# Assuring water quality along multi-barrier treatment systems for agricultural water reuse

Marius Mohr, Thomas Dockhorn, Jörg E. Drewes, Sybille Karwat, Susanne Lackner, Bryan Lotz, Andreas Nahrstedt, Andreas Nocker, Engelbert Schramm and Martin Zimmermann

## ABSTRACT

Based on three pilot- and demonstration-scale projects investigating agricultural irrigation practices with reclaimed water, risks associated with these water reuse practices are highlighted and processes and strategies to minimize associated microbial risks were evaluated. A number of treatment processes and combinations were tested regarding their efficacy for pathogen removal, representing the biggest threat to the quality of products from reuse irrigation practices. In addition, the importance of regrowth potential and different methods for monitoring risks associated with pathogens were discussed. One method for online monitoring is flow cytometry. The results of an exemplary quantitative microbial risk assessment (QMRA) were discussed to determine the significance of microbial risks. Multi-barrier approaches comprised of technical and administrative barriers can reduce the risks of water reuse significantly. Quality management also needs to address all stakeholders involved in a reuse project, starting from source control in the sewershed to marketing of the final products. In addition, environmental risks of water reuse need to be addressed by quality management as well.

**Key words** | agricultural irrigation, multiple barriers, pathogens, QMRA, water quality management, water reuse

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## HIGHLIGHTS

- Multi-barrier approaches comprised of technical and administrative barriers can reduce the risks of water reuse significantly.
- A number of treatment processes and combinations were tested regarding their efficacy for pathogen removal (ultrafiltration, ozone, UV, ponds, biological processes, and biofilters).
- Flow cytometry as a method for online monitoring is discussed.
- The importance of regrowth potential is shown.
- An exemplary QMRA is carried out, differentiating between risks from bacteria, viruses, and protozoa.



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## INTRODUCTION

Global challenges such as climate change, population growth, and an increasing shift toward urbanization are resulting in an increased demand of water resources for agricultural and industrial purposes. Thus, the gap between the growing need for water supply and the existing limited water resources is widening continuously. With this growing demand, fresh water supplies are becoming an increasingly scarce resource in many regions worldwide including areas with a high population density, e.g. mega cities. Due to perceptible climate change, the lack of available water resources will be of even greater importance worldwide in the near future, as the frequency of extended droughts and heavy rainfalls is becoming a more widespread phenomenon even in moderate climate zones. According to the FAO (2017), agricultural irrigation needs an average of 70% of fresh water supplies worldwide. Thus, water reuse focusing on reducing the amount of conventional fresh water supplies is increasingly considered to avoid conflicts due to local supply shortfalls while implementing a sustainable water resource management with a water quality appropriate to the intended use (Becerra-Castro *et al.* 2015; Becker *et al.* 2017; Maassen 2018; Salgot & Folch 2018; WRI 2019).

## MICROBIAL RISK ASSESSMENT AND MANAGEMENT

Water reuse for agricultural irrigation is associated with an elevated risk due to the presence of pathogens in municipal wastewater. Thus, it is paramount that the microbial risk in these applications is properly managed. Microbial risk assessment combines scientific knowledge about water-based pathogens, the effect of natural and engineered barriers, and monitoring strategies. Risk assessment consists of four steps: problem formulation, exposure assessment, dose–response assessment, and risk characterization (WHO 2016; Drewes *et al.* 2019). The combination of scientific findings with mathematical models enables a quantitative microbial risk assessment (QMRA). The risk characterization, therefore, contains information on the probability of an exposed person being endangered by specific pathogenic microorganisms with regard to infection or disease.

The measure of Disability-Adjusted Life Years (DALYs) represents the extent of suffering in the form of the sum of years that a person lives impaired by a disease, Years Living with a Disability, and the years of life lost due to the disease through premature death, Years of Life Lost (Prüss-Üstün 2003; Scheierling *et al.* 2011). DALYs can be calculated for various health hazards and differ depending on the health hazard under consideration. The risk assessment is carried out by comparing the maximum reasonable exposure to infection with the expected risk of infection. With regard to the raw consumption of food irrigated by reclaimed water, the World Health Organization (WHO) determines the maximum acceptable additional burden of an individual from waterborne diseases at $10^{-6}$ DALY per person per year (WHO 2006, 2016; Drewes *et al.* 2019).

The WHO (2006) recommends that achieving 'health-based targets' for a given reuse application should be determined by means of QMRA. The overall 'health-based target' can be accomplished by determining individual 'health-based goals', which represent the necessary cumulative reductions of pathogen concentrations in raw wastewater by individual barriers, typically expressed in logarithmic reduction steps (Log10) steps.

In order to properly manage the overall risk and to achieve the health-based target, a multiple barrier approach can be adopted which is based on a number of measures that together avoid or reduce the risk of contamination by the use of recycled water (WHO 2016). This is achieved by control measures at various locations along the entire production chain to establish effective barriers against the spread of pathogens (NHMRC 2011; Health Canada 2013).

## QUALITY MANAGEMENT IN AGRICULTURE

In the technical debate, it is assumed that the 'fit for purpose' approach entails the production of reclaimed water to a quality that meets the needs of the intended end-use (ISO 20670:2018). In traditional commercial water supply sectors, quality control typically involves monitoring of the quality of the water produced at one or more locations in the process. For this purpose, the supplier either sets quality standards itself or negotiates them with its customers. Another possibility is that the standards are specified by technical norms or legal requirements. In the commercial agricultural business context, quality management is increasingly built up very systematically and comprehensively. However, extending these protocols to water reuse applied in the context of agricultural irrigation has not been developed systematically. A successful water reuse project not only depends on reliable treatment processes employed but also on proper organizational procedures and strict compliance with regulatory requirements while also meeting customer expectations continuously. These different aspects can be addressed under the framework of quality assurance. Quality assurance is comprised of an early identification of hazards or the avoidance of hazards. If this is practised in an organized fashion, it is referred to as quality management. Quality management comprises the initiation and safeguarding of organizational measures that serve to ensure the provision of services and the underlying procedures and processes – and thus also the quality of the final product. Guaranteeing desired quality requirements while also involving all stakeholders in quality management is, therefore, a central prerequisite for the consideration of water reuse in agriculture. The EU Commission has stressed this point as a major obstacle to further growing agricultural water reuse across Europe (Council of the EU 2019).

