DEMOWARE

Deliverable 2.7
Process monitoring and performance control of water recycling schemes

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Deliverable Title	**D2.7** **Recommendations for monitoring in water reuse schemes integrating work of all partners and their activities in the WP. Contributions cover microbial and chemical contaminant characterisation as well as operational aspects.es**
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Abstract	The report at hand seeks to extract the essential findings from the demonstration work on process monitoring and performance control. I seeks to derive some suggestions for improved monitoring and operational control. It integrates the work of all partners and their activities in relation to microbial and chemical contaminant characterisation as well as operational aspects, i.e.: • Flow cytometry and qPCR methods to assess treatment process effectiveness and to control microbial contamination in water reuse • On-line particle monitoring to monitor membrane integrity • Chemical fingerprinting as refined and optimises procedure for chemicals' detection and charac-terisation of their fate) • Effect based bioassays to detect the integrated effects of chemical constituents • Various approaches to investigate the effect of disinfection on biofilm formation

Table of contents

List of figures

List of tables

Executive Summary

Part of the work of the DEMOWARE project aimed at demonstrating advanced monitoring and control options for water recycling schemes which enable better understanding of contaminant fate and safe operation of full-scale systems. The inherent risk in water recycling stems from the wide range and potential negative impact of contaminants in wastewater. Treatment technologies and appropriate use practices are able to mitigate those risks but the range of contaminants of concern gets wider with an ever growing spectrum of organisms and chemicals detected in reclaimed wastewater as the analytical capacities increase. Based on a better understanding of contaminant occurrence and fate operators should be enabled to monitor their water recycling processes more effectively.

The general objective of work package 2 thus was to demonstrate approaches for the assurance of desired routes/fate of reclaimed water constituents (pathogens, contaminants as well as nutrients) in various reuse applications covering the full scheme from treatment through distribution to actual application. This specifically includes

- The demonstration and testing of advanced monitoring and control options for microbial and chemical contaminants.
- To test advanced technologies to control the integrity of treatment barriers.
- To provide end-user specific suggestions on process monitoring and performance control of water reuse schemes.

Based on that work we have provided a set of characterised approaches, namely

- Flow cytometry and qPCR methods to assess treatment process effectiveness and to control microbial contamination in water reuse
- On-line particle monitoring to monitor membrane integrity
- Chemical fingerprinting as refined and optimises procedure for chemicals' detection and characterisation of their fate)
- Effect based bioassays to detect the integrated effects of chemical constituents
- Various approaches to investigate the effect of disinfection on biofilm formation

The report at hand seeks to extract the essential findings from this demonstration work and to derive some suggestions for improved monitoring and operational control.

Not all tools tested turned out to be suited for routine operational or verification monitoring. However, they enable a detailed and site-specific analysis and characterisation of the effectiveness of an installed treatment train. They can help to identify points of attention and to increase overall understanding for the functioning and interdependencies between process steps and components. This can be a valuable contribution to risk assessment work in future water reuse safety planning.

Particularly the molecularbiological methods provided insight into the effects of different control measures, such as membranes and disinfection on the microbial community in the down-stream part of the reuse scheme.

An overview of their main characteristic and applicability is compiled in the table below.

Table Overview table of main characteristic of applied tools and methodologies

*commercial particle counters give results immediately, the tested device data could not be read in real-time and required a lot of manual processing

Tool	Online / offline	Time to result	State of development Application by /when	Suited for monitoring
FCM	Mostly offline Online under development and testing	Minutes to < 1 h	Fully commercialised Operator (water utility) or external lab Routinely, within regular sampling programes	Operational confirming distribution network maintenance effectiveness
qPCR based indicator system	Offline	Hours to 1 day	Underlying methods well established, targeted development, Specialised water lab	Operational, exceeding of threshold values
Chemical fingerprinting	Offline	2-5 days	In-house expertise, service to be provided in planning phase / impact assessment phase or follow up studies	Long term changes
Submicron particle counting	Offline and online	Minutes to hours or days*	Prototype requiring improved data management and calibration	Operational,
Effect based bioassay	Offline and Online under testing	Minutes - < 1h	Commercialised / in stage of commercialisation	Operational, Verification

1 Introduction

1.1 Monitoring requirements in water reuse

Today, all national water reuse legislation in Europe requires monitoring to verify that water quality complies with limit values. Depending on the final use such standards are defined for microbial contaminants or chemical constituent posing a risk to workers, end-users or the environment. This means water quality monitoring is mainly performed as control of the end product when it leaves the water reclamation plant (Point VE in Figure 2), using indicator organisms and most frequently bulk parameters.

