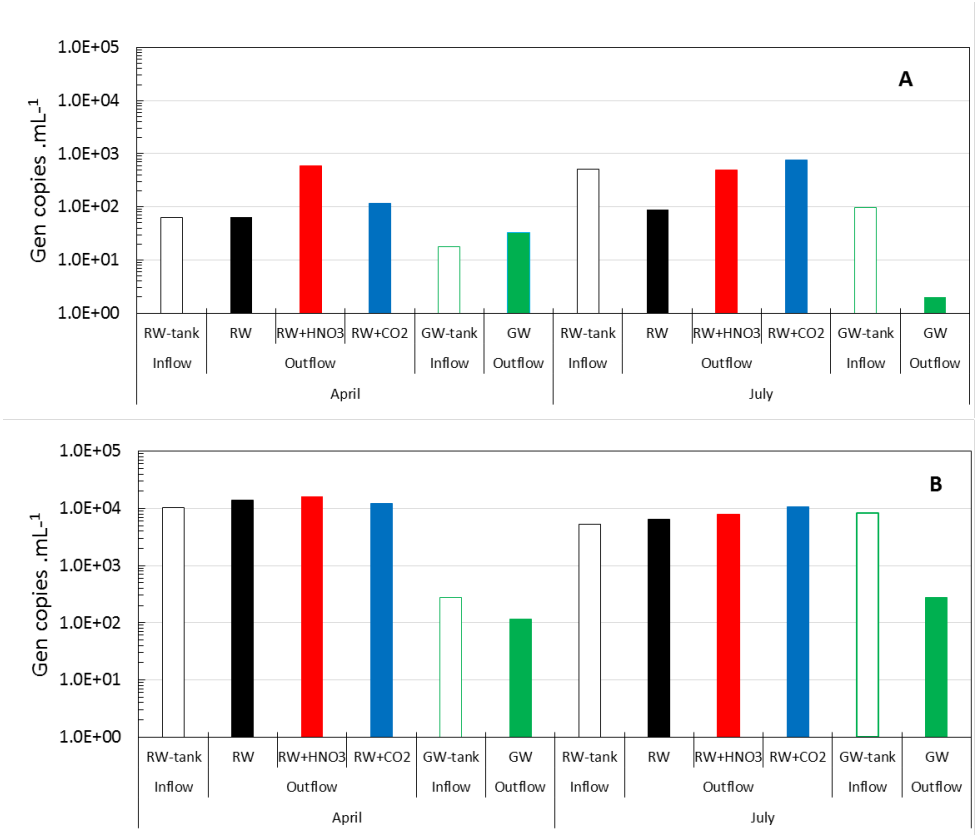

Figure 49 Quantitative and relative abundance of potential human pathogens, *Legionella* (A) and *Mycobacterium* (B), in water samples determined by combining 16S rRNA gene sequencing (Miseq) and qPCR of 16S rRNA gene.

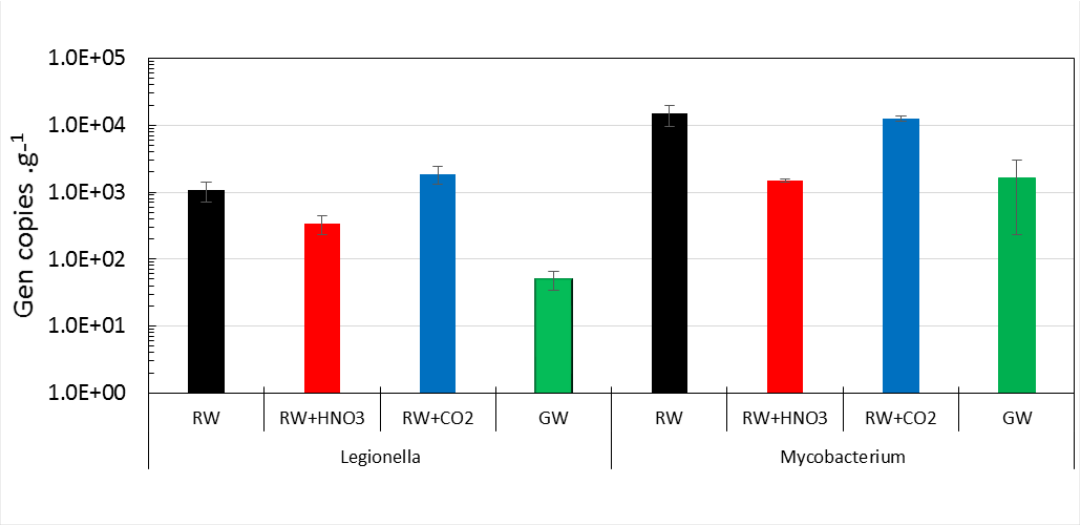

Figure 50 Quantitative and relative abundance of potential human pathogens, Legionella and and Mycobacterium, in biofilm dripper samples in July, determined by combining 16S rRNA gene sequencing (Miseq) and qPCR of 16S rRNA gene

4.3.4 Fouling composition

Fouling was characterized in integral drippers at the end of the demonstration study in order to account for the fouling nature, either biofouling or inorganic scaling (for methodology details see section 4.2.3.4). Results are shown in Table 11 and Figure 51.

Table 11 Fouling composition in integral drippers with reclaimed wastewater and groundwater

Fouling constituent	Dripper with RW		Dripper with GW	
	(mg/dripper, average ± deviation, n=4)	*% of total fouling in RW*	*(mg/dripper, average ± deviation, n=4)*	*% of total fouling in GW*
Organic matter (TOC x 1.8[a])	0.18 ± 0.02	1.37	0.07 ± 0.04	0.26
Insoluble particles at pH 1.3 (HCl 0.05M)	2.25 ± 0.41	16.8	3.69 ± 4.07	14.3
Chloride	<0.03	-	<0.03	-
Sulphate	0.23 ± 0.19	1.74	0.26 ± 0.10	1.03
Nitrate	<0.03	-	0.07 ± 0.07	0.28
Phosphate	1.95 ± 0.34	14.6	0.05 ± 0.02	0.18
Ca^{2+}	2.94 ± 0.61	21.9	8.53 ± 2.70	33.2
Mg^{+}	0.23 ± 0.09	1.69	0.13 ± 0.03	0.51
Na^{+}	0.98 ± 1.53	7.28	0.03 ± 0.02	0.12
K^{+}	0.26 ± 0.41	1.91	<0.03	-
NH_4^{+}	0.009 ± 0.011	0.07	<0.006	-
Carbonates[a]	4.39 ± 2.44	32.7	12.9 ± 4.1	50.1
Total fouling	13.41	100%	25.72	100%

a Emeterio Iglesias Jiménez and Victor Pérez Garcia, 1992. Relationships between Organic Carbon and Total Organic Matter in Municipal Solid Wastes and City Refuse Composts. Bioresource Technology 41, 265-272.