In this paper, we discuss how effectively water quality can be assured in a multi-barrier approach by analyzing the practices of different agricultural water reuse practices as part of three large-scale research projects ('HypoWave', 'EPoNa', and 'MULTI-ReUse') funded by the German Federal Ministry of Education and Research (BMBF). For these water reuse case studies, an overview of the technical and administrative barriers is given and their effectiveness is assessed. As far as process-related barriers are concerned, we investigated to what extent quality management can improve the process results. In addition, risk management is applied to assess the entire chain of wastewater collection, treatment, and conveyance up to the final application to agricultural products.

## MATERIALS AND METHODS

### Case studies and analytical methods applied

(A) Treatment technologies tested at pilot-scale in the 'HypoWave' project included an expanded granular sludge bed reactor (EGSB), a sequencing batch reactor (SBR), a biological activated carbon (BAC, empty bed contact time: 3.3 h) filter, and an ozone reactor (OZONE, ozone dose approximately 0.8 mg $O_3$/mg DOC (dissolved organic carbon)) (Bliedung et al. 2020). The feed water to the pilot was primary effluent (influent to the aeration tank, AT) of the wastewater treatment plant (WWTP) Wolfsburg-Hattorf, Germany (a treatment capacity of 10,500 PE). Three parallel treatment trains were employed as illustrated in Figure 1 in order to produce reclaimed water of different qualities.

The analysis of microbial parameters included the determination of the indicator organism *Escherichia coli* (EC) as well as the bacteria groups of total coliforms (C), Enterobacteriaceae (EB), and total viable aerobic count (TC). The aim was to evaluate the microbiological contamination of wastewater before and after using different treatment processes in order to estimate their effectiveness as procedural barriers along the water treatment chain. The methods employed used ready-to-use selective culture media (Petrifilm) of the 3M company followed by incubation and counting of colony-forming units (CFU/ml) according to the manufacturer's specifications: C/EC at $37 \pm 1\,^{\circ}C$ for

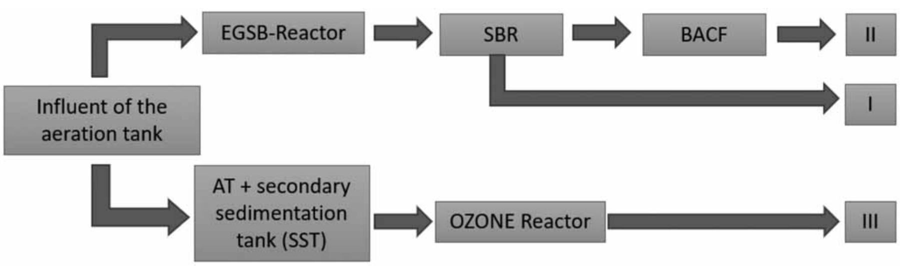


**Figure 1** | Flow schematic of the three pilot-scale treatment combinations within the HypoWave project at WWTP Wolfsburg-Hattorf.

24 ± 2 h, respectively, 48 ± 2 h (NMKL method 147.1993); EB at 37 ± 1 °C for 24 ± 2 h and TC at 30 ± 1 °C for 72 h. Dilution was done by deionized water (after 0.2 μm sterile filtration). Three replicates per sample and per bacteria group examined were generated, and corresponding blank samples were collected for each day of analysis. The analysis was based on eight sampling days. Taking the replicates into account, a total of 24 measuring results of each sampling point and each bacteria group was obtained.

How the HypoWave concept can be implemented has been examined in the context of a case study in Germany. The wastewater of the village of Weißenberge (Gifhorn county, approximately 500 inhabitants, 26,400 $m^3$/a) is currently being discharged into a system of treatment ponds (Figure 2). In case of dry weather conditions, the residence time in the system is more than 90 days. The effluent of the plant still contains relatively high concentrations of nutrients such as nitrogen and phosphorus, but due to the long residence time only low levels of pathogenic microorganisms. In an activated carbon biofilter, the nitrogen, which is largely present as ammonium-nitrogen, will be oxidized to nitrate by aeration and thus be made more readily available to plants. In order to reduce the risk of pathogens, the UV-disinfection process will be used after the biofilter. The treated water will then be used in hydroponic vegetable production, which also will utilize and remove the remaining nutrients. Eggplants, zucchini, tomatoes, peppers, and lettuce are potential crops for this application. For the case study of Weißenberge, a QMRA was carried out.

(B) In the EPoNa project, the town of Outapi in Northern Namibia has been selected. The town has flush toilets and a gravity sewer system in its formal districts. The household wastewater is discharged to waste stabilization ponds (WSP). Population growth and the overflowing of the ponds during the rainy season are challenges for the functionality of the system. Since the mere evaporation of the water is effectively wasting a valuable resource, water reuse for irrigation purposes is considered to be a resource-efficient option in the semi-arid region according to the Namibian Water Resources Management Act (Act No. 24 of 2004).

