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Supersaturated-oxygen aeration effects on a high-loaded membrane bioreactor (HL-MBR): Biological performance and microbial population dynamics

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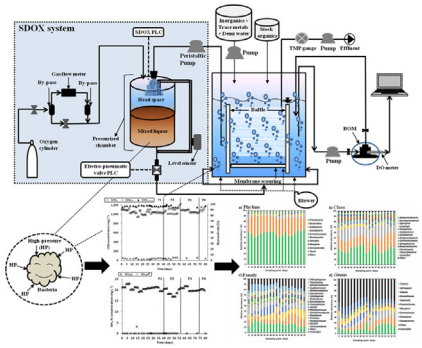
HIGHLIGHTS

- The biological performance of an MBR equipped with a supersaturated-oxygen aeration system was not affected negatively.
- Insignificant changes were observed in the microbial communities when switching from bubble diffusers to the supersaturated aeration system.
- Supersaturated-oxygen aeration technologies are presented as a promising alternative for the provision of dissolved oxygen in MBR systems.

GRAPHICAL ABSTRACT



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ABSTRACT

Conventional diffused aeration systems (such as fine-bubble diffusers) exhibit a poor oxygen transfer in wastewater treatment plants (WWTPs), particularly when operating at sludge concentrations higher than 15 g L^{-1}. The supersaturated dissolved oxygen (SDOX) system has been proposed as an alternative for supplying dissolved oxygen (DO) at high mixed liquor suspended solids (MLSS) concentrations. The advantages introduced by such technology include the possibility of operating WWTPs at much higher than usual MLSS concentrations, increasing the treatment capacity of WWTPs. Recent studies have demonstrated that the SDOX system has higher oxygen transfer rates (OTRs) and oxygen transfer efficiencies (OTEs) relative to fine-bubble diffusers. However, it is unknown if the high-pressure conditions introduced by SDOX may possibly impact the biological performance of WWTPs. In this study, the effects of SDOX technology on the biological performance of a membrane bioreactor (MBR) were evaluated. The MBR was operated at an MLSS concentration of approximately 15 g L^{-1} in four phases as follows: (P1) with bubble diffusers, (P2) with an SDOX unit, (P3) with the bubble diffusers, and (P4) with the SDOX unit. The performance of the MBR was assessed by monitoring the sludge concentration, as well as changes in the particle size distribution (PSD), sludge activity, organic matter removal and nitrification performance, and changes in the microbial community within the MBR. The operational conditions exerted by the SDOX

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technology did not affect the concentration of active biomass during the study period. The biological performance of the MBR was not affected by the introduction of the SDOX technology. Finally, the microbial community was relatively stable although some variations at the family and genus level were evident during each of the study phases. Therefore, the SDOX system can be proposed as an alternative technology for DO supply in WWTPs increasing the overall treatment capacity.

1. Introduction

Operating an activated sludge WWTP at the highest possible active MLSS concentration is highly desirable, since the treatment capacity of the WWTP can be increased almost proportionally to the increase on the active MLSS concentration. The higher the active MLSS concentration, either the higher the influent flowrate that a WWTP can handle (at a given footprint), or the lower the footprint needs (at a given flowrate); in addition, the higher the MLSS concentrations, the lower the sludge production. However, there are some limitations for reaching high MLSS concentrations in activated sludge WWTPs, mostly introduced by commonly used aeration technologies (e.g. fine and coarse bubble diffusers).

Membrane bioreactors (MBRs) are arguably the most suitable and available activated sludge wastewater treatment (WWT) technology to operate at high MLSS concentrations. MBRs combine biological processes with membrane filtration. The operation of MBRs is not affected by the settling characteristics of the sludge as in conventional activated sludge (CAS) processes. Therefore, MBRs are mostly operated at higher MLSS concentrations of approximately 10 g L^{-1} (Hai et al., 2019) compared to CAS systems regularly operated at approximately 3 g L^{-1}. In addition, advantages of MBRs compared to CAS systems include: a consistently high quality solids-free effluent, the capacity to handle high organic loading rates, a low footprint, and low sludge production, among others (Bagheri et al., 2019; Kim et al., 2019).

Operating MBRs at even higher than usual MBR MLSS concentrations (i.e., higher than 10 g L^{-1}) increases their treatment capacity and reduces even further the system footprint and sludge production (Barreto et al., 2017; Livingston, 2010). Furthermore, this may encourage the design of containerized and movable MBR systems suitable for the provision of on-site decentralized WWT (Zakaria et al., 2015). Such concept of an MBR operated at higher than usual MBR MLSS concentrations (from approximately 15 to 40 g L^{-1}) was introduced by Kim et al. (2019) as the high-loaded MBR (HL-MBR). The HL-MBR exhibits all the advantages previously described of an MBR operated at high MLSS concentrations. However, there are severe limitations for reaching such high MLSS concentrations when applying aeration technologies commonly used in activated sludge WWTPs (fine and coarse bubble diffusers).

Innovative aeration technologies have been developed for achieving more effective and efficient oxygen transfer when working at high MLSS concentrations of above 20 g L^{-1}. Among them, the supersaturated dissolved oxygen (SDOX), a supersaturated-oxygen aeration technology, has demonstrated promising advantages. The SDOX system consists of a pressurized chamber (operated at approximately 0.8 MPa) connected to a high-purity oxygen (HPO) source. The mixed liquor to be oxygenated is recirculated through the pressurized chamber where it gets in contact with pure oxygen under high-pressure conditions. A large gas-liquid interface is created between the mixed liquor (reaching the SDOX at the top of the pressurized chamber) and the pure oxygen; such high-pressure conditions in the SDOX allow DO concentrations to reach up to 350 mg L^{-1} in clean water (Jones, 2010). Kim et al. (2020) evaluated the oxygen transfer performance of the SDOX system at MLSS concentrations from approximately 4 to 45 g L^{-1}. Slightly lower oxygen mass transfer rate coefficients (K_La) were observed in clean water with the SDOX system (2.6 h^{-1}) compared to diffused aeration systems (4 h^{-1} and 11 h^{-1} for coarse and fine-bubble diffusers, respectively); but, the SDOX system showed higher oxygen transfer rates (OTRs) (14 g O_2 L^{-1} d^{-1}) compared to fine-bubble diffusers (2.4 g O_2 L^{-1} d^{-1}). Also, the SDOX system reached oxygen transfer efficiencies (OTEs) of approximately 100% in clean water, much higher than the approximate 5% per meter of submergence usually reported for fine-bubble diffusers. In particular, at MLSS concentrations higher than 20 g L^{-1}, the SDOX system exhibited considerably higher alpha factors (mass transfer ratio of process water to clean water) and demanded less energy than fine-bubble diffusers. Such advantages position the SDOX technology as a promising alternative for supplying DO in activated sludge WWT systems operated at high MLSS concentrations. However, the evaluations carried out so far with the SDOX system did not assess the potential negative effects on the biological activities (Kim et al., 2019; Kim et al., 2020). For instance, the shear forces and high-pressure conditions at which the sludge is exposed in the SDOX system may affect the biological activity, thus influencing the performance of WWTPs.

High-pressure conditions may negatively influence the cell structure of bacteria as well as their metabolic processes and survival capacity (Bartlett, 2002). Microorganisms are adversely impacted by the high-pressure conditions, depending on the intensity of such pressure (Picard and Daniel, 2013). However, certain studies have reported the effects of high-pressure conditions on the performance of diverse biological WWT systems indicating that the biological removal processes were not affected but even improved at high-pressure conditions (Ellis et al., 1992; Jin et al., 2010; Xu et al., 2016; Zhang et al., 2014; Zhang et al., 2016). Nevertheless, most such studies were conducted in pressurized batch reactors exposing the biomass continuously and completely to high-pressure conditions, using diffused aeration systems, and treating specific types of wastewater (e.g. industrial wastewater or synthetic saline wastewater). Neither any of the previously reported studies were carried out in WWT systems operated at high MLSS concentrations. Moreover, no such studies included a supersaturated oxygen aeration technology (such as the SDOX system) as the main source of DO. The SDOX technology exposes part of the sludge to the high-pressure conditions and shear effects in a completely different manner than diffused aeration systems. Thus, the impacts of such technology on the sludge activity and microbial population dynamics may be completely different and need to be evaluated.

In this study, the effects of the SDOX technology on the performance of a biological WWT system were evaluated. An MBR was operated at a relatively high MLSS concentration of approximately 15 g L^{-1}. For comparison purposes, the MBR system was evaluated in four different phases with either fine-bubble diffusers or using an SDOX unit for DO supply. The performance of the MBR system was assessed by monitoring changes in the sludge concentration and particle size distribution (PSD), the sludge activity in terms of the oxygen uptake rate (OUR), the organic matter removal and nitrification performance, and overall variations in the microbial population communities when exposing the sludge to the SDOX technology. The SDOX technology has already demonstrated a much better oxygen transfer performance (e.g. alpha factors, OTEs, and energy consumption) at high MLSS concentrations compared

to diffused aeration systems. If successful, this research will demonstrate the absence of major negative effects on the biological performance of a WWT system equipped with an SDOX system. Therefore, positioning the SDOX technology as a feasible and energy efficient alternative for operating WWTPs at higher than usual MLSS concentrations increasing the WWTPs treatment capacity (i.e., increasing the receiving wastewater flowrates at a given footprint, or lowering the footprint requirements at a giving flowrate).

