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Bacterial community composition and dynamics during reclaimed wastewater storage

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Highlights:

- **Indicator microorganisms did not regrow during treated wastewater storage over 15 days**
- **Resistance genes *mcr-1*, bla_{TEM}, *ermB* and bla_{NDM-1} did not increase over 15 days of storage**
- **The bacterial community undergoes major shifts during the 15-day storage period after ozonation, filtration and UV disinfection**

Keywords: water reuse; bacterial regrowth; antibiotic resistance

INTRODUCTION

The fast-paced climate change relentlessly urges the world to fundamentally reconsider water supply processes. In the context of wastewater (WW) reuse in agriculture, wastewater treatment plants (WWTPs) must ensure that the quality of treated WW does not increase the risk of transmitting pathogens to humans and animals e.g. through crop contamination. Therefore, the EU introduced a water reuse regulation (EU 2020/741) that applies since June 2023, delineating guidelines for the secure use of treated WW. WW quality is typically determined by chemical, physical, and microbial parameters that reflect the immediate condition of a WW sample. The reuse regulation considers storage in risk management but lacks precise microbial target values for treated WW post storage, overlooking potential microbial community shifts that could lead to regrowth of certain microorganisms, particularly pathogens. This study investigates microbial regrowth and community shifts during storage of WW treated by a pilot-scale WWTP. Specifically, we aimed at investigating whether extended storage times have an adverse effect on water quality. The investigation involved the analysis of different treatment stages, including the influent and effluent of the full-scale WWTP as well as effluents of a pilot plant that uses a combination of ozonation, filtration and UV disinfection.

METHODOLOGY

The pilot plant consists of a combination of ozonation (~8 m^3/h), filtration (~2 m^3/h), and UV disinfection (~1.5 m^3/h). It was used to further treat the secondary effluent of the Steinhof WWTP

(Brunswick, Germany), which has a treatment capacity of about 350.000 population equivalents. A total of three sampling campaigns were conducted after each treatment step. Samples were analyzed the same day of sampling (T_0) and subsequently incubated at 22°C. Follow-up analyses were performed at three (T_3), seven (T_7) and fifteen (T_{15}) days. Cultural analyses included somatic coliphages (EN ISO 10705-2:2001), Clostridia spp. (EN ISO 14189:2016) intestinal enterococci and E. coli (DIN EN ISO 7899-1/2:1998 and 9308-1/3:1998). Molecular quantification of antibiotic resistance genes (ARGs) such as *bla*$_{TEM}$, *bla*$_{NDM-1}$, *mcr-1* and *ermB* with normalization to the *16S* rRNA housekeeping gene, was performed using qPCR. Lastly, the *16S* rRNA V3-V4 hypervariable region was sequenced (Illumina MiSeq). We evaluated the α-diversity of the bacterial communities based on richness, Shannon and Simpson indices. The β-diversity was estimated using the Bray-Curtis dissimilarity metric. The metric was visualized by principal coordinate analysis (PCoA) biplot.

RESULTS AND CONCLUSIONS

Overall, there was a reduction in all indicators along the treatment chain, with further decreases during storage. Somatic coliphages, *Clostridium* spp. spores, *E. coli*, and intestinal Enterococci decreased by ~6, 4, 7 and 7 log, respectively, from the WWTP influent to the effluent of UV disinfection. No regrowth of the analyzed indicators was observed, except for somatic coliphages in the WWTP influent, which persisted at stable levels during storage. Clostridia declined along the treatment chain but persisted during storage (Fig. 1). ARGs showed a consistent decline along

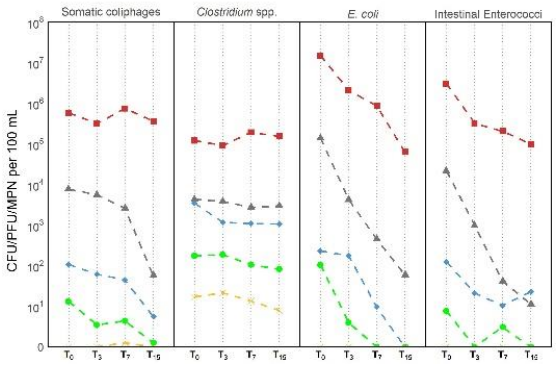

Fig. 1 Arithmetic mean concentration of somatic coliphages (PFU/100 mL), *Clostridium* spp. spores (CFU/100 mL), *E. coli* (MPN/100 mL), intestinal Enterococci (MPN/100 mL) estimated by culture of triplicate samples. Influent (square), effluent (triangle), ozone (diamond), filter (circle), UV (cross).

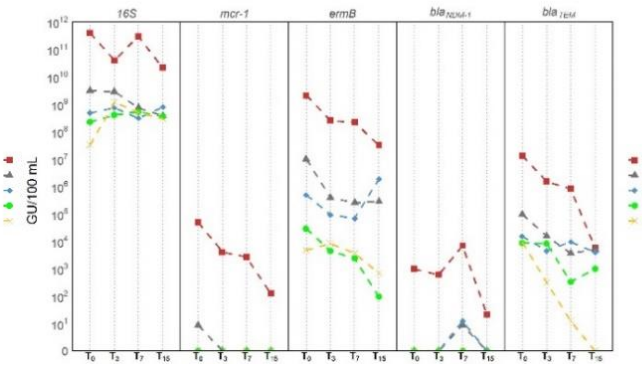

Fig. 2 Arithmetic mean concentration of the housekeeping gene *16S* rRNA and the antibiotic resistance genes *mcr-1*, *ermB*, *bla*$_{NDM-1}$ and *bla*$_{TEM}$ estimated by qPCR of triplicate samples. Influent (square), effluent (triangle), ozone (diamond), filter (circle), UV (cross).