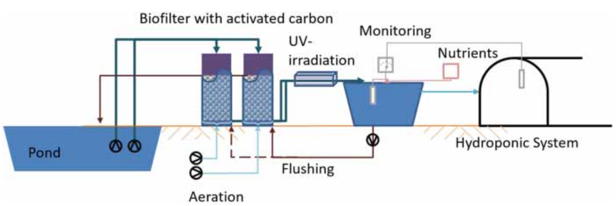


**Figure 2** | Flow chart of treatment concept for Weißenberge (HypoWave).

The WSP system comprises two parallel treatment trains (trains A and B) consisting of four ponds each (one primary facultative pond and three maturation ponds; Figure 3). All eight ponds together have a surface area of approximately 40,000 $m^2$ and a volume of 55,000 $m^3$. The treated water reaches an evaporation pond (surface area: 41,000 $m^2$; volume: 20,000 $m^3$) as the final stage. The system's average inflow was approximately 753 $m^3$/d. To enable water reuse, one train (A) of the WSP was equipped with a pretreatment (two options were tested: an up-flow anaerobic sludge blanket reactor (UASB) and a micro sieve) to reduce the organic load (Sinn *et al.* 2019). Additionally, guiding walls helped optimizing the flow conditions in pond A1. A rock filter served as posttreatment in pond A4. These measures aim at reaching an effluent water quality suitable for irrigation purposes to produce animal fodder (Lackner *et al.* 2017).

Microbial samples were analyzed regularly for total coliforms, *E. coli* and enterococci as indicator organisms for pathogens. Total coliforms and *E. coli* were detected by using the IDEXX Colilert-18 test (DIN EN ISO 9308-2:2012), enterococci by the Enterolert 250 and quantified with a Quanti-Tray/2000 system based on the most probable number (MPN) model.

(C) Near the North Sea coast between the Jade bay and the river Weser, drinking water supply is under the regional stress of strong limited water resources with a sufficient low salinity, longer periods with high temperatures (spring, summer, and autumn), and a growing industry. For several decades, the local water supply company Oldenburgisch-Ostfriesischer Wasserverband (OOWV) secures the water supply on the basis of water resources from neighboring administrative districts in competition with the water demand for agricultural irrigation. Long-term forecasts indicate a raise of use conflicts in the near future.

For this reason, the OOWV started working on concepts for a more efficient water supply to deliver their customers with a water quality adapted to their needs. Beside drinking water, this includes different advanced treated water types of a defined quality using tertiary effluent from the municipal WWTP Nordenham (36,000 population

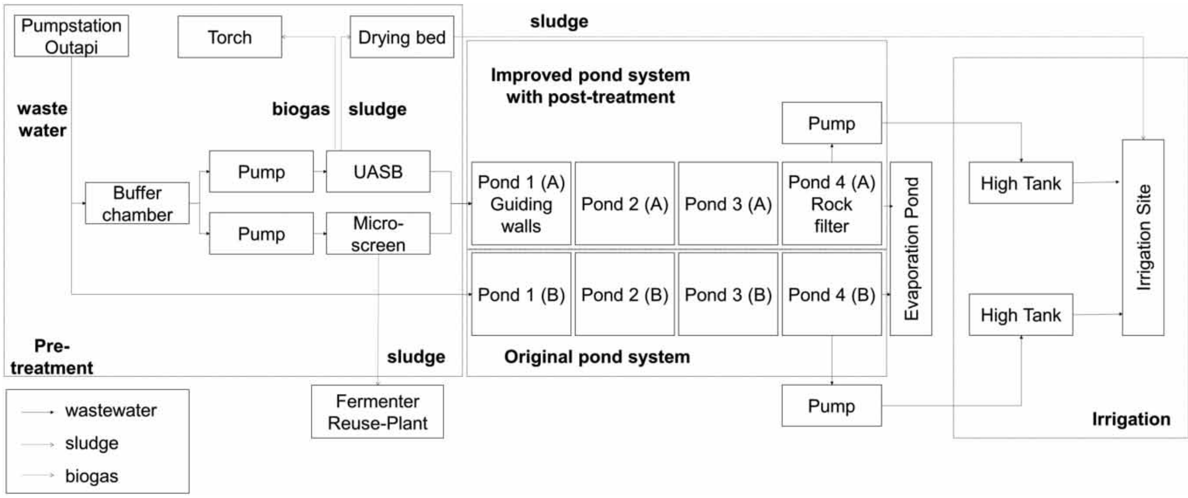


**Figure 3** | Flow schematic of the waste stabilization pond system within the EPoNa project (Sinn *et al.* 2019).

equivalents, Q = 3,000–15,000 $m^3$/d). The different qualities targeted agriculture needs (irrigation for non-food crop) as well as industrial processes for cooling and boiler feed water.

In MULTI-ReUse, a pilot-scale facility with three different trains of modular process units was built to produce three different water qualities (Figure 4): (1) pre-filtration, (optional) powdered activated carbon (PAC) dosage, flocculation, ultrafiltration (UF, each line with a capacity 5.6 $m^3$/h), and UV disinfection; (2) pre-filtration, flocculation, UF, UV, sand filtration, and granulated activated carbon (GAC) filtration; and (3) pre-filtration (sieve), PAC dosage (optional), flocculation, UF, monochloramine dosage, reverse osmosis (RO, in two trains to compare several low-pressure modules), and UV disinfection.

Log reductions in the treatment processes of the MULTI-ReUse project were monitored by applying traditional culture methods to quantify important microbiological indicator organisms: colony counts at 22 and 36 °C (both DIN EN ISO 6222:1999-07), *E. coli* and coliforms (both DIN EN ISO 9308-2:2014-06), intestinal enterococci (DIN EN ISO 7899-2:2000-11), *Clostridium perfringens* (DIN EN ISO 14189:2016-11), and *Legionella* spp. (DIN EN ISO 11731:2017-05). The culture methods were supplemented