2. Materials and methods

2.1. Design of the experiment

A bench-scale MBR was continuously operated for 80 days and fed with synthetic wastewater. The MBR was equipped with either fine-bubble diffusers or with a bench-scale SDOX unit, for the provision of DO. The MBR was inoculated with fresh activated sludge from the municipal WWTP of the city of Zagreb (Zagreb, Croatia) and concentrated up to approximately 10 g L^{-1}. The system was operated in four phases: phase one (P1) using bubble diffusers (days 0 to 40); phase two (P2) with the SDOX unit (days 41 to 56); phase three (P3) bubble diffusers (days 57 to 74); and, phase four (P4) SDOX unit (days 75 to 80).

Thus, after inoculation, in P1 the MBR was operated with the fine-bubble diffusers for 40 days to acclimatize the biomass. On day 41, P2, the SDOX unit was introduced and the MBR was operated under identical operational conditions to P1 for 16 days; P2 aimed at investigating the potential influence of the high-pressure conditions and shear effects on the biomass. Then, in P3, the SDOX unit was taken out, and the MBR operated again with fine-bubble diffusers for 18 days. P3 was included to investigate the effects of the sludge and of the MBR system when exposed again to bubble diffusers (since the sludge could have been damaged after being exposed to the SDOX unit). Finally, in P4, the aeration system was replaced one more time by the SDOX unit to confirm the effect of high-pressure conditions for a further six days. In P3 and P4, full stabilized reactor conditions cannot be entirely claimed. These phases were carried out mostly to confirm the previous trends observed in P1 and P2.

A solution containing the concentrated organic components of the synthetic wastewater (glucose, acetate, peptone, and yeast) was fed to the MBR by gravity drips using a gravity medical infusion unit operated at a flowrate of 1 L d^{-1}. A second solution containing the inorganic components of the synthetic wastewater was added through a piston fluid-metering pump (FMI PM6014 RHV, Fluid Metering Inc., USA) at a flowrate of 39.6 L d^{-1}. That is, the total influent flowrate to the MBR was set at 40.6 L d^{-1} delivering the wastewater composition of the synthetic wastewater to the MBR system described in Table 1. Such a flowrate established a total hydraulic retention time (HRT) of approximately 4 h and a membrane flux of 15 L m^{-2} h^{-1}. The solid retention time (SRT) was set at 10 days by withdrawing 0.65 L d^{-1} of sludge from the MBR.

Table 1
Characterization of the synthetic wastewater reaching the MBR system.

Chemical compounds	Concentration (mg L^{-1})	Chemical compounds	Concentration (mg L^{-1})
$C_6H_{12}O_6$	421.88	$FeCl_3 \cdot 6H_2O$	19.36
$C_2H_3NaO_2$	571.28	$C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$	30.00
Peptone	260.00	$MnCl_2 \cdot 4H_2O$	0.74
Yeast	40.00	$ZnSO_4 \cdot 7H_2O$	2.50
NH_4Cl	65.69	$CuSO_4 \cdot 5H_2O$	0.61
KH_2PO_4	48.33	$CoCl_2 \cdot 6H_2O$	2.09
$NaHCO_3$	251.95	$Na_2MoO_4 \cdot 2H_2O$	0.26
$CaCl_2$	40.37	H_3BO_3	0.13
$MgSO_4$	65.65	$NiSO_4 \cdot 7H_2O$	0.29

Samples of the MBR sludge, influent, and effluent were regularly collected and analyzed for the determination of water quality parameters, sludge characteristics, and changes in the microbial populations.

2.2. Experimental setup

The setup consisted of an MBR equipped with either fine-bubble diffusers (Fig. 1a) or the SDOX unit (Fig. 1b), for introducing the DO. The MBR basin was made of transparent acrylic glass with a total volume of 30.6 L (16 × 25.5 × 75 cm), and it was operated at a working volume of 6.5 L. A flat-sheet membrane (XJ3 module by Kubota) made of chlorinated polyethylene was immersed in the middle and lower part of the MBR basin. The membrane had an effective filtration area of 0.11 m^2 with a nominal pore size of 0.4 μm. A coarse bubble diffuser (Uxcell, model number: US-SA-AJD-231698, Hong Kong) was placed at the bottom of the MBR for membrane scoring. Air was supplied by a blower (HIBLOW HP 80, Techno Takatsuki, Japan) which was operated to satisfy the specific membrane scouring aeration needs of 2 m^3 m^{-2} h^{-1}. The permeate was taken out of the MBR system through a piston fluid metering pump (FMI PM6014 RHV, Fluid Metering Inc., USA).

During P1 and P3, the DO was supplied by fine-bubble diffusers (Hydrofarm, Inc., USA) placed at the bottom of the MBR basin (Fig. 1a). The fine-bubble diffusers were operated at air flow rate (AFR) values of approximately 0.5 m^3 h^{-1}. Also, two baffles were placed, one at either side of the immersed membrane, to provide a uniform distribution of the aeration. During P2 and P4, the DO was supplied by a bench-scale SDOX unit (Fig. 1b). The bench-scale SDOX unit consisted of a pressurized chamber connected to an HPO source (oxygen cylinder (MESSER, Croatia)). The pressurized chamber had a total volume of 2.75 L. Approximately 20% of that volume (0.55 L) was occupied by the sludge solution to be oxygenated, while the 80% remaining (2.20 L) consisted of the headspace. The pressure in the SDOX unit was set at 0.69 MPa. The pressurized chamber operated with two analogic pressure gauges (McDaniel Controls, USA). Moreover, both a pressure digital sensor (SICK AG, Germany) and a level digital sensor (Setra Systems, USA) were placed inside the pressurized chamber. An electro-pneumatic valve (NVF3-MOH-5/2-K-1/4-EX, FESTO, Germany) was introduced at the effluent drainage of the pressurized chamber. The pressure sensors, level sensors, and the electro-pneumatic valve were used to monitor and control the level and pressure of the pressurized chamber by the aid of a program logic controller (PLC) system (SIMATIC S7-1200, Siemens, Germany). Pure oxygen was supplied into the system through a gas flowmeter equipped with a mass totalizer (Model # 32908-59, Cole-Palmer, USA). The sludge stream was introduced into the SDOX system through a 6 mm orifice by a high-pressure peristaltic pump (EW-74203-24, Cole-Palmer, USA) operated at a flowrate of 0.3 L min^{-1}. The supersaturated sludge stream was then released back into the MBR basin, thus introducing oxygen into the MBR system. The sludge from the pressurized chamber was released at the bottom of the MBR basin, contributing to the mixing of the MBR system.

2.3. Analytical methods

The MBR effluent and influent samples were analyzed to determine the chemical oxygen demand (COD), NH_4-N, NO_2-N, NO_3-N, total nitrogen (TN), PO_4-P, and total phosphorus (TP) using the Hach Lange Cuvette Tests (LCK 238, 303, 304, 314, 339, 342, 350, 514). Composite permeate samples were collected within 24 h and were analyzed daily. The MLSS and mixed liquor volatile suspended solids (MLVSS) concentrations were determined following the standard methods for the examination of water and wastewater (APHA, 2017). The temperature and DO of the MBR system were measured using a DO probe (WTW Oxi 3310, Germany). The pH was measured with a pH probe (SI Analytics GmbH, Germany). Both the DO and pH determinations were

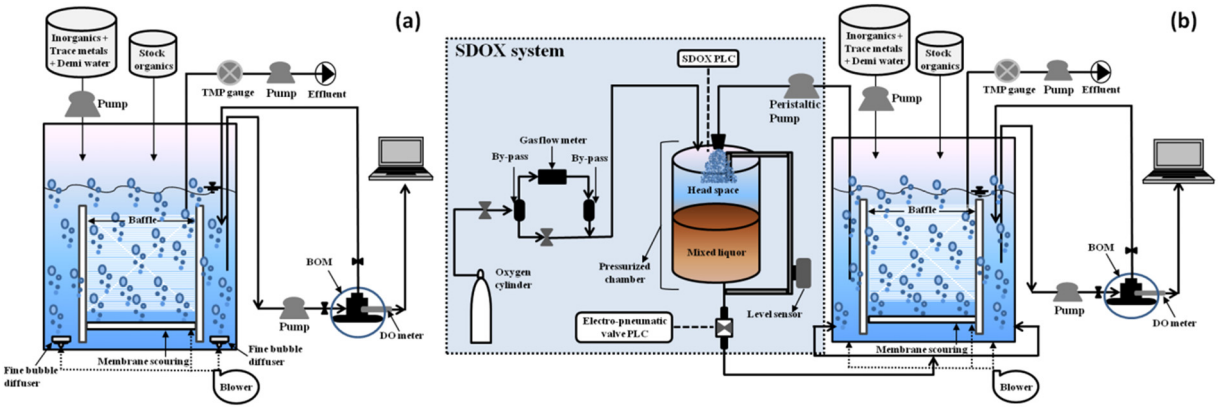

Fig. 1. Experimental setup of the MBR system equipped with (a) bubble diffusers and (b) the SDOX system.

corrected by the actual temperature. The PSD was measured using a Malvern Mastersizer 2000 laser diffraction particle counter (Malvern Instruments Ltd., Malvern, UK). The Mann-Whitney *U* test was carried out to assess significant differences on the performance of the MBR between P1 and P2 for the evaluated key performance indicators such as the PSD, specific OURs (SOURs), COD, NH_4, and PO_4; in addition, the average values and standard deviations for such parameters were also determined.