With respect to health risk related parameter, the microbial parameters most often used are faecal indicators, such as faecal coliforms, total coliforms and *E. coli*. (Alcalde-Sanz and Gawlik, 2014). Various national water reuse regulations not only specify the numerical values but also the frequency and method of sampling and analysis. Observing chemical parameters

Currently the European Commission undertakes to develop EU-wide minimum quality requirements for water reuse in agricultural irrigation and aquifer recharge. The latest draft version addresses three types of monitoring that are suited to assure safe water reuse: Verification monitoring, validation monitoring and operational monitoring

Verification monitoring is the routine monitoring of reclaimed water quality, usually at the end of the treatment process. It is to prove compliance with requested minimum reclaimed water quality limits.

Validation monitoring aims at demonstrating the removal efficacy of a treatment train for a defined set of indicator organisms – or substances. It is to validate a specified reduction performance for different groups of pathogens (bacteria, virus and protozoa). Performance targets are specified as log removal values (LRV), i.e a LRV of 3 equals a 99.9% removal.

Operational monitoring refers to surveillance of treatment process components or other control measures (barriers) in place. The purpose is to confirm the intactness and proper functioning of these measures. Any such monitoring is quite specific to a process or the site and therefore requirements are usually not specified in legislation. However, best-practice recommendation and guidance documents can advice on suitable approaches (parameters and methods). Operational monitoring is a key component in any type of risk management based water safety concepts, such as the Sanitation Safety Plan (WHO, 2016).

1.2 Water reuse scheme components

Water recycling encompasses the treatment and processing of wastewater to fit-for-use quality and its distribution and application for beneficial purposes. Such system hence includes treatment technology, storage tanks, transport and distribution networks and appliances at the point of use. A very schematic illustration of these elements is given in Figure 1.

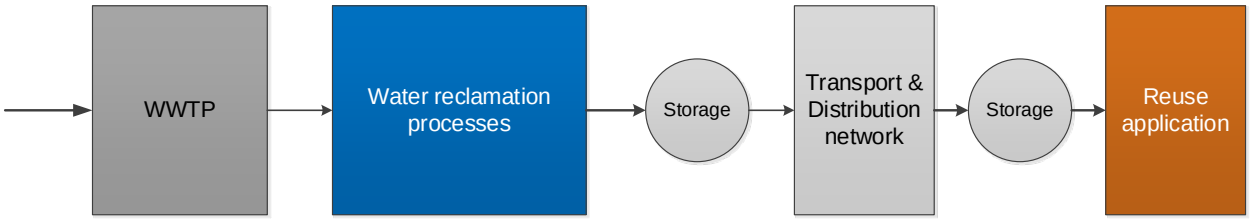

Figure 1 Schematic illustration of water reuse scheme components (WWTP = wastewater treatment plant)

Each component of such system has either the purpose or potential to alter the water quality. Whilst dedicated treatment processes shall remove or reduce target compounds and parameters, storage and distribution can bring rather uncontrolled changes of water quality, by e.g. regrowth, dissolution or infiltration.

1.3 Scope of testing

Figure 2 further exemplifies the water reuse scheme components with some arbitrary processes, mentioning those that were considered in the project. The yellow triangles indicate possible monitoring or control points. Treatment steps such as membranes and disinfection are strong barriers for microbial contaminants. If installed in flowsheets, their proper functioning is key to achieve required water quality in the verification monitoring point (VE).

Alteration of water quality in storage and piping elements of the distribution network might require additional post-treatment before actual reuse - a measure to be decided upon based on monitoring data.

A list summarizing the precise treatment trains in the demonstration sites is presented in Table 1.

