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Sewage sludge minimisation by OSA-MBR: A pilot plant experiment

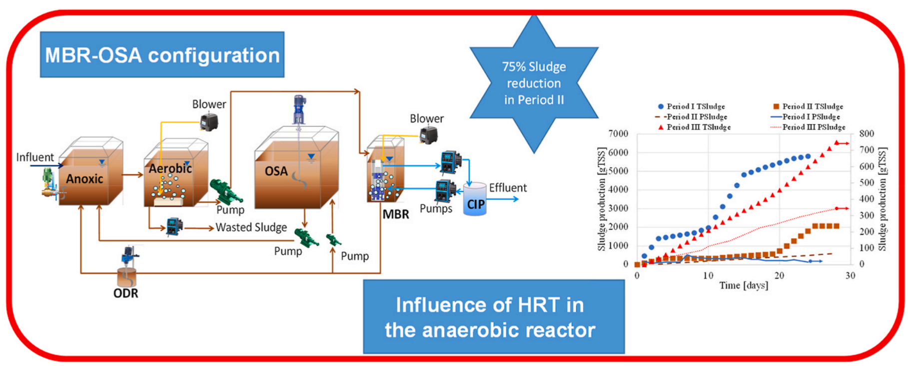
Alida Cosenza, Daniele Di Trapani, Paulo Marcelo Bosco Mofatto, Giorgio Mannina *

Engineering Department, Palermo University, Viale delle Scienze, Ed. 8, 90128, Palermo, Italy

HIGHLIGHTS

- MBR-OSA configuration reduced biological sludge of 75% with HRT of 4 h in ASRR.
- EPS destructuration worsened the sludge settling properties.
- No significant evidence of effluent quality deterioration due to OSA implementation.
- Nitrifiers were affected by prolonged starvation under non aerated conditions.
- The HRT increase in the ASSR promoted a N_2O–N increase inside the unaerated reactors.

GRAPHICAL ABSTRACT



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ABSTRACT

This study presents the excess sludge minimisation in a Membrane Bioreactor (MBR) system by an Oxic Settling Anaerobic (OSA) process. The pilot plant was fed with real wastewater and OSA was operated with two different hydraulic retention times (HRT), respectively 4 (Period II) and 6 h (Period III) and compared to an MBR (Period I). Multiple parameters/variables were monitored: sludge minimisation, nitrogen and carbon removal, membrane fouling, and biokinetic behaviour through respirometry. With respect to the current literature, greenhouse gas emissions were also here monitored, often neglected. Results demonstrated that combining MBR and OSA systems can significantly reduce excess sludge production (89.7%, in Period III and 59.7% in Period II, compared to Period I). However, Period III presented better PO_4–P removal efficiencies but worse performances in the other parameters (COD, NH_4 and Total Nitrogen). No substantial variation in membrane fouling was obtained over the experimental periods. Finally, the HRT increase in the anaerobic reactor promoted a N_2O–N increase inside the unaerated reactors, highlighting the need for a trade-off between sludge minimisation and GHG emission.

1. Introduction

Excess sewage sludge production is a significant economic and environmental concern due to treatment, transportation and final disposal (Collivignarelli et al., 2021). Indeed, managing and disposal of excess sludge can reach 60% of the total plant operation costs. Moreover, in Italy the total amount of sewage sludge was estimated at about 4.3 million tons in 2019 (ISPRA, 2021). Landfilling, incineration and agricultural reuse are the main options for sludge disposal (Mannina et al., 2023). However, such options can create potential risks for human

* Corresponding author.
E-mail address: giorgio.mannina@unipa.it (G. Mannina).

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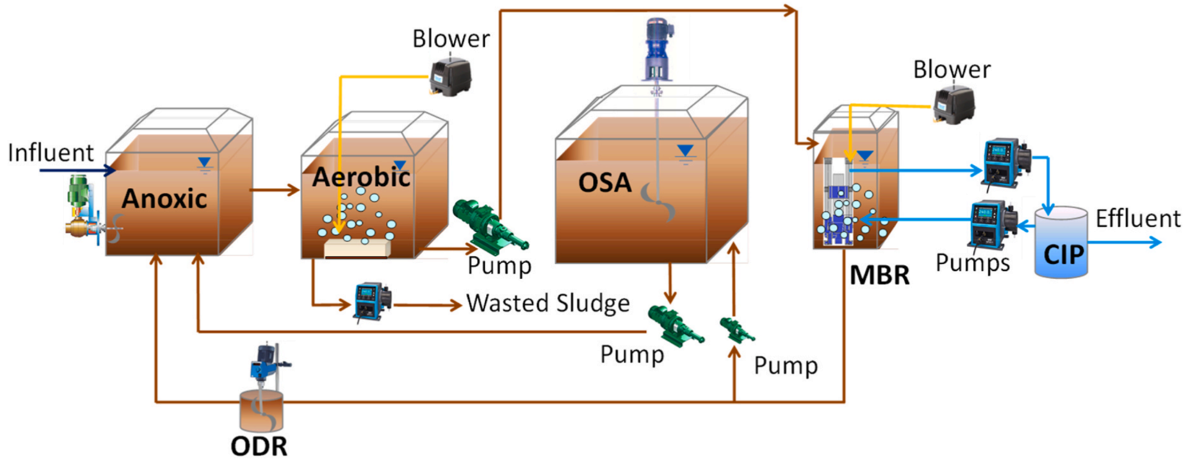