2.4. Oxygen uptake rate

The OUR determinations were carried out with a biological oxygen meter (BOM) based on the batch respirometric method (Kim et al., 2020). The BOM consisted of a glass container equipped with a DO probe (WTW Oxi 3310, Germany) and a stirring plate (IKA® COLOR SQUID, Germany). A Master flex peristaltic pump (Cole-Parmer, USA) recirculated the sludge from the MBR under evaluation through the BOM. When the BOM was filled with the activated sludge, the pump was stopped and the decrease in the DO as a function of time was monitored and recorded by the DO probe. After determining the OUR values, the sludge was returned to the MBR. A DO range from 6.5 to 2.5 mg L^{-1} was used to calculate the OUR values. OURs were determined in triplicate, and an average value of the calculated OURs was reported.

2.5. High-throughput sequencing analysis

2.5.1. DNA isolation, PCR, and sequencing

A total of 19 sludge samples were analyzed throughout P1 and P2 to explore the response of the microbial community structure when switching between the two aeration systems. The initial sample (day 0) represented the sludge taken from the WWTP. Eight sludge samples were collected during P1 (on days 16, 28, 30, 32, 34, 36, 38, and 40) to assess the MBR operation with fine-bubble diffusers, while ten sludge samples were collected in P2 (on days 41, 43, 45, 47, 49, 50, 51, 53, 54, and 55) to study the operation of the MBR with the SDOX unit.

The samples for deoxyribonucleic acid (DNA) extraction were obtained by pelleting 2 mL of the sludge by centrifugation (10,000g for 5 min) and removing the supernatant. The DNA was extracted from the pellets (0.25–0.30 g pellets) using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, CA, USA). The sludge samples were added to a bead beating tube provided with the PowerSoil DNA Isolation Kits. The cell lysis was achieved by multidirectional beating in a homogenizer set, following the manufacturer's recommendations (30 s at 5.0 m s^{-1}). The DNA was eluted in 100 μL solution of C6. After the extraction, the DNA integrity was checked by running 1 μL of all the samples using 0.8% agarose gel and storing the extracted DNA at −20 °C. Negative controls were included in this study to account for background noise from possible material and reagent contamination.

The bacterial DNA was analyzed at the Genomic Sequencing and Analysis Facility (GSAF) at the University of Texas at Austin (Austin, TX, USA) for Illumina® paired-end (2250) sequencing on the MiSeq platform. The first-round polymerase chain reaction (PCR) (19 cycles) was used to amplify the V4/V5 regions of the 16S ribosomal ribonucleic acid (rRNA) gene using the primers 515F (5′-GTGYCAGCMGCCGCGGTA-3′) (Baker et al., 2003) and 909R (5′-CCCCGYCAATTCMTTTRAGT-3′) (Wang and Qian, 2009). These primers included appropriate Illumina adapters with reverse primers which also had an error-correcting 12-bp barcode unique to each sample to permit multiplexing of the samples. After the PCR amplification, samples were prepared for their Illumina® sequencing run. This first round of PCR amplification was run in triplicate for each sample, pooled, and then cleaned using AMPure beads (New England Biolabs, Ipswich, MA). A second-round PCR amplification (11 cycles) was performed with hybrid primers that added sample-specific barcodes. Both rounds of the PCR amplification used Taq polymerase NEB Q5 (New England Biolabs, Ipswich, MA). The final PCR products for each sample after both rounds of amplification were again size-purified by removing amplicons less than 300 bp in length using AMPure beads (New England Biolabs, Ipswich, MA) and quantified using PicoGreen (Life Technologies, Carlsbad, CA). Samples were then normalized by amplicon mass and pooled for the Illumina® run. In addition, a random subset of samples was assessed on an Agilent BioAnalyzer (Agilent Technologies, Santa Clara, CA) to ensure correct amplicon size. Negative PCR controls (negative template) were included to test for contamination during amplification and sequencing processes. However, no sequences were obtained from these controls.

2.5.2. Sequence processing and statistical analysis

Bacterial DNA sequences were processed and analyzed in QIIME v.1.8 (Caporaso et al., 2010). Sequences were demultiplexed and forward and reverse reads were merged using FLASH v.1.2.11 (Magoč and Salzberg, 2011) with maximum overlap of 250 bp. Sequences were quality-filtered (-q 19), and chimeras were removed via QIIME and USEARCH (Edgar, 2010). High-quality sequences were clustered into operational taxonomic units (OTUs) at 97% similarity using QIIME's USEARCH-based open-reference OTU clustering workflow (pick_open_reference_otus.py). Global singleton OTUs were removed, and OTU proportions were standardized to the total number of high-quality reads. Taxonomy was assigned using the Ribosomal Database Project (RDP) classifier (Wang et al., 2007) with the reference database Greengenes13_8 16s rRNA (McDonald et al., 2012). All the samples were rarefied to the least number of sequences present in any individual sample as is commonly done in microbiome studies. All statistical analyses were performed in the R environment (www.r-project.org). Pair-wise dissimilarities between communities were calculated using both unweighted and weighted UniFrac (Lozupone and Knight, 2005). The Mann-Whitney *U* test was carried

out to assess significant differences on the taxonomic ranks between P1 and P2.

3. Results and discussion

3.1. Effects of the SDOX system on the sludge concentration

The effects of the SDOX unit on the sludge concentration were evaluated. Fig. 2 describes the changes in the MLSS and MLVSS concentrations, and the MLVSS/MLSS ratio as a function of the exposure time. After inoculation, the initial MLSS concentration was approximately 10 g L^{-1}. The operational conditions in the MBR (including the composition of the synthetic wastewater, HRT, and SRT) were designed to achieve a sludge concentration of approximately 15 g L^{-1}, which was reached during P1.

On the operational day 41, the SDOX unit was introduced (P2), and a decrease in the sludge concentration was immediately observed. It continued to decrease for the next three consecutive days until reaching an MLVSS concentration of 12.6 g L^{-1}; after that, it stabilized (and even slightly increased) until the end of phase P2 (day 56). The reduction in the MLSS concentration observed when incorporating the SDOX unit in P2 mostly occurred due to a dilution observed in the MBR basin. When the SDOX unit was introduced, it was filled with sludge, thus reducing the level in the MBR basin. This resulted in more influent wastewater coming into the MBR to reach the operational MBR level setpoint established by the automatization system, thus diluting the sludge in the MBR basin. Such an effect could have contributed with approximately a 0.85 dilution factor to the lowering of the MLVSS concentration to approximately 12.7 g L^{-1}, similar to the observed MLVSS concentration of 12.6 g L^{-1}on operational day 43 (Fig. 2). Therefore, the decrease in the MLVSS concentration was mostly due to the dilution rather than to a deleterious effect caused by the SDOX unit (e.g., cell lysis that could have reduced the sludge concentration).

Thereafter in P3 (days 57 to 74), the SDOX unit was removed, and the MBR was operated again with only bubble diffusers. During this phase, the sludge concentration returned back up to approximately the original MLVSS concentration of 15 g L^{-1}. In P4 (days 75 to 80), the SDOX unit was again introduced; however, during this phase a decrease in the sludge concentration was not observed. When moving from P3 to P4 (that is, when switching again from the bubble diffusers to the SDOX unit), the level setpoint control in the automatization system was modified to avoid such a dilution effect as observed when moving from P1 to P2.

In addition, the MLVSS/MLSS ratio was monitored (Fig. 2). During the first 10 days of operation in P1, when the sludge in the MBR was getting acclimated to the new synthetic wastewater and operational conditions, the MLVSS/MLSS ratio increased from approximately 0.76 to 0.90. In this study, a readily biodegradable synthetic wastewater was provided to the system. So, during such a period, fresh active biomass grew contributing to the increase in MLVSS and the MLVSS/MLSS ratio. The MLVSS/MLSS ratio remained constant during the entire evaluation period, indicating that the SDOX unit did not induce major damage to the cells. If this had happened then a consequent reduction in the MLVSS concentration would have been observed, contributing significantly to lowering the MLVSS/MLSS ratio.

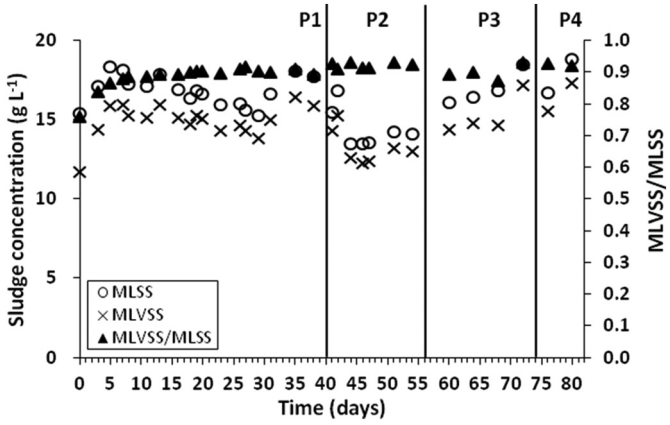

Fig. 2. Sludge MLSS and MLVSS concentrations and MLVSS/MLSS ratio (P1: aerated with diffusers, P2: aerated with SDOX, P3: aerated again with diffusers, P4: aerated again with SDOX).