Absolute values of total fouling were higher in drippers with GW (26 mg/dripper) than drippers with RW (13 mg/dripper). A minority of the total fouling had an organic origin in both water types. The organic fouling represented only the 1.4 and 0.3% for RW and GW, respectively. An important fraction in both water types corresponds to particles not solubilized at pH 1.3, which probably are silicate minerals.

Chloride, nitrate, sodium, potassium and ammonium usually form rather soluble salts, thus the residual concentrations found in both dripper types probably come from the residual water remaining in the dripper.

Main constituents in drippers with GW are calcium and carbonates. Calcium carbonate was estimated to constitute the 83% of the total fouling in these drippers (21.3 mg $CaCO_3$/dripper). Other minority salts representing less of 1% of the total fouling are magnesium carbonate and magnesium sulphate.

Calcium carbonate is less important in drippers with RW, even though is still the major constituent representing the 43% of the total fouling (5.8 mg $CaCO_3$/dripper), followed by other salts such as calcium phosphate (1.72 mg/dripper, 13%), magnesium carbonate (0.29 mg/dripper, 2%), and magnesium phosphate (0.24 mg/dripper, 2%). Salt forms were not analysed but these salt amounts were estimated from the ionic composition of the extracts.

To sum-up, most of the fouling found in both dripper types has an inorganic origin, with calcium carbonate being the most abundant scale-forming salt in GW and in RW. In principle, organic matter and

nutrients could lead to the formation of organic fouling and in principle was a fact expected in drippers with RW. However, TOC and nutrients may be low in RW and this formation has not been favored. In GW, there is an unexpected high content of nitrates but these have not lead to bacterial growth inside the irrigation pipes.

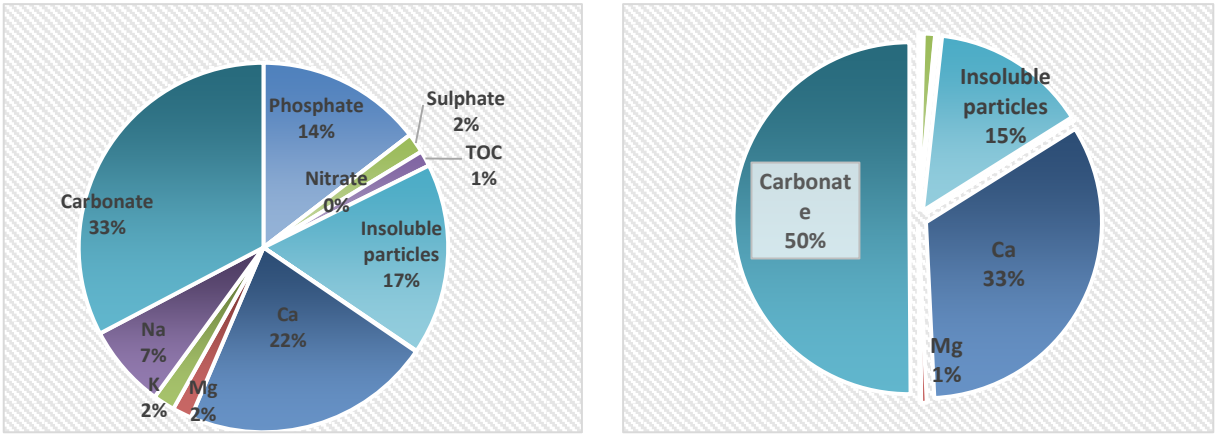

Figure 51 Fouling composition in integral drippers with RW (left) and GW (right)

4.4 Conclusions

The chemical quality characterization showed different features for untreated reclaimed wastewater and groundwater. Some features such as content of nitrates, sodium and scaling ions can have implications for crops irrigation and fouling formation in the irrigation network.

Reclaimed wastewater (RW) had a high content of sodium, what has to be considered when using this water for irrigation purposes because could have severe implications for plant nutrients/water uptake and soil salinization. In contrast, groundwater (GW) had a high content of nitrates (due to aquifer contamination episodes), that should be taken into account when calculating fertilization dosages. Both RW and GW had a considerable amount of scaling ions that may lead to inorganic scaling, but being scaling in RW less likely than in GW.

A period of approximately 2 years was not enough to achieve enough biofouling formation in pipes/drippers that led to issues on the irrigation performance, using either RW or GW, probably due to the low content of biodegradable carbon that both water types had. We observed only dripper clogging in GW with clear effects on the irrigation system performance (higher pressure variations along the pipe, variations in the dripper flows and lower coefficient of uniformity). However, this clogging in GW had mainly an inorganic origin (calcium carbonates). Since we had not enough development of biofilm, was not possible to evaluate if CO_2 and HNO_3 treatments were efficient methods for biofilm mitigation in RW by means of loss of irrigation performance. Both CO_2 and HNO_3 treatments achieved a pH reduction in RW (from 8.0 to 6.5), what may have prevented scaling formation in pipes and drippers using RW.

At the end of the study, fouling was characterized in integral drippers to confirm that biofouling was not quantitatively relevant in the present demonstration study and instead scaling formation was the cause of clogging in GW. Fouling in drippers with GW had mainly contributions of silicate minerals (14%) and calcium carbonate (83%). Fouling in RW also had mainly an inorganic origin but was lower than in GW and did not cause problems in the irrigation system performance.

Even though biofouling did not cause clogging of drippers in the present demonstration study, biofilm and dripper water inflows and outflows were characterized by means of qPCR and high-throughput molecular approaches. Molecular assessment by NGS showed that microbial communities were different between water inflow (water tanks) and outflows. Moreover, microbial community structure on dripper biofilm was dependent on water source. Furthermore, the high temperature (>37 °C) inside the pipes/drippers seemed to exert a selective pressure on final microbial population. This fact promoted the selective enrichment of thermophilic microbiota outcompeting the enrichment of pathogenic microbes.

