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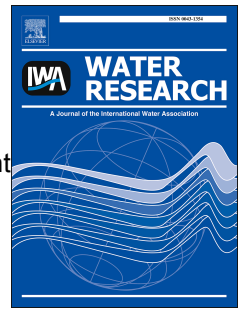

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Evaluation of a membrane bioreactor system as post-treatment waste water treatment for better removal of micropollutants

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Abstract

Organic micropollutants such as pharmaceuticals are persistent pollutants that are only partially degraded in waste water treatment plants (WWTPs). In this study, a membrane bioreactor (MBR) system was used as a polishing step on a full-scale WWTP, and its ability to remove micropollutants was examined together with the development and stability of the microbial community. Two stages of operation were studied during a period of 9 months , one with (S1) and one without (S2) the addition of exogenous organic micropollutants. Ibuprofen and naproxen had the highest degradation rates with values of 248 $\mu g/g_{VSS} \cdot h$ and 71$\mu g/g_{VSS} \cdot h$, whereas diclofenac was a more persistent OMP (7.28 $\mu g/g_{VSS} \cdot h$). Mineralization of ^{14}C-labeled organic micropollutants' in batch kinetic experiments indicates that higher removal rates (~0.8 $ng/mg_{SS} \cdot h$) with a short lag phase can be obtained when artificial addition of organic micropollutants was performed. Similar microbial populations dominated S1 and S2, despite the independent operations. *Hydrogenophaga*, *Nitrospira*, p55-a5, the actinobacterial *Tetrasphaera*, *Propionicimonas*, *Fodinicola*, and *Candidatus* Microthrix were the most abundant groups in the polishing MBR. Finally, potential microbial candidates for ibuprofen and naproxen degradation are proposed.

1. Introduction

Emerging Organic Micropollutants (OMPs) have become a major environmental health issue in terms of sewage treatment quality due to their potentially harmful impact on the environment (Sahar et al., 2011). OMPs include pharmaceuticals, personal care products (PPCPs) and their byproducts, of which some are endocrine disrupting compounds (EDCs). The use of pharmaceuticals has increased greatly in recent years with analgesic/anti-inflammatory compounds and antibiotics as those most commonly consumed (Guerra et al., 2014). Most of these OMPs finally end up in the waste water and can usually be found in trace levels up to a few µg/L or ng/L. A large portion of the OMPs are relatively persistent and only partially degraded in waste water treatment plants (WWTP) and can therefore be detected in the effluents and receiving waters. The increasing interest within the scientific community and water authorities in optimizing removal of OMPs in waste water treatment plants has led to several treatment technologies that appear prudent for improved environmental protection and reuse of waste water (Camacho-Muñoz et al., 2012). WWTPs based on conventional activated sludge processes (CAS) are designed and optimized to remove organic material (COD), pathogens, and nutrients from waste water, but not to remove OMPs. Operational parameters that can be regulated, such as solid retention time or hydraulic retention time, do not offer sufficient aptitude to improve microbiology for better removal of OMPs (Kagle et al., 2009; Camacho et al., 2012; Guerra et al., 2014). Thus, future challenges of CAS technologies require additional efforts in order to improve the removal efficiency of OMPs. Initiatives taken to improve OMP removal in CAS cover the addition of surfactants, intense mixing, and aeration, supplementation with inorganic nutrients, and bioaugmentation (Alexander, M., 1999; Semrany et al., 2012; Zhou et al., 2014). Other techniques involve additional treatment steps (post-treatment or polishing

steps) applied as add-ons, which do not alter the other reactions in the plant. Post-treatment steps for OMPs removal based on advanced oxidation processes (AOP) such as ozonation, photooxidation, and photocatalytic degradation, and physicochemical methods (e.g. nanofiltration and activated carbon adsorption) have been investigated (Sipma et al., 2010; Siegrist and Joss, 2012). However, energy demand, investment costs, energy and chemical consumptions, and removal efficiencies of OMPs are substantial concerns for these approaches to become sufficient for efficient micropollutant removal due to the large volume of effluents in WWTP (Krzeminski et al., 2012; Köhler et al., 2012). However, biological methods such as MBR systems are cheap and space-saving alternatives.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among some of the pharmaceuticals most frequently found in sewage with typical values reported for diclofenac (269-1260 ng/L), ibuprofen (510-8600 ng/L), naproxen (8.8-6280 ng/L), and ketoprofen (289-589 ng/L) (Alidina et al., 2014; Guerra et al., 2014; Santos et al., 2013; Luo et al., 2014). Removal efficiencies for these compounds have been reported in the ranges of 20-57%, 73-97%, 47-93%, 66-94%, and 51-89%, respectively (e.g. Sipma et al., 2010; Camacho-Muñoz et al., 2012; Luo et al., 2014). Somewhat better removal efficiencies (20-60%) have been reported in MBR systems relative to CAS for many of these and other OMPs (Kagle et al., 2009; Luo et al., 2014). The long solid retention times and high accumulation of active biomass found in MBR systems make it possible to create an adapted microbial community with high ability to remove OMPs (Sipma et al., 2010; Siegrist and Joss, 2012). MBR systems implemented as an end-of-pipe polishing step in the effluent of existing WWTPs benefit by not interfering with the overall treatment processes in the mother plant. However, little is known about how efficient such post polishing can become in terms of removal of micropollutants and

stability under *in situ* conditions. The aim of the present work was to evaluate the long-term effect of an advanced biological posttreatment in the form of a MBR system functioning as a polishing step for more efficient removal of OMPs. The effect of amending micropollutants during upstart of the MBR system was examined by the ability to mineralize selected micropollutants. The study also explored the evolution and stability of the involved microbial communities and their correlation with the removal of OMPs.

2. Materials and methods

2.1 Reagents

Ibuprofen, naproxen, diclofenac, ketoprofen, and gemfibrozil were purchased from Sigma-Aldrich with a purity of >97%. Ibuprofen RS-[Carboxyl-14C] (Specific activity 55 mCi/mmol), Naproxen [O-methyl-14C] (Specific activity 55 mCi/mmol), and Diclofenac [carboxyl-14C] (Specific activity 55 mCi/mmol) (American Radiolabeled Chemicals, ARC Inc.). Stock solutions of both labeled and unlabeled compounds were prepared in deionized water.