3.2. Effects of the SDOX system on the PSD

The effects of the SDOX system on the PSD of the sludge were determined. Fig. 3a indicates the changes in the PSD in the sludge from the inoculation of the MBR until the end of P2 (SDOX evaluation). When inoculated, the sludge exhibited an average particle size (Dv50) of 114 μm (at day 0) which was reduced down to 39.8 μm towards the end of P1 (day 40). During P1 the sludge was mostly acclimating to the MBR system conditions and to the new synthetic wastewater. Such a reduction in the PSD and average particle size was caused by the effects of the turbulence and mixing provided by the bubble diffusers in the MBR basin, and possibly by the effects of the pressure exerted on the surface of the membrane. Zhang et al. (2015) reported that the sludge flocs get smaller in aerobic basins due to the shear forces generated by diffused aeration systems. This means that a noticeable reduction in the average size of the particles was observed without taking the effects of the SDOX system into account. After introducing the SDOX unit (P2), a further decrease in the average particle size was observed down to 27.8 μm at the end of P2 (day 54 in Fig. 3a). The Mann-Whitney *U* test indicated significant differences ($p \leq 0.02$) in the average particle size between P1 and P2. The changes in the PSD from day 40 to day 54 were attributed to the effects of high-pressure and shear exerted by the SDOX unit. Fig. 3b shows the changes in the PSD and average particle size immediately after using the SDOX unit on the first five consecutive operational days in P2 (day 41 to day 45). The PSD and average particle size of sludge decreased from 43.0 μm (day 41 right after introducing the SDOX unit) until 24.9 μm (day 45). The average particle size stabilized at the end of P2 as observed in Fig. 3a at an average particle size of 27.8 μm (day 54). The high-pressure conditions and shear effects introduced by the SDOX system contributed to reducing the PSD of the sludge; however, such a decline in the PSD occurred right after the SDOX unit was introduced and the effects stabilized with the exposure time. When the SDOX unit was removed and the MBR was operated one more time with the bubble diffusers (P3), the PSD shifted back to larger particles, and an average particle size of 49.3 μm was reported at the end of P3 (day 65) (Fig. 3c). As the high-pressure conditions and shear effects imposed by the SDOX unit ceased, the sludge flocs became larger again. In phase P4, the MBR system was operated again with the SDOX unit, and the average particle size decreased to 44.7 μm (day 76) (Fig. 3c). Overall, the SDOX system tended to decrease the PSD of the sludge. In particular, the effects were more pronounced during the first days after introducing the SDOX system until the PSD stabilized. The reduction in the PSD did not have any critical effect on the sludge concentration of the MBR system or on the MLVSS/MLSS ratio. Consequently, it can be considered that the high-pressure conditions and shear effect did not appear to produce any substantial cell lysis and/or cell inactivation with subsequent losses of volatile suspended solids in the sludge.

3.3. Effects of the SDOX system on the sludge activity

The effects of the SDOX unit on the biological sludge activity in the MBR were also evaluated. Fig. 4 presents the OUR and SOUR when the MBR was provided with bubble diffusers (P1) and with the SDOX unit (P2) until the operational day 56. In the first

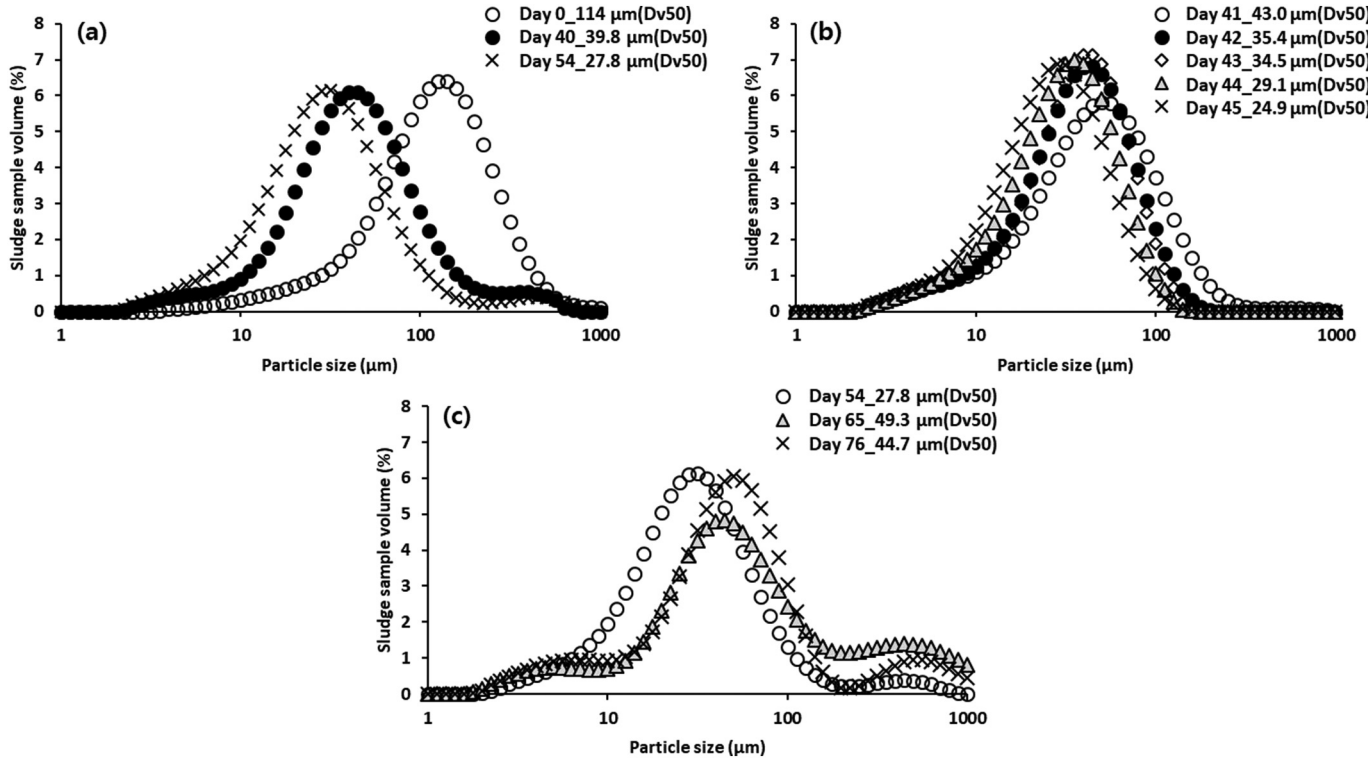

Fig. 3. Changes in PSD: (a) when acclimating the sludge and switching from bubble diffusers to the SDOX unit (phases P1 and P2); (b) after introducing the SDOX (phase P2); and (c) when switching from the SDOX unit to bubble aeration and back to the SDOX unit (phases P2, P3, and P4).

20 days of operation, both the OUR and SOUR halved. During P1, the sludge was adapting to the MBR operational conditions and the synthetic wastewater. In particular, the SRT of the sludge changed from 5 days (operational SRT at the local WWTP) to 10 days (SRT set for the MBR evaluation), explaining such a reduction in the OUR and SOUR values. After the operational day 20, the OUR and SOUR stabilized until the end of the phase. No major differences were observed in the reported values when switching the aeration systems from the bubble diffuser to the SDOX unit (i.e., from P1 to P2) as indicated in Fig. 4. Average SOUR values of 8.0 ± 1.6 and 7.2 ± 1.0 mg O_2 L^{-1} h^{-1} were obtained for P1 and P2, respectively. In addition, the Mann-Whitney *U* test indicated no significant differences ($p \leq 0.02$) in the SOUR values between P1 and P2. There was a slight decrease in the OUR values due to the losses of sludge at the beginning of P2 due to the dilution effect previously described. However, the SOUR did not considerably change. Then, as determined by the OUR, switching to the SDOX system did not affect the biological activity.

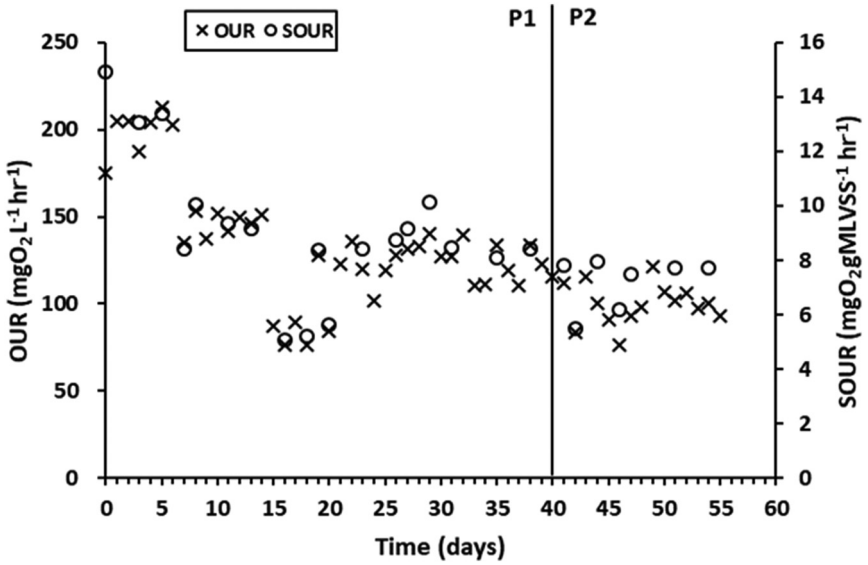

Fig. 4. OUR and SOUR for the experiments with bubble diffusers and SDOX (P1: aerated with diffusers, P2: aerated with SDOX).