Fig. 1. Schematic layout of the MBR-OSA system.

health and the environment, since they may contain heavy metals, pathogens, and organic contaminants (Collivignarelli et al., 2021). As suggested by Wang et al. (2023) further studies to identify strategies to prevent human health risks of sewage sludge reuse are required in literature.

Nowadays, several technologies have been proposed to minimise the production of excess sludge based on chemical, physical, thermal and biological processes (Ferrentino et al., 2019; Li et al., 2021; Zhang et al., 2021; Cheng et al., 2022). Biological processes are interesting from an environmental point of view, because are more sustainable than chemical processes (Collivignarelli et al., 2021). The oxic-settling-anaerobic (OSA) process represents one of the most potentially cost-effective and low-impact solutions to achieve excess sludge minimisation (Foladori et al., 2010). Moreover, the OSA process can reduce sludge production using the anaerobic reactor and depending on its hydraulic retention time (HRT) (Collivignarelli et al., 2021).

The OSA process consists of the installation of an anaerobic reactor along the sludge recirculation line of a Conventional Activated Sludge (CAS) system (Chudoba et al., 1992). In this process, the total sludge production reduces due to the alternation of aerobic and anaerobic conditions and the absence of exogenous organic sources inside the anaerobic side-stream reactor (ASSR). It stimulates the catabolic activity of the microorganisms (Semblante et al., 2014). Indeed, Sodhi et al. (2020) by comparing CAS, CAS-OSA and Anoxic modified (An) CAS configurations at the lab scale found that the sewage sludge production of CAS-OSA was lower than that of An-CAS (around 21% lower).

In the OSA process, under long ASSR HRTs, many mechanisms can contribute to excess sludge production, such as endogenous decay, extracellular polymeric substance (EPS) destruction, cryptic growth, uncoupling metabolism, etc.) (Wang et al., 2013). The adoption of the OSA process also has the advantage of promoting phosphorus removal from the liquid phase in systems not conceived for this purpose. Indeed, the alternation of anaerobic and aerobic conditions typical of the OSA process is favourable for the growth of phosphorus accumulating organisms (PAOs) (Mannina et al., 2024). Mannina et al. (2024) obtained up to 68% of PO_4–P removal in a CAS – OSA full-scale plant.

However, long-term exposures under anaerobic conditions could worsen the sludge settling properties if simultaneous dissimilatory sulphate reduction occurs (Morello et al., 2022). Furthermore, high HRT under low oxygen availability could impact nitrification, thus altering the fundamental mechanisms of biological nitrogen removal (Loh et al., 2023). The reduction of nitrification efficiency may promote the production/emission of N_2O, which is recognised as a major greenhouse gas (GHG), due to its high global warming potential (GWP) (Mannina et al., 2018).

Several studies have been carried out about the combination of the OSA process with other technologies to improve sludge minimisation, for instance, by using methods such as ultrasound, ozone, chemical uncouplers, etc. (Ye and Li, 2010; Vitanza et al., 2019). Although these technologies cause a significant reduction in excess sludge production (Foladori et al., 2010), they can be quite expensive and detrimental to the effluent quality (Wang et al., 2008).

A combination of a membrane biological reactor (MBR) with the OSA process can be interesting for sludge yield reduction (Fida et al., 2021). Indeed, MBR is an interesting process proposed as an alternative to the conventional activated sludge systems thanks to its capability to achieve higher effluent quality with smaller volumes and a lower amount of excess sludge production (Di Trapani et al., 2018).

According to Pronk et al. (2015), MBR is considered one of the most promising technologies for increasing biological nutrient removal and minimising excess sludge production. Several authors have noted a high reduction in the production of excess sludge using an MBR-OSA combination, such as 64% and 74% by using synthetic wastewater (Morello et al., 2022). In the study by Fida et al. (2021) a sludge reduction of 58% was achieved in an MBR system coupled with side-stream anoxic reactors and fed with synthetic wastewater. However, most of the studies have been carried out using an MBR-OSA pilot plant and fed with synthetic wastewater and very few use real wastewater (Vitanza et al., 2019).