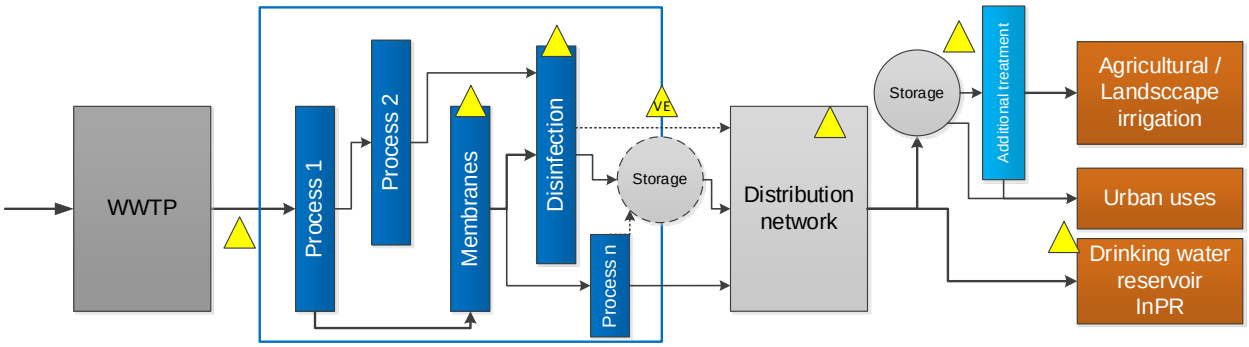

Figure 2 Schematic illustration of water reuse scheme components and possible monitoring points (yellow triangles).

Table 1 Summary of demonstration sites in which monitoring methods were applied

CAS: conventional activated sludge, GAC: granular activated carbon, SF: sand filtration, UV: ultraviolet irradiation, SAT: soil aquifer treatment

No	Train	Reuse site	Type of reuse	Scale
1	Membrane bioreactor + GAC + Chlorination (treatment & distribution network)	Old Ford Water Recycling Plant (UK)	Urban reuse (toilet flushing, park irrigation)	Full
2	CAS + SF + Ultrafiltration + UV	Capitanata (IT)	Agricultural irrigation	Pilot
3	Membrane bioreactor + Chlorination + UV	Sabadell (ES)	Urban reuse (park irrigation, street cleaning)	Full / pilot
4	Sequencing batch reactor + UF	Basel (CH)	none	Pilot
5	CAS + water acidification Agricultural irrigation network	Torre Marimon (ES)	Agricultural irrigation	Pilot
6	Biofiltration + Ozonation + SAT	Shafdan (IL)	Agricultural irrigation	Pilot
7	Ultrafiltration + Reverse Osmosis + SAT	Shafdan (IL)	Agricultural irrigation	Pilot
8	Ultrafiltration + Reverse Osmosis + infiltration	Torreele (BE)	Indirect potable reuse	Full
9	Not specified	Vendée Greenfield site	Indirect potable reuse	planned

2 DEMOWARE monitoring tools and methods

This section summarises the results of our testing focussing on the lessons learnt and pointing at application options. The characterisation covers a short description of the tool or method specifying what it is technically. Mentioning the application area in which we have tested it or where it could be deployed, the advantages over alternative methods are summarised.

The tools under investigation are listed in Table 2. Detailed information on the experiments and campaigns can be found in the quoted deliverables (see also Table 4).

Table 2 Monitoring tools applied in different demonstration sites (for numbers see Table 1)

Tool	Targeted aspect	Tested in demonstration site	Related deliverable
Flow cytometry	Disinfection effectiveness, Microbial water quality & regrowth	1 - 4	D2.5
qPCR based indicator system molecularbiological methods	Disinfection effectiveness, microbial water quality Detection of new indicators	(1, 2, 3, 4)	D2.5
(Submicron) particle counting	Membrane integrity	3 + 4	D2.2
Bioassay	Integrated effects of chemical water quality,	6 - 8	D2.6
Chemical fingerprinting	Impact of wastewater discharge / reuse on surface water quality	9	D2.3
Biofilm mitigation techniques		3, 5	D2.4

2.1 Flow cytometry

2.1.1 Description (from D2.5)

FCM offers real-time monitoring (within 15-20 minutes from sampling), ease of use (limited specialisation of the personnel), transportability, high precision, high reproducibility, low cost, and amenable of automation. The FCM represents a generic technology that counts and measures the optical properties of individual particles of 0.1 – 50 μm in a flow stream. The analysis is performed by measuring the scattered laser light (both forward (FSC) and side scatter (SSC)) and the fluorescence signals through photomultiplier detectors (PMTs). Approximately 1,000 events per second can be detected within any sample that can be reduced to a suspension of mono-dispersed particles with a mass suitable for analysis.

