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Long-term performance evaluation of a membrane bioreactor for slaughterhouse wastewater reclamation and reuse

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ABSTRACT

Increasing water scarcity is highlighted as a major threat to human development. Food processing industry, which is a traditional pillar of many economies worldwide, is an important water consumer facing increasing pressure towards new supply strategies. The slaughtering industry, producing large volumes of wastewater characterized by a high content in organic matter and pathogens, stands as one of the most promising and challenging sectors for the implementation of water reclamation technologies. In this sense, membrane bioreactors (MBR) are an emerging strategy for slaughterhouse wastewater (SWW) treatment to achieve treated water quality suitable for water reuse within the food industry. In this study, the performance of a pilot MBR treating SWW has been investigated for 3 different periods accounting for over 600 d of operation, from a singular approach evaluating both technical and regulatory factors determining the technology applicability in an industrial environment for water reclamation and reuse purposes. For the studied parameters, the bioreactor showed an efficient performance at a hydraulic retention time (HRT) above 1.5 d, with removal efficiencies higher than 95 % and 99 % for chemical oxygen demand and ammonium, respectively. At HRT under 1.5 d, the MBR performance was compromised, showing imbalanced microbial populations, partial nitrification driving to nitrite accumulation and increased membrane fouling rate. Regarding the quality of the MBR permeate, the HRT of 2 d was the only one fitting the required parameters for water reuse as process and cleaning water within the food industry, according to the Spanish water reuse regulation in force (RD 1085/2024).

1. Introduction

A fast decrease in freshwater resources quality and availability is currently ongoing parallel to an exponential growth in their demand for urban, industrial and agricultural uses [1,2]. Water scarcity, which is already affecting almost half of the world's population, arises as a major challenge faced by 21st century societies across all continents [3]. Traditional economic activities and means of living are becoming increasingly endangered by this water crisis, which must be addressed through new approaches in sustainable water management and water reuse. Food processing is a particularly water-intensive industry with major significance in many economies worldwide. According to Food Drink Europe [4], the food and beverage industry is the largest manufacturing sector in the EU both in terms of employment and turnover (15.5 % of share), as well as regarding water consumption (34 % of share). Meat processing is the leading industry in the food and beverage sector, accounting for 20 % of the turnover and 32 % of the employment related to the European food industry, as well as almost 30 % of the water use in the sector [5]. Regionally, the economic weight of the sector can be much larger. Spain is the European leader in meat production, with the meat sector representing 24 % of the country's food and beverage industry, which is also the leading industrial activity accounting for 24 % of the industrial turnover and employment [6]. Even further, the slaughtering and meat processing activity is significantly focused on some regions such as Catalonia, concentrating up to 35–40 % of the total Spanish slaughtering activity. Water consumption in this activity can reach from 1.5 m^3 per tonne of pork carcass to 15 m^3 per tonne of beef carcass [7], with the same large amounts of wastewater generated, characterised by a high load in organic matter, nutrients as well as pathogenic microorganisms [8,9]. According to IPPC (2005),

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most of the water consumption in slaughterhouses is related to cleaning activities (up to 35–45 % of the water use), intended to attain the strict sanitary requirements, followed by cooling water (6–12 %), hide removal (7–13 %), vehicle washing (5–7 %) and lairage (3–5 %).

Conventional activated sludge (CAS) process is the most frequent technology for the treatment of slaughterhouse wastewater (SWW), providing operational flexibility and high efficiency for the removal of organic matter and nitrogen, both required for public sewer discharge [10], [11]. However, the CAS process is linked to high sludge production and energy requirements associated to aeration [12]. Moreover, limited efficiency in suspended solids removal in the clarifier keeps important pathogenic risks, preventing the achievement of treated water quality suitable for reuse purposes [10]. Replacing the final settler by a membrane filtration allows a complete separation of the microorganisms and suspended solids from the treated water, leading to exceptional water quality regarding microbiological parameters [13]. Thus, membrane bioreactors (MBR) are rising as the major alternative system for wastewater treatment, combining a compact configuration and enhanced biological treatment and effluent quality. The membrane function for complete retention of the biosolids within the reactor enables superior removal of suspended solids and pathogens, as well as increased control over the concentration of mixed liquor suspended solids (MLSS) and sludge retention time (SRT), which is decoupled from the hydraulic retention time (HRT). Concurrently, more efficient and stable nitrification and organic matter oxidation are achieved even at lower aeration rates and lower sludge production than CAS [11].

The use of MBRs is still limited in the slaughtering sector, as its implementation closely depends on the ability to achieve a water reclamation and reuse system, a practice still marginal and especially sensitive in sectors such as food industry [14]. Increased investment and operation and maintenance costs compared to CAS are some of the major drawbacks hampering a wider implementation of MBRs [15]. Membrane permeability loss due to fouling, naturally occurring and boosted under disturbed operating conditions, is generally recognised as the main factor behind all these obstacles for further membrane technology implementation [16]. Also, a knowledge gap on membrane fouling mechanisms, which are influenced by multiple interdependent factors such as feed and biomass characteristics, module design, hydrodynamic conditions and operational parameters, makes it difficult to standardize MBR operating strategies [17].

In spite of those drawbacks, increasing MBR implementation experiences demonstrate the system reliability for a safe supply of reclaimed water of excellent and stable quality, both in urban and industrial environments [18]. Water reuse is proving to be the most consistent and attractive strategy for water saving in industries, decreasing their dependence and pressure on external and conventional freshwater resources by diversifying supplying sources from increasing circularity of their own processes [7]. Thus, MBRs stand up as the most promising technology for the transition from conventional wastewater treatment to water reclamation for reuse purposes, and the most notable advance in water sustainability in the industries [19,20].

As far as we are aware, there is a significant lack of studies assessing the long-term performance of the MBR technology in an industrial environment for slaughterhouse wastewater reclamation and reuse both from a technical and regulatory perspective. In this study, a long-term operation of an MBR was conducted at pilot scale in an industrial slaughterhouse for wastewater treatment. The work aimed to evaluate the efficiency and resilience of the technology to validate its potential implementation for water reclamation in the slaughtering industry. To this end, the potential reuse of reclaimed water in the slaughterhouse is discussed, considering the potential water uses in the installation and the quality requirements established in the Spanish legislation in force (RD 1085/2024) on water reuse in the food industry.

2. Materials & methods

2.1. MBR configuration

Fig. 1 represents the pilot plant used in this study, including the wastewater pretreatment unit, consisting of a Decanter Unit (DU) and an Air Flotation (AF) unit, the Homogenization Tank (HT) for the pretreated wastewater storage, and the MBR. The pilot plant was installed at MAFRICA S.A., a pig slaughterhouse and meat processing company located in Sant Joan de Vilatorrada (Catalonia, Spain) with a slaughtering capacity of approximately 2000 animals per working day.