In this light, the present study aims to evaluate the sludge minimisation plant by adding an ASRR in the sludge return line, thus realising an OSA configuration using real wastewater from the University of Palermo's Campus. Sludge minimisation, nitrogen and carbon removal, membrane fouling tendency, biokinetic parameters and nitrous oxide production/emission were assessed. Three experimental periods were investigated: Period I, by using an MBR reactor; and Periods II and III, by using an MBR-OSA reactor with two different HRTs of ASRR, 4 and 6 h. The novelty of this work, compared with previous studies relies on the use of real wastewater instead of synthetic and the assessment of GHG emissions from the system. The achieved results could provide useful insights in view of the potential application of MBR-OSA systems at full scale.

2. Material and methods

2.1. The pilot plant

An MBR pilot plant (Fig. 1) was realised at the Water Resource Recovery Facility of Palermo University (Mannina et al., 2021). The initial MBR configuration was conceived for carbon and nitrogen removal using a pre-denitrification scheme. It was subsequently modified by adding an ASSR in the sludge return line, thus realising the OSA

Table 1
Average values of the main influent and operational features for each experimental period; SD = Standard Deviation.

Parameter	Symbol	Units	Period I		Period II		Period III	
			Average	SD	Average	SD	Average	SD
Total COD	TCOD	[mg L^{-1}]	1964	914	1352	175	1152	207
Soluble COD	sCOD	[mg L^{-1}]	420	141	213	74	142	46
Biochemical oxygen demand	BOD	[mg L^{-1}]	473	67	305	95	314	100
Total suspended solids	TSS	[mg L^{-1}]	718	300	550	100	540	200
BOD/COD ratio	BOD/COD	[–]	0.24	0.1	0.22	0.1	0.27	0.07
Total Nitrogen	TN	[mg L^{-1}]	42	8	38	6	29	3
Ammonium	NH_4–N	[mg L^{-1}]	34	5	32	4	27	3
Phosphate	PO_4–P	[mg L^{-1}]	12	2	14	3	9	3
Flow Rate	Q_{IN}	[L h^{-1}]	16.5	0.4	17.5	2.5	17.9	0
Hydraulic retention time	HRT	[h]	26	0.4	25	3	24	0
Experimental duration	–	[d]	25	–	28	–	28	–
Average Temperature	T	[°C]	24	3	27	2	29	1
Sludge Retention Time	SRT	[d]	18	9	41	24	53	18

configuration. The pilot plant was characterised by one anoxic reactor (V = 110 L) and one aerobic reactor (V = 240 L) followed by a membrane bioreactor (V = 48 L) with an ultrafiltration hollow fibres membrane module (0.03 μm nominal pore size, 1.4 m^2 membrane area) (Fig. 1). The membrane reactor was equipped with a clean-in-place (CIP) system for ordinary backwashing (Fig. 1). An oxygen depletion reactor (ODR) (V = 53 L) was inserted in the internal recycling line between the aerobic and anoxic reactors to reduce the mass of dissolved oxygen before entering the anoxic reactor (Fig. 1). The liquid volume of the ASSR inserted in the RAS line varied based on the established HRT (V = 175 L for HRT = 4 h; and V = 275 L for HRT = 6 h).

Real wastewater collected from the student dormitory and canteen of the campus of Palermo University was used to feed the pilot plant.

The average influent flow rate was 17.3 L h^{-1} (Q_{in}). An 80 L h^{-1} flow rate (Q_{R1}) of mixed liquor was pumped from the aerobic to the MBR compartment, whilst an RAS flow rate equal to 62 L h^{-1} (Q_{RAS}) was recycled from the MBR to the anoxic reactor through the ODR. With the OSA configuration implementation, 45% of the RAS flow rate from the MBR was pumped to the ASSR reactor and further pumped to the anoxic compartment, depending on the HRT in the ASSR reactor (4 or 6 h). The remaining 55% was directly recycled from the MBR to the anoxic reactor through the ODR. Sludge wasting operations were carried out from the aerobic reactor by means of a peristaltic pump, whose flow rate was periodically modified based on the TSS concentration in the system.

2.2. The experimental campaign and analytical methods

The experimental campaign was divided into three periods, namely Period I, Period II and Period III, respectively. In Period I (25 days) the plant was operated according to a MBR configuration. In Period II (28 days), an MBR-OSA configuration was realised by inserting an ASSR reactor in the RAS line, the latter characterised by an HRT of 4 h. Finally, in Period III (28 days), the plant layout was the same as the previous Period II, but the HRT of the ASSR was increased to 6 h. It is important to stress that the switch from one period to the next was carried out based on system performance regarding pollutant removal efficiency, rather than on the convention to wait a period equal to 3 times the sludge retention time (SRT) value.

