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Assessing the potential of a membrane bioreactor and granular activated carbon process for wastewater reuse – A full-scale WWTP operated over one year in Scania, Sweden

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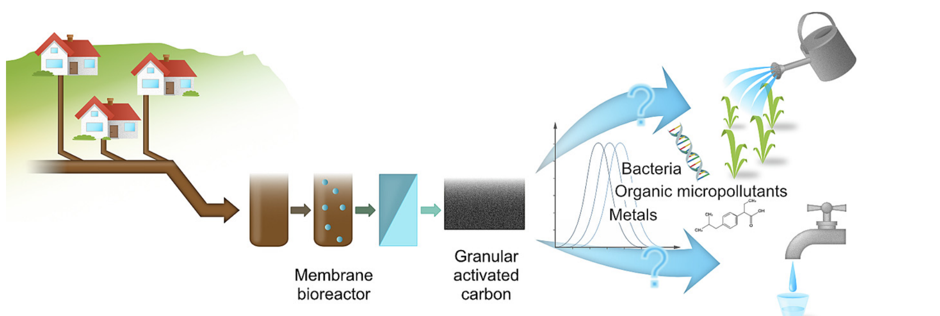
HIGHLIGHTS

- Wastewater reuse with a full-scale MBR and GAC process was investigated.
- DNA sequencing and analysis of bacteria, metals, and organic micropollutants
- Low effluent concentrations of *E. coli*, metals, and organic micropollutants
- Drinking water quality was not achieved.
- Water quality for irrigation was generally achieved.

GRAPHICAL ABSTRACT



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ABSTRACT

A full-scale membrane bioreactor (MBR) with ultrafiltration, followed by granular activated carbon (GAC), was examined to determine the potential of reusing treated water as a source of drinking water or for irrigation. The major part of the bacteria removal took place in the MBR, whereas the GAC removed substantial amounts of organic micropollutants. Annual variations in inflow and infiltration resulted in a concentrated influent during summer and a diluted influent in the winter. The removal of *E. coli* was high throughout the process (average log removal 5.8), with effluent concentrations meeting the threshold for class B water standards for irrigation (EU 2020/741) but exceeding those for drinking water in Sweden. The total bacterial concentration increased over the GAC, indicating the growth and release of bacteria; however, *E. coli* concentrations declined. The effluent concentrations of metals met the Swedish criteria for drinking water. The removal of organic micropollutants decreased during the initial operation of the treatment plant, but after 1 year and 3 months, corresponding to 15,000 bed volumes, the removal increased. Maturation of the biofilm in the GAC filters might have resulted in biodegradation of certain organic micropollutants, in combination with bioregeneration. Although there is no legislation in Scandinavia with regard to many organic micropollutants in drinking water and water for irrigation, the effluent concentrations were generally in the same order of magnitude as to those in Swedish source waters that are used for drinking water production.

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1. Introduction

Investments have been made in wastewater treatment for removing organic micropollutants in several countries (Audenaert et al., 2014; VSA, 2022a; KomS, 2021, Svenskt Vatten, 2021), to protect the receiving aquatic environment and drinking water sources. Water scarcity, in combination with such investments have in some places resulted in the goal of reusing the treated effluent for irrigation, industrial use, and indirect and direct potable use, raising the issue of the water quality requirements.

Regulations on water reuse for drinking water or irrigation often include limits on pathogens, due to the acute risk of disease, but criteria for organic pollutants, including pharmaceutical residues, are sometimes lacking. Sweden and Scandinavia have no legislation on many water reuse applications. Comparisons with accepted concentrations in recipient bodies that also serve as drinking water sources can act as a proxy for such missing guidelines to understand what concentrations are accepted in source waters and advance these technologies, despite the absence of legislation.

Water reuse treatment processes often comprise microfiltration (MF) or ultrafiltration (UF), followed by reverse osmosis (RO) and disinfection (Drewes and Horstmeyer, 2016; Jeffrey et al., 2022; Reungoat et al., 2012). Treatment processes that involve RO can be costly, due to the energy demand and the handling of brine residue (Kehrein et al., 2021), prompting the growing interest in advanced treatment processes without RO (Hogard et al., 2021; Reungoat et al., 2012). Implementation of new processes, however, must consider the potential environmental and health effects from the resulting water if it is to be used for long-term irrigation, artificial infiltration, or drinking water production. Consumption of water or food that contains antimicrobial residues can have negative health effects, and irrigation with water that harbors these substances could impact the development of the irrigated plants (Adeel et al., 2017; Janeczko and Skoczowski, 2005; Treiber and Beranek-Knauer, 2021). Concerns have been expressed over the potential connection between environmental exposure to pharmaceutical residues and adverse health effects (Miarov et al., 2020), and poly- and perfluorinated alkyl substances (PFAS) can negatively impact health (US EPA, 2022).

This study examined the first Swedish full-scale treatment plant comprising a membrane bioreactor (MBR) with ultrafiltration followed by granular activated carbon (GAC). Ultrafiltration constitutes an efficient microbial barrier (Drewes and Horstmeyer, 2016) and helps remove particle-bound metals (Saleh et al., 2022). GAC can be an effective chemical barrier and in combination with processes such as ozonation, biofiltration, and UV, a promising option for water reuse (Hogard et al., 2021; Reungoat et al., 2012).

In GAC filters, a biofilm develops (Gibert et al., 2013; Weber et al., 1978), of which certain groups of bacteria in the water attach to the GAC, whereas others disperse in the water (Kantor et al., 2019; Piras et al., 2022). The effect of this biofilm on the effluent water quality is not well understood. Thus, the aim of this study was to determine the suitability of the MBR and GAC process for the production of source water for drinking water production (with further treatment at a drinking water treatment plant) or irrigation, through examination of the effluent microbial and chemical water quality and of the development of the bacterial community in the GAC biofilm over time from the startup of the process in December 2020. If the water is to be used as a source water for drinking water production, additional treatment steps at a drinking water treatment plant are needed. The water quality was compared to legislative and guideline standards from Sweden, EU, the US, and Australia, with information on Swedish source water quality to complement existing legislation.

The GAC biofilm can contribute to the removal of some organic micropollutants and of DOC through biological degradation (Altmann et al., 2016; Reungoat et al., 2010), and can affect the effluent water quality through release of cells and alteration of the water bacterial composition (Kantor et al., 2019; Miller et al., 2020; Piras et al., 2022; Stewart et al., 1990; Vignola et al., 2018). Because of this, concerns have been raised over the growth and release of opportunistic bacteria from GAC filters and other media filters (Kantor et al., 2019; Miller et al., 2020; Vignola et al., 2018; Wullings et al., 2011).

2. Materials and methods

2.1. Study site

In 2018, the Simrishamn municipality acquired funds from the Swedish Environmental Protection Agency to implement treatment for removal of organic mircopollutants from wastewater (Svenskt Vatten, 2022). This helped financing the reconstruction of a wastewater treatment plant (WWTP) in Kivik, which became operational in December 2020. Kivik is a town in southeast Sweden with approximately 890 inhabitants. The treatment plant receives wastewater from Kivik and the towns of Vitaby (~350 inhabitants), Vitemölla (~100), and Södra Mellby (~100) and is dimensioned for a maximum of 7500 population equivalents. This high dimensioning is due to the popularity of Kivik as a tourist area and its density of vacation homes, particularly in June, July, and August – increasing its population during summers.

Kivik lies in an agricultural landscape (SCB Statistical Database, 2022) and has experienced water shortages in recent years. The summer of 2021, included in this study, was described as “extreme”, with low groundwater levels and drinking water that had to be delivered by tanker trucks from a nearby town, 19 km away (Vodopija Stark, 2021).

The treatment plant consists of a pretreatment (screening, grease trap, sand trap), chemical precipitation, with an aluminum based chemical, and disc filters, followed by an MBR that comprises an anoxic zone and an aerated zone, followed by 2 parallel ultrafiltration membranes (pore size 0.038 μm) (Fig. 1). Two parallel GAC filters follow the MBR, after which the water is released to the recipient: Hanö Bay in the Baltic Sea.

One reason behind the choice of the MBR + GAC process was a limitation of space at the treatment plant. Further, treatment for the removal of organic micropollutants had been implemented at another treatment plant in the municipality (with ozonation, sand filtration and GAC filtration), and there was a wish to do so also in Kivik, but with a different technology to enable comparison between the two processes.