CO_2 and HNO_3 treatments reduced considerably the presence of the phytopathogen *Cladosporium spp.* and active human pathogens such as *Legionella* and *Mycobacterium* in acidified RW. In conclusion, treatments based on CO_2 or HNO_3 applied on reclaimed water are key factors to prevent the presence of microbial pathogens in water.

5 General conclusions

This work presents a study of different maintenance strategies for biofilm mitigation in water system networks for urban and agricultural reuse water applications. Two different types of reclaimed water were used in this study:

- Effluent from a pilot MBR located at Riu Sec WWTP
- Secondary effluent from Caldes de Montbui WWTP

The potential to promote biofilm growth of each type of reclaimed water with different post-treatments was tested in two pilot water networks.

The effluent from the pilot MBR was tested on a pilot irrigation network simulating a urban application of reclaimed water. Four different effluents were tested: effluent with no treatment and effluent disinfected with: (1) sodium hypochlorite, (2) sodium hypochlorite plus ultraviolet and (3) ultraviolet. Only effluents treated with sodium hypochlorite decreased the potential of biofilm growth on pipes.

Specifically, the use of an electrochemical sensor to predict biofilm growth on urban networks was investigated in Sabadell site. The use of the electrochemical sensor ALVIM, gave promising results under constant flow conditions in laboratory and field tests.

Secondary effluent from Caldes de Montbui was tested on a pilot irrigation network simulating an agricultural reuse application. Three different effluents were tested: secondary effluent with no treatment and secondary effluent treated with: (1) CO_2 and (2) HNO_3. Besides, groundwater was used as a control. Biofilm and scaling formation and composition on pipes and drippers were evaluated. Eventually, drip clogging occured in GW instead of RW although both water types had high concentrations of scaling ions. Treatments with CO_2 and nitric acid allowed getting acidified waters and avoid scaling formation. We couldn't assess if nitric acid and CO_2 were good measures to mitigate biofilm formation because we had not enough development of biofilm throughout the duration of the demonstration study in order to have a substantial worsening of the irrigation system performance with RW. However, biofouling composition was evaluated. CO_2 and HNO_3 treatments achieved a considerable reduction of microbial pathogens in the water outflow, such as the phytopathogen *Cladosporium spp.* and human pathogens such as *Legionella* and *Mycobacterium*. Acidification treatments applied on reclaimed water, besides their use for scaling prevention, are key factors to prevent microbial pathogens. Concretely for the present study, the use of reclaimed wastewater led to a better drip irrigation performance, in terms of clogging reduction and drip uniformity, and less scaling formation compared to groundwater.

Table 12 presents a comparison of main results obtained with each water treatment.

Table 12 Comparison of maintenance strategies

Demonstration site	Treatment	Advantages	Disadvantages
Riu Sec WWTP (Sabadell)	Effluent MBR + Chlorination	• reduction of microbial population in water • reduction of biofilm growth in pipes	• presence of resistant microorganisms • toxicity
	Effluent MBR + Ultraviolet	• low toxicity	• favors microbial regrowth in water under certainconditions • favors biofilm growth on pipes
Torre Marimon (Caldes de Montbui)	RW + CO_2	• low toxicity • reduces pH efficiently and potentially avoids scaling	

Demonstration site	Treatment	Advantages	Disadvantages
		• reduces plant and human pathogen in dripper water outflow	
	RW + HNO_3	• reduces pH efficiently and potentially avoids scaling • reduces fungal and human pathogens in dripper water outflow	• toxicity • addition of nitrate in the irrigation water, thus it has to be accounted for the fertilisation dosage

6 References

ALVIM Biofilm Monitoring System brochure.

Ayers, R.S. and Westcot, D.W. (1994). Water quality for agriculture. FAO Irrigation and Drainage Paper 29. Rome, Italy.

Asano, T., Burton, F., Leverenz, H., Tsuchihashi, R., and Tchobanoglous, G. (2007). Water reuse. McGrawHill, New York, USA.

Boe-Hansen, R., Martiny, A. C., Arvin, E., and Albrechtsen, H. J. (2003). Monitoring biofilm formation and activity in drinking water distribution networks under oligotrophic conditions. Water Science and Technology, 47(5), 91-97.

Bucks, D. A. (1979). Trickle irrigation management for grape production.

Caporaso, J. G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello E.K., Fierer N., Gonzalez A.-Pena,. Goodrich J.K, Gordon J.I., Huttley G.A., Kelley S.T., Knights D., Koenig J.E., Ley R.E., Lozupone C.A., McDonald D., Muegge B.D., Pirrung M., Reeder J., Sevinsky J.R., Turnbaugh P.J., Walters W.A., Widmann J., Yatsunenko T., Zaneveld J., Knight R. (2010). QIIME allows analysis of high-throughput community sequencing data. Nature Methods. 7: 335-336.

Caporaso, J.G., K. Bittinger, F.D. Bushman, T.Z. DeSantis, G.L. Andersen, R. Knight. (2010). PyNAST: A flexible tool for aligning sequences to a template alignment. Bioinformatics. 26: 266-267.

Capra, A., and Scicolone, B. (1998). Water quality and distribution uniformity in drip/trickle irrigation systems. Journal of Agricultural Engineering Research, 70(4), 355-365.

Capra, A., and Scicolone, B. (2004). Emitter and filter tests for wastewater reuse by drip irrigation. Agricultural water management, 68(2), 135-149.

Capra, A., and Scicolone, B. (2005). Assessing dripper clogging and filtering performance using municipal wastewater. Irrigation and drainage, 54(S1).

Capra, A. (2016). Management of micro irrigation using wastewater to reduce emitter clogging: a review. In: Wasterwater management for irrigation. Principles and practices. Goyal MR and Tripathi VK (eds.). Chapter 5, pp. 97-144. CRC Press and Apple Academic Press, Inc.

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72(7): 5069-5072.

Dosoretz, C.G., Tarchitzky, J., Katz, I., Kenig, E., and Chen, Y. (2011). Fouling in microirrigation Systems applying treated water efluents. IN: Treated water in agriculture. Levy GL, Fine P and Bar-Tal A (eds.) Chapter 10. Blackwell Publishing Ltd. Pp 328-350.

Edgar, R.C. (2010). Search and clustering orders of magnitude faster than BLAST, Bioinformatics, 26: 2460-2461.