The DU consisted of two 1000 L High Density Polyethylene (HDPE) tanks, where a coagulant was dosed and mixed with the screened (<1 mm) SWW, followed by decantation before transferring the supernatant to the AF unit. The AF unit consisted of two 1000 L fibreglass tanks in which the aeration and flotation were performed to remove fats, oils and greases (FOG). Each tank was set with an air diffuser system at the bottom, connected to a compressed air line.

The HT and the MBR consisted of two 1000 L tanks coupled by a peristaltic pump, with 3 and 6 membrane air diffusers, respectively, to provide air and agitation in each tank. The MBR incorporated a submerged ultrafiltration membrane module consisting of hollow fibre membranes (DuPont Products, S.A.) using Polyvinylidene fluoride (PVDF) as material. The membranes had a pore size of 0.04 μm and a total filtration area of 5.5 m^2. The membrane module was connected by a filtration pump both to the permeate storage tank and to the cleaning in place tank. A blower was connected to both the HT, the MBR and the membrane module, being the aeration rate at each line regulated by manual valves. The MBR was equipped with liquid level and pressure sensors and pH, redox and temperature probes.

2.2. Pilot plant operation and monitoring

The wastewater fed to the pilot plant was collected after the 1 mm grid at the entry of the industrial wastewater treatment plant (WWTP). Once per week, the screened wastewater was pumped to a 10,000 L storage tank, from where batches of 1000 L were processed in the pretreatment unit (details in Supplementary material S1). The pretreated wastewater was then transferred to the HT, where an aeration flow rate of 3 $m^3\ h^{-1}$ was maintained for wastewater homogenisation. As only nitrification process was performed in the MBR (only aerobic conditions were established during its continuous operation), sodium bicarbonate was dosed to the pretreated wastewater to achieve an alkalinity to ammonium nitrogen ratio value of 7.1 g $CaCO_3\ g^{-1}$ N-NH_4^+ needed for complete nitrification [21], considering the typical N-NH4+ concentration in the influent SWW as well as its low alkalinity.

A summary of the operational periods and strategies undergone throughout the MBR performance is summarised in Table 1. The MBR operation was divided into three different operational periods (I, II and III), each starting with a new MBR inoculation. Inoculations were done with activated sludge collected from the secondary settler of the municipal urban WWTP at a volatile suspended solids (VSS) concentration of 4–5 g L^{-1}. The concentration of total suspended solids (TSS) in the MBR was controlled through purges under 16 g L^{-1} during period I, and under 12 g L^{-1} during periods II and III.

For each operational period, several stages were defined according to the operational strategies (i.e., HRT). HRTs in the typical range for CAS reactors and MBRs in the slaughtering industry were assessed [22], beginning the MBR start-up at each period at HRT of 3 d, analogous to that used in the CAS reactor implemented in the industrial WWTP. The MBR was operated in continuous mode at a working volume of 750 L. The permeate flux was adjusted to the target operation HRT whereas the feeding was activated depending on the volume of the reactor, monitored by the level sensor. The membrane module was operated in cycles, depending on the HRT set, by adjusting the filtration and relax stages duration. An aeration rate of approximately 6 $m^3\ h^{-1}$ was maintained at

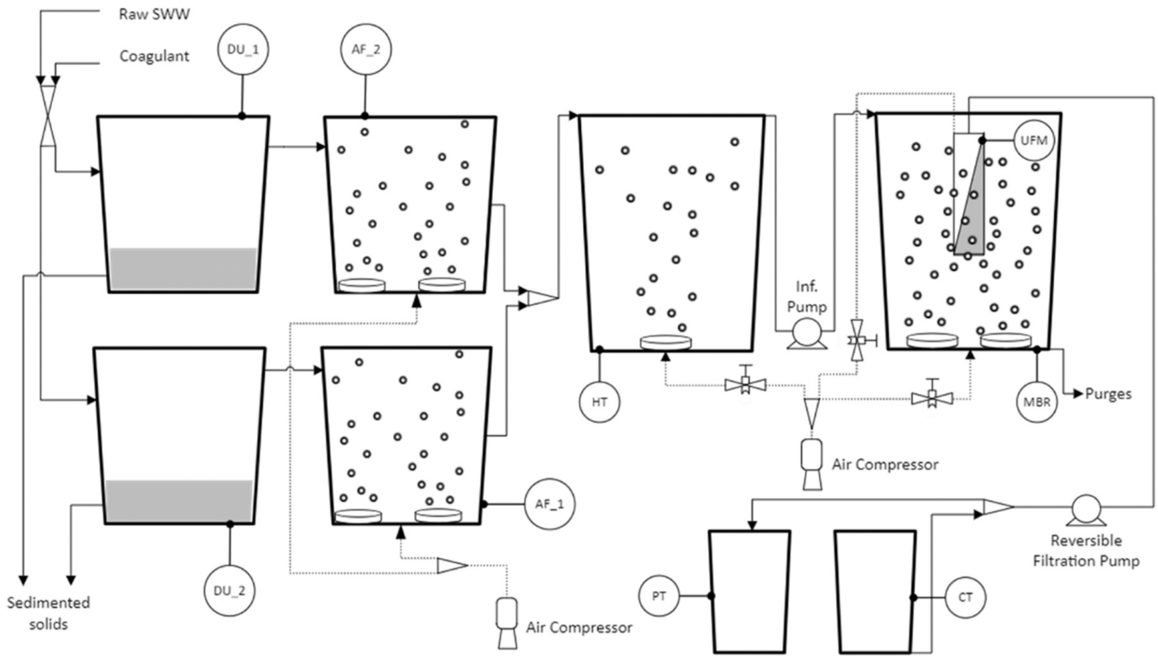

Fig. 1. Schematic diagram of the MBR pilot plant. DU: Decanter Unit; AF: Aeroflotation Unit; HT: Homogenization Tank; MBR: Membrane Bioreactor; UFM: Ultrafiltration module; PT: Permeate Tank; CT: Cleaning in place Tank.

Table 1
Summary of the operational strategies for the pilot MBR.

Year	Months	Operational days	Operational period	Stage	HRT (d)
2022	March-May	1 – 56	I	I-A	3
	May-July	57 – 122		I-B	3
	July-August	123 – 149		I-C	3
	August-September	150 – 196		I-D	2
	September-December	197 – 272		I-E	1.5
	December-February	273 – 323		I-F	1.5
2023	March-May	1 – 62	II	II-A	3
	May-June	63 – 96		II-B	2
	June-July	97 – 118		II-C	1
	July-August	119 – 153		II-D	1.5
	August-September	154 – 183		II-E	2
	September	184 – 192		II-F	1.5
2024	February-March	1 – 39	III	III-A	3
	March-May	40 – 97		III-B	2

the MBR, to ensure aerobic conditions (ORP >300 mV) needed for organic matter oxidation and nitrification processes. An additional 3 m^3 h^{-1} constant air flux connected to the membrane module was maintained for the generation of the air pulses for scouring purposes.