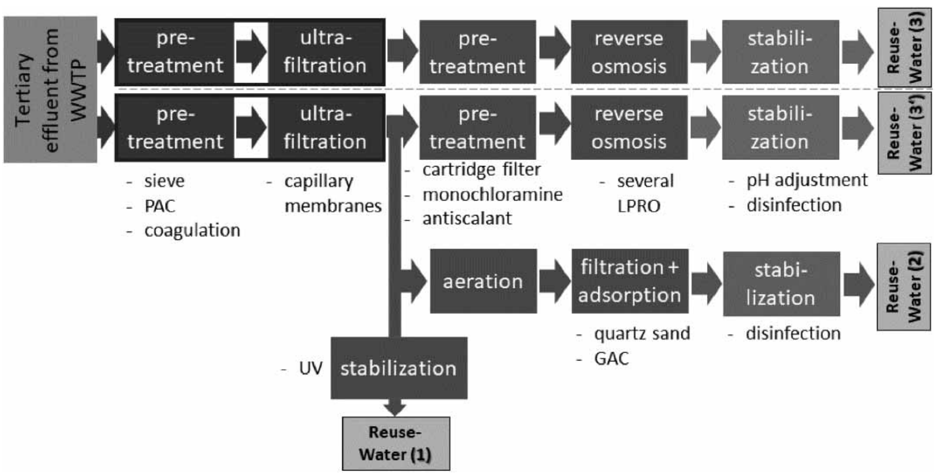


**Figure 4** | Treatment train of the pilot plant in the MULTI-ReUse project to produce three different water qualities. The treatment train producing water quality 3 was duplicated for comparison of the effects of process alterations.

by the cultivation-independent assessment of total cell counts (TCC) and intact cell counts (ICC) using flow cytometry in combination with fluorescent staining. Whereas culture values only detect the small proportion of culturable bacteria in the water, flow cytometry allows monitoring of the entire bacterial population independent of the fact whether they form colonies on a given medium under the defined conditions. Samples were transported cooled to the laboratory to ensure that the resulting data represent the microbiological status of the water at the time of sampling (day 0). Obtained values were referred to as $TCC_{day\ 0}$ and $ICC_{day\ 0}$. After analysis of aliquots, water samples were subsequently incubated at 22 °C for 7 days to allow bacterial growth followed by repeated measurement of intact cell count ($ICC_{day\ 7}$). The increase in ICC in these 7 days (i.e. the extent of growth) was determined by the amount of assimilable organic carbon (AOC) present in the sample. $ICC_{day\ 7}$ values represent the maximal concentrations of intact cells supported by the AOC contained in the sample. The offline flow cytometry was supported by online analysis, which allowed high-resolution data. An Online Bacteria Analyzer (OBA Metanor, ONTRONIX AG, Switzerland) was installed after UF which was considered a critical control point.

## RESULTS AND DISCUSSION

### Multi-barrier systems

Table 1 provides a summary of barriers employed for the projects EPoNa, HypoWave, and MULTI-ReUse. In addition to wastewater treatment, the barriers in the area of wastewater collection and agricultural application are also displayed. In addition to technical barriers, administrative barriers were also used in all projects; for example, in some applications, the risk of hazardous substances in the sewage is reduced by monitoring the connections to the sewer network, and in other applications, the crops that are irrigated are not for human use. In the following section, different technical barriers regarding pathogens are discussed, an exemplary QMRA is executed followed by a brief discussion of quality management and administrative barriers.

**Table 1** | Barriers along the water chain in the analyzed projects

| Project/case study | Collection | Treatment | | | Irrigation | Harvest |
|---|---|---|---|---|---|---|
| MULTI-ReUse | | Tertiary treatment | UF | Disinfection (UV-radiation) | Energy and industrial crops | |
| HypoWave Standard case | Controlled sewage collection: No industrial indirect dischargers, no hospitals | Biological treatment (ASP, SBR) | Biofilter/activated carbon | Disinfection (Ozone, UV-radiation) | Water only in contact with roots, plastic cover between root and shoot of the plants | Specific hygiene measures by the quality assurance system agreed with the trade or purchaser |
| HypoWave Weißenberge | Controlled sewage collection: No industrial indirect dischargers, no hospitals | WSP (natural UV-radiation, cold temperatures) | Biofilter/activated carbon | Disinfection (UV-radiation) | Water only in contact with roots, plastic cover between root and shoot of the plants | Specific hygiene measures by the quality assurance system agreed with the trade or purchaser |
| EPoNa | Controlled sewage collection: No industrial indirect dischargers | Pretreatment line A: Up-flow anaerobic sludge blanket reactor or micro sieve | WSP (natural UV-radiation) | Rock filter in pond A4 | Animal fodder | |

## Performance of treatment processes regarding pathogens

The main goal of these investigations was to highlight how water could be reused in agriculture while properly managing associated health risks. Thus, treatment processes as technical barriers have been tested with a focus on pathogen removal.

The feed water used in the HypoWave project was characterized by the following bacteria concentrations: total aerobic count ranging from $10^6$ to $10^7$ CFU/ml, EB and total coliforms from $10^5$ to $10^6$ CFU/ml, and *E. coli* from $10^4$ to $10^5$ CFU/ml. These concentrations are representative of municipal raw wastewater exhibiting typical *E. coli* concentration of $10^3$ to $10^6$ CFU/ml and $10^5$ to $10^7$ CFU/ml for total coliforms, respectively (WHO 2006; Asano *et al.* 2007; Guy *et al.* 2010; DWA 2017; Cornel *et al.* 2018). The efficacy of the different treatment barriers is summarized in Figure 5.

Based on these findings, the removal efficacy of the EGSB appeared to be the lowest in terms of the reduction of all four bacteria groups measured. With a mean *E. coli* removal of 0.09 log units and a maximum of 0.42 log units, its performance was considerably lower than expected. As the EGSB is considered to be a high-efficiency UASB, a mean bacterial removal of at least 1–2 log units was expected (WHO 2006; DWA 2008, 2017). However, during this study, the performance of the EGSB was far from being stable and regrowth in all four bacteria groups has been observed. Contrary to this, the SBR installed after the EGSB removed *E. coli* at least by 1.8 log units and at most by 2.7 log units. This means that even the lower margin of treatment performance of the SBR achieved a log removal efficiency equal to the upper range of the usual bacterial log removal of a conventional activated sludge (CAS) process including secondary clarification, i.e. 1–2 log units, whereas the SBR maximum performance exceeds a CAS process by almost 1 log unit (WHO 2006; DWA 2008, 2017).