2.2 Biomass sampling

Effluent and sludge samples were sampled from the aeration tank and two pilot-scale membrane bioreactors (polishing MBR and MBR plant, Table 1) at the Aalborg West (AAW) WWTP (57.049422° N, 9.864735° E) in Denmark from February 2014 through August 2015. Sampling permission was granted by Aalborg Forsyning, Kloak A/S. All samples were transported to the laboratory within 1 hour after sampling and used for kinetic experiments and microbial composition analysis. AAW WWTP treats primarily domestic waste water with 30% industrial contribution (avg. 195,000 population

equivalents) and includes an advanced enhanced biological phosphorous removal system with very stable performances.

2.3 MBR systems for waste water treatment

Two MBR pilot scale systems have been implemented at the AAW WWTP, one fed with primary sewage (MBR plant) and functioning as an end-of-pipe polishing MBR (polishing MBR) fed with effluent from the WWTP. The main characteristics of the two MBR systems are listed in Table 1.

The aerobic immersed polishing MBR-(Biosep^{TM}) consists of a submerged hollow-fiber ultrafiltration membrane module (configuration is shown in Fig. 1). The membrane module is continuously aerated to minimize fouling, and is combined with a recirculation line from the permeate tank to the membrane module, which periodically refluxes to remove fouling layers.

The MBR plant system, which consists of an alternating aerobic/anaerobic process, was originally designed for phosphorous removal with an SRT of 20-25 days (Table 1).

2.4 Adaptation of biomass to micropollutants

The adaptation of the biomass to remove the micropollutants was studied only in the polishing MBR system; two stages (S1 and S2) of operation were evaluated. S1 and S2 lapsed 70 days during February-May 2014 and 320 days from October 2014 to October 2015, respectively. Prior to initiating S1 and S2, the polishing MBR was totally emptied, washed with chlorine, carefully rinsed, and restarted. The start-up inoculums were effluent from the WWTP for both stages of operation.Throughout the 70 days of incubation in S1, an artificial mixture containing 20 mg naproxen, 20 mg ibuprofen, and 20 mg diclofenac was added daily directly into the 250 L polishing MBR reactor at a

continuous rate. No accumulation of the amended compounds were observed at any time during the incubation. The objective was to improve the ability of the biomass to degrade the micropollutants. S2 was operated without any exogenous addition of micropollutants.

2.5 Analysis of micropollutants

Analysis of degradation of five selected anionic pharmaceuticals were performed by extraction, derivatisation and quantification by GC-MS for the kinetic experiment described in section 2.6 and removal of a broader palette of organic micropollutants in the investigated systems were performed using quantifications by LC-MS of samples taken from influent and effluents from each system.

GC-MS: Kinetic experiments were performed, using 200 mL of sludge samples from the MBR systems and the effluent of the WWTP. The samples were passed through a 0.2 µm mixed cellulose ester filter (Advantec MFS, Inc.) and acidified with 1M phosphate buffer (pH 2.2) for the subsequent analysis. Analyses were performed as described elsewhere (Hansen at al., 2016). Briefly, sample aliquots were extracted with Oasis HLB cartridges, and the extract was derivatized with BSTFA (N,O-bis-trimethylsilyl-trifluoroacetamide, Sigma, Denmark) before analysis by GC-MS. The membrane filters of each sample filtered were stored at -20°C for subsequent DNA extraction for the microbial population analysis.

LC-MS: Removal efficiencies were determined, using LC/MS analysis of samples taken at day 33 during S1 and days 13 and 98 during S2. Removal efficiencies were determined as the differences between incoming water and treated water of the MBR systems. Analysis was performed by LC-tandem MS via direct injection as described elsewhere (Rühmland et al., 2015). The instrument consisted of an Agilent 1200 series HPLC, equipped with a ZORBAX Eclipse Plus C18 column (150 x 2.1 mm, 3.5 µm

from Agilent Technologies) coupled via ESI to a Qq-LIT-MS (API5500 QTRAP, Sciex) with ESI in positive ionization mode.

2.6 Kinetics of primary degradation of micropollutants

Degradation of five micropollutants (naproxen, ibuprofen, diclofenac, ketoprofen, and gemfibrozil) was carried out using sludge samples from the aeration tank in the CAS and the two MBR systems. These kinetic experiments were carried out in 1 L batch tests under aerobic conditions; each sludge sample was supplemented with 100 µg/L of each micropollutant. The reactor was covered with aluminum foil in order to eliminate the photo oxidation. The sludge was maintained fully aerated and in suspension by using a magnetic stirrer (200 rpm) and injection of 1.3 L_{air}/min by a porous stone diffuser. Liquid samples were obtained every 4 h for 24 h. The specific removal rates of the five micropollutants, the SS, and VSS were obtained disregarding sorption to sludge as the considered organic micropollutants are known to sorbe insignificantly to activated sludge (Hörsing et al., 2011)

2.7 Kinetics of mineralization of micropollutants

Samples of effluent water from the WWTP, which is also the inlet of the polishing MBR, and sludge from the two MBR systems (MBR plant and polishing MBR) were used for determining the degradation kinetics of each micropollutant (diclofenac, ibuprofen, and naproxen).

The mineralization experiments were performed in triplicates in 20 mL serum flasks closed with rubber stoppers and crimp-sealed. Two mL of sludge sample was amended with 0.2 µCi and adjusted to a final concentration of 0.2 µg/L by addition of unlabeled micropollutant. This chosen concentrations allowed for sufficient sensitivity and reliable measurements without depletion of substrate during incubations (data not shown). A