Membrane backwashing, consisting of 2 cycles of 5 min of cleaning solution pumping followed by 15 min of relax each cycle, was conducted at increasing frequency while decreasing HRT (one, two or three times per week for HRT of 3, 2 and <2 d, respectively), in order to mitigate increasing fouling rates at decreasing HRTs. The cleaning solution consisted of a sodium hypochlorite solution at a free chlorine concentration of 200 mg L^{-1}. Following the provider's recommendations, *ex situ* chemical cleaning of the membrane was performed, at least, once every 6 months, with additional chemical cleanings performed when a transmembrane pressure (TMP) threshold value of 750 mbar was reached. For the chemical cleaning, a special backwash was firstly conducted at 10 cycles of 1 min of pumping followed by 5 min of relax. Then, the membrane module was disassembled from the MBR and soaked in hypochlorite solution at free chlorine concentration of 1000 mg L^{-1} for 3–6 hours (depending on the water temperature).

Samples from the MBR inlet, outlet and sludge were collected weekly to monitor the MBR performance. TSS and VSS were measured for inlet and sludge samples. Chemical oxygen demand (COD) and nitrogen (N) species (ammonium (N-NH_4^+), nitrite (N-NO_2^-), and nitrate(N-NO_3^-)) were measured for influent and permeate samples. Turbidity was measured for permeate samples. Several sampling campaigns at different year periods and under different operational conditions were also conducted for pathogens determination at the different streams (inlet, sludge and permeate).

2.3. Biomass activities

Heterotrophic, nitrification and nitrite-oxidation activities of the MBR biomass were determined at each operating stage by means of respirometric tests [23]. Sludge collected from the MBR was washed to remove the remaining COD/NH_4^+/NO_2^- traces before the respirometric tests by means of 3 serial centrifugation and pellet resuspension on phosphate (for heterotrophic tests) or bicarbonate (for nitrification and nitrite-oxidation tests) buffer. The buffers composition is described by López Fiuza et al. [23]. Finally, washed biomass was left on aeration overnight before the respirometric tests for activity maintenance and diluted with tap water to achieve concentrations among 1–2 g VSS L^{-1}. Tests were conducted at room temperature (20–25ºC) within 500 mL Erlenmeyer flasks magnetically stirred, equipped with an aeration system and a dissolved oxygen (DO) probe (HI98193, HANNA Instruments S.L.). Once the sludge is saturated in oxygen, aeration is stopped and DO is measured before (for endogenous activity) and after (for exogenous activity) the addition of sodium acetate (420 mg mL^{-1}), ammonium chloride (300 mg mL^{-1}) or sodium nitrite (100 mg mL^{-1}), for heterotrophic, nitrification and nitrite-oxidation activities, respectively.

Oxygen uptake rate (OUR) was obtained from the DO consumption slope according to Eq. (1) [24] and used for the determination of the specific heterotrophic (SHA), nitrification (SNA) and nitrite oxidation (SNOA) activities following Eqs. (2), (3) and (4), respectively. Stoichiometric oxygen consumption values for ammonium and nitrite oxidation were used for the relation between specific OUR and corresponding activities referred as N consumption.

$$OUR\left(mgO_2 \quad L^{-1} \quad h^{-1}\right) = -\frac{d[O_2]}{dt} \tag{1}$$

$$SHA(mgO_2 \quad gVSS^{-1} \quad h^{-1}) = \frac{OUR_{ex} - OUR_{en}}{VSS} \tag{2}$$

$$SNA\left(mgN - NH_4^+ \quad gVSS^{-1} \quad h^{-1}\right) = \frac{OUR_{ex} - OUR_{en}}{VSS} \; x \; \frac{1molO_2}{32g \quad O_2} \; x \; \frac{1molNH_4^+}{2molO_2} \; x \; \frac{14 \quad g \quad N - NH_4^+}{1molNH_4^+} \tag{3}$$

$$SNOA\left(mgN - NO_2^- \quad gVSS^{-1} \quad h^{-1}\right) = \frac{OUR_{ex} - OUR_{en}}{VSS} \; x \; \frac{1molO_2}{32g \quad O_2} \; x \; \frac{1molNO_2^-}{0.5molO_2} \; x \; \frac{14 \quad g \quad N - NO_2^-}{1molNO_2^-} \tag{4}$$

where VSS is the concentration of volatile suspended solids inside the test flask (in g L^{-1}), and OUR_{en} and OUR_{ex} are the endogenous (before substrate addition) and exogenous (after substrate addition) oxygen uptake rate measured in the flask.

2.4. Microscopic examination

At the end of each operational stage, a sludge sample was collected from the MBR for its fresh examination under microscope (ISCOPE 2060448; Euromex, Duiven, Netherlands) techniques according to Eikelboom [25] procedures. For the examination, the sample was gently shaken before the setting of one drop over a microscope slide. Two different slides were examined for each sample. No tinction nor sample fixation procedures were applied. Slides were systematically examined following a zigzag pattern from one end of the slide to the other, using the 100X objective for sludge overview and flocs description, and 200X objective for identification of specific genera and structures. Along the slide, recorded parameters were shape (rounded or irregular), structure (compact or open), strength (firm or weak), size (small, medium or large) and diversity (low, medium or high) regarding flocs morphology. Besides, relative quantification was done for filamentous bacteria, protozoa (ciliates, flagellates and amoeba), metazoan (rotifer, tardigrade, nematode and worms) and others (algae and fungi), as indicator microbiological criteria for sludge qualitative assessment.

2.5. Microbial community analysis

Sludge samples were collected during the operational period II at the end of each operational stage described in Table 1, to determine the microbial community evolution. Each sample was centrifuged, and the pellet kept at −80°C prior to DNA extraction and analysis. Genomic DNA extraction was performed using a commercial kit (DNeasy PowerSoil, Qiagen) following the manufacturer's instructions and DNA concentration was quantified with Qubit® dsDNA HS Assay Kit and a Qubit Flex fluorometer (Thermo Fisher Scientific).

The phylogenetic composition of the sludge was analysed using 16S rRNA gene amplicon sequencing of the V3-V4 region, using primers 341 F (CCTACGGNGGCWGCAG) and 805 R (GACTACHVGGGTATCTAATCC) [26,27]. Sequencing was conducted by Macrogen (Seoul, Korea) on an Illumina MiSeq platform. Cutadapt (v3.2) was utilized to remove adapter and primer sequences from the raw data and for forward and reverse reads trimming to 250 bp and 200 bp. DADA2 (v1.18.0) was used for error-correction, merging and denoising to generate Amplicon Sequence Variants (ASV) sequences. ASV were aligned to the most similar organism in NCBI_16S Database using BLAST+, and QIIME (v1.9.0) was utilized for downstream analysis.