With a maximum *E. coli* removal of 2.1 log units and a minimum of 1.1 log units, the purification performance of the BACF is in the range of the usual purification performance of slow sand filtration and to the middle range of fast sand filtration (WHO 2006; DWA 2008, 2017). In the HypoWave project, OZONE performed unusually poor with a mean treatment efficiency far below common values, and even a potential of regrowth regarding EB and total bacteria count was observed. During the ozone process, *E. coli* was removed at least by 0.1 log units and at most by 2.25 log units (Figure 5). This means that the purification performance of the installed OZONE was significantly lower than the usual bacteria removal performance of such a treatment process being 2–6 log units (WHO 2006; DWA 2008, 2017; Seis *et al.* 2016). Moreover, the low purification performance may be due to a deliberately chosen ozone dose which was

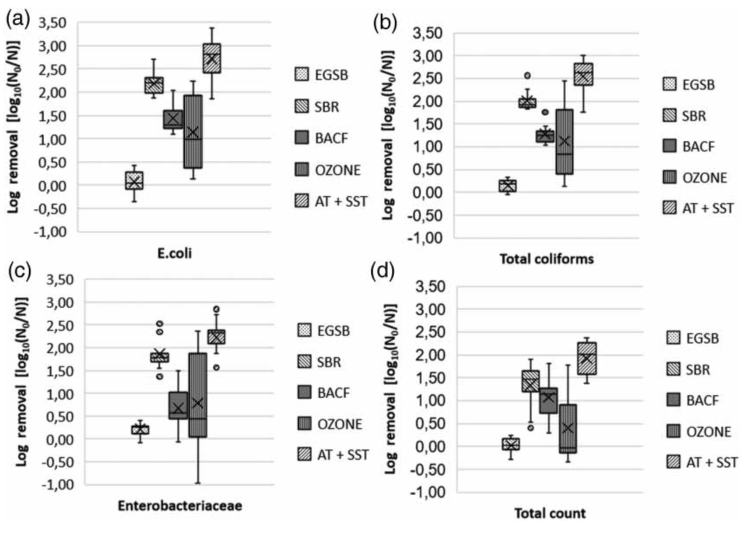


**Figure 5** | Performance of each treatment process employed at pilot-scale within the HypoWave project regarding the four measured bacteria groups.

about four times lower than the usual dose for disinfection. A second reason may be the very low concentration of *E. coli* of $10^1$ to $10^3$ CFU/ml in the influent of ozone. Nevertheless, with a mean *E. coli* removal performance of 2.87 log units, the activated sludge process including secondary sedimentation (AT + SST) shows a performance slightly superior to other data (WHO 2006; DWA 2008, 2017).

In view of fecal coliforms (EC), only irrigation water of treatment II complies with the requirements of the WHO concerning the food production suitable for raw consumption, i.e. $\leq 10^4$ CFU/l. Finally, as *E. coli* is an indicator organism only for bacteria, it has to be stressed that the data cannot be used to make reliable statements on the treatment processes of the pilot reactor regarding protozoa, viruses, and helminths.

The EPoNa project studied WSP for their capabilities to remove pathogens. Properly designed WSP are capable of removing large numbers of fecal bacteria. Aside from sedimentation and decay in the ponds, naturally occurring disinfection by solar UV radiation takes place. However, the effluent quality of WSP often does not comply with common Quality Standards. In the Namibian Code of Practice (DWAF 2012), filtration and disinfection are part of the tertiary treatment steps required for water reuse. However, due to the high amounts of organic solids and ammonia, conventional disinfection (e.g. chlorination) is often not effective and the effluent cannot be reused for unrestricted irrigation. In 2012, the Code of Practice was amended to allow the irrigation of fodder without the requirement of disinfection in exceptional cases. A permit for exemption from the obligation to disinfect wastewater can be granted if it can be proven that the non-disinfected wastewater will not come into contact with any humans or animals.

Data from the sampled WSP indicate that already the overloaded WSP (train B) was able to result in a significant reduction in *E. coli* with 3.90 log units reduction (from the influent to the effluent of B4) and 2.34 log units for enterococci (Figure 6). The upgrade of train A improved the reduction of *E. coli* to 4.29 log units (3.32 log units for enterococci) and concentrations of *E. coli* in the effluent of A4 of $6.9 \times 10^2 \pm 1.0 \times 10^3$ MPN/100 ml. The effect on total coliforms was, however, much less, with a reduction of only 0.94 log units for train A and 0.80 log units for train B. With concentrations of still $2.2 \times 10^4 \pm 5.0 \times 10^4$ MPN/100 ml for *E. coli* in the effluent of B4 (see also Figure 6), the original pond system without any upgrades was not able to meet the WHO guidelines for safe water reuse of $1 \times 10^3$ CFU/100 ml for fecal coliforms and application for fodder crop irrigation. Additionally, the reduction of other pathogens might not be as high in the tested technologies, as the effect on enterococci in both lines with and without pre- and post-treatment seemed to be less compared to *E. coli*.

To evaluate the pathogen reduction of the WSP system (with additional pre- and post-treatment) in comparison to only the WSP, the reduction of *E. coli* was analyzed in depth. Pretreatment and upgrading of pond A1 resulted in an *E. coli* reduction of 97% (inflow – A1), compared to 91% reduction without pretreatment. Further reductions by the ponds (from pond 2 to 4) were similar for both, trains A and B, with 99.8%. However, also considering the reuse water storage tanks, concentrations of *E. coli* were still $>1.0 \times 10^3$ MPN/100 ml in line B, and there might even be the danger of regrowth.