186 CO_2 trap was made by placing a glass microtube containing 300 µL of 0.1 M NaOH in
187 each flask. The flasks were maintained at room temperature at 200 rpm on a stirring
188 plate. The kinetic experiments lasted 24 hours, after which they were terminated by
189 analyzing ^{14}C-activity in the CO_2 sorbed in NaOH and liquid phases from before and
190 after centrifugation (10,000 *x*g for 8 minutes). A carbon mass balance was performed in
191 order to obtain the total CO_2 produced in each flask. The total ^{14}C-labelled CO_2
192 production was determined in the batch experiments after 24 hours by measuring the
193 percentage accumulation of precipitated radioactivity, using a liquid scintillation
194 counter (Packard 1600 TR; Packard) as follows. Samples from the NaOH solution
195 contained in the microtube placed inside the flasks and samples from the culture
196 medium of the flasks were taken separately; then, these were directly transferred to 3
197 mL scintillation liquid (Ultima Gold XR; Packard) to measure the total radioactivity of
198 the culture. All incubations were carried out in triplicates.
199 The mineralization rate (calculated as the amount of micropollutant degraded and found
200 in the gas phase and biomass relative to the amount added and the labeling of the tracer)
201 of the micropollutants was normalized to the SS concentration of the sludge.
202
203 *2.8 Wastewater analysis techniques*
204 Sludge suspension samples from the main effluent of WWTP and for the MBR systems
205 were characterized in terms of suspended solids (SS), volatile suspended solids (VSS),
206 Chemical Oxygen Demand (COD), pH, conductivity, and salinity. In brief, 200 mL of
207 sludge samples were filtered through 0.6 µm glass fiber filters (Advantec MFS, Inc.).
208 Then, suspended solids (SS) and volatile suspended solids (VSS) were measured
209 according to standard methods at 105°C and 550°C, respectively (APHA, 2005). SS and
210 VSS were expressed in terms of mg/L. Conductivity and salinity were measured with a

conductivity meter (VWR CO 310) using the raw sludge sample. Total and dissolved COD from sludge samples were measured using a Dr. Lange cuvette test kit LCK 314 and a DR 3900 spectrophotometer (Hach Lange GmbH). The pH of filtered sample was measured with a pH meter (Eutech Instruments).

2.9 DNA extraction and 16S rRNA gene amplicon sequencing

Biomasses for DNA extraction were collected by filtering 200 mL of polishing MBR sludge and WWTP effluent onto a 0.2 µm mixed cellulose ester (Advantec MFS, Inc.). The filters were cut into small pieces (~4 mm^2) and added directly into lysis solution from the Fast DNA SPIN Kit for Soil (MP Biomedicals, USA). DNA extractions were performed according to manufacturer's recommendations with an amendment to the bead-beating step to 4 x 40 seconds at 6 m/s. Purity of the DNA extracts was evaluated by determining $A_{260/230nm}$ and $A_{260/280nm}$ using Nanodrop1000 (Thermo Fisher Scientific, USA). The quality of the extracted DNA was evaluated using the Tapestation 2200 and Genomic DNA ScreenTapes (Agilent, USA). DNA concentration was determined using Quant-iT BR DNA Assay (Thermo Fisher Scientific, USA) on an Infinite M200 PRO (TECAN, Switzerland) plate reader.

The procedure for bacterial 16S rRNA gene amplicon sequencing targeting the V1-3 variable region was performed as described elsewhere (Caporaso et al., 2012). Amplicon library PCR was performed using 10 ng of extracted DNA as template per 25 µL PCR reaction (400nM of each dNTP, 1.5mM $MgSO_4$, 2mU Platinum Taq DNA polymerase HF and 1X Platinum High Fidelity buffer (Thermo Fisher Scientific, USA) and 400 nM of bar-coded library adapter pair). V1-3 primers: 27F AGAGTTTGATCCTGGCTCAG and 534R ATTACCGCGGCTGCTGG. Thermocycler settings: Initial denaturation at 95°C for 2 min, 30 cycles of 95°C for 20

s, 56°C for 30 s, 72°C for 60 s, and final elongation at 72°C for 5 min. All PCR reactions were run in duplicate and pooled. The amplicon libraries obtained were purified using AMpure XP bead protocol (Beckmann Coulter, USA), with the following amendments: the sample/bead solution ratio was 5/4, and the purified DNA was eluted in 23 µL of nuclease free water. Library concentration was measured with Quant-iT HS DNA Assay (Thermo Fisher Scientific, USA) and quality evaluated using D1000 ScreenTapes (Agilent, USA). Samples were pooled in equimolar concentrations, and the library pool was sequenced on a MiSeq (Illumina, USA) according to previous published procedure (Caporaso et al., 2012), with the exception of 20% PhiX control library (Illumina, USA) spike-in and a final library concentration of 20 pM.

All sequenced sample libraries were subsampled to 50.000 raw reads, trimmed, and bad reads were removed using trimmomatic (v0.32) (Bolger et al., 2014). Reads were merged using FLASH (v1.2.7) (Magoč et al., 2011). Reads were then formatted for use with the UPARSE workflow and screened for chimeric sequences (Edgar, 2013). Usearch7 was used to de-replicate reads, screen for Phi-X contamination and clustering into Operational Taxonomic Units (OTUs) at 97% sequence similarity. Taxonomy was assigned using RDP classifier (Wang et al., 2007) as implemented in QIIME (Caporaso et al., 2010) using MiDAS taxonomy version 1.20 (McIlroy et al., 2015), which is based on SILVA taxonomy (Quast et al., 2013). The obtained raw sequence data is available at the European Nucleotide Archive (ENA) under project accession number PRJEB14551.

2.10 Statistical analyses

All statistical analyses were performed in RStudio (version 0.99 (http://www.rstudio.com) using R version 3.2 (R core team, 2015)) using the R CRAN

packages: ampvis (v1.13) (Albertsen et al., 2015), vegan (Oksanen et al., 2013), ggplot2 (Wickham, 2009), and the Bioconductor package Phyloseq (McMurdie, 2013). Microbial community composition and structure were explored using heatmaps. Microbial richness and evenness were visualized using Chao1 and Shannon-Weaver indices. Beta diversity was investigated using principal component analysis on square root transformed abundance counts. Constrained redundancy analysis (RDA) was applied to the polishing MBR sequence data in order to identify correlations between removal data and OTU abundances.

3. Results and Discussion

3.1 Reactor performance

In this study, we have investigated the effect of implementing membrane technology in waste water treatment for improved removal of pollutants. Focus has been on the long-term effect of implementing an MBR as a polishing step on a full-scale WWTP and the establishment of a stable microbial community and an improved ability to biodegrade micropollutants. The end-of-pipe polishing MBR experimental setup was a 250 L MBR system implemented as a side stream directly on the effluent of the full-scale WWTP, which has been operating under stable conditions for years. To determine whether it is possible to accelerate the development of an active OMP degrading biomass two independent periods were investigated: one with amendment of OMPs (S1) into the treated water (effluent from the WWTP) and one without amendment of exogenous OMPs (S2).