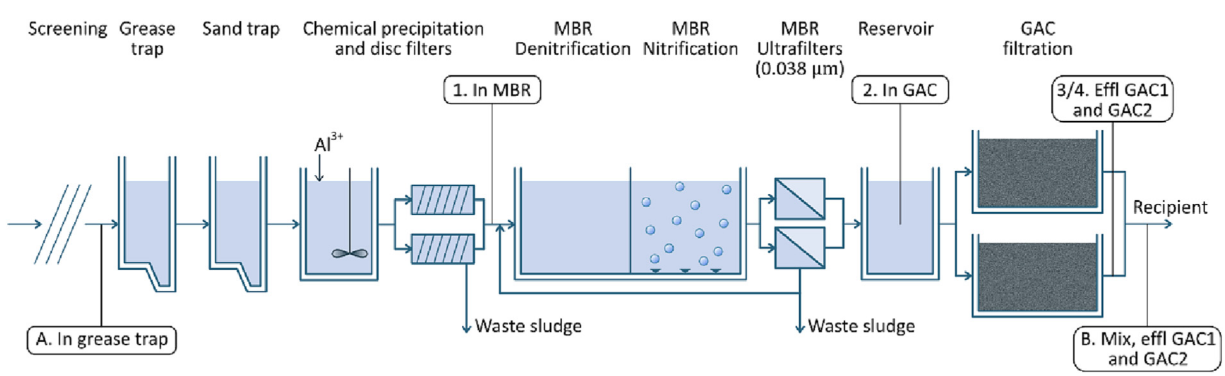

Fig. 1. Process scheme of the studied full-scale treatment plant. Sampling points are indicated in the boxes.

The GAC is comprised of Jacobi Aquasorb 6100 and has a filter area of 15 m^2 per filter and a depth of 1.2 m, resulting in a volume of 18 m^3 for each filter bed. Since March 2022, simultaneous precipitation has been performed in the MBR. The technical specifications of the MBR and the GAC filters are summarized in Table S1, Supplementary material.

The treatment plant is designed for a hydraulic load of 180 m^3/h, and the limit is 0.4 mg/l (average per quarter) on the effluent total phosphorus concentration and 8 mg/l (average per quarter) on the effluent BOD_7 concentration.

2.2. Evaluation of water reuse potential

The potential to reuse the effluent water for irrigation was assessed against Regulation (EU) 2020/741 of the European Parliament and of the Council of 25 May 2020 on minimum requirements for water reuse, and US EPA (2012) guidelines for water reuse. The potential to reuse it as a drinking water source was evaluated using Swedish drinking water regulations (LIVSFS 2017:2).

Because these guidelines do not include regulations on many organic micropollutants, these contaminants were compared against Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption (recast), the *Australian guidelines for water recycling* (2008), Reungoat et al. (2012), and California EPA (2018) water quality control policy for recycled water (the latter 3 concern the augmentation of drinking water supplies). See Tables S2 and S3 (Supplementary material) for a summary of the legislations and guidelines. References for the concentrations of organic micropollutants for wastewater recipients that serve as drinking water sources included the Göta Älv river in southwest Sweden (Tröger et al., 2020) and the 3 largest Swedish lakes – Vänern, Vättern, and Mälaren (Malnes et al., 2020), as well as lake Ringsjön (Svahn and Björklund, 2017). These values reflect the concentrations that are accepted in source water that is used for drinking water production and can be used to evaluate water quality in the absence of legislative thresholds.

2.3. Sampling

The project consisted of 2 sampling periods that overlapped and comprised different sampling points. Sampling Period 1, from December 2020 to December 2021, in which bacteria, metals, and organic micropollutants were analyzed, was initiated by Lund University together with Kristianstad University and the water utility in Kivik (Österlen VA AB). Sampling Period 2 aimed to analyze only organic micropollutants, starting in May 2019 (before reconstruction of the plant) and remains ongoing. This analysis was initiated by the county board in Scania, as a requirement for the funds that the municipality received for reconstruction of the treatment plant. Until March 2021, only effluent samples were analyzed, and from April 2021, influent and effluent samples were analyzed. From Period 2, only samples from April 2021 to May 2022 were included in this study.

During Period 1, grab water samples were collected approximately once per month (14 occasions from December 2020 to December 2021; Table S4, Supplementary material) at 4 points throughout the treatment process (1. influent MBR, 2. influent to GAC filters, 3. and 4. effluent from each GAC filter; Fig. 1). Granules from each filter were also collected at the same time. In Period 2, 24-hour flow proportional composite samples were collected from the influent to the grease trap, and from a mixture of GAC1 and GAC2 effluents (A. In grease trap, and B. Mix effl. GAC1 and GAC2; Fig. 1).

Flow cytometry, *E. coli* and total coliform analyses were conducted within 24 h and completed within 48 h, with storage of the samples at 4–7 °C when necessary. DOC, PO_4^{3-}-P, NO_3^--N, NO_2^--N, and NH_4^+-N were analyzed within 48 h from the sampling (samples stored at 4–7 °C) (Section 2.7 – Standard parameters). A control sample for the *E. coli* and total coliform analysis was collected from the drinking water tap at the treatment plant. Samples for flow cytometry were collected in sterile Falcon tubes, and those for the organic micropollutant and metal analyses were collected in HDPE bottles and 50 ml Falcon tubes, respectively, and stored at −20 °C.

Water samples for 16S rRNA gene amplicon sequencing of the bacterial community in the GAC influent and the GAC effluents were collected in glass bottles that had been cleaned for 10 min with diluted (1:10) sodium hypochlorite (6–14 % active chlorine before dilution), rinsed with MilliQ water 5 times, and rinsed with the sample water 3 times. 150 ml of each water sample were passed through 0.22 μm Isopore filters to collect bacteria for DNA extraction (Merck Millipore, PC Membrane, 47 mm). GAC granules were collected from the top layer of both filters and stored in sterile Falcon tubes. All filters and granules were stored at −20 °C until sequencing.

2.4. Microbial analysis

2.4.1. E. coli and total coliforms

Total coliforms and *E. coli* were analyzed using IDEXX Colilert 18 and Quanti-tray 2000, following manufacturer's instructions. The MBR influent was diluted 1:100,000 with MilliQ water prior to analysis, and the GAC influent and effluents were diluted 1:10 on the first 2 sampling occasions. Because the concentrations of *E. coli* in the GAC influent and effluents were 0 this dilution, they were considered below the limit of detection (LOD, 10 CFU/100 ml at a dilution of 1:10) and were not diluted on subsequent occasions.

2.4.2. Flow cytometry

Total cell concentration (TCC, including intact cells and damaged cells) and intact cell concentration (ICC) were analyzed using flow cytometry (FCM). Most samples were analyzed on a BD C6 Accuri flow cytometer. However, due to maintenance, samples from November and December 2021 were run on a BD C6 Plus Accuri, the results of which were adjusted to allow them to be compared with those from the BD C6 Accuri, using Spherotech 8-peak validation beads (FL1 - FL3) as a validation sample. Fluorescence was read at 533 ± 30 nm (FL1, green fluorescence) and >670 nm (FL3, red fluorescence).

The influent to the MBR was passed through a 10 μm filter before FCM analysis, to protect the instrument from clogging, and diluted 1:10 due to its high cell concentrations. SYBR® Green I, diluted to a concentration of 100× in dimethyl sulfoxide, was used for the TCC analysis. A mixture of propidium iodide (PI) and SYBR® Green I was used to differentiate intact cells from damaged cells. The final concentration of SYBR® Green I in the stained samples was 1× for the TCC and ICC analyses, and the concentration of PI was 0.3 mM. Samples were incubated for 15 min at 37 °C after staining and analyzed in duplicate; the average of the duplicates was used in the data analysis. A volume of 50 μl was analyzed at a flow of 35 ml/min, and the results were analyzed in FlowJo (v 10.6.2).

2.4.3. 16S rRNA gene amplicon sequencing

Full-length 16S rRNA gene amplicon sequencing was performed on DNA that was extracted from the GAC influent, GAC1 effluent, and granules from GAC1 on 12 occasions from December 2020 to December 2021 (no sequencing was performed on samples from February 23 and March 23, 2021). Because the microbial communities in the effluent from GAC1 and GAC2 were similar, based on flow cytometry results, amplicon sequencing was performed only for GAC1. Three samples of GAC (0.5 g wet weight) from three time points were dried and used to calculate its dry weight.