3.4. Effects of the SDOX system on COD removal, nitrification, and phosphorus removal

The effects of the SDOX unit on the biological performance of the MBR system were determined by evaluating the COD removal performance. Fig. 5a indicates the COD removal for the entire evaluated period. The system was fed with synthetic wastewater at an influent concentration of approximately 1000 mg L^{-1}. The effluent COD concentration remained below 60 mg L^{-1}, showing an average COD removal efficiency higher than 95%. As observed in Fig. 5b, after the introduction of the SDOX unit, the effluent COD concentration remained unchanged compared to the performance in P1 at approximately 40 mg L^{-1}. Later on, towards the end of P2, a slight increase in the effluent COD up to approximately 60 mg L^{-1} was observed. This increase in the COD in the effluent could have been caused by changes in the PSD of the sludge, producing possibly some colloidal materials with a particle size lower than the pore size of the microfiltration membrane (0.4 μm) that could have escaped the MBR. Average COD removal values of 96.8 ± 0.8 and 96.2 ± 1.5% were obtained for P1 and P2, respectively. In addition, the Mann-Whitney *U* test indicated no significant differences ($p \leq 0.02$) in the effluent COD concentration between P1 and P2. Certainly, it can be concluded that the introduction of the SDOX unit did not affect the performance of the MBR system regarding the removal of COD, and the MBR exhibited overall an excellent COD removal efficiency of above 95% on average. Therefore, this suggests that the functionality of the microbial community responsible for the decomposition of organic matter was not affected when introducing the SDOX system.

The effects of the SDOX unit on the biological performance of the MBR were also evaluated by determining the nitrification performance of the system. Fig. 6a and b shows the influent and effluent ammonia and nitrate concentration, respectively, for the evaluated period. An influent ammonia concentration of approximately 20 mg L^{-1} was continuously added to the MBR system. Complete ammonia removal was observed (Fig. 6a) with the subsequent formation of nitrate (Fig. 6b), already immediately after inoculating the MBR with the sludge from the WWTP (P1). This indicates the presence of nitrifying microorganisms in such sludge. Moreover, when switching to the SDOX unit (P2), the ammonia removal performance and nitrate generation of the system remained unchanged. Average NH_4 removal values of 98.6 ± 3.1 and 99.4 ± 0.2% were obtained for P1 and P2, respectively. In addition, the Mann-Whitney *U* test indicated no significant differences ($p \leq 0.02$) in

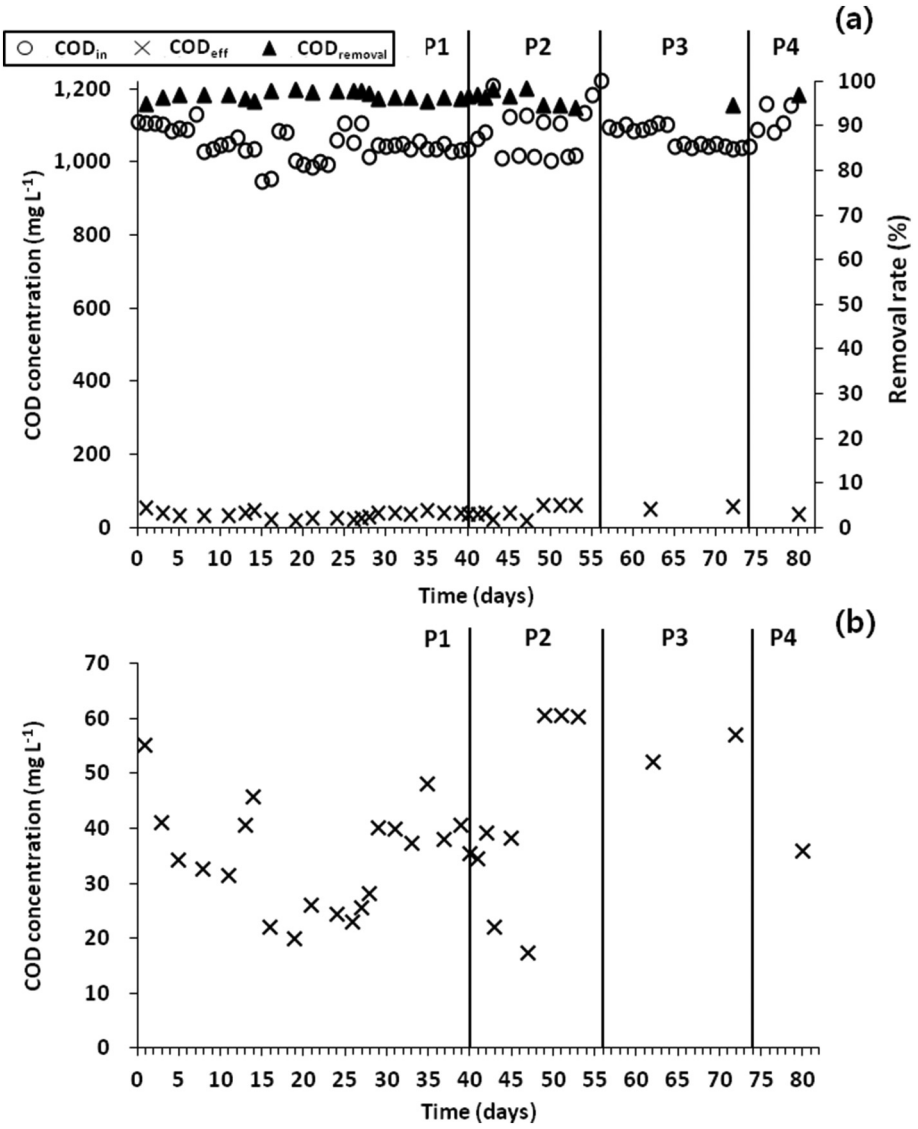

Fig. 5. (a) Influent and effluent COD concentration and removal efficiency, (b) effluent COD concentration (P1: aerated with diffusers, P2: aerated with SDOX, P3: aerated again with diffusers, P4: aerated again with SDOX).

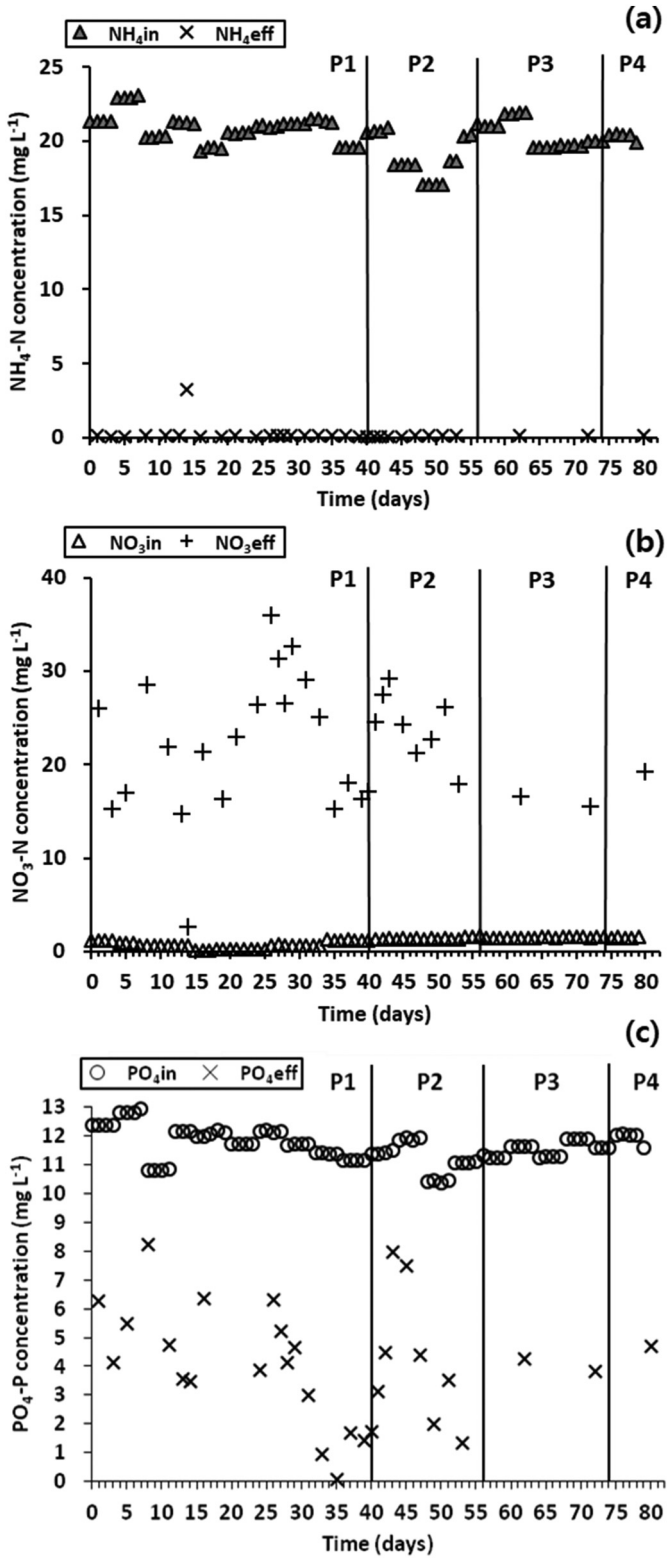

Fig. 6. Influent and effluent concentration of NH_4-N (a), NO_3-N, and PO_4-P (c) (P1: aerated with diffusers, P2: aerated with SDOX, P3: aerated again with diffusers, P4: aerated again with SDOX).

the effluent NH_4 concentration between P1 and P2. Similar observations were observed in P3 and P4. Therefore, as observed with the organic matter removal organisms, there is a clear indication that the ammonia oxidizing populations were not affected by the high-pressure conditions and shear effects that the SDOX system could have created and led to biomass lysis.

Fig. 6c shows the concentrations of phosphate in the influent and effluent of the MBR. An influent phosphate concentration of approximately 12 mg L^{-1} was fed to the MBR system. The MBR system was not designed for enhanced biological phosphorous removal (EBPR), and chemical phosphate removal was not applied. Therefore, phosphate was mostly removed for biomass growth requirements. Moreover, phosphate release was not observed after introducing the SDOX unit. Thus, this is another indication that the SDOX system did not contribute significantly to cell lysis that could have released phosphate into the system. Average PO_4 removal values of 71.1 ± 16.0 and 67.1 ± 16.7% were obtained for P1 and P2, respectively. In addition, the Mann-Whitney U test indicated no significant differences ($p \leq 0.02$) in the effluent PO_4 concentration between P1 and P2.