Gasol, J.M., Moran, X.A.G. (2015). Flow cytometric determination of microbial abundances and its use to obtain indices of community structure and relative activity, 159e187. doi:10.1007/8623_2015_139.

Haas, B.J., Gevers, D., Earl, A.M., Feldgarden, M., Ward, D.V., Giannoukos, G., et al. (2011). Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Gen. Res. 21: 494-504.

Hallam, N.B., West, J.R., Forster, C.F. and Simms, J. (2001). The potential for biofilm growth in water distribution systems. Water Research, 35 (17), 4063-4071.

ISO 8199:2005. Water quality - General guidance on the enumeration of microorganisms by culture.

Nakayama, F. S., & Bucks, D. A. (1991). Water quality in drip/trickle irrigation: a review. Irrigation Science, 12(4), 187-192.

Nakayama, F. S. (2007). Microirrigation for crop production: design, operation, and management. Elsevier, Amsterdam.

Nakayama, F. S., and Bucks, D. A. (1981). Emitter clogging effects on trickle irrigation uniformity. Transactions of the ASAE, 24(1), 77-0080.

Nakayama, F.S. and Bucks, D.A. (1991). Water quality in drip/trickle irrigation. Irrigation Science, 12:187-192.

Parsons, L.R., Sheikh, B., Holden R., York, D.W. (2010). Reclaimed Water as an Alternative Water Source for Crop Irrigation. Hortscience, 45(11), 1626-1629.

Pavanello, G., Faimali, M., Pittore, M., Mollica, A., Mollica, A., Mollica, A. (2011). Exploiting a new electrochemical sensor for biofilm monitoring and water treatment optimization. Water Resarch, 45 (4), 1651-1658.

Raudales, R.E., Parke, J.L., Guy, C.L., and Fisher, P.R. (2014). Control of waterbone microbes in irrigation: a review. Agricultural Water Management, 143: 9-28.

Reeder, J., Knight R. (2010). Rapidly denoising pyrosequencing amplicon reads by exploiting rank-abundance distributions. Nature Methods 7, 668-669

Van der Kooij, D., Veenendaal, H.R. (2001). Biomass production potential of materials in contact with drinking water: method and practical importance. Water Science Technology. Water Supply 1 (3), 39-45.

Varaprasad, B.J.S. & Viswanadh G.K. (2012). A study on scale formation in water distribution systems. IJWREM 3(1):129-136

Velji, M.I. and Albright, L.J. (1986). The dispersion of adherent marine bacteria by pyrophosphate and ultrasound prior to direct counting. IFREMER, Actes de Colloques,3, pp 249-259.

Wilcox, J. C., and Swailes, G. E. (1947). Uniformity of water distribution by some under tree orchard sprinklers. Scientific Agriculture, 27(11), 565-583.

7 Annex

7.1 MBR pilot plant set-up

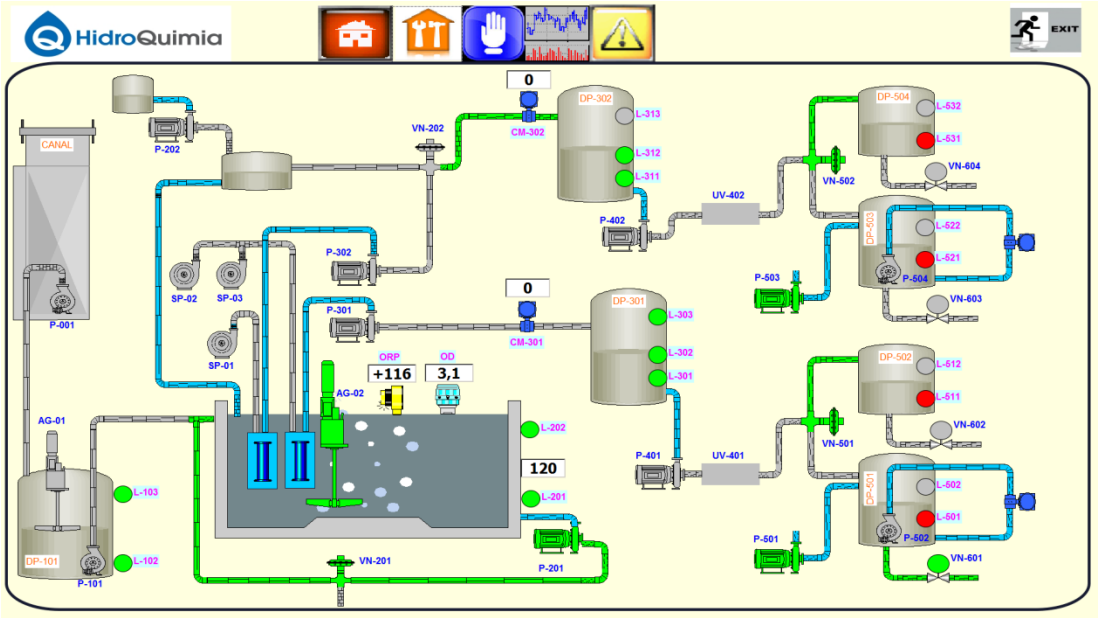

Figure 52 Screenshot of the SCADA system controlling the MBR pilot plant operation

7.2 Review of electrochemical sensors

Introduction

Serious wide-range technological problems can be caused by fouling development on any apparatus or material exposed to natural water (fluid flow systems, water distribution lines, sensors, etc), involving highly negative economic repercussions.

Biofilms are made up of free floating microorganisms that weakly adhere themselves to a surface. If they aren't immediately removed, they can bind more strongly to the surface, allowing further species to attach and colonise into a biofilm (see Figure 53).