The operational parameters, such as DO, pH and oxidation-reduction potential (ORP), were monitored daily using specific probes connected to a multimeter (WTW 3340). Specifically, the following probes were used: SenTix® 940-3 (pH), FDO® 925-3 (DO), and SenTix® ORP-T 900 (ORP).

Moreover, the following parameters were measured according to Standard Methods (APHA, 2012) two times per week: chemical oxygen demand (COD), ammonia nitrogen (NH_4^+-N), nitrate (NO_3^--N), nitrite (NO_2^--N), orthophosphate (PO_4^{-3}-P), total suspended solid (TSS) and volatile suspended solid (VSS) concentrations, biological oxygen demand (BOD) and Total Nitrogen (TN). Sludge settling properties were assessed by the sludge volume index (SVI). The extracellular polymeric substances (EPS) and the soluble microbial products (SMP) were extracted according to the two-step extraction method reported in the literature (Le-Clech et al., 2006) and following characterised of proteins (Lowry et al., 1951) and carbohydrates (DuBois et al., 1956) content. The total suspended solids concentration was measured every day. Membrane fouling has been monitored by measuring the total membrane filtration resistance (R_T) according to Judd (2011).

2.3. Respirometric tests for assessing autotrophic and heterotrophic biomass kinetics

Periodic respirometric batch tests assessed stoichiometric and kinetic parameters of suspended biomass at a standard temperature of 20 °C (Mannina et al., 2017). Specifically, the endogenous decay coefficient (b_H), the maximum growth rate (μ_H), the maximum yield coefficient (Y_H) and the active fraction of the heterotrophic biomass (f_{XH}), as well as the maximum growth rate (μ_A) and the maximum yield coefficient (Y_A) of the autotrophic biomass were carried according to literature (Di Trapani et al., 2018). The respirometric assays were performed by measuring the oxygen utilisation rate (OUR) for the consumption of a readily biodegradable substrate (e.g., acetate for heterotrophic and ammonium chloride for autotrophic bacteria).

2.4. Excess sludge production quantification

The daily excess sludge production (ΔX) [kgSS d^{-1}] was evaluated by summing the amount of TSS removed from the system with the wasted sludge and the samples. The amount of TSS removed with the treated water was neglected since the membrane guaranteed the absence of TSS in the permeate. ΔX accounts for both settleable suspended solids in the influent wastewater and secondary sludge (biological). Settleable suspended solids in the influent wastewater were measured. At the same time, secondary sludge was calculated as the difference between ΔX and settleable suspended solids in the influent wastewater.

The observed yield coefficient (Y_{obs}) was calculated according to Equation (1) by dividing the cumulative mass of TSS produced by the cumulative mass of COD removed (Gardoni et al., 2011).

$$Y_{obs} = \frac{\Delta X}{Q_i \bullet (TCOD_{in} - TCOD_{out})} \left(gTSSgCOD^{-1}\right) \tag{1}$$

where $TCOD_{in}$ and $TCOD_{out}$ are the COD concentrations in the influent and effluent (gCOS L^{-1}), respectively. Q_i is the daily influent flow rate (L d^{-1}) and ΔX is the daily excess sludge production (gTSS d^{-1}).

The observed yield coefficient corrected with respect to the standard temperature of 20 °C ($Y_{obs,20}$) was calculated according to Vitanza et al. (2019) (eq. (2)).