Ensure target reduction rates can be achieved by extending the multi-barrier approach to the irrigation system. Besides the WSP and corresponding treatment

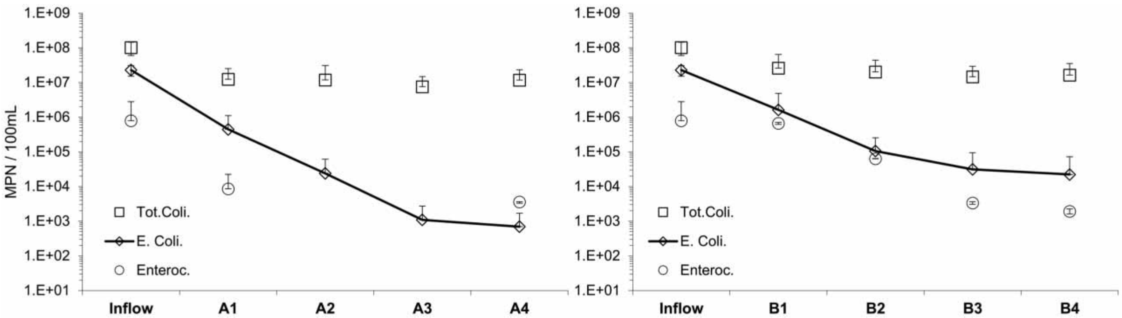


**Figure 6** | Reduction of total coliforms, *E. coli* and enterococci through both lines of the WSP (average values include all data with pretreatment (784 days)).

steps, a specific irrigation technology can also act as another barrier. Drip irrigation of low-growing crops would for instance further reduce pathogens by 2 log10 units (WHO 2006). This would bring the values way below the required log removal requirement.

In the MULTI-ReUse project, a membrane integrity concept was applied to assure proper and efficient removal of viruses, bacteria, and protozoa. To validate the initial virus removal efficiency of the UF, modules were tested with MS2 phages yielding a log removal value >4. This indicates a suitable pore diameter distribution and the integrity of the membrane system. During the treatment operation, it was necessary to monitor and ensure this integrity. Single defects are a pathway for feed water bypassing the active membrane (defect flow). But, a critical drop of virus retention will only occur if the ratio of defect flow rate and total flow rate through the active membrane reaches a critical value. For critical defect flow rates in full-scale UF modules, single defects (fiber break, holes in the membrane) must have a size of some micrometer (EPA 2005; DVGW 2007). Defects of this size will also lead to bypassing bacteria. Therefore, the monitoring of a log reduction value (LRV) of 5 or 6 for bacteria retention was used to ensure an LRV >4 for virus retention. With the online measured TCC using flow cytometry, it was possible to monitor an LRV of 3–4 for bacteria nearly in real time. The upper detection limit for the LRV reached is strongly dependent on the filtrate quality, which is affected by the release of bacteria into the filtrate from regrown biofilms in pipes and valves. The culturing method for *E. coli* and coliforms ensures an LRV >6 with a time offset of several hours. To exclude the risk from membrane ageing which can occur in a process under the stress of chemicals and can potentially affect its pore diameter distribution, challenge tests with phages or with (small!) viruses already present in the effluent of a WWTP should be repeated.

To follow the multi-barrier approach, virus and bacteria retention was enhanced by UV inactivation in accordance to the German standards DVGW W 294 Series for drinking water supply and DWA (M)205 for WTP effluent. By meeting these standards, a 4 log inactivation for waterborne pathogens is guaranteed. The flow rate and UV transmission (in a water after UF free of TSS) were monitored online. Furthermore, certified reactors monitor the intensity of UV light inside by an integrated UV sensor.

The hygienic status of treated water was monitored by traditional cultivation of indicator bacteria. UF as the first treatment barrier resulted in complete elimination of *E. coli*, coliforms, intestinal enterococci, *C. perfringens,* and *Legionella* spp. The concentrations of these bacteria in the secondary effluent of a WWTP were subject to strong variations with average values of 79,000 *E. coli*/100 ml, 164,900 enterococci/100 ml, 64,300 *C. perfringens*/100 ml, and >100 *Legionella* spp./100 ml. These indicators were absent also in samples from subsequent treatment steps confirming the hygienic safety of the advanced treated water at the time of sampling. Colony counts in raw water were on average 24,600 CFU/ml at 22 °C and 42,600 CFU/ml at 36 °C and were reduced by UF to 2 and 120 CFU/ml, respectively. UF was therefore seen as a critical control point.

TCC and ICC measurements exhibited strong reductions by UF; however, the UF filtrate side was as expected not sterile. Bacteria colonizing surfaces in the filtrate compartment are the source of freely suspended bacteria in the freshly filtered water. The integrity of the UF membrane was not compromised. Regrowth on the filtrate side could be distinguished from breakthrough events by monitoring relative proportions of low nucleic acid (LNA) bacteria and high nucleic acid (HNA) bacteria using offline flow cytometry. Whereas LNA bacteria tended to be dominant in UF feed water (or at least be present in equal numbers as HNA bacteria), they were absent in the UF filtrate. In case of compromised membrane integrity, it would be primarily LNA bacteria that would be expected in the filtrate as they tend to be smaller in size and have a higher probability of membrane passage. The fact that UF filtrate nearly exclusively contained HNA bacteria indicates clearly that the bacterial population in filtrate originated from regrowth.

Flow cytometry was, therefore, seen as a valuable diagnostic tool to assess whether bacteria on the filtrate side originated from regrowth or from a breach of UF membrane integrity. Online flow cytometry can greatly assist in monitoring the functionality of this important barrier. The use of a fluorescent stain mixture allowing to distinguish between intact and damaged bacteria also provides a continuous monitoring strategy to assess disinfection efficiency in case an oxidative disinfectant (e.g., hypochlorite or monochloramine) is added as an additional barrier (for details see Nocker *et al.* 2020).