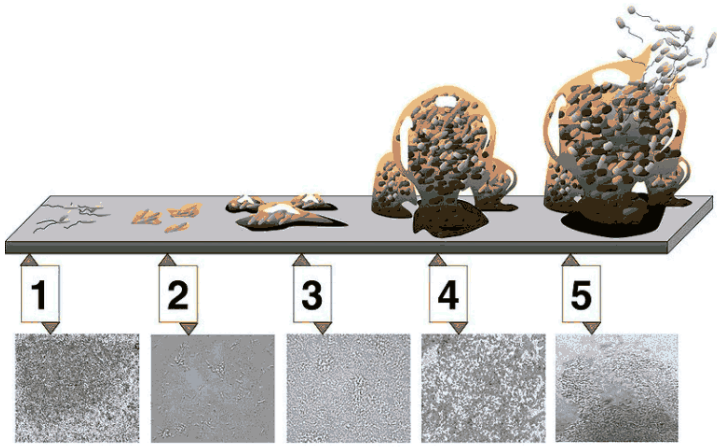

Figure 53 The five stages of biofilm development: (1) Initial attachment, (2) Irreversible attachment, (3) Maturation I, (4) Maturation II, (5) Dispersion [1]

In the water lines of industrial plants, large amounts of disinfectants and other chemical substances are usually employed as a countermeasure against biofilm. The continuous monitoring of the biofilm can be extremely useful to prevent these technological problems by applying hindering treatments as soon as the fouling appears.

Recently, the electrochemical activity of natural aquatic biofilms was proven to be proportional to the surface area covered by bacteria; therefore, measuring the biofilm electrochemical signal is possible to know the amount of biofilm covering on the surface [2].

Figure 54Figure 1 shows, on the left side, the evolution of the bacteria mean population density on stainless steel surfaces exposed in a loop in which natural seawater flowed. On the right side of the figure the trend of the current generated by the sensor put in series in the same loop is plotted. Therefore, from these two graphs it can be concluded that electrochemical sensors can provide correct information on the incubation time and on the growing rate of the biofilm [3].

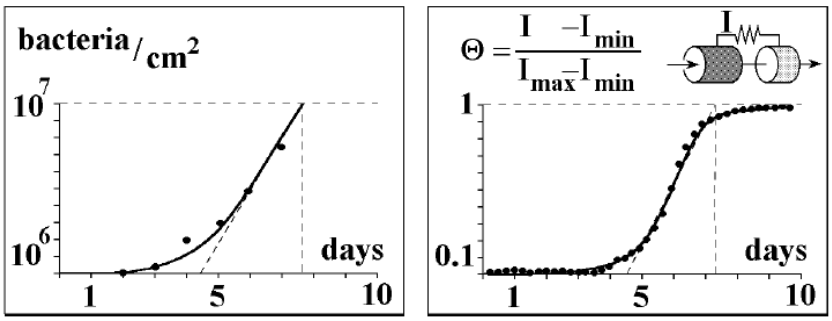

Figure 54 Amount of bacteria settled and the corresponding electrochemical signal obtained in a seawater loop [3]

New generation of on-line monitoring systems based on electrochemical biofilm sensors provides a signal that can be used to rationalise the application of antifouling procedures in plants saving efficiency, decreasing corrosion and concurrently minimising chemicals addition in water systems.

The main objective of this document is to identify the different electrochemical sensors that are currently available to measure the biofilm growth in water distribution networks. The following paragraphs provide

the main characteristics of each sensor found. With this information, the best sensor to be used in Task 2.4 of the DEMOWARE project will be selected.

BIOX Electrochemical probe

The BIOX probe was developed by CESI in collaboration with CNR-ICMM of Genova, and then applied since 1996 in Italian power plants in order to optimise the antifouling treatments based on oxidant biocides, mainly chlorination.

BIOX system works applying a quasi-intentionstatic cathodic polarisation to the stainless steel element. Its response is correlated to the changes of electrochemical kinetic processes on metal surfaces induced by bacteria settlement or by oxidant agents. The response of the sensor permits to have a direct index of biofilm growth and the effective oxidants concentration on the sensor surface.