2.6. Analytical methods

TSS and VSS were determined according to standard methods procedures 2540 D and 2540E [28], respectively. COD was determined using HANNA COD Medium Range Reagent Vials (HI94754B-25), based on the dichromate method (standard methods procedure 5220 C) [28]. Turbidity was measured using a portable turbidity meter (Model HI 98713, HACH). Inorganic nitrogen content (N-NH_4^+, N-NO_2^- and N-NO_3^-) was measured according to standard methods, procedures 4500-NO_3^- A and 4500-NO_2^- A [28] for nitrate and nitrite nitrogen, respectively, and spectrometric method for ammonium [29]. Organic nitrogen (ON) content was calculated by subtracting N-NH_4^+ measured values to Total Kjeldahl Nitrogen (TKN) values, measured in MBR influent and permeate samples according to standard methods procedure 4500-N_{org} B [28]. Total Nitrogen (TN) was also measured using HACH Total Nitrogen High Range Reagent Vials (Laton LCK 338), based on Peroxodisulphate digestion and photometric detection with 2.6-Dimethylphenol [30].

Several sampling campaigns for analysis of pathogens included in the Spanish Royal Decree 1085/2024 [31] for water reuse in the food industry were conducted during the operation of the MBR on influent, permeate, and mixed liquor samples. Pathogens were analysed by an external ISO 17025 accredited laboratory (Calitec Lab S.L.), following procedures UNE-EN ISO 9308–1:2014 for *E. coli* and ISO 11731 for *Legionella spp.*

3. Results & discussion

3.1. Pretreated wastewater characterisation

Details on the optimization of the pretreatment for the screened SWW can be found in Supplementary material S1. Pretreated wastewater entering the MBR was characterised in terms of physicochemical parameters during the whole operation, providing a representative profile for the pollutants load ranges in MBR influent. Table 2 summarises the obtained data grouped by operational stage.

The results showed a large variability in all the analysed physicochemical parameters, not only occurring at different stages or seasons but also between consecutive sampling campaigns. pH and conductivity values were mostly comprised (interquartile range, IQR) between 7.5 – 8.2 and 4900 to 7100 µS cm^{-1}, respectively. pH values above 9 were exceptional, as well as conductivity measurements above 8000 µS cm^{-1}. Regarding organic loading, COD content was mostly found among 1700 to 2600 mg L^{-1}, with several punctual measurements differing up to two times the common values. N-NH_4^+ content was the most variable of the measured parameters, with IQR comprised between 70 and 170 mg L^{-1}. On the other hand, negligible N-NO_2^- and N-NO_3^- concentrations were always measured in the pretreated SWW. Quite variable contents were also found regarding both TSS and VSS. High variability in SWW composition is commonly reported and explained by its dependence on several factors, such as the number, species and feeding of slaughtered animals, industry size and workflow, cleaning strategies and complementary meat processing activities [8,32]. Thus, the obtained values and detected variability are aligned with SWW characterization in the literature, ranging from 500 to 15,000 mg L^{-1} for COD, 20–300 mg L^{-1} for N-NH_4^+ and up to 6400 mg L^{-1} for TSS [8,22].

Seasonal variability regarding organic matter and ammonium content in the pretreated SWW could also be observed. Remarkable seasonal differences in animal characteristics and the composition of their wastes and by-products within the meat processing industry have been described in literature [33], so similar trends could be expected in SWW composition. Specifically, larger and fatter animals result from winter breeding and processing, as well as the highest meat processing activity occurs in these months. Accordingly, lower COD content (from 1600 to 2200 mg L^{-1}) was found in all the periods during the summer months (June to September), compared to mean values from 2100 to 3000 mg

Table 2
Summary of the physicochemical characterization of the pretreated SWW corresponding to each operational stage assessed by the MBR. The ranges represent the maximum and minimum values determined at each stage.

Stage	T (°C)	pH	Conductivity (μS cm^{-1})	COD (mg L^{-1})	$N\text{-}NH_4^+$ (mg L^{-1})	TSS (mg L^{-1})	VSS (% on TSS)
I-A	11–20	6.5–7.2	4032 – 9713	1245 – 4790	33.5–205.5	30–380	86–99
I-B	20–25	6.8 – 8.0	3890 – 8326	1570 – 3165	99.2–198.5	80–300	55–99
I-C	25–28	7.0–7.8	4965 – 7429	1980 – 2642	116.8–203.9	30–320	60–99
I-D	20–28	7.4–8.1	4574 – 8107	2180 – 3850	140.5–262.9	170–860	71–99
I-E	14–24	7.4–8.4	4433 – 8921	1155 – 4205	87.1–276.7	120–1000	58–99
I-F	10–17	7.4–7.8	6461 – 7088	2025 – 3280	69.7–171.1	120–470	75–99
II-A	15–21	6.9–8.7	4814 – 7795	1790 – 3545	26.1–109.2	40–510	57–99
II-B	19–24	7.6–8.1	4631 – 8636	1410 – 2885	42.4–201.8	30–100	28–98
II-C	24–26	7.8–7.9	4108 – 4751	2045 – 2320	88.8–121.1	200–250	81–86
II-D	24–26	8.1–8.9	5274 – 7429	1235 – 1700	81.6–180.2	100–230	54–93
II-E	23–24	7.3–8.8	4904 – 6494	1870 – 2270	78.2–110.6	90–200	83–92
II-F	23–22	8.4–8.9	5245 – 5934	1670 – 1830	70.8–93.8	200–260	68–70
III-A	13–16	8.0–9.1	4337 – 5794	1620 – 2800	92.8–192.7	100–250	83–89
III-B	16–17	8.4–9.2	3614 – 4969	1150 – 1755	74.9–145.3	70–140	84–95

COD L^{-1} for the rest of the year. Even clearer trends could also be observed in $N\text{-}NH_4^+$, which showed marked yearly increases coinciding with the warmest months, reaching values among 100–210 mg $N\text{-}NH_4^+$ L^{-1} at summer months compared to 60–150 mg $N\text{-}NH_4^+$ L^{-1} for the rest of the year.

3.2. MBR performance

3.2.1. Physicochemical parameters

The results on the MBR performance in terms of organic matter and ammonium removal recorded for all the stages among the 3 operational periods are summarized in Table 3. Detailed data on the permeate turbidity values can be found in Table S2. Additionally, the evolution on COD and N concentrations and removal efficiencies during period II (as the most representative operational period) is shown in Fig. 2.