DNA was extracted from filter papers or from 0.5 g wet GAC using the FastDNA Spin kit for soil (MP Biomedicals, Denmark), following the manufacturer's recommendations. Quant-iT HS DNA Assay kit (Thermo Fisher Scientific, USA) was used to determine the DNA concentration. The full-length 16S rRNA gene was amplified using the primer 27F: 5′-AGAGTTTGATCCTGGCTCAG-3′ and 1492R: 5′-GGTTACCTTGTTACGACTT-3′ (Lane, 1991). Duplicate PCR reactions of 25 μl (PCRBIO 1× Ultra Mix, PCR BIOSYSTEMS), 400 nM of each primer, 10 ng of template DNA, and nuclease-free water, were run under the following conditions: an initial denaturation at 95 °C for 2 min, 30 cycles of 95 °C for 15 s, 50 °C for 15 s, and 72 °C for 90 s, and a final elongation at 72 °C for 5 min.

A nontemplate control and a sample of known content were included to assess the quality of the amplicon generation. The amplicon libraries were purified using the CleanNGS (CleanNA, the Netherlands), according to manufacturer's recommendations. The library concentration was measured with the Quant-iT HS DNA Assay (Thermo Fisher Scientific, USA) and quality checked with a TapeStation 2200 using D1000 ScreenTapes (Agilent, USA).

Samples were bar-coded, pooled equimolar, DNA was repaired and end-prepped, adapter ligation and clean-up was performed, and finally priming and loading of the flowcell using the SQK-LSK109 with the EXP-PBC096 following the manufactures recommendations (Oxford Nanopore Technologies, United Kingdom). The resulting library was loaded onto a single MinION R9.4.1 (106) flow cell and sequenced for 72 h.

Raw reads were based called using Guppy v.6.0.6 (Guppy is available only to ONT customers via their community site, https://community.nanoporetech.com) and mapped against the MiDAS v.4.8.1 database using minimap2 (Li, 2016) and SAMtools (Danecek et al., 2021). All data were visualized in R version 4.1.2 (https://www.r-project.org/), using Rstudio version 1.4.1717 (https://www.rstudio.com/). The R package decontam (https://github.com/benjjneb/decontam) (method = "frequency") was used to identify and remove contaminating DNA from the extraction kit and from the reagents (Davis et al., 2018). In the decontam analysis, 2 samples were removed, because their DNA stock concentration was negative. The R package ampvis2 (v.2.7.27) was then used to generate heat maps (Andersen et al., 2018).

2.5. Organic micropollutants

In Sampling Period 1, 38 organic micropollutants were analyzed, 14 of which are listed in Option 1 in Proposal for Directive (EU) 2022/0344 (COD). In Sampling Period 2, 13 indicator organic micropollutants were analyzed (atenolol, carbamazepine, ciprofloxacin, citalopram, clarithromycin, diclofenac, estrone, metoprolol, naproxen, oxazepam, tramadol, trimethoprim, venlafaxine), 3 of which are listed in EU 2022/0344 and 6 of which are on the Swiss list of 12 indicator substances for verifying the performance of measures for the abatement of organic micropollutants (VSA, 2022b).

Samples were purified and concentrated using solid-phase extraction (SPE, Oasis HLB, 200 mg) and analyzed with ultra-performance liquid chromatography (UPLC), followed by tandem mass spectrometry (MS/MS), described by Svahn and Björklund (2016, 2019). Briefly, 50 ml of sample was purified and concentrated on an SPE column. After being dried, eluted, desiccated, and reconstituted in 1 ml 1:10 methanol:MilliQ, the sample was injected to the UPLC-MS/MS (Waters Acquity UPLC H-Class, Xevo TQS Waters Micromass, Manchester, UK) using 3 different volumes (1 μl, 1 μl respective 10 μl) in 3 different methods, all of which have been validated according to the standard method, 1694, published by the US EPA (2007). The limit of quantification (LOQ), standard deviations, and internal standards for the 38 analyzed micropollutants are presented in Table S5, Supplementary material.

2.6. Metals and other trace elements

Metals and other inorganic trace elements were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES) (Perkin Elmer, Optima 8300) and, when necessary due to low concentrations, inductively coupled plasma mass spectrometry (ICP-MS) (Bruker, Aurora Elite) (Table S6, Supplementary material).

2.7. Standard parameters

PO_4^{3-}, NO_3^-, NO_2^-, and NH_4^+ were analyzed on a Metrohm Eco Ion Chromatograph. DOC was analyzed using HACH Lange LCK 385 cuvettes and a HACH DR2800 spectrophotometer. Prior to the analysis of nitrogen, phosphorus, and DOC, the samples were passed through a 0.45 μm syringe filter (GVS). Dissolved oxygen and temperature were measured in the reservoir and in each GAC filter (HACH portable HQ40d). Turbidity was measured using a HACH 2100P ISO turbidimeter.

3. Results and discussion

3.1. General performance

Annual variations in the flow to the treatment plant were observed, with higher flow from approximately October until April, due to greater precipitation and runoff and thus higher infiltration and inflow into the sewer system (Fig. 2). Together with a high number of visitors during the summer months, this resulted in a more concentrated influent in summer and a more diluted influent in late fall, winter, and early spring, affecting the concentrations of *E. coli* and total coliforms in the influent to the MBR (Fig. 2). The average empty bed contact time (EBCT) in the GAC filters was 51 min and correlated inversely with flow. The average hydraulic retention time was 4 h in the anoxic zone of the MBR, and 8 h in the aerobic. The GAC filters were backwashed for the first time in November 2021 (week 46), thereafter approximately once every other week during the spring of 2022, and thereafter more seldom. The UF membranes were backwashed every fourth minute, and cleaned with sodium hypochlorite approximately once per month.

The effluent DOC concentration was on average 5.3 mg/l from GAC1 and 4.8 mg/l from GAC2, with highest removal over the MBR, but also over the GAC filters.

The effluent concentration of nitrate (88 mg NO_3^- /l, corresponding to 20 mg N/l) exceeded the limit for acceptable drinking water (50 mg NO_3^- /l), whereas that of nitrite (0.07 mg NO_2^- /l, corresponding to 0.023 mg N/l) met the requirements (acceptable: 0.50 mg NO_2^- /l, acceptable but concerning: 0.10 mg NO_2^- /l) (LIVSFS 2017:2). The general performance parameters are summarized in Table S7, supplementary material.

3.2. Microbial water quality

There was a substantial removal of *E. coli* and total coliforms throughout the treatment process, to which the MBR process contributed most (Fig. 3). In certain cases, the concentration was below the LOD due to excessive dilution of the samples (<10 CFU/100 ml). Thus, a concentration of 5 CFU/100 ml was assumed in the calculations. This adjustment was applied to the *E. coli* concentration in 4 samples (effluent GAC2 December 17, 2020, influent GAC February 2, 2021, effluent GAC1 February 2, 2021, and effluent GAC2 February 2, 2021) and to the total coliform concentration in 1 sample (effluent GAC1 in February 2021).

Based on the average of the GAC1 and GAC2 effluents, the yearly average log removal values (LRVs) for *E. coli* and total coliforms by the entire treatment plant were 5.8 log units and 5.5 log units. The removal varied throughout the year, peaking in July 2021 (7.4 log units for *E. coli*, and

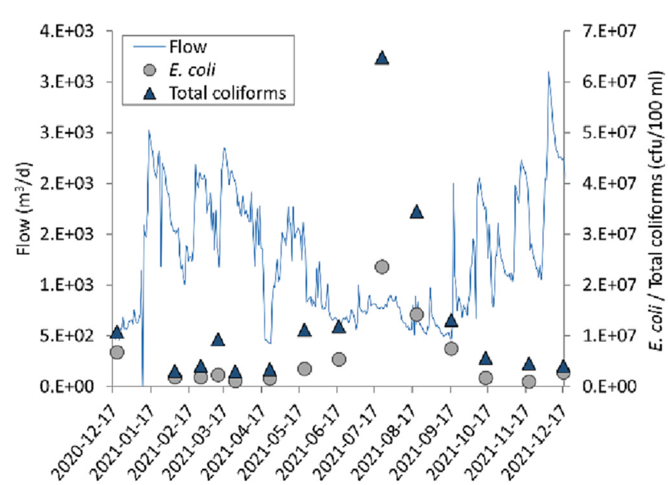

Fig. 2. Flow and concentrations of *E. coli* and total coliforms in the MBR influent.