3.5. Effects of the SDOX system on the microbial community in the MBR system

The effects of the SDOX system on the microbial community structure in the MBR system were evaluated via high-throughput DNA sequencing. The similarities between the different microbial communities when switching from diffused aeration to the SDOX unit (P1 and P2, respectively) were visualized via principal coordinate analysis (PCoA) of the unweighted and weighted UniFrac dissimilarities as shown in Fig. 7a and b, respectively. Several sludge samples were analyzed covering the entire P1 (bubble diffusers – days 0 to 40) and P2 (SDOX unit – days 41 to 56). During P1, when the MBR was equipped with bubble diffusers, the sludge was acclimating to the operational conditions and synthetic influent wastewater in the MBR system. As time progressed during P1, the sludge sample communities shifted in both the weighted and unweighted PCoA diagrams (Fig. 7). Such trends suggest a slight change in microbial community structures, although the variability in the ordinates represents only 11.4 and 19.8% of the total variability for the unweighted and weighted PCoA diagrams, respectively. Some changes in the microbial communities were expected during this time since the sludge was acclimating to the environmental conditions within the MBR. However, when the aeration systems were switched from bubble diffusers to the SDOX system (from P1 to P2), more significant shifts in the microbial community were immediately evident in both the weighted and unweighted plots. These results suggest that the introduction of the SDOX unit yielded more substantial shifts in the membership (unweighted) and structure (weighted) of the bacterial community than the acclimatization of the sludge did to the MBR operational conditions. However, such differences become less pronounced at the end of P2, when the systems are becoming acclimated to the SDOX aeration system.

The relative abundances of the taxon assignments at the level of phylum, class, family, and genus for each sludge sample collected throughout P1 and P2 are presented in Fig. 8. With respect to the phylum level, a total of 24 different phyla were detected in the 19 analyzed sludge samples corresponding to P1 (diffused aeration – days 0 to 40) and P2 (SDOX unit – days 41 to 56). As indicated in Fig. 8a, only a small number of bacterial phyla constituted the majority of the bacterial communities in P1 and P2. The dominant phyla were the *Proteobacteria* and *Bacteriodetes* bacteria, comprising between 49.2–64.8% and 23.4–39.8% of the total bacterial sequences, respectively. These two phyla have been reported to be dominant in biological WWT systems regardless of the specific WWT technology deployed (Nascimento et al., 2018; Xu et al., 2018). The phyla composition did not change after switching the aeration system from diffused aeration to the SDOX unit (on day 41). After introducing the SDOX unit the *Proteobacteria* and *Bacteriodetes* abundance remained at 86% of the total bacterial sequences compared to the 89% reported for the diffused aeration. In addition, the Mann-Whitney *U* test indicated no significant differences ($p \leq 0.02$) in the abundance between P1 and P2 for

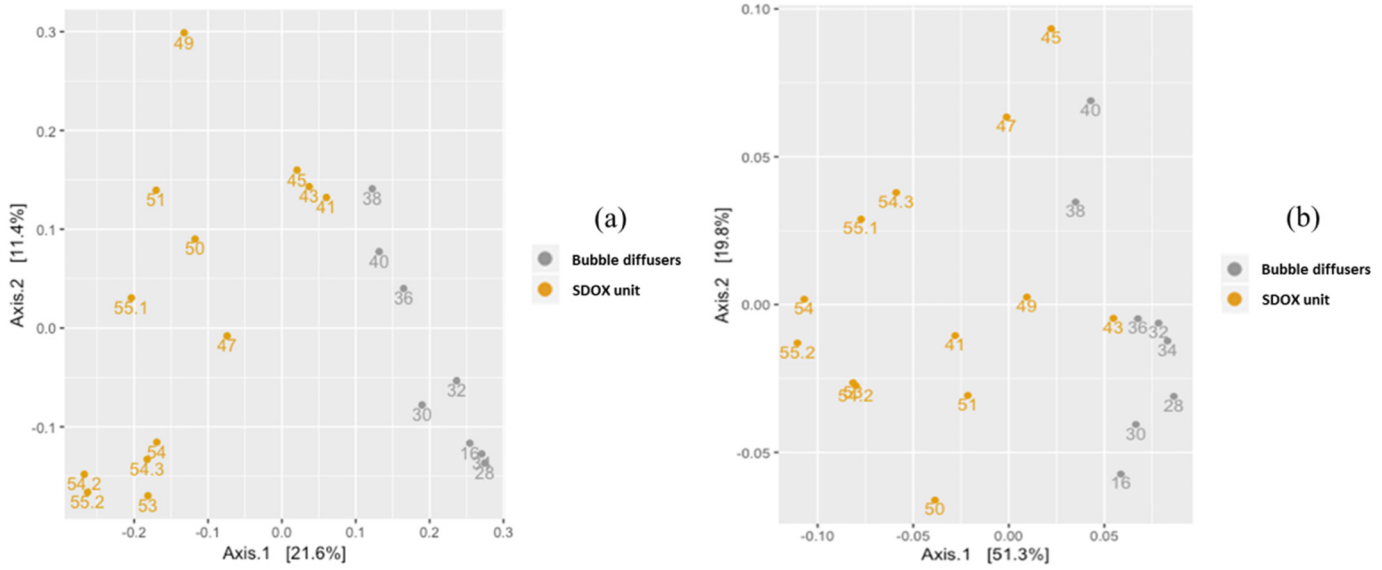

Fig. 7. (a) PCoA unweighted UniFrac, and (b) weighted visualization of the microbial community structure of sludge samples under two different aeration systems. The numbers in the data points in the figures represent the day of operation. When more than one sample was analyzed for the same operational day, a dot followed by the sample number was added. For instance, the data points 55.1 and 55.2 in panel (a) describes the first and second samples taken on the operational day 55, respectively.

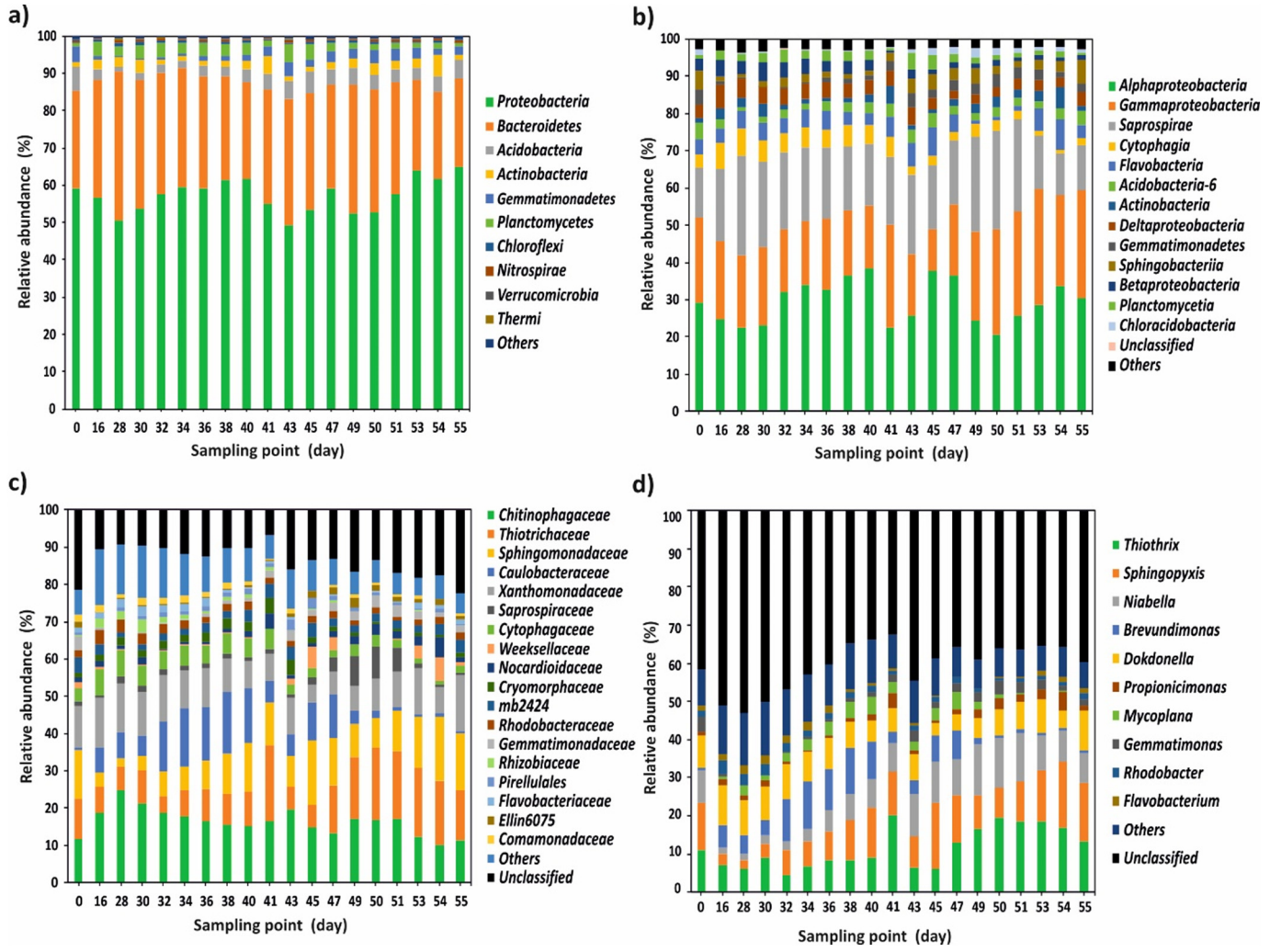

Fig. 8. - Bacterial community abundance for the first 55 days of the MBR operation at the level of (a) phylum, (b) class, (c) family, and (d) genus. Minor taxa classified as less than 1.0% of total sequences are grouped as "others", and those not classified are noted as "unclassified".