The polishing MBR was operated until establishment of stable running conditions as indicated by the constant dry matter content, pH, conductivity and salinity (Figs. S1 and S2). The operational conditions were set to provide a constant flux, short hydraulic retention time, and without removal of biomass. Despite these running conditions, the

biomass did not accumulate to more than 31 ± 3 mg SS/L and a VSS of 19 ± 1 mg/L during S1 and 20 ± 2 mg SS/L and a VSS of 14 ± 0.6 mg/L during S2. The COD in the effluent from the WWTP (which was also inlet of the polishing MBR) was measured on flow-weighted composite samples (sampled over 24h; n=6) and constituted 170± 8 mg/L (total) and 40 ± 1 mg/L (dissolved), while the treated water after the polishing step was 35 ± 0.4 (total) and 45 ± 2 mg/L (dissolved), respectively. These numbers indicate a slight underestimation of the suspended matter measured by the standard method, but the low values are confirmed by the COD measurements. The implementation of an MBR as part of a polishing step of the effluent therefore significantly reduces the residual compounds and especially particulates that can be retained by the membrane bioreactor system.

Implementation of MBR technologies has been reported elsewhere to yield high removal efficiencies of COD of up to 90%, while AOP systems are less efficient with removal efficiencies (RE) between 5 to 32 % (Köhler et al., 2012; Krzeminski et al., 2012). In the present study, total COD after the polishing MBR step was 80% (35 mg/L /170 mg/L) lower than the COD content in the effluent of the WWTP. The energy consumption for a final ozonation would therefore be significantly reduced by implementation of the membrane system. Furthermore, as the MBR polishing step primarily removes particulate matter, it also reduces the cost of removal and inactivation of pathogenic bacteria. However, another effect observed by the implementation of the polishing MBR was that the pH increased to about 8.5 which was about 0.5 pH units higher than the WWTP effluent (Fig. S2). Although an increase in pH can negatively influence the ozone doses required to remove micropollutants it could also result in a faster reaction which allows a smaller ozone contact tank (Hansen et al., 2016). Thus, MBR system applied as a polishing step after conventional activated

312 sludge systems, but before a final ozone treatment step, presents advantages in terms of
313 energy consumption and quality of effluent over AOP processes. However, further
314 studies are needed to establish a full understanding of the economical and energetic
315 benefits by implementing an end-of-pipe polishing step in combination with advanced
316 oxidation processes.
317
318 *3.2 Micropollutant removal performance*
319 The performance of the removal of the OMPs in the main effluent of the Aalborg West
320 WWTP is shown in Fig. 2. Thirty one OMPs, including a herbicide (diuron), fungicides
321 (fluconazole, climbazol); and several types of pharmaceuticals such as analgesics
322 (codein, diclofenac, tramadol, and human metabolites hereof: O-desmethyl-tramadol
323 (O-DM-tramadol), N-desmethyl-tramadol (N-DM-tramadol), N,O-didesmethyl-
324 tramadol (N,O-DDM-tramadol), antidepressants (oxazepam, venlafaxin, and the
325 metabolites hereof: N-desmethylvenlafaxin, N,O-didesmethylvenlafaxin, O-
326 desmethylvenlafaxin), antibiotics (sulfamethoxazole (SMX), clarithromycin,
327 erythromycin, trimethoprim), antivirals (acyclovir), β-blockers (sotalol, metropolol,
328 atenolol), radio contrasts (diatrizoat, iomeprol), and antiepileptic (carbamazepine (CBZ)
329 and metabolites hereof: 2-hydroxy-carbamazepine (2-OH-CBZ), 3-hydroxy-
330 carbamazepine (3-OH-CBZ), Dihydro-hydroxy-carbamazepine (DHH-CBZ), Dihydro-
331 dihydroxy-carbamazepine (DH-DH-CBZ) were detected. Acesulfam, an artificial
332 sweetener, and benzotriazol, a corrosion inhibiter, were also present. All the OMPs
333 detected in the Aalborg West WWTP main effluent are among the most common
334 pollutants found worldwide in WWTPs (Luo et al., 2014).
335 The removal efficiencies (RE) of OMPs in the polishing MBR system were determined
336 at three sampling dates (day 33 in the MBR receiving exogenous OMPs (S1), and at day

208 and 320 in the MBR without receiving exogenous OMPs (S2), see Fig. 2). In general, the polishing performed by the polishing MBR increased the removal of OMPs. In the experiment with amendment of ibuprofen, naproxen, and diclofenac (S1), nine micropollutants, which were not added as exogenous OMPs, showed a RE larger than 30% already after 33 days of operation. This could indicate a similar stimulation pattern for the degradation of these compounds or the presence of microbiota with multiple degradation capabilities towards these micropollutants. In the polishing MBR not receiving exogenous OMPs (S2), more pollutants were removed after 208 days of incubation, these included DH-DH-CBZ, benzotriazol, sotalol, metoprolol, diatrizoate, erythromycin, climbazol, iomeprol, clarithromycin, antenolol, acyclovir, trimethoprim, DHH-CBZ, and codeine, with RE increasing to more than 20%. However, at day 320, the better removal performance continued and even increased in terms of removal efficiency as well as the number of pollutants being removed with 24 OMPs having a RE larger than 20% relative to the WWTP effluent. The removal of the antibiotics (erythromycin, clarithromycin, and trimethoprim) on Day 320 to below detection levels is a significant improvement relative to the more general observations in the literature, in which RE between 40 to 90% have been reported in other types of membrane bioreactors treating municipal wastewater (Sipma et al., 2010; Dolar et al., 2012). Very similar RE in MBR reactors have also been reported for the removal of anti-inflammatory compounds and atenolol (90-100% RE), but also other OMPs as those investigated in this study (Luo et al., 2014; Trinh et al., 2012; Kovalova et al., 2012).

A few compounds such as SMX, diclofenac, and CBZ showed negative RE mainly during early sampling dates. Negative values of RE have been reported elsewhere (Kovalova et al., 2012, Falås et al., 2012b) and are usually explained by the fact that

they were linked to conjugate compounds that convert back to the parent compound during the treatment, re-dissolution of OMPs or problems associated to inappropriate sampling or analytical measurements (Guerra et al., 2014; Jelic et al., 2011; Göbel et al., 2007).