Another efficient barrier in MULTI-ReUse was RO as another membrane filtration step to further increase water quality. Both membrane filtration steps led to a profoundly different bacterial community, which was also confirmed by Illumina Sequencing. Sand filtration and granular activated charcoal (GAC) on the other hand greatly increased bacterial diversity and led to biological stabilization of the water. The resulting increase in bacterial numbers greatly diminished the regrowth potential compared to water after UF or RO with filtered, bacteria-stripped water showing high regrowth potentials (Nocker *et al.* 2020). The presence of an indigenous microbial community (as present on sand or GAC filter) has been reported to be antagonistic to the growth of pathogens (Camper *et al.* 1985; Hibbing *et al.* 2010). For drinking water treatment, biological stabilization has been reported to shape the bacterial community composition with lasting effects also during water distribution (Pinto *et al.* 2012; Lin *et al.* 2014; Prest *et al.* 2016). Van Nevel *et al.* (2013) summarized that the bacterial invasion potential in water is determined by nutrient availability and the indigenous bacterial population. The same can also be assumed for reuse water adding another microbiological barrier to the treatment process.

Nutrient depletion as a microbiological barrier is the basis for drinking water treatment when following the philosophy of distributing biologically stable water (instead of employing a disinfectant with residual). Apart from using flow cytometry for process monitoring, this diagnostic method could also provide valuable insight into the change in regrowth potential along treatment. Regrowth was assessed by measuring intact cell concentrations obtained from samples incubated for 7 days at 22 °C. The bacterial concentrations obtained after stagnation represent the maximal numbers supported by the available nutrients contained in the sample. The advantage of this approach lies in the sensitivity in the low nutrient range. After RO, where DOC and traditional AOC (van der Kooij 1992) were near the detection limits of the corresponding methods, the permeate still allowed regrowth to $ICC_{day\ 7}$ concentrations between $10^5$ and $10^6$ bacteria/ml. The lower end of this range is comparable to bacterial concentrations typically contained in drinking water, the upper range to that of stagnated potable water in household plumbing systems (Hammes *et al.* 2007; Lipphaus *et al.* 2014). As bacterial concentrations in this range are typically not associated with hygienic problems, the quality of the produced recycled water can be considered very good. The critical question is whether the regrowth potential in filtrate or permeate, which has not yet been converted into planktonic suspended biomass (like after GAC), will be deposited in the form of biofilms. Another critical question is whether the regrowth potential can be used by pathogens that accidentally enter the treated water. It is important to realize that the microbial population in water is highly dynamic and changes rapidly over time. The water quality should also be known at the time of water use and not just directly after treatment.

## QMRA to assess overall treatment efficacy

The aim of the QMRA undertaken for the HypoWave case study in the county of Gifhorn was to assess whether the suggested concept concerning cultivation and consumption of hydroponically grown food raised by using reclaimed water complies with the maximum additional tolerable health risk specified by the WHO (2006). The QMRA method utilized was based on the approaches of Eregno *et al.* (2017), who followed the provisions of the WHO guideline (2006, 2016) very closely, but included assumptions on hydroponic cultivation such as a retained and ingested exposure volume of $1.0 \times 10^{-7}$ l of irrigation water per gram of lettuce. In addition, by using lettuce as an example for an agricultural product provided for raw consumption, the focus of the QMRA was on a high exposure to health risks for the consumer. If the residual risk of the Gifhorn concept is below $10^{-6}$ DALYs, every other hydroponically grown product in the same system could be considered to be hygienically safe as well.

Under the assumption that 100 g of lettuce is consumed in a 2-day rhythm, the risks of infection with Cryptosporidium, Campylobacter, and Norovirus are shown in Figure 7. While the risk of infection with Campylobacter is lowest, the risk of an exposed person becoming infected by a virus seemed to be highest.

In order to verify whether these QMRA results concerning the consumption of hydroponically grown lettuce complied with the maximum additional health risk of $10^{-6}$ DALY, the DALY value was converted into a maximum tolerable risk of infection per person and year (pppy) for each pathogen according to the WHO Guidelines (2006, 2016). The corresponding values for the reference pathogens Cryptosporidium, Campylobacter and Norovirus – standing for each pathogen group, i.e. protozoa (P), bacteria (B), and viruses (V) – are also shown in Figure 7. Thus, for the three selected reference pathogens, the potential risk of infection for the consumer of lettuce does not cause a higher risk than the WHO (2006) tolerates. For viruses, the calculated risk is very close to the maximum tolerable health risk, though.

Thus, it may be useful to implement further preventive barriers against viruses, i.e. barriers to reduce the risk of virus infection by additional treatment processes, especially when using the water in hydroponics, where plants are in direct contact with irrigation water via their roots (Steele & Odumeru 2004; Uyttendaele *et al.* 2015; Moriarty *et al.* 2019). For example, an UF unit after the BACF could significantly reduce the risk of virus infection of the Weißenberge concept.

Due to a lack of data on the case study Weißenberge, the risk assessment was based mainly on assumptions

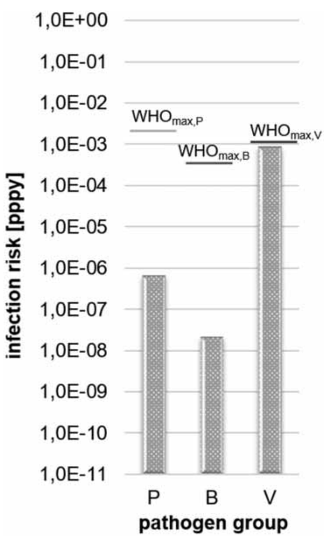


**Figure 7** | Mean infection risk per person per year (pppy) for consumption of lettuce for protozoa (P), bacteria (B), and viruses (V) compared with tolerable infection risks of the WHO (2006, 2016).

and thus only shows trends. The assumptions include mean standard pathogen concentrations per liter of raw wastewater for each of the selected reference pathogens (i.e. $5.0 \times 10^3$/l regarding Cryptosporidium and Campylobacter and $5.5 \times 10^5$/l regarding Noroviruses) and mean standard log reductions to estimate the performance of the treatment methods envisaged in the concept: In the WSP, the LRV is assumed to be 0.5 for protozoa and 1.5 for bacteria and viruses (WHO 2006; Fuhrmann 2013; DWA 2017). Based on the mean *E. coli*-reduction of the BACF in the HypoWave pilot plant, an LRV of 1.44 for all pathogens has been chosen for this treatment process. For the successive UV-disinfection process, an LRV of 3.0 for protozoa and bacteria and of 2.0 for viruses has been assumed (WHO 2006; Seis *et al.* 2016; DWA 2017). Based on the literature on hydroponic plant production based on wastewater (Garland 2000; Ottoson *et al.* 2005) and on results of the HypoWave pilot plant, a mean LRV of 1.0 for all pathogens in the hydroponic system has been used for the calculations.