After each inoculation, the biomass needed around 10 d to acclimatise to the new operational conditions established for the MBR. During the start-up period, COD removal was between 75 % and 80 %, reflected by a COD concentration in the permeate around 500 mg L^{-1} (Fig. 2a). After the biomass acclimatisation, high COD removals of 92–97 % were always achieved at almost all the operational periods, even at stage II-C, which operated at the lowest HRT (1 d) and the highest organic loading rate (OLR) of 2.2 g COD L^{-1} d^{-1}. The lowest COD removal efficiency (86 %) was observed for stage II-D (Fig. 2a), which, despite working at an HRT of 1.5 d, showed partial nitrification with $N\text{-}NO_2^-$ accumulation, started at the previous stage when HRT was decreased to 1 d (Fig. 2b). Former COD and nitrite removal efficiencies were only restored after a partial MBR reinoculation (10 % in volume) performed on day 154 (beginning of stage II-E). The results were in line with similar studies with submerged MBRs treating SWW. COD removal efficiencies up to 98 % were achieved by Keskes et al. [11] treating abattoir wastewaters with 2000 mg COD L^{-1} at the influent, working at 20 h of HRT and an OLR of 2.5 g COD L^{-1} d^{-1}. Common COD removal efficiencies between 80 % and 95 % have been reported for MBRs processing pretreated SWW at COD influent of 1000–3000 mg L^{-1}, working at 5 h – 3.2 d of HRT [34,35]. The COD removal efficiency of the MBR showed little affectation by the OLR variations, neither by punctual peaks in COD content in the MBR influent nor by the decreasing HRT. The MBR biomass activity at each stage was usually near the SHA obtained from the respirometric batch tests (Fig. S1a), which would be indicative of near-the-limit working conditions. However, it was found that the SHA shifted to much higher values (from 5.4 to 11.7 mg COD g^{-1} VSS h^{-1}) after the highest OLR applied at stage II-C (Fig. 3a), showing the biomass adaptability to the tested OLRs. On the other hand, both the SHA and the MBR heterotrophic activity decreased again (4.8 mg COD g^{-1} VSS h^{-1} in both cases) at stage II-D when nitrite accumulation was detected (Fig. 2b), which was aligned with the decrease in COD removal observed at the MBR in that stage. All the measured heterotrophic activities were in well accordance with other reported values in MBRs treating municipal wastewater [36,37].

Regarding N species (Fig. 2b), the MBR showed almost complete nitrification for most of the operational stages during the three operational periods. Ammonium concentrations in MBR permeate were found lower than 3 mg $N\text{-}NH_4^+$ L^{-1} for almost all the stages, most of them being under 1 mg $N\text{-}NH_4^+$ L^{-1}, with removal efficiencies mostly above 95 %. Under complete nitrification conditions, TN mass balances between influent and permeate were almost complete, being $N\text{-}NO_3^-$ in the MBR permeate always higher than $N\text{-}NH_4^+$ in the MBR influent and almost the same to TKN, indicating complete nitrification of all the N species, including organic nitrogen. Ammonium removal efficiencies are in line with those reported by Gürel and Büyükgüngör [34], reaching 99 % efficiency working with a submerged MBR treating SWW at an HRT of 3 d.

Similarly to COD removal, MBR showed sufficient nitrification capacity for all the ammonium loading rates (NLR) (up to 130 mg $N\text{-}NH_4^+$ L^{-1} d^{-1} at stage I-E (HRT of 1.5 d)) assessed, including punctual peaks in influent ammonium concentration. Accordingly, SNA found in respirometric batch tests were always found significantly higher (0.65–2.10 mg $N\text{-}NH_4^+$ g^{-1} VSS h^{-1}) than MBR nitrification activities for all the stages (0.1–0.5 mg $N\text{-}NH_4^+$ g^{-1} VSS h^{-1}) (Fig. S1b), and also compared to common values found in literature [36,37]. However, ammonium removal was only compromised at stage II-C, working at the lowest HRT (1 d) and the highest NLR (107 mg $N\text{-}NH_4^+$ L^{-1} d^{-1}) in period II, being up to three times the previous NLR in stage II-B (38 mg $N\text{-}NH_4^+$ L^{-1} d^{-1}). Decreased ammonium removal efficiencies (50–60 %) were observed

Table 3
Summary of the MBR performance regarding organic matter and ammonium removal.

Stage	HRT (d)	COD removal (%)	$N\text{-}NH_4^+$ removal (%)
I-A	3	83 ± 7	N.D.*
I-B	3	75 ± 5	91 ± 8
I-C	3	88 ± 5	> 99
I-D	2	96 ± 1	98 ± 1
I-E	1.5	96 ± 2	95 ± 5
I-F	1.5	92 ± 4	96 ± 4
II-A	3	93 ± 6	> 99
II-B	2	97 ± 1	> 99
II-C	1	92 ± 4	59 ± 39
II-D	1.5	86 ± 6	98 ± 1
II-E	2	96 ± 3	> 99
II-F	1.5	94 ± 1	> 99
III-A	3	92 ± 6	> 99
III-B	2	95 ± 3	98 ± 1