The method is based on staining microorganisms with fluorescent dyes which target a particular cell function and enable characterisation, e.g. cell viability, whilst differentiating cells from background particles in the same size range. Dyes typically intercalate into nucleic acids resulting in strong signals upon binding. Cells are subsequently focused in a stream of liquid (sheath fluid) and passed by an electronic detection system that records signals after exciting the corresponding dyes at suitable wavelengths. Fluorescent properties and scatter behaviour are recorded giving rise to 2-dimensional plots that allow the distinction of microbial sub-populations (every cell is visible as a dot in the plot).

2.1.2 Application areas

FCM was applied for microbial characterisation of a full-scale water reuse scheme. Both the treatment efficacy as well as the development of water quality in the distribution network were monitored. The results were found to be consistent with standard cultivation methods. Monitoring of any treated wastewater stream is easily possible.

2.1.3 Advantages

Sample processing and measurement is faster than standard cultivation methods. In principle, the measurement can also be performed on fixed samples and thus allows for some time between sampling and actual measurement.

Looking at the full range of bacteria with FCM can provide additional insights. It is e.g. possible to distinguishing whether contamination of the permeate side of a membrane is rather stemming from the feed side or is caused by regrowth or on which fraction of the microbial community a barrier acts.

2.1.4 Limitation

It is known and was reconfirmed that in high cell concentration matrices, such as activated sludge systems, the results might underestimate cell number due to insufficient disaggregation of flocs. However, monitoring requirements are usually not defined for these matrices.

2.2 qPCR based indicator system (molecular biological methods)

2.2.1 Description (from D2.5)

The real-time polymerase chain reaction is a laboratory technique based on the polymerase chain reaction (PCR). With the help of fluorescent dyes and a Real-Time Thermal Cycler it is possible to monitor the amplification of DNA molecules during the PCR (in real-time). With the application of specific predefined DNA-standards it is possible to quantify the DNA during this process (quantitative real-time PCR). There are several possible methods for real time PCR measurements, the one used in this project is based on non-specific fluorescent dyes that intercalate with any double-stranded DNA.

To quantify the amount of specific DNA-copies a custom primer set and standard DNA is needed for every organism of interest, but the approach (cloning and plasmid isolation) allows for easy extension of the spectrum of target microorganisms. The sample preparation can be standardized via existing DNA extraction protocols. In combination with the DNA stain PMA, it was possible to distinguish between viable and membrane-compromised cells. Real-time PCR with the PMA pre-treatment step can thus serve as a fast and applicable tool for risk management.

The idea here was to identify bacteria that withstand certain disinfection procedures and to develop a tailored detection and quantification method for those. Their increased occurrence would be a measure for changes in disinfection effectiveness.

2.2.2 Application area

The indicator set can be applied to water samples or resuspended biofilms. It was tested on samples from various set-ups to confirm and quantify the presence of the indicator organisms in disinfected effluents, storage tanks and after membranes.

Initial results for the qPCR method indicate that a monitoring of the success of disinfection treatments can be greatly enhanced by the application of molecular methods and can potentially lead to faster results than a cultural approach and can be applied to detect even non-cultivable hygienically relevant microorganisms.

Unlike FCM, real-time PCR analysis was possible for highly enriched biofilm samples with the procedure not considered overly time-consuming.

The tool now exists to allow for an easier assessment of specific maintenance and disinfection regimes and future results will help to understand the impact of specific treatments on the population dynamics of different wastewater treatment plants

2.2.3 Advantages

With the help of this method it is not only possible to reduce the time until results are available to a few hours, with the right equipment and trained personnel the tests can be performed directly on site. The more precise assessment of the microbiological risk of disinfected effluent waters, due to the possible inclusion of target organisms, which are non-cultivable, is an additional advantage. In order to distinguish between live and dead cells a pre-treatment step with the DNA binding dye propidium monoazide (PMA) was performed on the samples. This effectively removes bacterial DNA of compromised cells, from subsequent DNA amplification and detection steps.

The approach as such can be customised to target organisms of interest. In comparison with reference methods for microbial indicator detection this can deliver insight into the correlation of faecal indicators and more health relevant pathogens. Yet this would require a comprehensive data set.