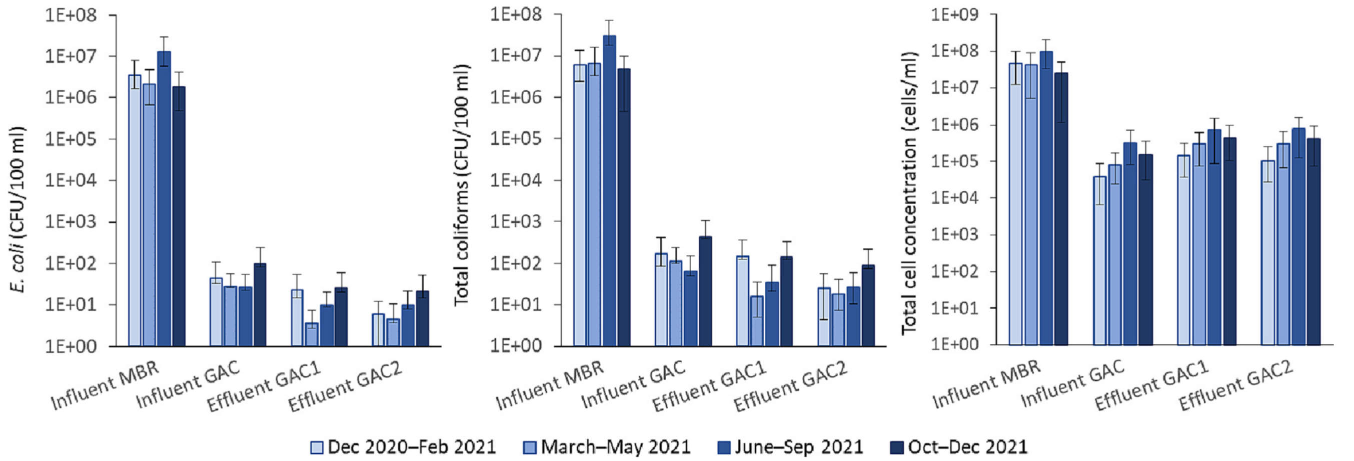

Fig. 3. *E. coli*, total coliform, and total cell concentration throughout the treatment process. Average concentrations together with 25th and 75th percentiles. When the effluent concentration was below the LOD, a concentration of 0.5 · LOD was assumed.

6.7 log units for total coliforms) and bottoming in December 2021 (4.7 log units for *E. coli* and 4.1 log units for total coliforms).

Several parameters might have contributed to the seasonal variations in this removal, such as the influent *E. coli* and total coliform concentrations, the influent DOC concentration, and variations in water temperature, flow, and hydraulic load. Maximum removal of *E. coli* and total coliforms occurred when their influent concentrations were high and in periods with high temperature and high influent DOC concentration. The influent temperature and DOC concentration might have affected the biological processes, such as the biological treatment in the MBR and the biological activity in the GAC filters. In the MBR, the average LRV for *E. coli* and total coliforms was 5.4 log units, and 5.2 log units, respectively.

In the GAC filters, the average LRV for *E. coli* and total coliforms was 0.37 log units and 0.29 log units. In July, the *E. coli* concentration in the GAC influent was 0 CFU/100 ml, resulting in invalid calculations of the LRV over the MBR and over the GAC filters; thus, these values were excluded from the yearly averages.

The average effluent concentration of *E. coli* during Period 1 was 14 ± 21 CFU/100 ml and 10 ± 14 CFU/100 ml for GAC1 and GAC2, respectively (Table S8, Supplementary material). The criterion with regard to *E. coli* for meeting class B water quality standards for irrigation in Regulation (EU) 2020/741 is <100 CFU/100 ml, which was achieved at each time point. Class B is the second strictest quality class out of four (class A, B, C and D), second only to class A (*E. coli* <10 CFU/100 ml). The class A limit was exceeded at 5 of 14 time points for GAC1 and at 3 time points for GAC2. In Regulation (EU) 2020/741, the indicative technology target for quality class B is secondary treatment as well as disinfection, and for class A secondary treatment, filtration and disinfection. The MBR and GAC treatment train is not directly comparable to these indicative technology targets. However, if disinfection, such as UV, would be added in order to reach the quality class A limit on *E. coli*, the indicative technology target would also be reached.

The average effluent concentration of total coliforms was 75 ± 136 CFU/100 ml and 37 ± 59 CFU/100 ml for GAC1 and GAC2, respectively, but there were large variations in concentration over time, resulting in high standard deviations.

Turbidity, measured in February 2022, was 0.37 NTU and 0.42 NTU in the effluent from GAC1 and GAC2, respectively, meeting the requirements for drinking water (0.5 NTU in finished drinking water and 1.5 NTU at the user or in bottled water, LIVSFS 2017:2) and class A standards for irrigation (Regulation, EU, 2020/741).

In the GAC influent, peaks in the concentrations of *E. coli* and total coliforms were observed at various time points (Fig. 4). These concentrations decreased slightly in the GAC effluents compared with the GAC influent. When adjusted for load, the patterns were similar, but with some peaks largely disappearing.

The total cell concentration (TCC) increased over the GAC filters (Figs. 3 and 4), indicating a growth of bacteria in the filters and a release to the effluent. However, this increase did not apply to *E. coli* and total coliforms, suggesting that the GAC filters created a new bacterial community that is partly released to the effluent water. The biofilm on GAC filters thus appear to be able to selectively remove *E. coli* and total coliforms, as supported by Chan et al. (2018), who reported removal of *E. coli* by biofilms in slow sand filters and increased total cell counts in the filter effluent.

Overall, the TCC in the GAC influent and effluents rose from April to October and declined in the winter, whereas the concentrations of *E. coli* and total coliforms did not follow this pattern (Fig. 4). The average intact cell concentration in the GAC influent and effluents was 79 % of the TCC.

When bacterial concentrations were adjusted to reflect changes in flow, the total cell load (TCL, cells/day) in the influent to and effluents from the GAC filters increased in October and November compared with the summer months, deviating from the pattern for TCC (Fig. 4). This result highlights that the dilution due to the infiltration and inflow during winter has a substantial impact on the variations in bacterial concentrations (Fig. 4). The peaks in TCL in October and November 2021 might be attributed to a release of bacteria from the GAC biofilm, due to changing environmental conditions in temperature or influent DOC concentration. In December 2021, the TCL decreased again, indicating possible adaptation of the biofilm to new conditions, but only to the levels that were observed in May–September. That the TCL did not return to the levels during the winter the previous year suggests that this cell concentration reflects a mature biofilm. This hypothesis is supported by an additional measurement that was made the following year, in February 2022, showing an effluent TCL of approximately $5 \cdot 10^{12}$ cells/day, indicating stabilization at this level. Longer studies are necessary to determine whether or how TCL varies as the GAC filter ages.

The amount of DNA that was extracted from GAC granules increased until approximately October 2021 but declined in November 2021, and in December 2021 it reached approximately the same levels as the previous spring (Fig. S1, Supplementary material), likely reflecting a decrease in the amount of biofilm bacteria on the granules. The peak content of DNA on the granules preceded that in TCL by 1 month, further supporting that biofilm growth was followed by an increase in the release of cells into the water.

The 16S rRNA gene amplicon sequencing analysis showed that the composition of the bacterial community in the GAC influent varied between time points (Fig. 5).