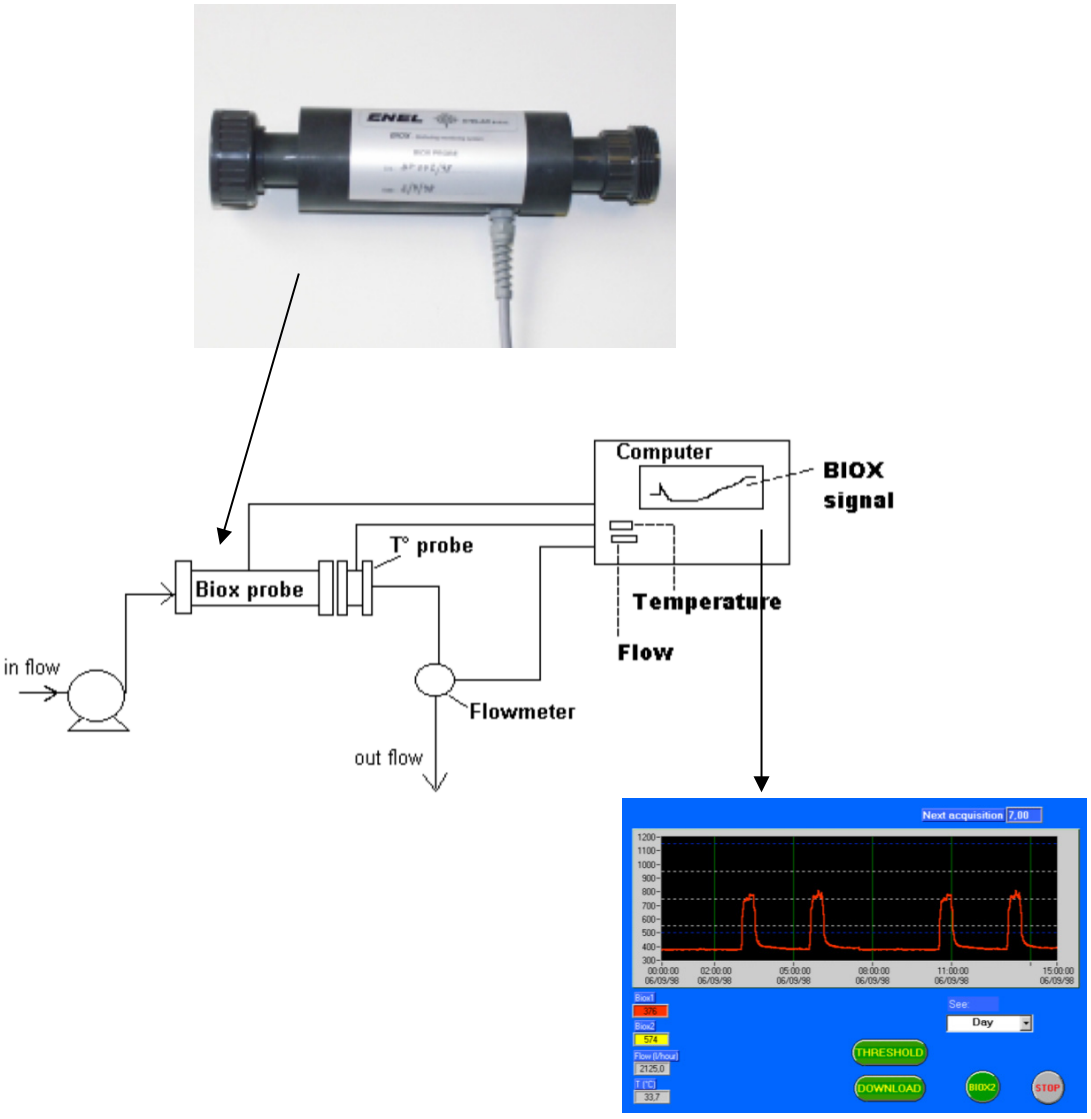

Figure 55 BIOX electrochemical sensor

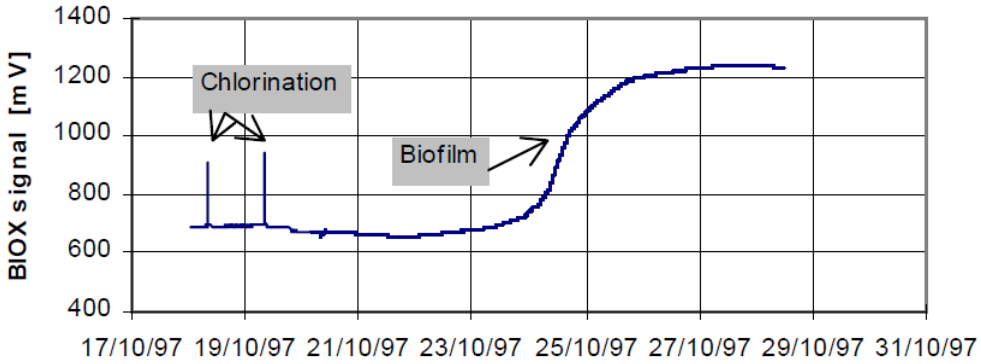

Figure 56 Signals provided by BIOX electrochemical sensor

On the basis of the information provided by the BIOX sensor, the plant personnel can easily deduce if the applied chlorination program can be reduced or must be increased, observing in real time the effect of the new disinfection program. An example is shown in Figure 57Figure 4, where it can be observed that the chlorination program initially applied in a power plant was correct because it was sufficient not only to avoid biofilm formation, but also was able to rapidly destroy the biofilm grown during a period in which the chlorination was temporarily stopped.

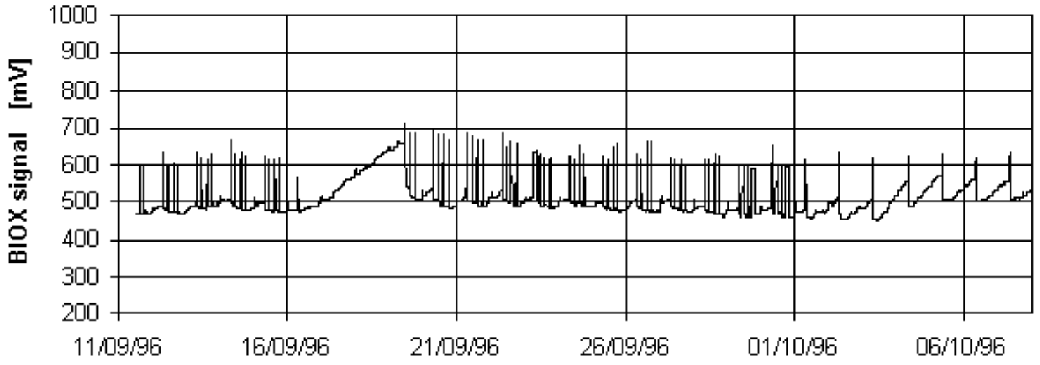

Figure 57 Chlorination and biofilm formation signalised by BIOX probes during two monitoring tests in a power plant

Related documentation:

Mollica and Cristiani, On-line biofilm monitoring by "BIOX" electrochemical prove, Water Science and Technology 47, 5 (2003) p. 45-49.

BioGEORGE – Biofilm activity monitoring system

The BioGEORGE system was developed to provide on-line and real-time indication of biofilm activity on typical metallic surfaces. The probe is designed and operated so that microorganisms in the environment are encouraged to settle on probe surfaces before they settle on piping.

By closely tracking biofilm activity on the probe, the operator is alerted to the need to treat the system, to assess the effectiveness of a treatment, to schedule maintenance activity or to optimize chemical treatments.

The system consists of a probe, which is installed directly in the pipe, its integrated electronics, interconnecting cable and display software.

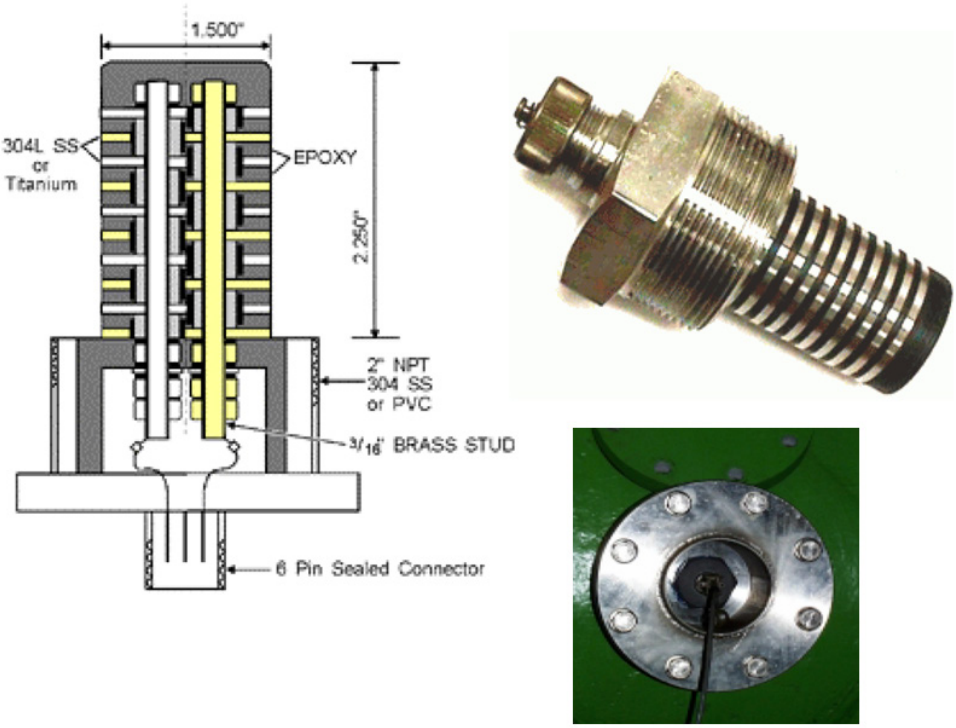

Figure 58 The BioGEORGE sensor

Related documentation:

http://www.alspi.com/biogeorge.htm

BioSense biofilm monitor

The BioSense biofilm monitoring sensor is a proven technology that monitors a water system and provides an alarm when conditions allow for the growth of a biofilm. The biofilm grows preferentially on the sensor surface encouraged by electrical simulation, and then can be detected.

Figure 59 The BioSense biofilm monitoring sensor

The BioSense sensor consists on a cylindrical sensor with a series of circular electrodes. The sensor is inserted into the water supply pipe at the point where biofilm is most likely to occur. It is connected to a set of electronics which applies a potential that causes microorganisms to settle on the surface of the probe before they would settle on the surface of the pipe. The biological activity of the biofilm creates a signal.

A BioSense controller collects and monitors that signal continuously, and when an increasing trend is observed it indicates the onset of biofilm activity on the probe. The controller can then take remedial action automatically by, for example, increasing or decreasing biocide levels.

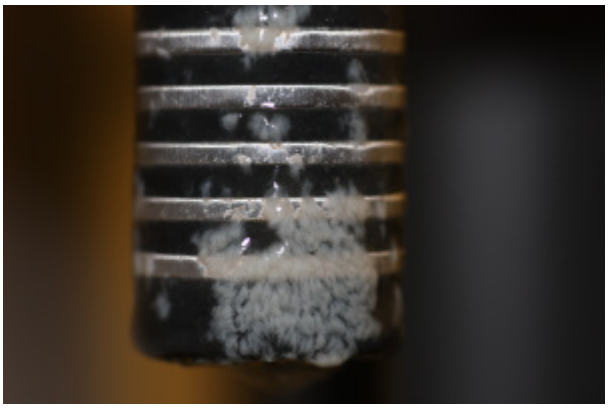

Figure 60 Biofilm growing in the BioSense sensor

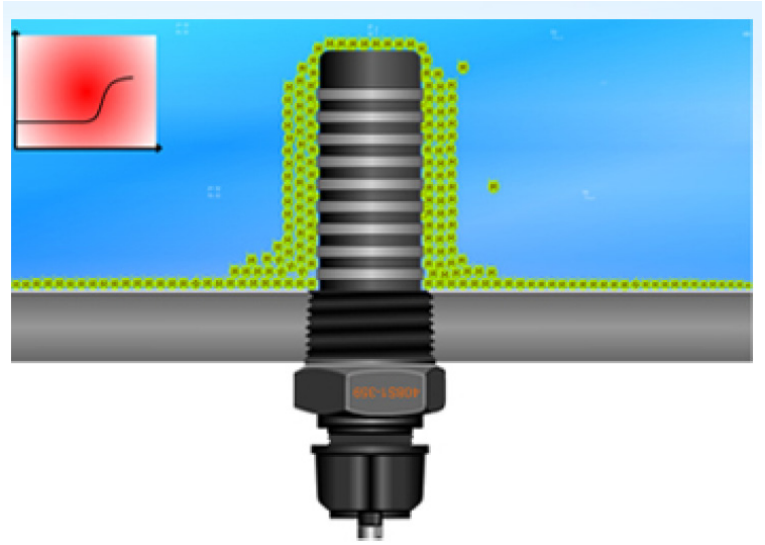

Figure 61 Diagram of the biofilm growth and the signal generated by the BioSense sensor

The BioSense biofilm monitor can be used in any water system that is subject to biofilm growth. It has remote access capability and process control, it does not require maintenance and can be combined with other sensors.

In order to demonstrate the efficiency of the BioSense sensor, it was tested against BioGEORGE sensor, which has been used to monitor biofilm formation in the Nuclear power industry for 20 years. The data obtained showed that BioSense responded to the biofilm formation in a concordant manner to that of BioGEORGE.

Related documentation:

BioSense – A biofilm monitor and controller (www.processintruments.net)

ALVIM sensor

The ALVIM system is based on a sophisticated biofilm electrochemical signal measuring technique. Figure 62 shows the correlation between ALVIM probe signal and increasing biofilm growth on the probe itself.

ALVIM probe allows a real-time measurement of biofilm growth rate and of its possible decrease due to biocide injection in the plant. The sensor can be easily inserted in any industrial plant thanks to a simple threaded lock, and is connected just to one cable, which is in charge of transporting data and powering the sensor. It is also important to mention that the sensor measure is not affected by temperature variations.

A part from revealing and monitoring the biofilm growth, ALVIM sensor is also sensitive to oxidizing substances; this allows a real-time monitoring of biocides application, providing additional information on disinfection plant functioning.