2.2.4 Limitations

The qPCR indicator system was a new development in the project. All procedures from identification of indicator organisms, extraction, primer selection, PCR conditions sample extraction protocol, had to be optimised. It is not yet a fully validated method. This entails some uncertainties about the results obtained. In relation to PCR, the current data is not sufficient to draw final conclusions about the effects of specific maintenance regimes or treatment processes on the population of chosen indicator microorganisms.

2.3 Submicron particle monitoring

2.3.1 Description

Particle counters detecting particles in the size range above 1 µm are available in the market. The technology is based on a light beam crossing a measuring cell through which a particle suspension is flowing. Light blockage is a widely used measuring principle that is typically applied for detecting particles in the 1 - 500 µm size range.

An alternative particle measurement technology that allows for the detection of small particles down to the nanometer range is the "Dark Laser Beam Technology". Similar to a light blockage system, its working principle is based on a flow-through cell in which suspended particles interact with a laser beam. However, in this specific case, the laser beam is structured as a "Dark Beam", yielding two separate peaks of light intensity on a photo-detector. Each time the beam hits a particle, the interaction creates a phase shift in the electric field of the "Dark Beam". The resulting change in the light intensity pattern is detected by two forward and two backscatter detectors and used to calculate the size of the particle. According to the manufacturer, the detectable range of particle sizes with this technology has been found to range from 20 µm down to 150 nm in the case of polystyrene latex particles and 80 nm in the case of gold.

It is to mention that not all particles in a fluid are analysed (other than in e.g. in flow cytometry). The results therefore do not give precise quantitative information. However, under comparable settings such as a constant water sampling flow, results are representing the particle distribution and its changes in a fluid.

It is a portable device that is operated in combination with an external water sampling pump and a computer that is run with a software provided by the manufacturer. The device is operated online as it requires a minimum feed flow. This can be supplied as a side-stream of the flow to be monitored. In our testing it was extracted directly from the permeate lines of the membrane filtration units.

2.3.2 Application area

The "Dark Laser Beam" measuring device "Pola 100X" was used within the DEMOWARE project to assess the permeate particle concentration and membrane integrity of ultrafiltration systems. The effect of different damages or operational states (chemical cleaning) was assessed by monitoring feed and permeate.

Pilot-scale experiments were performed to evaluate the potential of nanoparticle counting as a means to continuously assess the integrity of ultrafiltration systems in water reuse applications. Depending on the degree of membrane damage, a nanoparticle retention between 95% (intact membrane) and 70% (large damage) was measured, and even the smallest defect with a relative size of $1.7 \cdot 10^{-8}$ could be identified. This indicates that "Dark Laser Beam" particle counting is a suitable technology for sensitively detecting changes in permeate water quality due to losses of membrane integrity. The "Dark Laser Beam" device measured particles in size ranges between 0.2 and 21 µm in UF and MF membrane permeate and secondary treated effluent.

In the full-scale MBR the device was used to follow a regular chemical cleaning procedure. The removal of cakelayer and foulants resulted in an increased particles detection in the permeate. Analysis of *E. coli*, total heterotroph and flow cytometry measurements confirmed this trend. Yet, in order to utilize such info For operational purpose (disinfectant dosage) a more profound correlation of particles counts with hygienically relevant parameters needs to be established.

2.3.3 Advantages compared to existing approaches

Currently, the standard membrane test in the water treatment industry for the detection of small defects is the pressure decay test (PDT). Particle counting and turbidity monitoring are used to meet regulatory requirements and to detect larger defects. Direct integrity monitoring methods like the PDT are reliable and sensitive, but are time-intensive and, generally, non-continuous. They need to be performed off-line, which requires an interruption of normal operation and, in some cases, a draining of the vessels.

Turbidity measurement as an online method is not susceptible to small membrane defects. It would only respond to larger disturbances. In our testing even a cut fibre was not detected when observing this parameter.

The "Dark Laser Beam" measuring device was sensitive to detect small membrane damages which resulted in an increased number of particles detection. The sensitivity of the device was found to be higher than commercially available particle counters which detecting particles in the above 1 µm range. This way relative small membrane damages could be detected in pilot plant experiments (ultrafiltration of secondary treated wastewater).