One potential explanation for these variations is the large infiltration and inflow to the sewer system, potentially resulting in higher abundance of reads from soil bacteria with high precipitation and inflow. In the influent, the most abundant genera were *Romboutsia* and *Afipia* (Fig. S2, Supplementary material), which can reside in soil (Han et al., 2022; Pan et al., 2022), and several of the phyla abundant in the influent, such as *Proteobacteria*, *Firmicutes*, *Acidobacteria*, *Actinobacteria*, *Bacteroidota*,

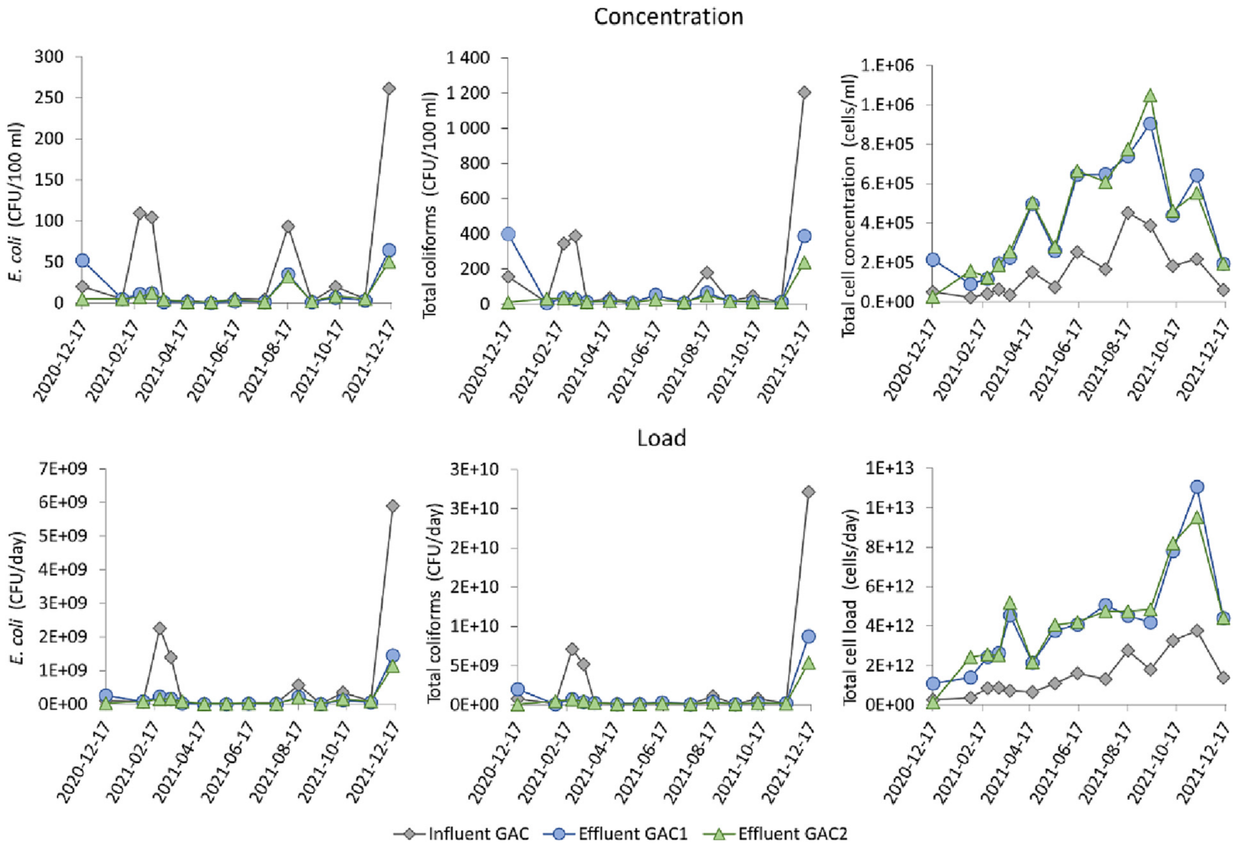

Fig. 4. *E. coli*, total coliform, and total cell concentrations in the GAC influent and effluents. When the effluent concentration was below the LOD, a concentration of 0.5 · LOD was assumed.

Myxococcota, and *Verrucumicrobiota* (Fig. S3, Supplementary material) have been observed in soil (Chen et al., 2021; Ehrhardt et al., 2022; Pan et al., 2022; Shami et al., 2022).

For some genera, the community on the granules appeared to affect that in the effluent, for example the genera *Rhodoferax*, *midas_g_3776*, *Denitratisoma*, and *Sulfuritalea* (Fig. 5). Several genera were more abundant on the granules compared with in the influent and effluent, such as *Afipia*, *Stenotrophobacter*, and *Hyphomicrobium*, suggesting an attachment of these to the GAC granules. The abundance of some genera on the granules decreased over time, including *Rhodoferax* and *Hydrogenophaga*, whereas those of others increased, such as *Bradyrhizobium* and *Denitratisoma* (Fig. 5). The patterns of phyla that attach or detach to GAC in Piras et al. (2022) were confirmed for *Acidobacteria* (which attaches to GAC), whereas *Bacteroidota* was somewhat more abundant on the granules versus in the effluent, implying attachment, rather than detachment, to granules, as observed by Piras et al. (2022) (Fig. S3, Supplementary material).

3.3. Chemical water quality

3.3.1. Metals and other trace contaminants

The removal of metals and other trace contaminants throughout the treatment process fluctuated between elements and time points, ranging from a negative removal to >90 %. The effluent concentrations were far below the guideline thresholds for irrigation (US EPA, 2012) and were below the Swedish limits for drinking water (LIVSFS 2017:2) for all but 1 time point, when manganese was detected at 59 μg/l in the effluent from GAC1 (December 2020), exceeding the limit for an acceptable but concerning concentration (50 μg/l) (Table S9, Supplementary material).

3.3.2. Organic micropollutants

In Sampling Period 2, the removal over the entire treatment plant in April 2021 was >90 % for 10 of 12 organic micropollutants for which a removal could be determined (Fig. 6). Based these compounds, the Swiss requirement (average removal >80 %) was met on all occasions but two (November and December 2021). In case the effluent concentration was below the LOQ, a concentration of 0.5 x LOQ was assumed.

Annual variations in the influent concentrations of organic micropollutants were observed, with lower concentrations in winter (~October–February) and higher during summer (~May–August) (Fig. 6), likely explained by the lower flow during summer, resulting in a concentrated wastewater (Fig. 2). Similar variations were observed in the GAC effluents, with concentrations of organic micropollutants peaking in May–August 2021 and then decreasing. The removal declined over time, with the lowest removal occurring in October–December 2021, after which it increased after approximately 15,000 bed volumes (BV) (February 2022), rising until the last study date in May 2022 at 20,000 BV (Fig. 6).

When only the GAC filters were examined, in Sampling Period 1, the removal was similar in GAC1 and GAC2 (Fig. S4, Supplementary material), even though the effluent concentrations were slightly lower and the removal slightly higher in GAC2 compared to GAC1. The initial removal in GAC1 was >80 % for 22 of 27 micropollutants for which a removal could be calculated. For 15 of these 27 compounds, the removal increased from the first sampling (December 2020) to second sampling (February 2021), after which it generally decreased over time and with increased bed volumes (Figs. 7 and S5, Supplementary material), although the removal patterns differed between organic micropollutants. For example, whereas paracetamol was removed completely throughout the entire study period, the removal of ciprofloxacin reached 100 % at the outset and the end but not in May–August 2021. The removal of losartan and sulfamethoxazole decreased, from over 80 % to <50 %, over the study, as did tramadol, trimethoprim, and venlafaxine but to a lesser extent.

The differences in sampling regimes during Period 1 (grab samples) and Period 2 (24-hour flow proportional composite samples) may have had effects on specific sampling occasions. For the long term general pattern, over