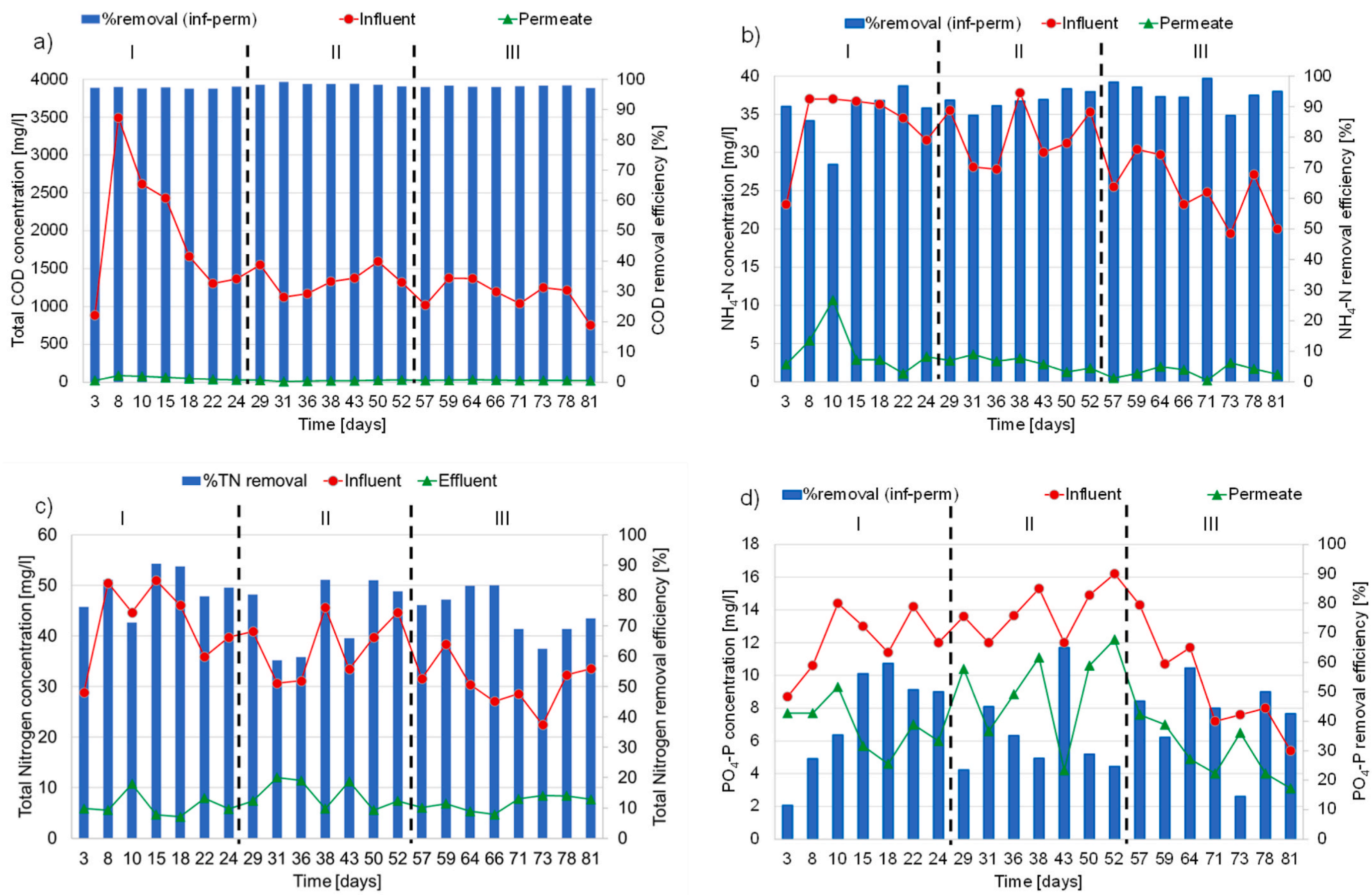

Fig. 2. Influent and effluent concentrations, as well as the removal efficiencies during experiments, of COD (Fig. 2a), ammonia (Fig. 2b), total Nitrogen (Fig. 2c) and phosphorus (Fig. 2d).

Table 2
Average values of total suspended solid concentrations in the reactors throughout experiments.

Section	Period I [gTSS L^{-1}]		Period II [gTSS L^{-1}]		Period III [gTSS L^{-1}]	
	Average	SD	Average	SD	Average	SD
Anoxic	6.02	2.21	2.59	0.65	2.92	0.82
Aerobic	5.83	2.01	2.72	0.73	2.93	0.77
OSA	–	–	3.11	0.75	3.49	0.89
MBR	8.23	3.75	4.02	0.74	3.96	0.97

$$Y_{obs\ T} = Y_{obs,20} * \theta^{(20-T)}\left(gTSSgCOD^{-1}\right) \tag{2}$$

where T = temperature (°C), and θ = 1.029 (–).

2.5. Greenhouse gas measurement

Dissolved and gaseous N_2O concentration was measured according to the procedure described by Mannina et al. (2018) by using a Gas Chromatograph (GC) (Thermo Scientific™ TRACE GC) equipped with an Electron Capture Detector (ECD).

The N_2O emission factor (EF_{N2O}) was calculated according to Equation (3) (Tsuneda et al., 2005).

$$EF_{N2O} = \frac{N_2O - N_g/HRT_{hs} + N_2O - N_d/HRT}{TN} \tag{3}$$

where N_2O-N_g and N_2O–N_d represent the nitrous oxide gaseous and dissolved concentration respectively, HRT is the plant hydraulic retention time, HRT_{hs} is the tank headspace hydraulic retention time and TN is the influent total nitrogen concentration.