2.3.4 Limitations

Compared to alternative techniques such as pressure decay testing or monitoring of microbiological permeate quality, nanoparticle counting was found to have the lowest detection limit. However, the "Dark Laser Beam" measurements yielded comparably low log removals and were of poor reproducibility in general. Overall particles counts were low compared to other particle detections devices and flow cytometry. Whilst few hundreds of signals per liter were detected with the device, flow cytometry analysed 10^5 to 10^7 counts per mL, respectively, in the pilot plant experiments. Counts in the permeate of the full-scale plant

were even lower (< 100/L). This is largely related to the measuring principle of the device which is not presenting a complete analysis of all particles present in a sample.

From the practical experience gained with the prototype device it requires further development and improvement especially with regard to measurement calibration, technical robustness as well as data processing.

2.4 Monitoring water quality with effect-based bio-assays

2.4.1 Description

The effect-based bioassay utilizes natural non-pathogenic marine luminescent bacteria as sensitive sensors for detecting chemical contamination in water. The light emitted by the bacteria reflects their well-being. The bacteria were found to be sensitive to a broad range of chemical toxicants (such as, heavy metals, pesticides, herbicides, chlorinated hydrocarbons, detergents, surfactants, etc). The light is measured with sensitive light detecting sensors called, photomultipliers. The test was designed for use as a portable manual kit and also as part of an online device (BMT200). BMT200 is designed to work online – connected via a bypass to the water source, every 15 minutes, fresh samples are drawn into a temperature-controlled assay chamber, into which a sensitizing assay buffer plus bacterial suspension aliquot are injected and mixed. Light is recorded and data is transmitted to a control center where it is analyzed and reported. In both cases, the indication provides a rapid alert regarding acute changes in water quality.

The manual test kit is suitable for lab work, for qualitative and semi-quantitative discrete testing of samples. BMT200 is designed to work online.

2.4.2 Application area

The manual test kit can be applied to test a broad range of water sources: chlorinated and non-chlorinated treated water, raw drinking water, recycled water; in a WWTP: from post 500 micron filtered-effluents up to RO-filtrate. RO-brine should be diluted before testing due to the high salt concentration. The same is true for the online sensor, except some precautionary measures (such as pre-filtration and more frequent preventive maintenance visits) may need to be taken when sampling non-lucid samples.

The manual test kit is routinely sold (mainly in China) to water service labs for use under emergency situations and also for routine testing. The BMT200 is still at a pre-commercial state.

Operators can use the tool themselves. The manual test kit requires some basic laboratory skills and expertise, together with a basic lab set up. The BMT200 is designed to operate unattended with basic maintenance (monthly replacement of refill reagents) that could be done by site operators. Sample measurements could also be purchased as service.

The sensitivity and responsiveness of the manual test-kit was verified in two demonstration sites. It was able to reflect the progressed purification along the treatment trains and to detect deviation from this performance. The toxicity test were able to follow the gradual removal of effluent toxicity through the different stages of the treatment trains. It highlighted the efficiency of the RO stage, as well as of the biofiltration and recharge steps. In the case of the RO stage at the Torreele site, the test was found to be useful in pointing at an unknown contamination problem in one of the skids

The bioassay was also found to be responsive and sensitive enough to detect the presence of residual cleaning agents dosed into reverse osmosis water at concentrations that were lower than those used for routine cleaning of membranes and filters. In summary this approach showed potential for application as early warning system and could improve detection and assessment of failures during operation.

2.4.3 Advantages

The test is rapid (results obtained within 15 minutes) and sensitive (within the range of 0.1-50ppm for many chemicals). Coverage of a broad range of toxic chemicals. The manual test is relatively easy to use for skilled-lab personnel.

Direct bioassays for toxicity in WWTP usually include the use of fish, Daphnia. These are usually not carried out on a daily or weekly basis due to their cost and complexity. Bioassay using bacteria are faster and easier to use, but most of them are not as sensitive as the BMT test. The online sensor enables continuous and automatic monitoring of water streams, thus, providing an early warning system for dramatic changes in water quality and hence taking timely corrective measures.

2.4.4 Limitations

Applying the BMT200 in a WWTP is not trivial. One has to take into account the proper set up in terms of pumps, pre-filtration (to protect sensitive tubings), especially adjusted preventive maintenance. As for the manual test kit, it is not the optimal choice when one is interested in detecting residual pharmaceuticals iterated n the effluent stream, due to lack of proper sensitivity.