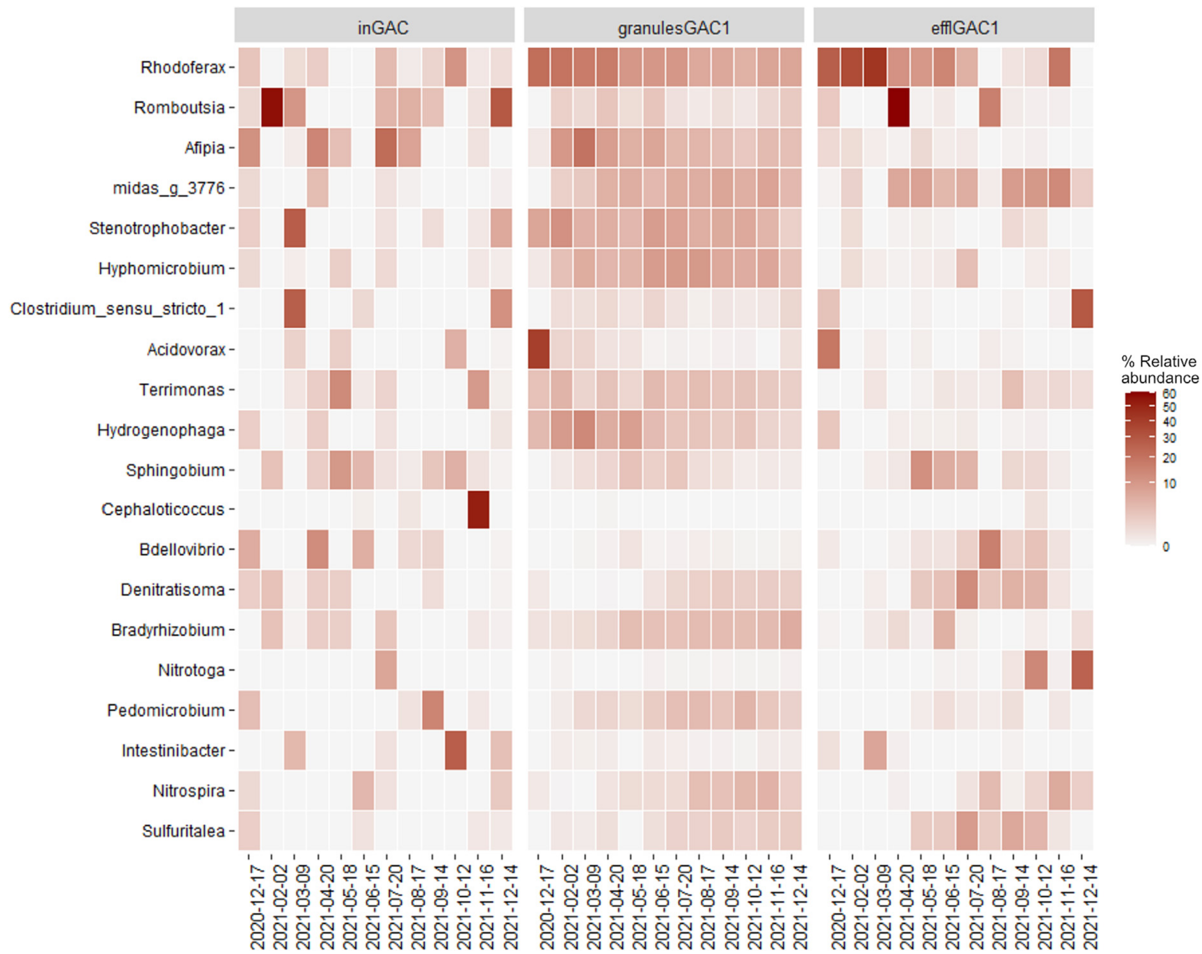

Fig. 5. Relative abundance of full-length 16S rRNA gene amplicon sequences from the 20 most abundant genera in the GAC influent, on the GAC1 granules, and in the GAC1 effluent.

the whole sampling period, differences in sampling regime probably had a minor effect.

The variations in the concentration in the GAC influent and effluents are likely explained in part by the annual variations in infiltration and inflow to the sewer system and in population size, resulting in a concentrated wastewater during summer. Other factors might also have impacted the effluent concentrations, such as a varying EBCT in the GAC bed due to flow variations, increased biological degradation of certain substances as the GAC biofilm matured, and desorption of organic micropollutants from the GAC.

Neef et al. (2022) have reported that micropollutant removal decreases during wet weather events, due to lower EBCT. At the dimensioning flow, the EBCT in the GAC filters in Kivik is 12 min, resulting in a low EBCT at flows that approximate the dimensioning. For adequate removal of organic micropollutants, the EBCT need to be roughly 30 min (Fundneider et al., 2021). At low flows, the retention time was high, such as in April 2021, with a calculated retention time of 123 min. The flow was highest during September until May, shortening the contact time in the GAC bed during this period and likely decreasing the removal of organic micropollutants.

Increased biodegradation could explain the increased removal of biodegradable organic micropollutants during Sampling Period 2 after 15,000 BV (Fig. 6). Whereas diclofenac and venlafaxine have been suggested to be persistent to conventional biological wastewater treatment (Falås et al., 2013; Joss et al., 2006), degradation of diclofenac has been observed in GAC filters (Betsholtz et al., 2021; Edefell et al., 2022) and with biofilm carriers (Falås et al., 2013), and degradation of diclofenac and venlafaxine has been observed in a submerged membrane bioreactor (Tiwari et al., 2021).

The concentrations of diclofenac, metoprolol, and venlafaxine in the GAC effluent were substantially lower in April and May 2022 compared with the same months in 2021 (1.2 ng/l, 2.9 ng/l, and 7.9 ng/l in May 2022 compared to 164 ng/l, 70 ng/l, and 92 ng/l in May 2021, respectively), whereas the influent concentrations were similar in both years. Thus, maturation of the GAC biofilm might have increased the biodegradation of these substances. This mechanism, however, does not explain the greater removal of carbamazepine (February 2022–May 2022), for example, since carbamazepine is known to be inert to biological wastewater treatment (Falås et al., 2013; Joss et al., 2006). This increased removal could be attributed to bioregeneration of the GAC, through the degradation of previously adsorbed DOC. Bioregeneration has been reported in several studies and can be used for the regeneration of used activated carbon (regeneration of activated carbon is necessary to maintain its adsorptive function) (El Gamal et al., 2018). Baresel et al. (2019) reported increased removal of carbamazepine, furosemide, and oxazepam after approximately 45,000 BV in an MBR and GAC pilot plant, and suggested bioregeneration as an explanation.

Effluent concentrations were compared with measurements for Swedish drinking water sources. Due to its resistance to biological wastewater treatment and biodegradation, carbamazepine has been suggested as a reference compound for analyzing concentration patterns (Björlenius et al., 2018; Edefell et al., 2022). The GAC1 and GAC2 effluent concentrations of carbamazepine (1–45 ng/l) in the present study were higher than the maximum concentrations in the Swedish raw water source Göta älv (13 ng/l; Tröger et al., 2020) in approximately half of the measurements (including Period 1 and Period 2). Across all compounds, the concentrations in the GAC effluents were in most cases on the same order of magnitude as those in the 3

Concentration, in grease trap

	Apr 2021 (5000 BV)	May 2021	Jul 2021	Aug 2021	Sep 2021	Oct 2021 (10000 BV)	Nov 2021	Dec 2021	Feb 2022 (15000 BV)	Apr 2022	May 2022 (20000 BV)
Atenolol	599	1002	1498	1000	544	384	181	195	309	483	562
Carbamazepine	8.6	153	202	267	215	29	7.7	9.4	48	80	104
Ciprofloxacin	301	709	1589	3689	698	456	196	142	207	319	1167
Citalopram	188	309	406	460	133	79	46	49	56	103	162
Clarithromycin	nd	nd	nd	nd	nd	2.9	1.4	1.9	<1	<1	19
Diclofenac	558	750	903	1246	397	133	102	115	223	636	449
Estrone	43	57	118	187	21	10	5.7	7.1	17	64	78
Metoprolol	1445	1748	2584	2569	1021	695	389	432	893	1527	1685
Naproxen	2958	4455	8834	9713	2904	1179	815	1010	1862	3636	3341
Oxazepam	410	394	553	663	200	119	51	55	143	285	326
Tramadol	216	458	530	411	108	103	74	35	33	162	134
Trimethoprim	19	13	181	224	29	8.7	3.6	2.9	9.2	402	91
Venlafaxine	530	728	1512	1199	537	233	135	213	401	469	547

Concentration, effl GAC1 and GAC2 mixed

	Apr 2021 (5000 BV)	May 2021	Jul 2021	Aug 2021	Sep 2021	Oct 2021 (10000 BV)	Nov 2021	Dec 2021	Feb 2022 (15000 BV)	Apr 2022	May 2022 (20000 BV)
Atenolol	<0.1	3.8	7.5	9.6	9.5	9.8	6.3	7.6	nd	0.2	2.0
Carbamazepine	3.1	26	45	39	31	7.3	5.8	5.6	3.3	3.7	4.0
Ciprofloxacin	nd	4.7	10	5.4	5.6	13	15	16	nd	nd	<5.0
Citalopram	5.9	17	22	27	11	8.5	8.4	7.8	1.0	<1.0	<1.0
Clarithromycin	nd	nd	nd	nd	nd	<1	1.1	<1	<1	<1	<1
Diclofenac	40	164	93	89	67	88	64	60	20	1.6	1.2
Estrone	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Metoprolol	26	70	82	102	55	31	29	31	4.8	4.6	2.9
Naproxen	1.4	1.5	1.0	nd	1.8	10	13	13	0.7	3.3	0.8
Oxazepam	35	57	67	75	54	39	34	34	26	24	14
Tramadol	12	31	43	55	15	18	14	11	<2.0	22	17
Trimethoprim	0.6	3.2	7.2	13	3.1	1.5	1.0	1.1	0.6	0.9	0.8
Venlafaxine	57	92	127	122	60	51	49	64	33	11	7.9