*N.D.: Not determined

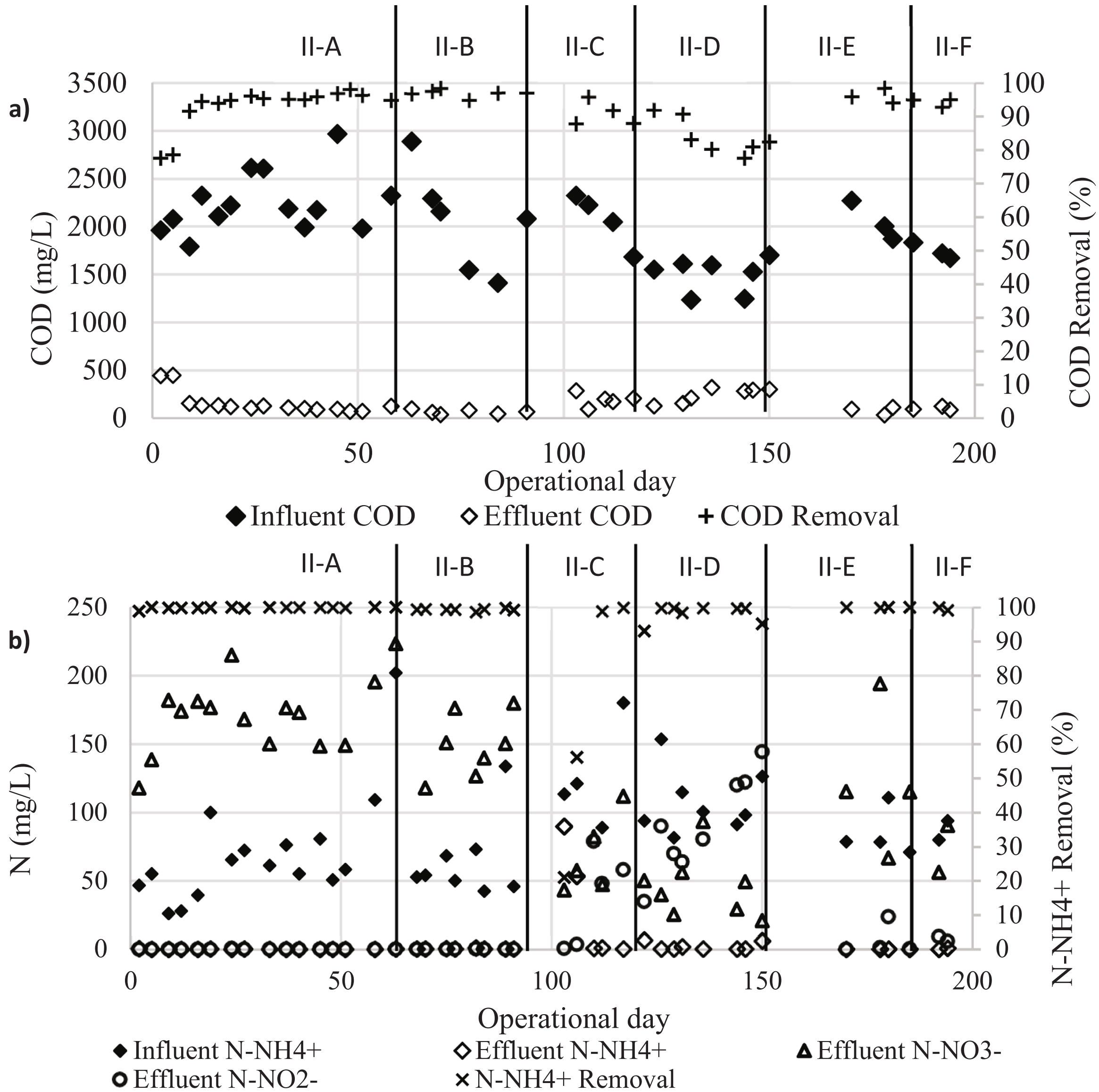

Fig. 2. Evolution of a) COD removal efficiency and COD concentration in MBR influent and effluent, and b) N-NH_4^+ removal efficiency and N-species concentration in MBR influent and effluent during the operational period II.

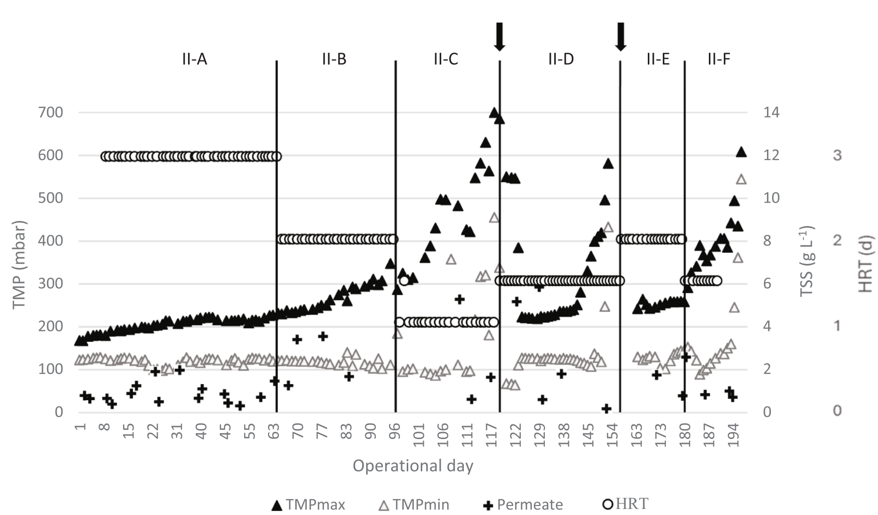

Fig. 3. Time evolution of TMP, TSS and HRT in the MBR during period II. The black arrows indicate the two chemical membrane cleanings performed.

from day 110 until the partial biomass reinoculation (10 % of the MBR volume) at day 154. During the following stage (II-D), ammonium removal efficiencies were variable, reaching > 99 % in some cases, but always remaining more than 50 % of the TN in the permeate under nitrite form, which reached concentrations up to 78 mg $N\text{-}NO_2^-$ L^{-1}. SNOA, measured in following stages, were always found in the range of bibliographic data [37] and higher than SNA (Fig. S1b), indicating no SNOA limitation at those stages. However, much lower SNOA values were found in stage II-E than in period III, reflecting reduced nitrite-oxidation activity at the end of period II. NOB activity and growth rates have been largely described to be detrimentally affected by factors such as moderate concentrations of ammonium and nitrite [38,39], which were conditions experienced in stages II-C and II-D. Together with rapid biomass growth experienced at low HRT and high OLR conditions in those stages, an unbalanced microbial population growth would have resulted in a decrease in the relative abundance (see Section 3.3 Biomass microbial communities) and activity of the NOB populations within the MBR sludge.

3.2.2. Membrane fouling

Fig. 3 shows the evolution of TMP at the beginning (minimum TMP, TMP_{min}) and the end (maximum TMP, TMP_{max}) of each filtration cycle, as well as TSS concentration maintained within the MBR during operational period II. TMP increase slope over time, usually referred as fouling rate (FR, mbar h^{-1}), was also considered as a useful indicator parameter of increased fouling phenomena [40,41]. No significant TMP increase was observed during stages II-A and II-B, working at HRT of 3 d and 2 d, respectively, even despite the significant increase in TSS within the MBR (from 4.8 g L^{-1} to 11 g L^{-1}). Conversely, TMP fastly shot up to maximum allowable values according to the membrane provider (750 mbar) when HRT was decreased to 1 d at stage II-C. TMP values similar to previous stages (<300 mbar) were recovered after a chemical cleaning of the membrane at day 120, followed by HRT increase to 1.5 d. However, after a stable performance from day 120 to day 140, TMP shot up again rising from 250 mbar to > 600 mbar in the next 10 d. After another chemical cleaning at day 150, a similar two-staged profile was observed in following stage II-E, working at HRT of 2 d, with stable values around 250 mbar for 20 d followed by a sharp increase up to 400 mbar. TMP rose again to > 600 mbar at the following stage II-F working at HRT of 1.5 d.

Increased operational fluxes at decreased HRT are also proportionally related to material retention on the membrane, and thus to increased fouling rate. Thus, to minimize fouling issues, working fluxes were settled between 9 and 16 LMH (L m^{-2} h^{-1}) as typical MBR critical fluxes at which FR boosts commonly range between 10 and 40 LMH [41]. Filtration fluxes were maintained quite stable throughout the whole operation, being lower HRTs achieved at higher filtration and lower relaxation times in the filtration cycles, with similar results on increasing FR at decreasing HRT and increasing operation flux. On the other side, physical membrane cleaning (backwashing) was applied at increased frequency for decreased HRTs as a fouling mitigation strategy (one, two and three times per week for HRTs of 3, 2 and <2 d, respectively). Chemical cleanings were minimised in the present work, as suggested to avoid membrane wear, and applied at an increased TMP threshold of 750 mbar. However, a more preventive approach with increased chemical cleaning frequency should also be considered, at least at the lowest HRT, to mitigate increased fouling rates and severe fouling episodes, compromising filtration performance and membrane lifespan [41].