While the use of such bioassay has the advantage of providing a quick response and a broad range of detection, one should not overlook some limitations. It would not be the choice of use when specificity of toxicity is required. In addition, this assay would not be the optimal one for detecting sub-ppm levels of residual pharmaceuticals. Furthermore, good workmanship is needed in reproducible sampling and test conductance in the lab to ensure comparability between sampling campaigns

2.5 Chemical fingerprinting

2.5.1 Description (from D2.3)

Chemical fingerprinting utilises high-end chemical analysis to screen samples for polar and non-polar compounds. This is possible thanks to the development of high throughput instrumentations such as gas chromatography (GC) and liquid chromatography (LC) coupled to high resolution mass spectrometers (HRMS). These technologies offer the possibility to perform both qualitative non-target screening and quantitative target analysis. Partner VERI has established a “suspect screening” approach for chemical fingerprinting: a list of more than 4000 compounds can be searched in environmental samples (from data issues from literature and open source database). A comprehensive in-house tools was developed within the DEMOWARE project for data processing, data interpretation and assessment of micropollutants levels derived from non-target screening of environmental and other water samples.

2.5.2 Application areas

Chemical fingerprinting is a method that can only be performed in specialised labs. Though considerable automation procedures have been developed it is still comparably time-consuming, taking days from sampling to result. Sample preparation, measurement, data analysis and interpretation require also need educated and trained personnel.

It is neither suited nor intended to directly support operation and operational monitoring. It has its value in characterizing water samples or compartments during a reuse scheme planning phase or impact assessment phase.

The method was applied in the planning phase of an indirect potable reuse scheme, i.e. the intended augmentation of a drinking water reservoir with reclaimed wastewater. A study was performed to assess the effects of regular WWTP discharge 20 km upstream a drinking water reservoir, reflecting a situation of

unplanned indirect potable reuse. By means of the chemical fingerprinting approach and statistical assessment it was elucidated that the water quality upstream the wastewater treatment plant resembled the one in the reservoir. Markers discharged by the WWTP were gradually less detected up into the reservoir pointing at elimination processes such as photooxidation, adsorption or biodegradation.

2.5.3 Advantages

Compared to target compound analysis as a classical, well-established method in specialised laboratories to monitor organic micropollutants, chemical fingerprinting provides a broader picture of changes in the chemical composition of water samples. In target compound analysis, samples are analysed for a set of known compounds (less than 100 substances in most cases) and quantitative results can be obtained. As the search is restricted to a short list of known compounds it does not give additional information about other micropollutants potentially present in the environmental sample.

The use of such a more global screening strategy can be a promising approach to better characterize chemical contamination and track the impact of sources, i.e. to assess the relative impact of WWTP compared to those stemming from land use or other tributaries. It is thus suited to characterise long-term effects of water reuse on receiving waters.

2.5.4 Limitations

The procedure is highly specialised and requires expert knowledge. The effort might be justified only in specific reuse cases such as indirect potable reuse that deserve such level of detail to distinguish background from effects of water reuse.

As the approach only allows a semi-quantitative measurement of ‘identified’ compounds, it is not sufficiently precise for environmental monitoring, e.g. with respect to environmental quality standards.

3 Conclusion

We cannot give fully consolidated recommendations for all of the tested approaches, as the data basis was not always sufficient or technical issues limited the validity of results.

However, the use of the various tools (FCM, effect based assays, particle monitoring) could detect alteration of water quality by treatment barriers or natural processes (chemical fingerprinting).

The application of tools presented here is not necessarily suited for routine operational or verification monitoring. However, they enable a detailed and site-specific analysis and characterisation of the effectiveness of an installed treatment train. They can help to identify points of attention and to increase overall understanding for the functioning and interdependencies between process steps and components. This can be a valuable contribution to risk assessment work in future water reuse safety planning.

Particularly the molecularbiological methods provided insight into the effects of different control measures, such as membranes and disinfection on the microbial community in the down-stream part of the reuse scheme.

Disinfection in water reuse does not aim at sterilization. The desired effect is a reduction in pathogens and their indicators. Yet, inevitably the whole microbiome is altered. Using the qPCR indicator system developed in the project we found hints, that the relative abundance of (potentially) pathogenic strains of *Pseudomonas spec*. and Legionella increases after disinfection. However, this observation requires further dedicated investigations. Also on the permeate side of ultrafiltration membranes quite some microbial contamination was found, though this could also have been related to the characteristics of the pilot plants.