WWTP and storage period. *16S* rRNA remained relatively stable over storage, with a notable increase post UV disinfection by 1.5 log (Fig. 2). Disinfection processes induced significant shifts in bacterial communities after storage, reflected in changes in α-diversity and relative abundances

of taxa (Fig. 3). While WWTP influent and effluent samples remained rather stable during storage (Fig. 3A, 3B), samples after ozonation showed steep losses in α-diversity after T_3, which tended to increase by T_7 and seemed rather balanced at T_{15} (Fig. 3C). UV disinfection strongly

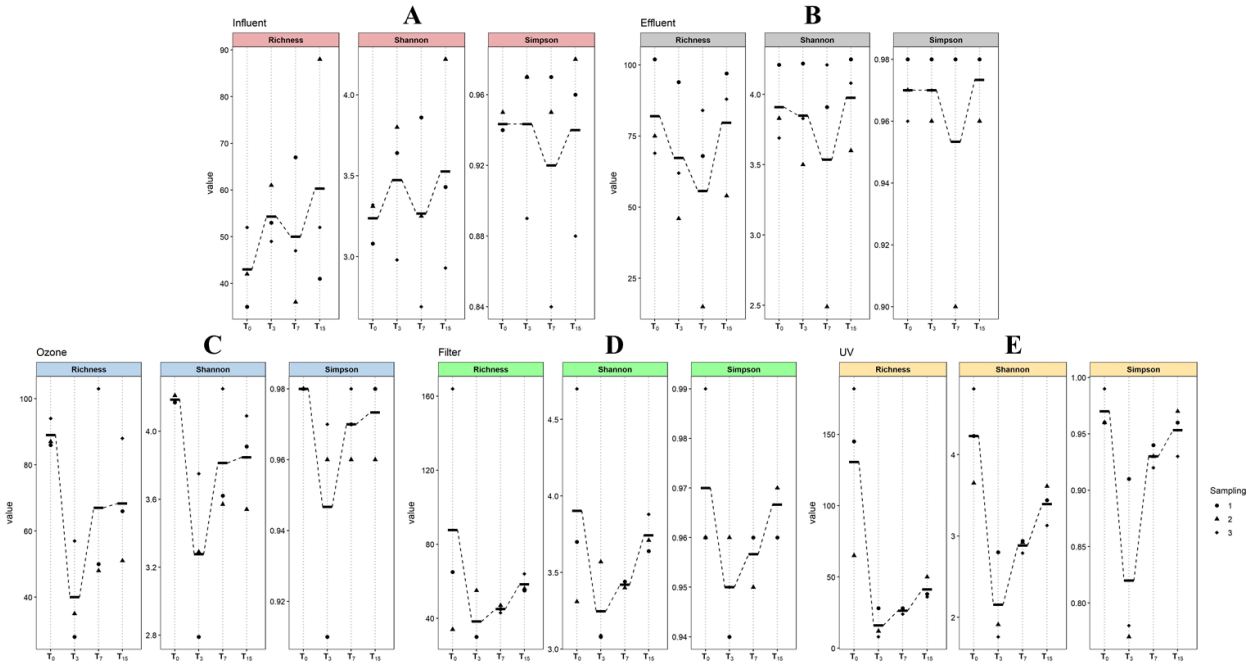

Fig. 3 α-diversity (left: richness, middle: Shannon index, right: Simpson index) of a) influent (red), b) effluent (gray), c) ozone (blue), d) filter (green), e) UV (yellow) from triplicate samples as circles and arithmetic mean as horizontal line

reduced α-diversity at T_3 but recovered gradually and did not reach original levels until T_{15} (Fig. 3E). Similarity of bacterial communities at different sampling sites and storage times is reflected in β-diversity and PCoA (not shown in abstract). Ozone, filtration and UV samples showed approx. similar bacterial composition at T_0, which changed extensively at T_3 and T_7 but converged at T_{15}, never returning to their original composition. Dominant bacterial taxonomic ranks in these communities have been identified (not shown in abstract). Storage did not cause regrowth of indicators or ARGs in the pilot plant. Nevertheless, ozonation, filtration and disinfection reduced α-diversity and altered bacterial communities extensively. Short-term changes (T_3, T_7) favored regrowth of certain taxonomies, indicating imbalances in bacterial communities that tended to be restored and converge, irrespective of treatment step (ozone, filter, UV) after T_{15}. Despite being rather unexplored, the decrease in environmental bacterial diversity could affect ecosystem stability and public health. Thus, water disinfection processes should consider their impact on regrowth of indigenous microbiota and imbalance between bacterial populations.

ACKNOWLEDGMENTS

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REFERENCES

EU 2020/741- Regulation (EU) 2020/741 of the European Parliament and of the Council of 25 May 2020 on minimum requirements for water reuse