Proteobacteria and *Bacteriodetes*. As such, the high-pressure conditions and shear effects introduced by the SDOX unit did not change the microbial community composition at the phyla level. Subdominant phyla accounting for more than 1% of the total bacterial sequences included *Acidobacteria* (1.4–6.2%), *Actinobacteria* (1.1–5.7%), *Plactomycetes* (1.0–4.6%), *Gemmatimonadetes* (0.1–4.1%), and *Chloroflexi* (0.2–1.1%). These phyla have also been proved to be abundant in biological WWTP systems, even though the percentage of each phylum has considerably varied among the different published studies (Hu et al., 2012; Nascimento et al., 2018; Xu et al., 2018; Yadav et al., 2014; Zhang et al., 2017).

At the class level, *Alphaproteobacteria* and *Gammaproteobacteria* (both *Proteobacteria*) were the dominant class at 20.6–38.4 and 11.2–31.1% of the total bacterial sequences, respectively. *Saprospirae* (*Bacteriodetes*) accounted for 11.0–26.9% of all the sequences. The abundance of these three classes was similar after switching the aeration system from diffused aeration to the SDOX unit. The Mann-Whitney *U* test indicated no significant differences ($p \leq 0.02$) in the abundance between P1 and P2 for these three classes. Classes above 1% included *Cytophagia* (1.0–7.1%), *Flavobacteria* (1.0–8.3%), *Acidobacteria-6* (1.2–4.4%), *Actinobacteria* (1.0–5.7%), *Deltaproteobacteria* (2.2–6.3%), *Gemmatimonadetes* (0.1–4.1%), *Sphingobacteriia* (0.9–6.2%), *Betaproteobacteria* (0.6–5.1%), *Planctomycetia* (0.6–4.3%), and *Chloracidobacteria* (0.1–2.4%). Some minor fluctuations of specific classes were noticed. However, most of the classes related to the phyla previously described supporting the presence of communities commonly abundant in municipal WWTP systems. The introduction of the SDOX unit (on day 41) did not induce changes at the class level as observed in Fig. 8b and as previously noticed at the phyla level.

At the family level, *Chitinophagaceae* was the subdominant group with an abundance of 10.3–24.7% of the total bacterial sequences, followed by *Thiotrichaceae* (4.5–20.3%), *Xanthomonadaceae* (4.8–15.0%), and *Sphingomonadaceae* (2.4–17.4%). The abundance of *Chitinophagaceae* remained mostly unchanged during the evaluated period, although a slight decrease was observed at the end of the SDOX period (on day 54 in P2). The abundance of *Caulobacteraceae*, gram negative bacteria affiliated to the *Proteobacteria* phylum, also gradually declined shortly after starting the MBR operation with the pressured aeration system, suggesting that these families could be vulnerable to the high-pressure conditions exerted by the SDOX unit. The opposite trend was observed for *Thiotrichaceae* and *Sphingomonadaceae* where the abundance of these two families increased after the introduction of the SDOX unit. Additionally, the abundance of *Saprospiraceae* (*Sphingobacteriales* class) increased after introducing the SDOX system. However, the Mann-Whitney *U* test indicated no significant differences ($p \leq 0.02$) in the abundance between P1 and P2 for the families previously described.

Finally, at the genus level, 4.5–20.3% of the total identified bacterial sequences corresponded to the genus *Thiothrix*, followed by *Sphingopyxis* (2.1–17.4%) and *Niabella* (1.6–13.4%). Overall, and as reported for the other bacterial community levels, there were no major changes when incorporating the SDOX unit, although some variations were observed as follows. The presence of the *Thiothrix* genus (*Proteobacteria*) slightly increased after introducing the SDOX unit towards the end of this phase. However, the Mann-Whitney U test indicated no significant differences ($p \leq 0.02$) in the abundance between P1 and P2 for this genus. The *Thiothrix* genus is characterized by a group of filamentous bacteria commonly found in WWTPs (Nielsen et al., 2000). The *Thiothrix* genus has been related to carbon removal and nitrification processes in WWTPs (Nierychlo et al., 2020). The *Thiothrix* genus may have better resisted the high-pressure conditions and shear effects exerted by the SDOX unit. The relative abundances of *Thiothrix* during P1 and P2 were on average 7.8 ± 1.9% and 14.3 ± 5.1%, respectively. The *Sphingopyxis* genus (*Sphingomonadaceae* class) exhibited a similar trend to the *Thiothrix* genus. The Mann-Whitney U test indicated no significant differences ($p \leq 0.02$) in the abundance between P1 and P2 for the *Sphingopyxis* genus. The *Sphingopyxis* genus can resist high osmotic pressure conditions (Verma et al., 2020); the authors also reported that *Sphingopyxis* have been involved in the degradation of aromatic compounds (Verma et al., 2020). The presence of *Niabella* originally decreased at the beginning of the MBR adaptation phase (P1), and then gradually increased even after introducing the SDOX unit. The relative abundances of *Niabella* during P1 and P2 were on average 4.4 ± 2.7% and 10.6 ± 2.3%, respectively. The Mann-Whitney U test indicated significant differences ($p \leq 0.02$) in the abundance between P1 and P2 for the *Niabella* genus. The presence of *Niabella* was detected in conventional WWTP systems provided with standard diffused aeration systems (Jiao et al., 2016; Starke et al., 2017), and they have been found to participate in the nitrification process (Bucci et al., 2020); however, this genus has not been reported when operating at high-pressure conditions (Zhang et al., 2016; Zhang et al., 2017).

Only a few other studies have evaluated changes in microbial communities under high pressure-conditions in the context of biological WWT (Zhang et al., 2016; Zhang et al., 2017). However, such studies were carried out in systems fed with high saline wastewater (approximately 3.0%). To the best of our knowledge, this is the first study reporting on the changes in microbial communities at high-pressure conditions in the context of biological WWT systems. The results of the current study indicate that at the phylum, class and family levels, the major components of the microbial community remained stable throughout the bubble diffusion and SDOX operating periods. However, the weighted and unweighted UniFrac analyses indicate that there was a shift in the microbial community over time, first during the acclimation period of P1 and more significantly following the change to the SDOX system during P2. Regardless, the overall performance of the WWT system remained consistent, indicating that while the high-pressure conditions and shear effects introduced by the SDOX system may have altered some membership within the microbial community, the changes were insufficient to change the overall performance of the system.

The SDOX technology exhibited both much higher alpha factors and energy efficiencies when operated at high MLSS concentrations compared to diffused aeration (Kim et al., 2020), and also did not affect the biological performance of the system. Therefore, the SDOX technology can be proposed as an alternative for DO supply in activated sludge WWTPs. A WWTP equipped with the SDOX technology can either increase the receiving wastewater flowrate (for a given footprint), or decrease the footprint needs (for a given flowrate). During this research, the system reached a pseudo steady-state conditions; particularly, in phase P2, the variation of key performance indicator parameters (e.g., MLSS, MLVSS, MLVSS/MLSS, SOUR, COD, NH_4, and PO_4) were lower than 10% in consecutive days. However, further research would be needed to explore the long-term effects of the SDOX unit on the biological activities of the sludge at a complete stabilized (steady-state) conditions. In addition, a synthetic wastewater simulating a municipal wastewater was used in this study for feeding the MBR; thus, further research would be needed to confirm such same effects on real municipal and/or industrial wastewater. In this context, additional research is needed to evaluate the impact of the operational conditions of the SDOX system on other biological processes such as denitrification and biological phosphorous removal (i.e., EBPR). Moreover, the impact of the SDOX system on the subsequent solid-liquid separation processes (such as sludge settling and/or membrane filtration) needs to be further evaluated. The experimental design of such future evaluations may consider also the possibility of evaluating biological systems in parallel equipped either with fine-bubble diffusers, or with an SDOX unit.

4. Conclusions

- The concentration of active biomass was not impacted by the introduction of the SDOX unit. The PSD was reduced by the action of the

SDOX unit, although the MLVSS concentration and the MLVSS/MLSS ratio were not affected by the introduction of the SDOX technology in the MBR system.
- The biological performance of the MBR system was not influenced by the introduction of the SDOX system. The COD removal, ammonia removal and nitrification activities were not modified by the introduction of the SDOX system. In addition, the sludge activity measured as the OUR and SOUR remained unchanged after introducing the SDOX unit.
- The microbial community in the MBR system shifted over time during the diffused aeration operating period and more substantially following the introduction of SDOX. However, the major taxa in the community (including many involved in key biological wastewater degradation processes) remained relatively stable throughout both operating periods, indicating that the community was sufficiently robust to handle the high-pressure conditions of the SDOX system.
- The SDOX technology is a promising technology for supplying DO in biological WWT systems, particularly when working at high MLSS concentrations. The treatment capacity of WWT systems can be eventually expanded by incorporating such aeration technology.