For agricultural irrigation networks molecularbiological methods revealed a change of microbial community in the application. It was found that the stagnant water in the pipes of the irrigation network differed from those in storage tanks. It was hypothesized that changes in redox and also extreme temperature select for different organisms.

From our work on distribution networks (using FCM, and m, we could

- Confirm the effectiveness of UV radiation as point-of use disinfection after storage.
- Observe that UV disinfection alone before a storage or distribution system is not sufficient to prevent regrowth
- Derive a residual disinfectant dose to control regrowth in network.

It also acknowledged that sophisticated operational monitoring might not be equally relevant in all types of reuse schemes.

In flow cytometry, total cell counts were a valid measure to assess the functioning and integrity of physical separation steps. Additionally, the dead-live ratio informs about the effectiveness of (chemical)disinfection steps. Both information can be translated into log reduction values and thus characterize a barrier.

Enumeration of viable cells via FCM within the non-potable distribution network was found, as expected, to correlate to a free chlorine residual enabling the determination of a bespoke residual for the network to limit cell viability and regrowth.

The additional value of FCM goes beyond the mere information on total cell counts and their dead-alive distinction. Particularly in membrane processes it can depict changes in microbial population caused by the separation step. To a limited extent it can be used to assess whether a permeate contamination stems from the feed side or is the result of regrowth.

In any case FCM provides a tool to characterize the base line water quality for an established scheme.

Chemical analyses provide info on the presence (and concentration) of specific compounds, whereas **effect-based bioassays** may indicate acute detrimental (toxic) effects that are not necessarily detectable by standard water quality monitoring procedures. They thus can indicate malfunctioning or failures in treatment or operation. The sensitivity and responsiveness of such a test system using bio-luminescent bacteria was verified in the two demonstration sites. The test was able to reflect the progressed purification along the treatment trains and to detect deviation from this performance. Results of our work clearly showed which process contributes most to reduction of effects (here the reverse osmosis membrane). The periodical routine monitoring of these treatment steps could ascertain its proper functioning. This might be advisable after upgrade or changes in the operational regime (membrane replacement, changes or failure in upstream treatment steps). In summary, this approach showed potential for application as early warning system and could improve detection and assessment of failures during operation.

In summary the technologies and techniques investigated have potential to support operators in

- characterizing disinfection effectiveness (beyond legal indicators)
- managing distribution network (maintenance regimes
    - residual disinfectant
    - flushing
    - biofilm formation observation & prevention
- observing membrane integrity

An overview of their main characteristic and applicability is compiled in Table 3.

Table 3 Overview table of main characteristic of applied tools and methodologies

*commercial particle counters give results immediately, the tested device data could not be read in real-time and required a lot of manual processing

Tool	Online / offline	Time to result	State of development Application by /when	Suited for monitoring
FCM	Mostly offline Online under development and testing	Minutes to < 1 h	Fully commercialised Operator (water utility) or external lab Routinely, within regular sampling programes	Operational confirming distribution network maintenance effectiveness
qPCR based indicator system	Offline	Hours to 1 day	Underlying methods well established, targeted development, Specialised water lab	Operational, exceeding of threshold values
Chemical fingerprinting	Offline	2-5 days	In-house expertise, service to be provided in planning phase / impact assessment phase or follow up studies	Long term changes
Submicron particle counting	Offline and online	Minutes to hours or days*	Prototype requiring improved data management and calibration	Operational,
Effect based bioassay	Offline and Online under testing	Minutes - < 1h	Commercialised / in stage of commercialisation	Operational, Verification

4 References

Alcalde-Sanz L and Gawlik B (2014) Water Reuse in Europe Relevant guidelines, needs for and barriers to innovation.

WHO (2016) Sanitation safety planning: manual for safe use and disposal of wastewater, greywater and excreta.

5 Annex

5.1 List of related deliverables

Table 4 WP2 deliverables with more detailed information of tested approaches

Deliverable	Title	Dissemination status
D2.1	Best practice recommendation on integrity monitoring	Public
D2.2	Report on membrane integrity testing protocol	Restricted
D2.3	Chemical fingerprinting as method to assess micropollutant levels and fate in the aquatic environment.	Restricted
D2.4	Good practice summary for biofilm mitigation in networks	Public
D2.5	Report on the applicability of FCM and qPCR methods to assess and control microbial contamination	Public
D2.6	Report on bioassay-monitor on-site testing	Restricted