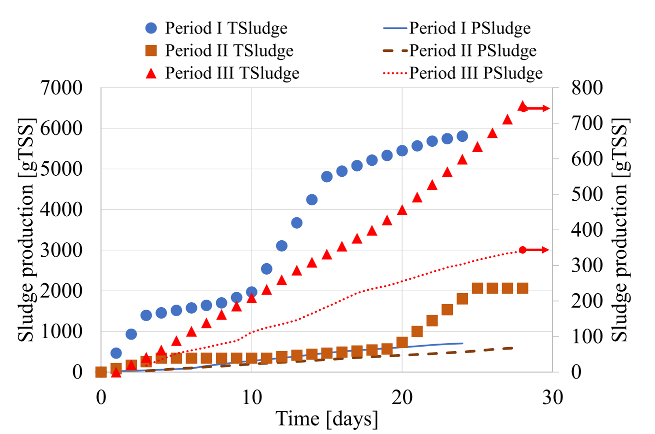

Fig. 3. Cumulative total sludge (TSludge) and settleable solids sludge (PSludge) production during each experimental period.

3. Results and discussion

3.1. Wastewater features and main operational conditions

Table 1 summarises the average features of the influent wastewater fed to the pilot plant and the operational conditions for each experimental period. Data reported in Table 1 show a variability of the real

Table 3
Values of observed biomass yield coefficient at the environment temperature ($Y_{obs,T}$) and 20 °C ($Y_{obs,20}$) and percentage of total sludge and biological sludge reduction for experimental Periods I, II and III.

	Period		
	I	II	III
$Y_{obs,T}$ [gTSS/gCOD]	0.22	0.20	0.17
$Y_{obs,20}$ [gTSS/gCOD]	0.26	0.25	0.21
T [°C]	24	27	29
Sludge reduction [%]	–	64	87
Biological sludge [%]	–	75	64

Table 4
Specific concentrations of SMP and EPS in each period for SMP and EPS for proteins and carbohydrates.

	Period I	Period II	Period III
SMP_P [mg/gTSS]	1.5	0.9	0.7
SMP_C [mg/gTSS]	1.8	2	2
EPS_P [mg/gTSS]	104.6	80.2	78.1
EPS_C [mg/gTSS]	10.2	7.8	6
SVI [mL/gTSS]	118	125	153

Table 5
Summary of the average values of the main heterotrophic kinetic and stoichiometric parameters (in brackets the standard deviation values).

Parameter	Symbol	Units	Heterotrophic		
			Period I	Period II	Period III
			MBR	MBR-OSA	MBR-OSA
Max. growth yield	Y_H	[gVSS g^{-1}COD]	0.37 (±0.02)	0.40 (±0.01)	0.37 (±0.04)
Decay rate	b_H	[d^{-1}]	0.53 (±0.06)	0.62 (±0.02)	0.77 (±0.05)
Max. growth rate	μ_H	[d^{-1}]	2.72 (±0.39)	2.27 (±0.61)	2.33 (±0.42)
Max. removal rate	ν_H	[d^{-1}]	7.38 (±1.23)	4.59 (±1.63)	6.46 (±1.87)
Net growth rate	μ_H- b_H	[d^{-1}]	2.25 (±0.37)	1.67 (±0.63)	1.55 (±0.39)
Active fraction	f_X	[%]	23.64 (±3.10)	30.51 (±4.96)	21.41 (±7.42)
Parameter	**Symbol**	**Units**	**Autotrophic**		
			Period I	**Period II**	**Period III**
			MBR	MBR-OSA	MBR-OSA
Max. growth yield	Y_A	[gVSS $g^{-1}NH_4$–N]	0.14 (±0.01)	0.16 (±0.01)	0.11 (±0.02)
Decay rate	b_A	[d^{-1}]	0.11 (±0.02)	0.14 (±0.01)	0.12 (±0.01)
Max. growth rate	μ_A	[d^{-1}]	0.14 (±0.01)	0.36 (±0.21)	0.25 (±0.04)
Max. removal rate	ν_A	[d^{-1}]	1.17 (±0.01)	2.21 (±1.07)	2.69 (±0.40)
Nitrification rate	N_R	[mgNH$_4$ L^{-1} h^{-1}]	3.03 (±0.02)	3.10 (±1.68)	5.95 (±0.76)

influent wastewater in terms of TCOD mainly during Period I. This variability is likely due to the TSS contribution, which increased the particulate organic matter. Indeed, the average TCOD was 1964 mg/L, 1352 mg/L and 1152 mg/L during Period I, II and II respectively. For the other compounds, the treated wastewater can be considered typical civil wastewater (Metcalf, 2015). In terms of soluble COD/total COD ratio, it was a general decreasing trend due to normal variations in the real wastewater features, depending on the students' habits. This aspect could have a certain impact on the system performance, but it was not feasible for the operation of two systems in parallel, one serving as control. Nevertheless, in view of dampening the peak concentration a slight influent flow rate (Q_{IN}) correction was applied. Indeed, during Period I the average Q_{IN} value was 16.9 L h^{-1}; this value increased to 17.5 L h^{-1} and 17.9 L h^{-1} during Periods I and II, respectively (Table 1). In this way, the F/M ratio over the three experimental periods was maintained almost constant and equal to 0.3 kgBOD/kg TSS day. The total HRT was kept almost constant over the three experimental periods ranging between 24 and 26 h (Table 1). Since it was decided to maintain almost constant the TSS concentrations in the biological reactors, the SRT values varied consequently depending on biomass growth.