Conc. (ng/l)
10000
7500
5000
2500
1000
500
100
50
25
0

Removal, treatment plant

	Apr 2021 (5000 BV)	May 2021	Jul 2021	Aug 2021	Sep 2021	Oct 2021 (10000 BV)	Nov 2021	Dec 2021	Feb 2022 (15000 BV)	Apr 2022	May 2022 (20000 BV)
Atenolol	100	100	99	99	98	97	97	96	100	100	100
Carbamazepine	64	83	78	85	86	75	25	40	93	95	96
Ciprofloxacin	100	99	99	100	99	97	92	89	100	100	100
Citalopram	97	95	95	94	92	89	82	84	98	100	100
Clarithromycin	N/A	N/A	N/A	N/A	N/A	83	26	74	N/A	N/A	97
Diclofenac	93	78	90	93	83	34	37	48	91	100	100
Estrone	100	100	100	100	100	100	100	100	100	100	100
Metoprolol	98	96	97	96	95	96	93	93	99	100	100
Naproxen	100	100	100	100	100	99	98	99	100	100	100
Oxazepam	91	86	88	89	73	67	32	39	82	92	96
Tramadol	94	93	92	87	86	82	81	69	97	86	87
Trimethoprim	97	75	96	94	89	82	72	64	93	100	99
Venlafaxine	89	87	92	90	89	78	64	70	92	98	99

Removal (%)
0
10
20
30
40
50
60
70
80
90
100

Fig. 6. Influent concentrations (In grease trap) (left), GAC1 and GAC2 effluent concentrations (middle), and removal (right) of organic micropollutants from sampling period 2. When the effluent concentration was below the LOQ, a concentration of 0.5 · LOQ was assumed. nd = not detected, N/A = not applicable.

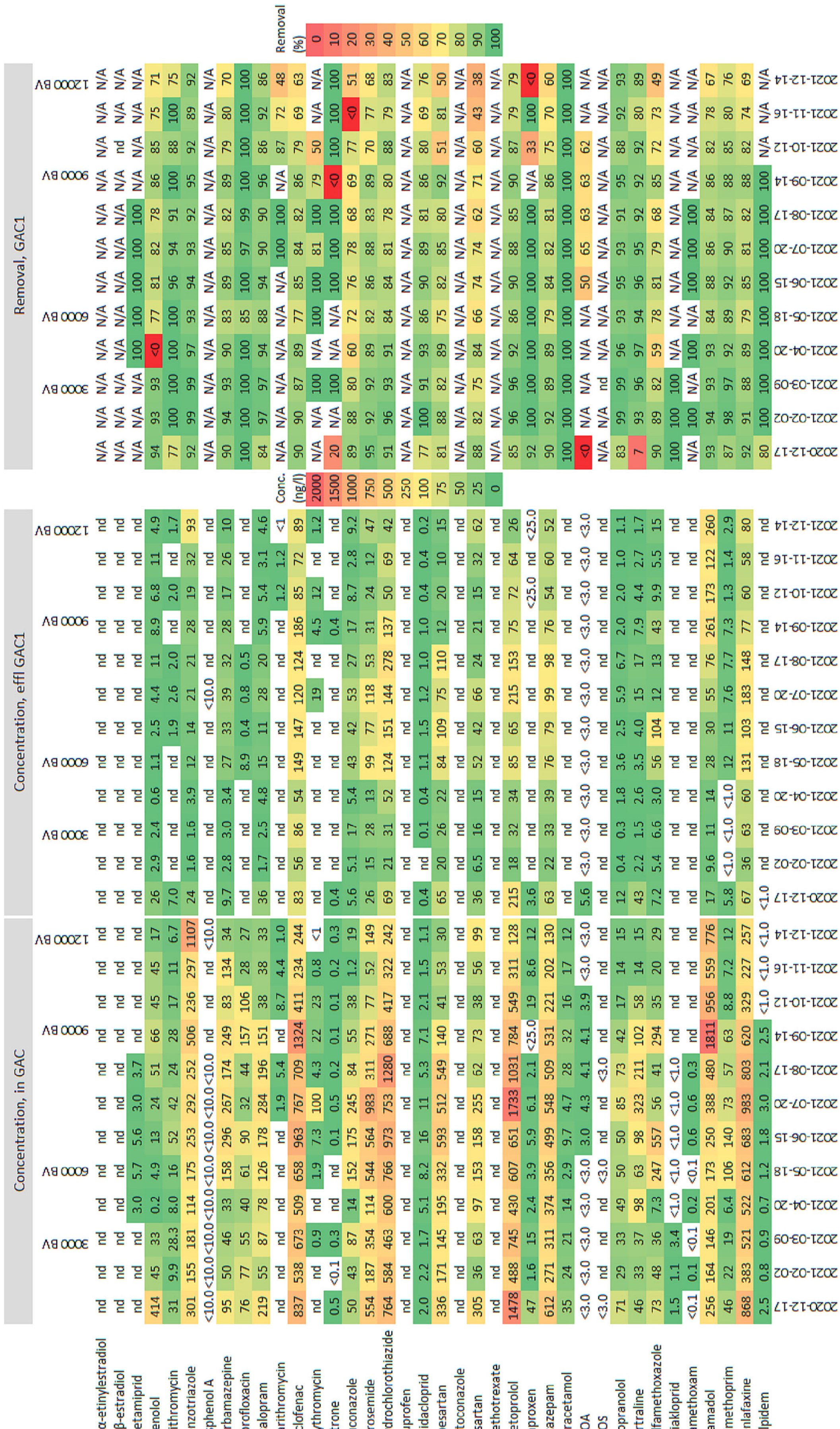

Fig. 7. GAC influent concentrations (left), GAC1 effluent concentrations (middle), and GAC1 removal (right) of organic micropollutants from sampling period 1. When the effluent concentration was below the LOQ, a concentration of 0.5 · LOQ was assumed. nd = not detected, N/A = not applicable.

largest Swedish lakes (Malnes et al., 2020) (an average of the concentrations from the 3 lakes) and in lake Ringsjön (Svahn and Björklund, 2017) (Table 1). Exceptions included 17β-estradiol (lower concentration in the GAC effluents) and citalopram, diclofenac, irbesartan, oxazepam, and sertraline (higher concentrations in the GAC effluents).

The concentrations of organic micropollutants in the GAC effluents were below the limits in the *Australian guidelines for water recycling* (2008), Reungoat et al. (2012), and Directive (EU) 2020/2184, but those of sulfamethoxazole exceeded the reporting limit in the Californian Water quality control policy for recycled water at 10 of 24 measurements (California EPA, 2018). In this regard, the Australian guidelines (10,000 ng for sulfamethoxazole/l and 175 ng for 17β-estradiol/l) differ significantly from other reporting limits and guidance values (10 ng sulfamethoxazole/l in California EPA, 2018, and 1 ng 17β-estradiol/l in Directive, EU, 2020/2184), necessitating further examination of safe concentrations.

4. The potential for wastewater reuse using MBR and GAC

According to Swedish legislation, drinking water is water intended for drinking, cooking, or preparing food (LIVSFS 2017:2), regardless of its origin. Thus, the quality of the water, not its origin, defines drinking water. A variety of water quality criteria exist worldwide, and a selection of them has been examined in this study through measurements and analyses of bacteria, organic micropollutants, and metals. However, difficulties arise regarding organic micropollutants, as guideline and reporting values differ widely between countries.

The concentrations of metals in the effluent from the studied MBR and GAC process met the criteria for irrigation (US EPA, 2012) and, in all cases but 1, Swedish drinking water criteria (LIVSFS 2017:2). The turbidity satisfied Swedish criteria for drinking water and the requirements for class A, B, C and D water quality standards for irrigation in regulation (EU) 2020/741, whereas the concentrations of *E. coli* were compatible with class B. Based on these parameters, the water can be reused for irrigation of "*crops consumed raw where the edible part is produced above ground and is not in direct contact with reclaimed water, processed food crops and non-food crops including crops used to feed milk- or meat-producing animals*" (EU 2020/741).

The bacterial concentrations fulfilled the criteria for class A water quality on most occasions, corresponding to irrigation of "*all food crops consumed raw where the edible part is in direct contact with reclaimed water and root crops consumed raw*." A disinfection step, such as UV or chlorination, downstream of the GAC filters could maintain *E. coli* concentrations below the class A limit. The concentrations of *E. coli* and total coliforms exceeded the limits for drinking water according to the Swedish criteria (LIVSFS 2017:2), although the concentrations of these bacteria are not 0 in traditional Swedish source waters (Swedish Agency for Marine and Water Management, 2022), and a disinfection step would be needed to obtain the concentrations for acceptable drinking water.