Nevertheless, the QMRA was applied repeatedly and proved to be a reliable and multifunctional method of risk forecast and risk assessment. Taking into account that the quality of the irrigation water is a decisive factor for the hygienic quality of the agricultural product, it is recommended not to exceed a value of $10^{-5}$ concerning the infection risk per person per year for all pathogen groups. Risks for the staff working in the greenhouses, e.g. for maintenance and harvesting, have to be taken into account as well.

These results suggest that conventional quality assurance must be supplemented by further measures to ensure that quality targets are reliably achieved in practice. In this context, flow cytometry is seen as a suitable technology for online quality and performance assurance due to its high data resolution and degree of automation. Although microbial signals are not specific and thus do not allow conclusions on the presence of pathogens, critical processes like UF membrane integrity or efficiency of chemical disinfection can be monitored with little time delay. The microbiological consequence of changes in the treatment process can be directly seen in the form of total and intact cell concentrations, the integrity of bacteria, and the ratio between HNA and LNA bacteria. Within the treatment process, flow cytometry further allows the fast assessment of log reduction of total and intact counts independent of their culturability.

Although flow cytometry has proven to be a sensitive tool to detect changes in treatment efficiency and process alterations and can thus be seen as a very useful tool for process monitoring, it does not exclude the necessity for the hygienic assessment of the end product. For the direct assessment of the hygienic status of water, either microbiological hygiene indicators or specific pathogens of interest have to be analyzed. Traditional hygiene indicators (like *E. coli*) have served well for this purpose, especially for fecal borne pathogens.

### Quality management as a tool for risk reduction

From the point of view of agricultural production, quality fluctuations in the irrigation water should be avoided as far as possible. For the same reason, downtimes of the water in the supply lines between the producer of the irrigation water and the agricultural producers are to be minimized if possible; it is possible (in the case of longer lines) that there is a monitoring point at the transfer point to the agricultural operator. Experience can lead to a reduction of the actual monitoring. In the same way, attention should also be paid to short retention times in reservoirs. Process stabilization in the field of wastewater treatment facilitates quality assurance of the subsequent water treatment for (agricultural) reuse in a special way. In this respect, it makes the tasks easier if it can be agreed that quality management measures are introduced in the wastewater treatment process, as this will stabilize the process control in the long run, e.g. through higher competence of the operating personnel and inherent process improvements.

The guarantee of desired quality requirements vis-à-vis the cooperation partners is a central prerequisite for the consideration of water reuse in agriculture. In the agricultural business context, quality management is increasingly built up very systematically and at the same time in a far more comprehensive way than is necessary for quality assurance. This approach has not yet been systematically developed for water reuse in agriculture (Schramm *et al.* 2019).

Risk assessments in accordance with QMRA can be incorporated into the quality assurance systems for food production introduced at the European level. This can be used to ensure the quality of the irrigation water. For the European Union, the regulation (in course of legislation) on water reuse will provide a uniform framework for the first time; disinfection barriers will now be mandatory for most applications (Council of EU 2019). The approach of risk-based and process-oriented management of EN ISO 9001 can be used as a basis.

With regard to the scope of quality management, it is necessary to make an explicit distinction between environmental prevention and health care. Environmental policy is primarily interested in environmental precautions. The minimization of environmental risks has so far been given only limited consideration in the existing instruments, as the EU regulation on water reuse makes clear.

## CONCLUSIONS

The prime focus of quality management in agricultural water reuse is on the removal of pathogens. A number of technical processes for the elimination of pathogens from water exist and have been applied in the involved research projects. Membrane filtration, e.g. UF, has proved to be a reliable barrier against pathogens. Multi-barrier approaches combine the advantages of single processes and make the reuse concept more resilient against disturbances. Especially aerobic treatment processes and stabilization ponds have a strong additional removal efficiency for pathogens, but also slow filtration processes, UV and ozone help reduce the concentration of pathogens in water. According to the individual reuse case, an appropriate combination of these processes should be chosen. In addition to technical barriers, administrative barriers are important to ensure efficient and safe water reuse. For administering and managing water quality, the question arises as to which institutional arrangements are needed in order to make quality management durable and with which processes it can be successfully coordinated. If municipal wastewater is reused in agriculture, different actors usually carry out quality assurance in parallel. In the long run, it will make sense to coordinate the quality assurance of the various actors along the emerging 'water chain'.

All three projects struggled with the question how to ensure a safe use of the treated wastewater. MULTI-ReUse chose a combination of elaborated treatment steps combined with innovative monitoring to technically minimize the risks. EPoNa reduces the risks from pathogens by upgrading the WSP, thereby adding additional barriers, and restricting the use of the treated water to animal fodder, while HypoWave limits the application of reuse to wastewater collected only in areas without industry and hospitals discharging potentially hazardous substances and stresses the importance of cooperation between the stakeholders involved.

Monitoring can be another barrier, too, especially if the results are available in time to react accordingly. The assessment of the microbiological water quality status directly after treatment should be supplemented by measurement of the regrowth potential. Regrowth potential is currently not assessed on a regular basis, but a standardized procedure for initial testing would prove highly beneficial. Despite standardization, sufficient flexibility should be provided as the relevant conditions (e.g. temperature) of the particular water application have to be considered. Flow cytometry is a valuable diagnostic tool to assess whether bacteria originate from regrowth or from the wastewater. Digital process control systems can contribute to quality management. QMRA is a valuable tool to identify potential threats for a given water reuse case. The barriers can be chosen based on the results of this analysis.

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