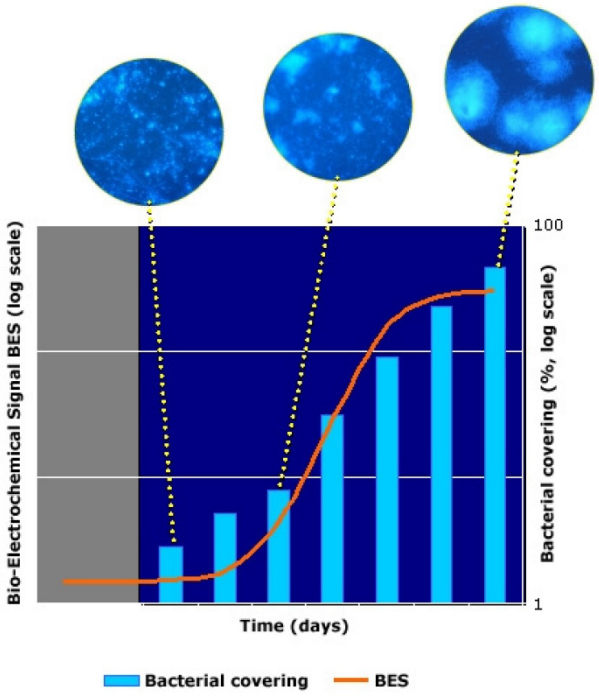

Figure 62 Correlation between ALVIM signal and biofilm growth rate

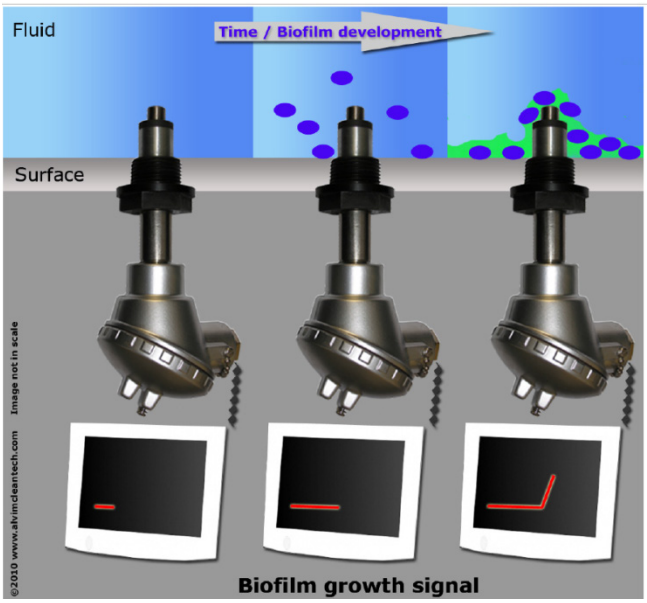

Figure 63 ALVIM sensor functioning

Finally, regarding the electronics for the control of the process, ALVIM has a fully digital management and its electronic is completely integrated within sensor housing. Furthermore, all the collected data can be viewed in real time remotely.

Related documentation:

Giovanni Pavanello, Marco Faimali, Massimiliano Pittore, Angelo Mollica, Alessandro Mollica, Alfonso Mollica; Exploiting a new electrochemical sensor for biofilm monitoring and water treatment optimization, Water Research 45 (2011) 1651-1658.

ALVIM Biofilm Monitoring System (www.alvim.it).

Neosens FS-series

A part from the electrochemical sensors previously described, a different kind of sensor based on thermal measurements has been also identified.

The Neosens FS-series fouling sensor continuously monitors the fouling phenomenon (including biofilm and scaling) within the water process enabling the optimization of treatment and confirmation of treatment effectiveness. The sensor is based on the hot wire method combined with measurements of heat flux and fluid and wall temperatures in a controlled hydraulic and thermal environment. It uses a patented thermal analysis technique which measures the minute changes in local thermal conductivity and heat transfer due to fouling of just a few microns in thickness.

Plugged directly inside your equipment, the probe monitors deposits in real-time in order to:

- Ensure water treatment efficiency,
- Trigger alerts in case of biofilm and/or scales abnormal increase
- Optimize and reduce chemical discharges
- Mitigate legionella risk

Figure 64 The Neosens FS-series fouling sensor

References:

[1] www.processinstruments.net

[2] Giovanni Pavanello, Marco Faimali, Massimiliano Pittore, Angelo Mollica, Alessandro Mollica, Alfonso Mollica, Exploiting a new electrochemical sensor for biofilm monitoring and water treatment optimization, Water Research 45 (2011) 1651-1658.

[3] Mollica A., Simple electrochemical sensors for biofilm and MIC monitoring, Instituto per la Corrosione Marina dei Metalli, Biofilm and MIC monitoring: State of the art.

Related documentation:

Fouling Systems, FS-1000 Series WT, Water-based applications (www.neo-sens.com)

Fouling Sensor by Neosens, Case studies (www.advantagecontrols.com)

Biofilm sensor comparison table

Sensor	Type of fouling	Measuring principle	Connection type	Electronics of the system	Where can be used?	For what can be used?
BIOX	Biofilm	Electrochemical signal measurement	It can measure the biofilm growth online, but it cannot be connected directly to the pipe.	The electrochemical probe is associated with a specific hardware/software to a computer. Device control and data reading are basically analogical.	Applied in systems with freshwater and also with seawater.	Monitors the biofilm growth and permits to optimize chlorination treatments.
BioGEORGE	Biofilm	Electrochemical signal measurement	Connected directly to the pipe	The control and data acquisition are housed in an external box, where the readings are stored in a database. System data can be downloaded to the user's PC. Software is included for analyzing the data.	Applied in the Nuclear power industry for 20 years.	The tracking of the biofilm activity on the probe alerts the operator to the need to treat the system and to optimize chemical dosing.
BioSense	Biofilm	Electrochemical signal measurement	Connected directly to the pipe	The signal generated by the biofilm growth is monitored and converted to a risk percentage. The control and monitoring can be done remotely.	It can be used in any water system that is subject to biofilm growth (cooling towers, hospitals, large leisure facilities, water circuits, swimming pools,	Monitors a water system and provides an alarm when a biofilm grows. It can be used to automatically instigate or change the chemical dosing, and also to reduce the risk of legionella growth.
ALVIM	Biofilm	Electrochemical signal measurement	Connected directly to the pipe	Data acquired by the sensor is collected by a PC or an external server and stored in a database (it is possible to access the data from different clients and to automatically carry out different operations on field acquired data).	It can be used in any field affected by biofilm-related problems (pipelines, tanks, heat exchangers, cooling towers, RO membranes, etc)	Precise real-time biofilm monitoring since its early stages. Evaluation of disinfection system effectiveness, automated biocides dosing in function of the needs and legionella risk prevention.
Neosens FS-series	Biofilm and scaling	Thermal measurement	Connected directly to the pipe	The sensor can be connected directly into a controller with the 4-20 mA input card option. Historical graphs, real time readings and email alarm notification can be done.	Cooling towers, heat exchangers, filtration & membranes, water treatment, pulp & paper. Resistant to harsh environments.	The probe monitors deposits in real-time in order to ensure water treatment efficiency, to trigger alerts in case of biofilm and/or scales increase, to optimize and reduce chemical discharges and to minimize legionella risks.