3.3 Micropollutant removal kinetics

In the present work, the microbial population able to degrade some of the OMPs detected in the effluent of the WWTP was established after 15 and 90 days of operation with (S1) or without (S2) amendment of exogenous OMPs, respectively (Figure 2 and 3). The necessity of an adaptation period for development of the microbial population to OMP degradation and thus a certain sludge age and concentration of biomass are crucial parameters for the optimal performance of an MBR system (Alexander M., 1999; Kagle et al., 2009; Luo et al., 2014). Longer sludge retention times allow more complete mineralization of biodegradable pollutants, but also an adaptation of microorganisms with specialized enzymes for less biodegradable compounds (Falås et al., 2012c; Clara et al., 2005).

Ibuprofen was the pollutant which presented the highest degradation rate with a value of 248 $\mu g/g_{VSS} \cdot h$, followed by naproxen (71 $\mu g/g_{VSS} \cdot h$), gemfibrozil (24 $\mu g/g_{VSS} \cdot h$), diclofenac (7.3 $\mu g/g_{VSS} \cdot h$), and ketoprofen (2.4 $\mu g/g_{VSS} \cdot h$). The specific consumption rates obtained here are in the same order of magnitude as the reported values for ibuprofen (2.4 to 20.2 $\mu g/g_{VSS} \cdot h$) and naproxen (0.19 to 2.66 $\mu g/g_{VSS} \cdot h$) obtained in MBR systems (Falås et al., 2012, Escola-Casas et al., 2015). The specific consumption rates obtained for the five OMPs tested (Table 2) indicate that the biomass enriched in the membrane bioreactor for the polishing of the effluent waste water was the most suitable system to degrade micropollutants relative to the CAS process and the MBR plant implemented in the waste water treatment. The rates of consumption for the OMPs

387 tested were at least 10-100 times greater in the polishing MBR, compared to the MBR
388 plant and CAS systems. So, despite the lower starting concentrations, polishing of the
389 effluent from a WWTP presents significant advantages for OMP degradation relative to
390 CAS processes. CAS processes were designed to remove COD, pathogens, and
391 nutrients from waste water, but not OMPs; the main drawback of CAS for OMP
392 degradation is the high HRT under normal conditions (~23 h), the limited ability to
393 allow the operation at longer SRT (~15 d) with risk of biomass washout and the low
394 biomass concentration (MLSS ~ 3 g/L) in the system (Weiss and Reemtsma, 2008).
395 This limits the formation of a microbial community able to degrade synthetic
396 micropollutants, and selection for fast-growing microorganisms and floc-forming
397 species (Sipma et al., 2010; Sahar et al., 2011; Luo et al., 2014). On the other hand,
398 MBR systems have advantages such as high biomass concentrations (MLSS 10-35 g/L)
399 and high solid retention time (20-100 d) with no biomass washout, which provide
400 conditions for the slower-growing species to proliferate and to adapt to the consumption
401 and mineralization of less biodegradable pollutants (Weiss and Reemtsma, 2008;
402 Quintana et al., 2005; Camacho-Muñoz et al., 2012).
403 The MBR plant fed with primary sewage and operated with an alternating
404 anaerobic/aerobic process for phosphorous removal did not provide similar high OMP
405 degradation rates as those found in the polishing MBR. The lower performance of the
406 MBR plant system is most likely related to the higher activity level and the limited mass
407 transfer of oxygen and micropollutants in an environment with high biomass content
408 (~9 g/L) relative to the low biomass content in the polishing MBR system.
409
410 The measurement of ^{14}C-labeled CO_2 from ^{14}C-OMPs amended (diclofenac, ibuprofen,
411 and naproxen) in samples collected during S1 and S2 reveals initial mineralization after

412 15 days (S1) of ibuprofen and naproxen with specific rates up to 1.2 ng/mg_{SS}·h (Fig. 3).
413 The mineralization rates were maintained stable at approximately 0.8 ng/mg_{SS}·h for at
414 least 50 days; except for an unusual episode that lasted one week, in which the rates of
415 mineralization decreased, probably due to the exchange of a pump in the MBR setup.
416 However, after this episode, the mineralization rates recovered to steady state values as
417 previously reached. Diclofenac was the most resistant micropollutant as it was not
418 mineralized during the entire operation of the polishing MBR, which is consistent with
419 previous reported studies (Luo et al., 2014; Quintana et al., 2005). The pH in the
420 polishing MBR sludge samples was relatively high, around 8.5 (Fig. S2), which can
421 provide problems with biodegradability for diclofenac due to changes in hydrophobicity
422 and adsorption properties (Radjenovic et al., 2008).
423
424 Control experiments carried out in the main effluent of the WWTP did not show
425 mineralization activity for the degradation of the OMPs added into the polishing MBR
426 during the entire period of sampling (70 days) (Fig. 3). This behavior was expected, as
427 the microbial population in the effluent of the WWTP is not adapted to the degradation
428 of OMPs.
429 The results obtained for the mineralization of micropollutants in the two time series
430 correlate well with the RE obtained for the thirty-three OMPs at day 33 (S1) and day 98
431 (S2). Thus, the development of a new microbial community able to degrade several
432 micropollutants was established after around three months of operation. The
433 mineralization of OMPs in the polishing MBR during S2 increases with time up to
434 values of 0.4 ng/mg_{SS}·h for ibuprofen and 0.2 ng/mg_{SS}·h for naproxen. The
435 mineralization rates of ibuprofen and naproxen obtained during S2 of operation were
436 two and three times lower than the ones obtained during S1, respectively.

Thus, the artificial addition of OMPs into the polishing MBR can be a good strategy to speed up the formation of biomass that was able to remove the OMPs added. If the results obtained herein are extended to real applications, the artificial addition of micropollutants could be restricted to only two weeks to develop an active biomass.