3.2. Nutrient removal performance

The results reported in Fig. 2 show the trend of the influent and effluent nutrient concentrations as well as the removal efficiencies during experiments, referring in particular to COD (Fig. 2a), ammonia (Fig. 2b), total nitrogen (Fig. 2c) and phosphorus (Fig. 2d).

In Fig. 2, a decreasing trend of all the nutrients in the influent, mainly noticed on Total COD, can be observed due to the number of students that use the campus. Period III was carried out in July when most of the students were on vacation.

Concerning the COD trend, in Period I, the average influent Total COD concentration was higher than in the subsequent periods (1964 mgCOD L^{-1} ± 915, 1352 mgCOD L^{-1} ± 175 and 1152 mgCOD L^{-1} ± 207, respectively in Period I, II and III. Despite this significant variation in the COD concentrations, the system showed similar performances throughout experiments (between 98.3% ± 0.4 and 97.0% ± 0.6 from Period I to Period III), demonstrating that the variation of the system layout, with the implementation of OSA configuration, did not significantly affect the system performance in terms of COD removal efficiency. This result is well in line with what was highlighted in previous literature studies, where no significant effects on organic matter removal were observed after the implementation of the OSA process (Vitanza et al., 2019; Fida et al., 2021).

Data reported in Fig. 2b showed that implementing the MBR-OSA configuration did not promote deterioration of nitrification; indeed, an average increase of nitrification efficiency from Period I to Period II (76% and 84% for Period I and Period II, respectively) was observed.

Data reported in Fig. 2c showed a decrease in the average nitrogen removal (82% ± 7 in Period I; 73% ± 12 in Period II and 74 % ± 7 in Period III) after implementing the OSA configuration (Periods II and III). This result was mainly due to the worsening of denitrification after the implementation of ASRR in the recycling line due to the decrease of influent carbon load. Nevertheless, the HRT increase in the ASRR reactor (from 4 h to 6 h) did not produce a further deterioration of TN removal.

Regarding PO_4^{3-}-P (Fig. 2d) no significant improvements in the removal efficiency were observed during OSA operation. Indeed, while in Period I the average PO_4^{3-}-P removal efficiency was equal to 41% in Period III it was close to 42%. These results are not by previous literature that suggests an improvement of phosphate accumulating organisms (PAOs) or denitrifying phosphate accumulating organisms (DPAOs) under OSA operation, due to the alternation of aerobic/anaerobic conditions (Fazelipour et al., 2021). However, the HRT values in the anaerobic reactor (4 and 6) of the present study could be insufficient to promote a significant growth of PAOs or DPAOs organisms. Indeed, previous studies highlighted a substantial increase in P removal when the HRT in the anaerobic reactor was equal to 12 h (Martins et al., 2020). Therefore, in the present study P removal could be likely ascribable to metabolic consumption.

3.3. Excess sludge production

Table 2 summarises the average TSS concentration inside each reactor and the standard deviation during the three experimental periods. As summarised in Table 2, during Period I, the average TSS