TSS concentration in the MBR is usually regarded as a major factor affecting membrane fouling, but it was not found directly proportional to fouling in this study. Wu and Huang [42] reported decreased sludge filterability at TSS above 10 g L^{-1}, with no correlation between fouling and TSS content at lower levels. Accordingly, TSS were maintained below 10 g L^{-1} in the present work for all the stages except for I-E and I-F (up to 18 g L^{-1} and 16 g L^{-1}, respectively, HRT of 1.5 d), showing severe fouling. OLR, F/M (Food to Microorganism ratio) and SRT are other working parameters commonly reported as major determinants of fouling phenomena, as they are directly related to biomass growth and metabolic stage, which are key factors determining sludge characteristics such as bioflocculation, compressibility or hydrophobicity [43]. Higher fouling rates are usually reported at higher values for OLR and F/M, as well as lower SRT [41]. Under similar water composition, these working conditions are related to decreased values in HRT, which was found the main operational factor related to fouling in the current study, with increased rates at lower HRT (unsustainable under 1.5 d). The FR threshold considered unsustainable depends on the maximum TMP of each system and the cleaning frequency. In the present study, stable TMP profiles at FR under 0.12 mbar h^{-1} were obtained working over 2 d of HRT, whilst FR over 0.5 mbar h^{-1} at lower HRT has led to exponential TMP increase. These results are aligned with literature data summarized by Le-Clech et al.[40], suggesting typical FR between 0.05 and 0.36 mbar h^{-1} driving to permeation time under stable TMP values between 2 and 6 weeks.

3.3. Biomass microbial communities

The success of activated sludge wastewater treatment strictly depends on the composition and functionality of the sludge microbial communities. Therefore, biomass characterisation over the different MBR working conditions provides valuable information on the system stress based on the observed consequences over the biomass communities.

Regarding the microscopic evaluation of the sludge, results on the qualitative description can be found in Table S3. Optical microscopic examination of sludge was assessed for each stage during operational periods II and III, with similar results in both cases regarding the trends and depictions obtained among the different MBR operating conditions. The sewage sludge used as inoculum, coming from an urban WWTP, showed the characteristics of a stable and lowly loaded process, with a well-established middle-aged sludge. Flocs showed a rounded shape, moderately compact structure and medium size, and apparent medium to high bacterial diversity, with moderate presence of filamentous bacteria. At the eukaryotic level, diversity was characterised by a moderately high presence of ciliates, a moderate presence of amoeba and some scarce rotifers. Quality changes were mainly observed at the eukaryotic macrodiversity level, being bacterial floc morphology and filamentous presence quite stable among the different HRTs assessed. However, mainly ciliate presence was dramatically affected by the HRT decrease, being extremely abundant at HRT of 3 d and 2 d and scarce when moving to HRT of 1.5 d. Surprisingly, rotifers' lowest abundance was found at HRT of 2 d, despite their stress tolerance similar to that of ciliates. Amoeba observations slightly decreased at each HRT decrease, whilst flagellates were never observed. Also, several worm observations were done at HRT of 1.5 d, which is indicative of high OLR and incomplete nutrient removal [25]. Low ciliated and rotifera populations in sludge are usually indicative of high F/M driving to decreased DO availability and sludge ageing, which compromises ciliates competitiveness against bacterial communities [25,44]. In addition to their indicator function, they are desired populations in activated sludge as, by their procaryotic predation feeding, their presence is related to enhanced COD, suspended solids and turbidity removal as well as decreased sludge production.

Notable phylogenetic differentiation in microbial communities among the different stages for period II was also found in the metagenomic analysis. The similarity analysis among samples, based on Bray-Curtis distance matrix (Fig. S2), showed the maximum microbial communities similarity being found in stages II-A and II-B, with significant differentiation of the MBR biomass compared with the inoculum, and also another important differentiation among the MBR biomass on that proper-performance stages (II-A and II-B) and after partial nitrification stages (II-D and II-F). Samples differentiation could also be observed

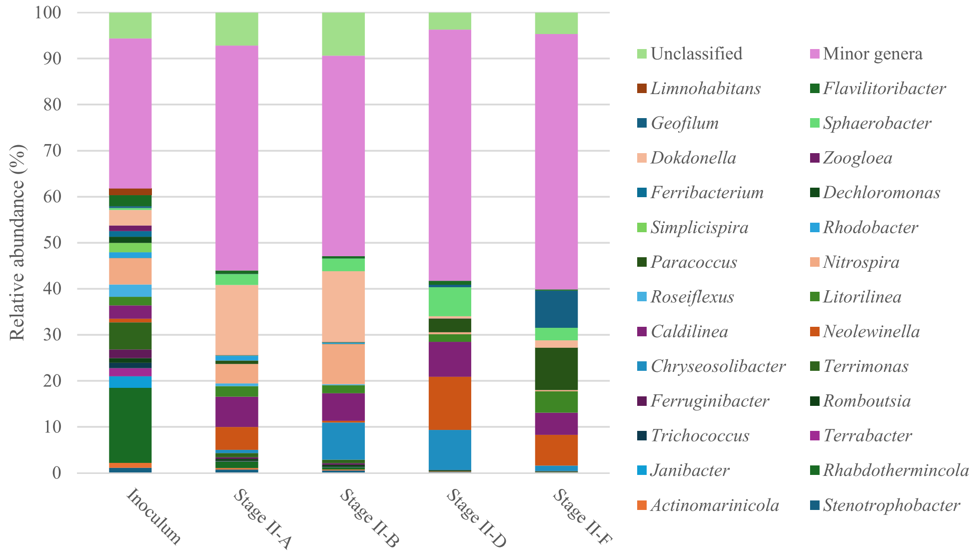

Fig. 4. Relative abundance of bacterial populations (at genus level) within the MBR sludge during operational period II. Genera observed at less than 5 % average abundance were grouped in "Minor genera".