CRediT authorship contribution statement

Sang Yeob Kim: Methodology, Validation, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Carlos M. Lopez-Vazquez:** Supervision, Visualization, Writing – original draft, Writing – review & editing. **Josip Curko:** Methodology, Validation, Formal analysis, Supervision, Writing – original draft. **Marin Matosic:** Conceptualization, Supervision, Writing – original draft. **Ivan K. Svetec:** Investigation, Resources. **Anamarija Štafa:** Investigation, Resources. **Chris Milligan:** Conceptualization, Resources. **Aridai Herrera:** Conceptualization, Resources. **Juan Pedro Maestre:** Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Kerry A. Kinney:** Methodology, Formal analysis, Supervision, Writing – original draft. **Damir Brdjanovic:** Conceptualization, Supervision, Funding acquisition. **Hector A. Garcia:** Methodology, Validation, Formal analysis, Supervision, Writing – original draft, Writing – review & editing.

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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References

APHA, AWWA, WEF, 2017. Standard Methods for the Examination of Water and Wastewater. 23rd edition. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D.C, USA.

Bagheri, M., Akbari, A., Mirbagheri, S.A., 2019. Advanced control of membrane fouling in filtration systems using artificial intelligence and machine learning techniques: a critical review. Process Saf. Environ. Prot. 123, 229–252. https://doi.org/10.1016/j.psep.2019.01.013.

Baker, G.C., Smith, J.J., Cowan, D.A., 2003. Review and re-analysis of domain-specific 16S primers. J. Microbiol. Methods 55 (3), 541–555. https://doi.org/10.1016/j.mimet.2003.08.009.

Barreto, C.M., Garcia, H.A., Hooijmans, C.M., Herrera, A., Brdjanovic, D., 2017. Assessing theperformance of an MBR operated at high biomass concentrations. Int. Biodeterior. Biodegradation 119, 528–537. https://doi.org/10.1016/j.ibiod.2016.10.006.

Bartlett, D.H., 2002. Pressure effects on in vivo microbial processes. Biochim. Biophys. Acta 1595 (1–2), 367–381. https://doi.org/10.1016/S0167-4838(01)00357-0.

Bucci, P., Coppotelli, B., Morelli, I., Zaritzky, N., Caravelli, A., 2020. Simultaneous heterotrophic nitrification and aerobic denitrification of wastewater in granular reactor: microbial composition by next generation sequencing analysis. Journal of Water Process Engineering 36, 101254. https://doi.org/10.1016/j.jwpe.2020.101254.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., et al., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7 (5), 335–336. https://doi.org/10.1038/nmeth.f.303.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26 (19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.

Ellis, K.V., Mortimer, G.H., Berktay, A., 1992. Biological wastewater treatment under the influence of pressure. Journal of Institution of Water and Environmental Management 6, 468–474. https://doi.org/10.1111/j.1747-6593.1992.tb00776.x.

Hai, F.I., Yamamoto, K., Lee, C.-H., 2019. Membrane Biological Reactors: Theory, Modeling, Design, Management and Applications to Wastewater Reuse. IWA Publishing, London.

Hu, M., Wang, X., Wen, X., Xia, Y., 2012. Microbial community structures in different wastewater treatment plants as revealed by 454-pyrosequencing analysis. Bioresour. Technol. 117, 72–79. https://doi.org/10.1016/j.biortech.2012.04.061.

Jiao, S., Chen, W., Wang, E., Wang, J., Liu, Z., Li, Y., Wei, G., 2016. Microbial succession in response to pollutants in batch-enrichment culture. Sci. Rep. 6, 21791. https://doi.org/10.1038/srep21791.

Jin, Z., Pan, Z., Yu, S., Lin, C., 2010. Experimental study on pressurized activated sludge process for high concentration pesticide wastewater. J. Environ. Sci. 22 (9), 1342–1347. https://doi.org/10.1016/S1001-0742(09)60260-6.

Jones, B., 2010. Introduction to SDOX®. LAKELINE, North American Lake Management Society.

Kim, S.Y., Garcia, H.A., Lopez-Vazquez, C.M., Milligan, C., Livingston, D., Herrera, A., Matosic, M., Curko, J., Brdjanovic, D., 2019. Limitations imposed by conventional fine bubble diffusers on the design of a high-loaded membrane bioreactor (HL-MBR). Environ. Sci. Pollut. Res. 26 (33), 34285–34300. https://doi.org/10.1007/s11356-019-04369-x.

Kim, S.Y., Garcia, H.A., Lopez-Vazquez, C.M., Milligan, C., Herrera, A., Matosic, M., Curko, J., Brdjanovic, D., 2020. Oxygen transfer performance of a supersaturated oxygen aerationsystem (SDOX) evaluated at high biomass concentrations. Process Saf. Environ. Prot. 139, 171–181.

Livingston, D., 2010. Beyond conventional mbrs. Oxygen transfer technology revolutionizing MBR applications. Paper Presented at the Membrane Technology Conference & Exposition.

Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71 (12), 8228–8235. https://doi.org/10.1128/AEM.71.12.8228-8235.2005.

Magoč, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27 (21), 2957–2963. https://doi.org/10.1093/bioinformatics/btr507.

McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DS, T.Z., Probst, A., Andersen, G.L., Knight, R., Hugenholtz, P., 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J. 6 (3), 610–618. https://doi.org/10.1038/ismej.2011.139.

Nascimento, A.L., Souza, A.J., PAM, Andrade, Andreote, F.D., Coscione, A.R., Oliveira, F.C., Regitano, J.B., 2018. Sewage sludge microbial structures and relations to their sources, treatments, and chemical attributes. Front. Microbiol. 9, 1462. https://doi.org/10.3389/fmicb.2018.01462.

Nielsen, P.H., De Muro, M.A., Nielsen, J.L., 2000. Studies on the *in situ* physiology of Thiothrix spp. present in activated sludge. Environ. Microbiol. 2 (4), 389–398. https://doi.org/10.1046/j.1462-2920.2000.00120.x.

Nierychlo, M., Mcllroy, S., Kucheryavskiy, S., Jiang, C., Ziegler, A., Kondrotaite, Z., Stokholm-Bjerregaard, M., Nielsen, P., 2020. *Candidatus* Amarolinea and *Candidatus* Microthrix are mainly responsible for filamentous bulking in Danish municipal wastewater treatment plants. Frontier in Microbiology https://doi.org/10.3389/fmicb.2020.01214.

Picard, A., Daniel, I., 2013. Pressure as an environmental parameter for microbial life – a review. Biophys. Chem. 183, 30–41. https://doi.org/10.1016/j.bpc.2013.06.019.

Starke, R., Bastida, F., Abadía, J., García, C., Nicolás, E., Jehmlich, N., 2017. Ecological and functional adaptations to water management in a semiarid agroecosystem: a soil metaproteomics approach. Sci. Rep. 7, 10221. https://doi.org/10.1038/s41598-017-09973-w.

Verma, H., Dhingra, G.G., Sharma, M., Gupta, V., Negi, R.K., Singh, Y., Lal, R., 2020. Comparative genomics of Sphingopyxis spp. unravelled functional attributes. Genomics 112 (2), 1956–1969. https://doi.org/10.1016/j.ygeno.2019.11.008.

Wang, Y., Qian, P.-Y., 2009. Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. PLoS One 4 (10), e7401. https://doi.org/10.1371/journal.pone.0007401.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73 (16), 5261–5267. https://doi.org/10.1128/AEM.00062-07.

Xu, R.X., Li, B., Zhang, Y., Si, L., Zhang, X.Q., Xie, B., 2016. Response of biodegradation characteristics of unacclimated activated sludge to moderate pressure in a batch reactor. Chemosphere 148, 41–46. https://doi.org/10.1016/j.chemosphere.2016.01.018.

Xu, S., Yao, J., Ainiwaer, M., Hong, Y., Zhang, Y., 2018. Analysis of bacterial community structure of activated sludge from wastewater treatment plants in winter. Biomed. Res. Int. 2018, 8278970. https://doi.org/10.1155/2018/8278970.

Yadav, T.C., Khardenavis, A.A., Kapley, A., 2014. Shifts in microbial community in response to dissolved oxygen levels in activated sludge. Bioresour. Technol. 165, 257–264. https://doi.org/10.1016/j.biortech.2014.03.007.

Zakaria, F., Garcia, H.A., Hooijmans, C.M., Brdjanovic, D., 2015. Decision support system for the provision of emergency sanitation. Sci. Total Environ. 512, 645–658. https://doi.org/10.1016/j.scitotenv.2015.01.051.

Zhang, Q., Liu, S., Yang, C., Chen, F., Lu, S., 2014. Bioreactor consisting of pressurized aeration and dissolved air flotation for domestic wastewater treatment. Sep. Purif. Technol. 138, 186–190. https://doi.org/10.1016/j.seppur.2014.10.024.

Zhang, X., Yue, X., Liu, Z., Li, Q., Hua, X., 2015. Impacts of sludge retention time on sludge characteristics and membrane fouling in a submerged anaerobic-oxic membrane bioreactor. Appl. Microbiol. Biotechnol. 99 (11), 4893–4903. https://doi.org/10.1007/s00253-015-6383-x.

Zhang, Y., Li, B., Xu, R.X., Wang, G.X., Zhou, Y., Xie, B., 2016. Effects of pressurized aeration on organic degradation efficiency and bacterial community structure of activated sludge treating saline wastewater. Bioresour. Technol. 222, 182–189. https://doi.org/10.1016/j.biortech.2016.10.005.

Zhang, Y., Jiang, W.-L., Qin, Y., Wang, G.-X., Xu, R.-X., Xie, B., 2017. Dynamic changes of bacterial community in activated sludge with pressurized aeration in a sequencing batch reactor. Water Sci. Technol. 75 (11), 2639–2648. https://doi.org/10.2166/wst.2017.147.