3.4 Microbial community analysis

The MBR polishing step was operated until establishment of stable running conditions as indicated by the constant dry matter content, pH, conductivity and salinity (Figs. S1 and S2). Amplicon sequencing of the 16S rRNA gene revealed the presence of microbial communities with high diversity and complexity (average richness: 2047±337 OTUs per sample, average evenness 5.43±0.64). The polishing MBR was initiated without inoculation and therefore started out resembling the WWTP effluent samples. Analysis of the microbial community evolution over the course of each time period revealed that the microbial community in the polishing MBR had developed into a specialized community, significantly different from the WWTP effluent which showed relative stability throughout the sampling period (Fig. 4). In the two time series (S1 and S2), the polishing MBR sludge contained 58 and 29 OTUs of consistent and significant relative abundance (> 0.1% of total reads in at least 90% of all samples), respectively, that could be considered the core population. In the effluent samples, the core communities following this distribution were 66 (S1) and 60 (S2) OTUs. The MBR sludge receiving continuous addition of exogenous OMPs was dominated by *Hydrogenophaga* (*Proteobacteria*) and an uncharacterized *Chloroflexi* (C10_SB1A) accounting for up to 31.9 and 9.7% of the total read abundance. In the reactor not

462 receiving exogenous OMP, the most abundant groups were p-55-a5 (*Firmicutes*) and
463 *Arcobacter* (*Proteobacteria*), which accounted for up to 26.3 and 16.4% of the total
464 abundance read, respectively. Each of these abundant groups was transient, and their
465 presence declined before the end of the experiments (Fig. 4A and B). However, the
466 microbial community dynamics showed a clear trajectory, where the effluent samples
467 can be seen clustering separately for both S1 and S2 (Figure 4C). The microbial
468 community present in the polishing MBR reactor during S1 migrates away from the
469 effluent after day 9 and begins to cluster closer together after day 15. During S2 the
470 microbial community can be seen to cluster separately from both the CAS effluent and
471 polishing MBR samples collected during S1 during day 8 to 69. However from day 155
472 onward, the microbial community approaches the composition found in the polishing
473 MBR during S1 and continues to resemble this until the end of the experimental period
474 at day 320 (Fig. 4). Despite being independent time series, the relative abundance
475 profiles of these two experiments were dominated by several similar populations:
476 *Hydrogenotropha*, *Nitrospira*, p55-a5, and the actinobacterial *Tetrasphaera*,
477 *Propionicimonas*, *Fodinicola,* and *Candidatus* Microthrix.
478 An interesting observation was that bacteria abundant in the effluent from the WWTP
479 did not constitute abundant groups in the sludge from the polishing MBR, probably due
480 to the lack of long term survival under the conditions prevailing in the polishing MBR.
481 The development of removal efficiencies during the biodegradation of OMPs seen in
482 Figs. 2 and 3 also indicates that microbial populations developed in the polishing MBR
483 need time to adapt before initiating a removal of anthropogenic levels of
484 micropollutants. The increased SRT in MBR systems commonly works to improve
485 biomass concentration and biodiversity, which increases the chances of adapting the
486 community in the reactor to degrade OMPs (Kagle et al., 2009).

The mineralization rates detected for the three ^{14}C-labeled micropollutants were fitted onto the PCA analysis data (Figs. 5, S3, S4 and S5). Goodness of fit testing revealed the removal rates of all three compounds to be significant groupings ($p = 0.001$ for Ibuprofen and Diclofenac, $p = 0.002$ for Naproxen). Constrained redundancy analysis was performed to extract the 25 OTUs that have the strongest correlation (loading) to the mineralization rates (Fig. 3). Only the removal rates for ibuprofen and naproxen were considered in the analysis, as their removal was significantly stronger than that of diclofenac. A number of OTUs were not considered as potential candidates for having a role in the removal of OMPs as their correlation is most likely based on their high read abundance, such as p-55-a5 and unclassified Chloroflexi C10_SB1A. Among the extracted OTUs, three bacterial families (*Sphingomonadaceae*, *Comamoadaceae*, and *Hyphomicrobiaceae*) may be of interest, as members of these families have been implicated and specifically the sphingomonads been shown to be involved in biodegradation of xenobiotic compounds like polycyclic hydrocarbons (Stolz, 2009).

4. Conclusion

Polishing of effluents from full-scale waste water treatment plants using a submerged MBR system was shown to be an efficient approach to improve the removal of organic matter and micropollutants.

Artificial addition of exogenous micropollutants during start-up was shown to significantly accelerate the adaptation of a biomass to remove the selected micropollutants. The ability to remove micropollutants were evaluated through direct measurements and by determining the mineralization using radiolabeled OMPs.

The removal efficiency and microbial community showed long term stability. Furthermore, statistical analysis of the microbial community and the removal of ibuprofen and naproxen provided a list of potential degraders involved in the mineralization of these compounds.

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Table 1. Design and operational parameters of the two MBR systems integrated into the configuration of Aalborg West WWTP.

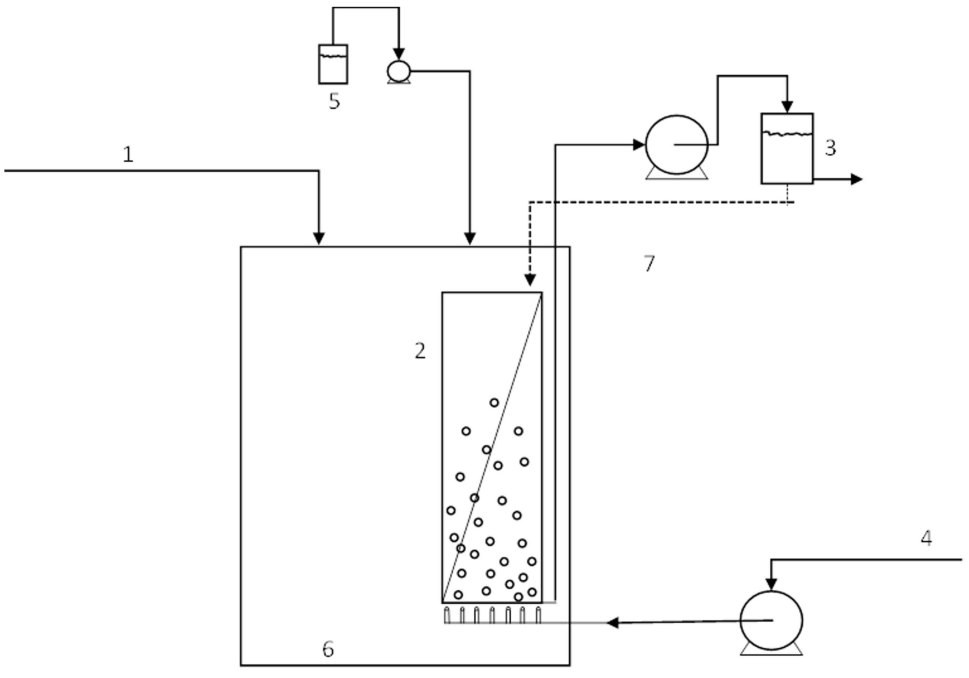
Parameter	Value	
	Polishing MBR	MBR plant
Volume	250 L	4.5 m^3
Feeding material	WWTP effluent Period S1: + OMPs*	Sewage (WWTP inlet)
Working mode	Constant flux	Constant pressure
HRT, h	10	12-15
SRT	-	20-25 days
TSS, mg/L	848±59	8900±105
Total COD, mg/L	170±7.8	1840±270
Membrane type/area	Hollow fiber, θ 0.9/1.9mm with 0.03-0.1μm pore size (GE Water & Process Technologies Canada, Inc.)	Hollow sheet, with pore size 0.2 μm PVDF membrane, Alfa Laval, Denmark