from microbial diversity distribution, represented in Fig. 4 (genus level) and Fig. S3 (phylum and class levels). The main distribution changes were observed for those phyla being the most abundant in all stages. *Pseudomonadota* went from being the dominant group (37–42 %) at higher HRTs (3–2 d) to almost equalizing or even being surpassed (25–35 %) by *Bacteroidota* at HRT of 1.5 d. In the inoculum, these were, respectively, the 2nd and 3rd most abundant phyla, behind *Actinomycetota* (26 % relative abundance), whose relative abundance was greatly reduced (down to 3–5 %) from stage II-A onwards. The results are aligned with common taxonomic distributions found in sewage sludge, where *Pseudomonadota* (36–65 %), *Bacteroidota* (3–16 %) and *Actinomycetota* (1–15 %) are usually the dominant communities [45,46]. Further beyond, the most remarkable change observed was that related to the near disappearance at HRT of 1.5 d of *Nitrospira* spp., which includes the major NOB species in CAS [47]. Microbial populations related to the nitrification process (ammonium oxidation and nitrite oxidation) are usually found among the minor phyla in WWTP sludge. In this study, an abundant NOB population (4–9 % relative abundance) was detected in the inoculum and MBR sludge at stages II-A and II-B (HRT of 3 d and 2 d, respectively), which were characterized by a complete nitrification performance. Conversely, after observing nitrite accumulation in stage II-D (Fig. 2b), the relative abundance of the NOB populations dropped to < 0.5 %. Ammonium and nitrite accumulation conditions detected at stages II-C and II-D, respectively, are known to be detrimental to NOB activity and growth rate [38,39], and thus a likely responsible for an unbalanced growth of those microbial populations (specifically *Nitrospira* spp. as the main NOB species in the bioreactor) compared to a higher growth rate for the other communities at lower HRTs.

3.4. MBR permeate quality

Table 4 summarises the MBR permeate quality in terms of microbiological and physicochemical parameters at working HRTs of 2 and 1.5 d. The results are compared to the required values for water reuse in the food industry, according to the in-force Spanish RD 1085/2024 (Spanish Presidency Ministry, 2024), for both cleaning and process water not in contact with foodstuffs (qualities Ia. and Ia. B/C, respectively).

Water quality parameters required for both intended uses were only continuously achieved at HRT of 2 d. Instead, permeate obtained at HRT of 1.5 d presented maximum measured values for *E. coli* and turbidity incompatible with non-foodstuff-contact cleaning uses (quality Ia. A), but still suitable for process water uses (qualities Ia. B/C). However, the permeate COD and BOD were also found above the allowable values for

Table 4
Comparison of permeate quality parameters with standard limits in the Spanish legislation.

Maximum Allowable Value RD 1085/2024		Permeate values			
		[a] Quality Ia. A	[b] Qualities Ia. B/C	HRT 1.5 d	HRT 2 d
E. coli		10 CFU 100 mL^{-1}	100–1000 CFU 100 mL^{-1}	< 960 CFU 100 mL^{-1}	< 4 CFU 100 mL^{-1}
[c] Legionella sp.		100 CFU L^{-1}	100 CFU L^{-1}	< 100 CFU L^{-1}	< 100 CFU L^{-1}
TSS		10 mg L^{-1}	35 mg L^{-1}	< 10 mg L^{-1}	< 10 mg L^{-1}
Turbidity		5 FNU	[d] N.E.	< 5.8 FNU	< 4.2 FNU
[e] Other pollutants	COD	125 mg L^{-1}	125 mg L^{-1}	[f] < 125 mg L^{-1} [g] < 320 mg L^{-1}	< 120 mg L^{-1}
	BOD_5	25 mg L^{-1}	25 mg L^{-1}	[f] < 25 mg L^{-1} [g] < 64 mg L^{-1}	< 24 mg L^{-1}

[a] Quality Ia. A: Cleaning water not intended to be in contact with foodstuff nor its raw materials, nor with surfaces, objects or materials to be in contact with foodstuff nor its raw materials.
[b] Quality Ia. B/C: Process water for refrigeration, steam or warm water, in closed system not in contact with foodstuff.
[c] According to RD 3/2023 on drinking water.
[d] N.E.: Not established.
[e] According to 91/271/EEC [48].
[f] Under non-observed MBR miss-functioning (nitrite accumulation and/or severe fouling).
[g] Under miss-functioning MBR performance (nitrite accumulation and/or severe fouling).

water reuse with qualities Ia. B/C in several cases at HRT of 1.5 d, mainly under miss-functioning episodes related to nitrite accumulation or interrupted performance due to severe fouling. Thus, despite permeate at HRT of 1.5 d could reach qualities Ia. B/C for process water reuse, suitable MBR performance and permeate quality parameters compliance could not be guaranteed under this HRT.

According to RD 1085/2024, the permeate obtained at HRT of 2 d could not be reused in the food industry for any use in contact with food, unless further permeate post-treatment and drinking water quality is achieved. However, the MBR permeate would be suitable for non-foodstuff-contact activities, which in the slaughtering industry include cleaning water used at outdoor facilities and those areas before livestock sacrifice, as well as process water used in closed systems. According to IPPC IPPC (2005), cleaning water used before livestock sacrifice is mainly related to lairage and livestock trucks cleaning, accounting for 8–13 % of the total water consumption, while another 6–13 % can be attributed to refrigeration/heating purposes. Thus, the reuse of reclaimed water obtained under HRT of 2 d in non-foodstuff-contact activities would enable freshwater savings in the slaughtering industry between 14 % and 26 % of the total water consumption.

4. Conclusions

This study investigated the performance of a pilot MBR with a submerged ultrafiltration membrane for more than 600 d treating SWW. The large data series on SWW characterisation showed some seasonal patterns beyond the large inherent variability in SWW characteristics. Despite the influent high variability, the MBR showed stable performance at HRT of 2 d, reaching permeate quality suitable for non-food-contact water reuse (i.e. outdoor facilities cleaning, process water) in the food industry according to the Spanish regulation (RD 1085/2024). At lower HRT (1–1.5 d), the MBR performance was compromised by most of the followed indicators, i.e. increased fouling rates, imbalanced sludge microbial population, and decreased removal of pathogens and organic matter, as well as partial nitrification. Under these conditions, MBR permeate quality was not guaranteed to comply with the requirements for water reuse in the food industry. Fouling mitigation strategies and mechanisms underlying microbial community drift stemmed as the main issues warranting further research. This study demonstrates from a practical perspective the reliability of the MBR technology for water reclamation of high quality in the food sector, promoting water circularity and decreasing the sector's water footprint and dependency on freshwater by up to 26 %. The joint engagement of industrial and political stakeholders in this study stands also as a key strength, facilitating an effective transfer of the results into both regulatory frameworks and industry practices.

CRediT authorship contribution statement

Bistué-Rovira Miquel: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Cantabella Daniel:** Writing – review & editing, Supervision, Methodology. **Martinez-Quintela Miguel:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Paredes Lidia:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization. **Mejias Laura:** Writing – review & editing, Validation, Supervision, Conceptualization. **Osegueda Oscar:** Supervision, Methodology, Data curation.

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2025.116144.

Data availability

Data will be made available on request.

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