Another factor affecting the possibilities for implementation of GAC and MBR for reuse purposes is the cost of the treatment. Cost estimations and life cycle assessments for various advanced treatment solutions at different plant sizes have been performed in a Swedish context. These studies show comparable costs to those presented by Pistocchi et al. (2022) and Rizzo et al. (2019). Kivik WWTP is under evaluation and costs will be summarized and presented when the lifetime of the carbon is established as well as the performance of regenerated carbon.

A biofilm developed on the GAC filters, releasing bacteria to the effluent and increasing the TCC. Annual variations in temperature, flow, and influent concentrations appear to have affected the TCC in the effluent but not the concentrations of *E. coli* or total coliforms and thus not the reuse potential directly. However, the operation of a disinfection step after the GAC filters will likely be affected by fluctuations in TCC, with the potential need for higher doses of UV or chlorine, for example, during periods with higher bacterial concentrations.

The biofilm on the GAC filters might also have contributed to the degradation of certain compounds, such as diclofenac, and to the bioregeneration of these filters, potentially increasing the overall removal of organic micropollutants. Further studies are necessary to investigate if biological degradation actually takes place, and of which compounds. Further studies are also necessary to investigate the connection between certain groups of bacteria and biological degradation of certain compounds.

Based on our measurements, the wastewater treatment plant, consisting of an MBR followed by GAC filters, attains a water quality that approximates drinking water. However, there are several aspects in the legislation for drinking water that were not analyzed, including microbial water quality parameters (such as *Actinomycetes*, heterotrophic microorganisms at 22 °C, slow-growing bacteria, and *Clostridium perfringens*) and chemical parameters (such as pH, smell, taste, chlorine, radon, sulfate, trihalomethanes, and tetrachloroethylene). These parameters, as well as viruses, also need to be analyzed.

As new pharmaceuticals and other chemicals are introduced to the market, drinking water legislation might have to adapt, especially if the source water is wastewater, and the collective effect (the so-called cocktail effect) of these pollutants – in addition to the concentrations of individual compounds – might need to be considered.

Swedish legislation for drinking water states that drinking water should be healthy and pure and is considered so if it: 1) does not contain

Table 1
Effluent concentrations of organic micropollutants from GAC1 and GAC2 and average of concentrations in the lakes Vättern, Vänern and Mälaren (Malnes et al., 2020) [1], and concentrations in lake Ringsjön (Svahn and Björklund, 2017) [2]. nd = not detected.

Parameter	Average (max) concentration in Swedish lakes (ng/l)	Effluent concentration (ng/l)					
		2020-12-17 (0 BV)		2021-07-20 (7700 BV)		2021-12-14 (12,600 BV)	
		GAC1	GAC2	GAC1	GAC2	GAC1	GAC2
17α-Etinylestradiol	<8.6 (<8.6)[1]	nd	nd	nd	nd	nd	nd
17β-Estradiol	2.8 (3.9)[1]	nd	nd	nd	nd	nd	nd
Acetamiprid		nd	nd	nd	nd	nd	nd
Atenolol	1.6 (4)[1]	2.3	2.5	4.4	4.6	4.9	4.6
Azithromycin	2.18 (3.6)[1]	7.0	nd	2.6	nd	1.7	nd
Benzotriazole		24	7.0	21	8.4	94	83
Bisphenol A		nd	nd	<10.0	nd	nd	nd
Carbamazepine	5.4 (22)[1] 12.4[2]	9.7	1.0	39	22	10	9.8
Ciprofloxacin	<10 (<10)[1]	nd	nd	0.9	nd	nd	nd
Citalopram	0.65 (4.2)[1]	36	1.4	28	10	4.6	3.7
Clarithromycin	0.68 (1.2)[1] 0.25[2]	nd	nd	nd	nd	<1	<1
Diclofenac	6.3 (23)[1] 1.5[2]	83	17	120	58	89	81
Erythromycin	3.5 (12)[1] 0.5[2]	nd	nd	19.0	11.2	1.2	2.1
Estrone	0.6[2]	0.4	nd	nd	nd	nd	nd
Fluconazole	2.9 (15)[1] 1.2[2]	5.6	1.1	53	40	9.2	8.7
Furosemide	<17 (<17)[1]	256	6.4	118	62	47	50
Hydrochlorothiazide	17 (61)[1]	69	63	145	86	42	39
Ibuprofen	<8.25 (<8.25)[1] nd[2]	nd	nd	nd	nd	nd	nd
Imidacloprid		0.5	nd	1.2	0.9	0.3	0.3
Irbesartan	0.85 (2.1)[1]	65	5.0	75	42	15	14
Ketoconazole	nd[2]	nd	nd	nd	nd	nd	nd
Losartan	6.9 (29)[1] 1.7[2]	36	3.9	66	47	62	58
Methotrexate		nd	nd	nd	nd	nd	nd
Metoprolol	4.1 (32)[1] 3.9[2]	215	11	215	104	26	23
Naproxen	3.1[2]	3.6	nd	nd	nd	<25	<25
Oxazepam	2.7 (10)[1] 4.7[2]	63	8.3	99	60	52	45
Paracetamol	<5.4 (<5.4)[1]	nd	nd	nd	nd	nd	nd
PFOA		5.6	<3.0	<3.0	<3.0	<3.0	<3.0
PFOS		nd	nd	nd	nd	nd	nd
Propranolol	0.79 (1.6)[1]	12	0.3	5.9	2.3	1.1	0.9
Sertraline	<1.1 (<1.1)[1] nd[2]	43	1.2	15	4.2	1.7	1.4
Sulfamethoxazole	3.0 (12)[1] 3.4[2]	7.2	3.1	12	8.4	15	15
Thiacloprid		nd	nd	nd	nd	nd	nd
Thiamethoxam		nd	nd	nd	nd	nd	nd
Tramadol	6.1 (59)[1] 1.2[2]	17	2.4	56	26	260	231
Trimethoprim	0.55 (2.8)[1] 0.6[2]	5.8	1.0	7.7	3.7	2.9	2.5
Venlafaxine	16 (43)[1]	67	9.8	183	110	80	72
Zolpidem	nd[2]	<1.0	nd	nd	nd	nd	nd

microorganisms, parasites, or substances in concentrations that imposes a risk on people's health and 2) meets the criteria therein. According to these stipulations, drinking water is considered healthy and pure not solely because all concentrations are below the defined limits: it cannot contain other compounds that may cause a risk towards people's health. Because wastewaters can harbor a variety of compounds, it will be important to combine advanced chemical analysis and other water quality assessment tools with legislative clarity.

5. Conclusions

A full-scale MBR and GAC wastewater treatment process was studied from December 2020 to May 2022 to examine the reuse potential of the treated water, including microbial and chemical water quality. The following main conclusions were drawn:

- Based on the concentrations of *E. coli* and total coliforms, the water cannot be used directly as drinking water, for which additional disinfection would be necessary. Water quality with regard to microbial parameters was achieved according to the class B criteria for irrigation in regulation (EU) 2020/741.
- The GAC filter increased the total bacterial concentration without increasing the concentrations of *E. coli* and total coliforms.
- The treated wastewater met the Swedish drinking water criteria with regard to metal concentrations.
- The MBR and GAC process achieved high removal of organic micropollutants, >90 %, for 12 of 13 compounds after 1.5 years, corresponding to 20,000 BV.
- Except in November and December 2021, the MBR and GAC process achieved an average removal of >80 % for the 13 measured organic micropollutants.
- The concentrations of organic micropollutants in the treated wastewater in this study were generally on the same order of magnitude as those in Swedish lakes and rivers used for comparison.

CRediT authorship contribution statement

Maria Takman: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization, Project administration, Funding acquisition. **Ola Svahn:** Conceptualization, Methodology, Resources, Data curation, Writing – review & editing, Funding acquisition. **Catherine Paul:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition. **Michael Cimbritz:** Conceptualization, Resources, Writing – review & editing, Supervision, Funding acquisition. **Stefan Blomqvist:** Conceptualization, Resources, Funding acquisition. **Jan Struckmann Poulsen:** Formal analysis, Data curation, Writing – review & editing. **Jeppe Lund Nielsen:** Resources, Writing – review & editing, Supervision. **Åsa Davidsson:** Conceptualization, Resources, Writing – review & editing, Supervision, Funding acquisition.

Data availability

All amplicon data is available at the European Nucleotide Archive (ENA), project accession number: PRJEB58614.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.165185.

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