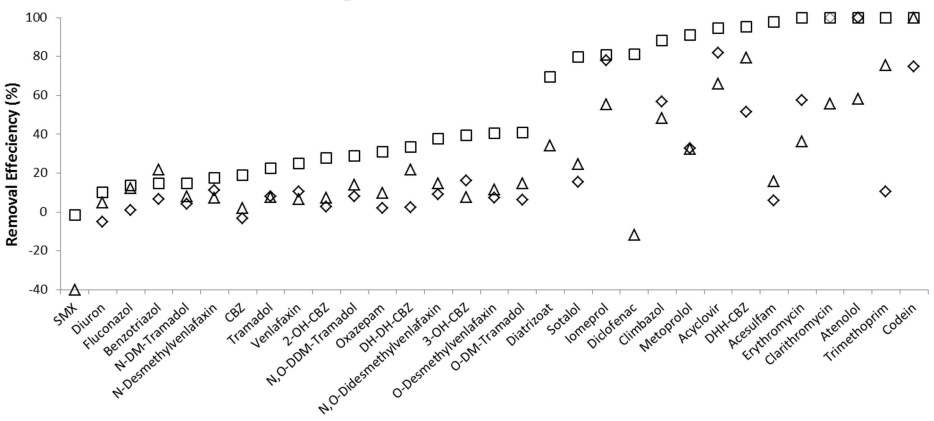
*OMPs: Organic Micropollutants.

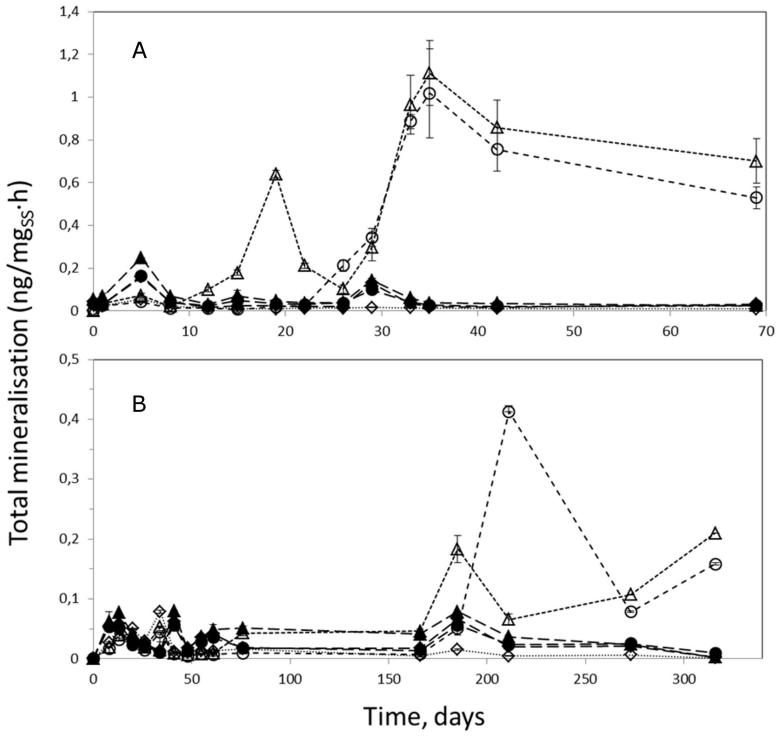
1 Table 2. Micropollutants consumption rates obtained from conventional activated sludge (CAS),
2 membrane reactor pilot scale plant (MBR plant) and a membrane reactor functioning as an
3 afterpolishing step (Polishing MBR).

Process	Micropollutant degradation rate, ($\mu g/g_{VSS}h$)				
	Ketoprofen	Diclofenac	Ibuprofen	Naproxen	Gemfibrozil
CAS	0.014	0.0	2.555	0.775	0.034
MBR plant	0.198	0.112	2.731	0.440	0.153
Polishing MBR	2.388	7.285	247.952	71.110	24.198

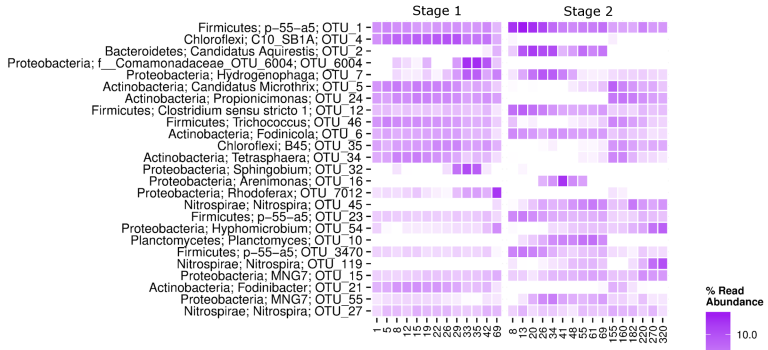
4







A.



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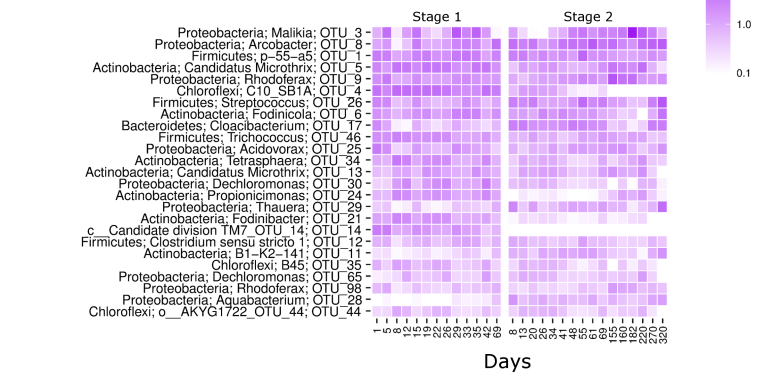

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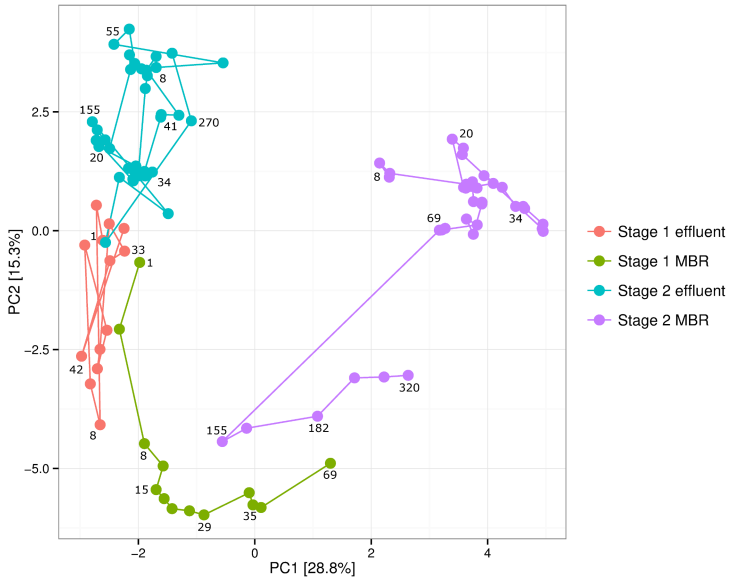

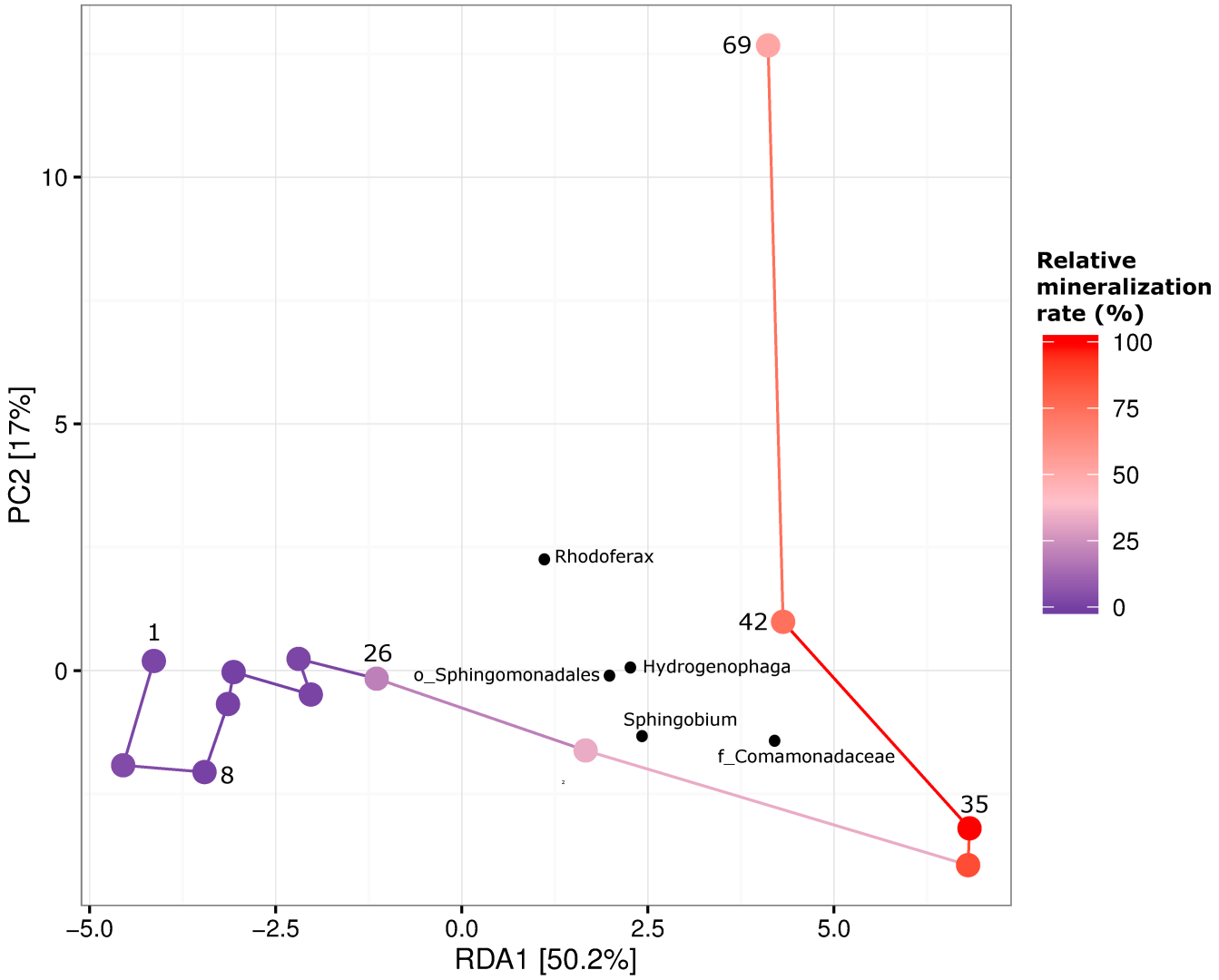
69
10
5
0
PC2 [17%]
Rhodoferax
42
1
26
o_Sphingomonadales
Hydrogenophaga
Sphingobium
f_Comamonadaceae
8
35
−5.0
−2.5
0.0
2.5
5.0
RDA1 [50.2%]
Relative mineralization rate (%)
100
75
50
25
0

Highlights

- Polishing of WWTP effluent by MBR can efficiently remove micropollutants
- Biomass actively removing pollutants can be accelerated by amendment of exogenous pollutants
- Microbial community structure development reveals removal candidate organisms
- Polishing by MBR efficiently removes COD under stable operation at full scale WWTP