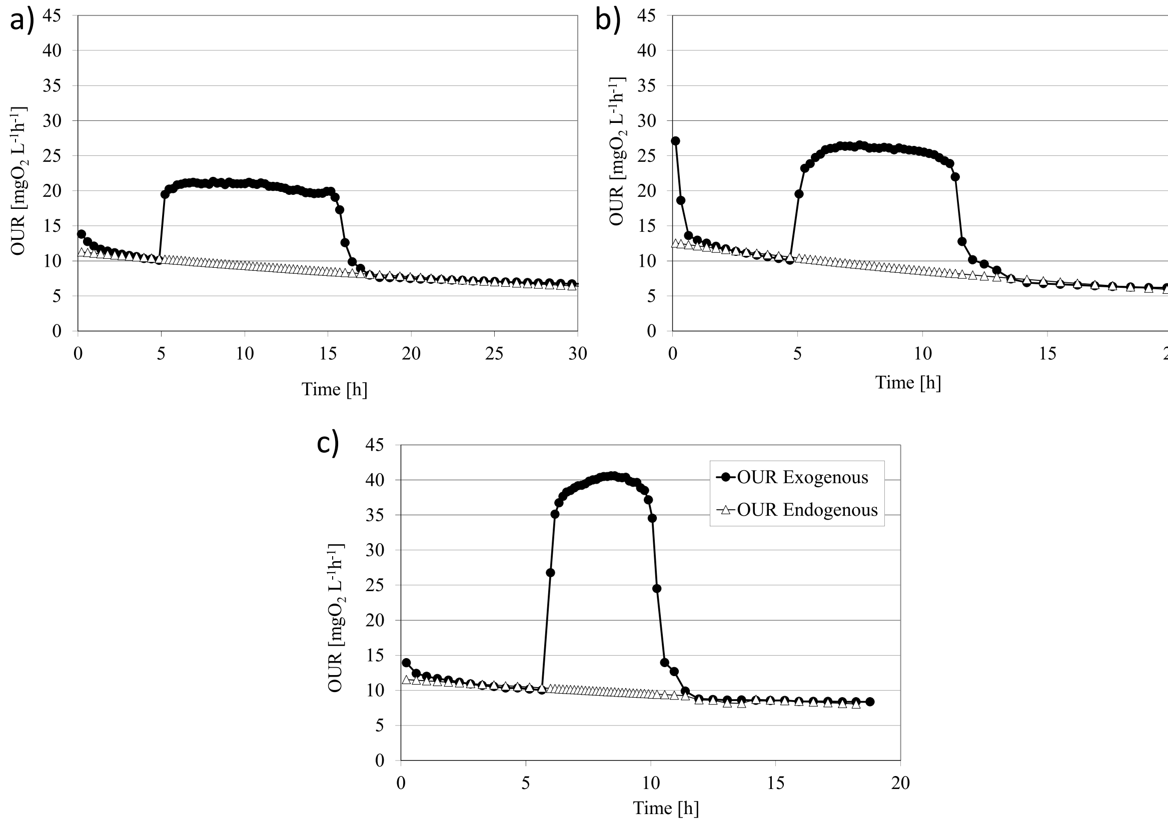

Fig. 4. Profile of respirogram chart for autotrophic species in Period I (a), Period II (b) and Period III (c), respectively.

concentration inside the tanks (6.02 gTSS L^{-1} - anoxic, 5.83 gTSS L^{-1} - aerobic and 8.23 gTSS L^{-1} – MBR) was higher than that of the other two experimental periods; this was due to the highest influent TSS concentration during this period. During the other two experimental periods, the average TSS concentration inside each tank was maintained almost constant (Table 2).

Fig. 3 shows the trend of cumulative total (TSludge) and settleable solids sludge (PSludge) produced during each experimental Period. In Table 3, the values of the observed biomass yield coefficient at the environment temperature ($Y_{obs,T}$) and at 20 °C ($Y_{obs,20}$) and the percentage of total sludge and biological sludge reduction for experimental Periods I, II and III are summarised.

Despite it being often neglected in the literature, it is imperative to discriminate settleable and biological sludge in an MBR system to evaluate the better effect of inserting the ASRR in the RAS line since all the solids are retained inside the system (Guo et al., 2020). The biological sludge has been calculated here as the difference between TSludge and PSludge. Data in Fig. 3 show a progressive decrease in sludge production (total and biological) from Period I to Period III. The TSludge production at the end of Period I was 5804 gTSS day^{-1} (as cumulative value) (15.78 gTSS day^{-1} as PSludge and 5788 gTSS day^{-1} as biological). The insertion of ASRR leads to the reduction of sludge production. More in detail, when the ASRR was operated under HRT of 4 h (Period II), 2066 gTSS day^{-1} of sludge was produced (as cumulative value) (600 gTSS day^{-1} as PSludge and 1466 gTSS day^{-1} as biological). When the ASRR was operated under HRT of 6 h (Period III) 750 gTSS day^{-1} of sludge was produced (as cumulative value) (340 gTSS day^{-1} as PSludge and 410 gTSS day^{-1} as biological). Moreover, the percentage of settleable solids sludge in the three periods is quite different. More in detail, in Period I the settleable solids sludge accounted for 0.27%. In contrast, in Period II and Period III the settleable solids sludge accounted for 30% and 45% of the total sludge, respectively, thus suggesting that the insertion of ASRR strongly influenced the biological growth of biomass and, consequently the biological amount of sludge. Moreover, sludge production was strongly influenced by the ASRR HRT. Under 4 h HRT operation of the ASRR (Period II), the highest reduction of biological sludge production (75%) took place (Table 3). While, under 6 h HRT operation (Period III) of the ASRR the biological sludge production reduced by 64% compared to Period I (Table 3).

The results discussed above were corroborated by the calculated observed yield values, both at the environment and 20 °C temperature ($Y_{obs,T}$ and $Y_{obs,20}$, respectively) as reported in Table 3. From Period I to Period II a slight reduction of $Y_{obs,T}$ took place (from 0.22 to 0.20 gTSS $gCOD^{-1}$). A substantial reduction occurred in Period III. Indeed, data from Table 3 suggest that $Y_{obs,T}$ decreased from 0.22 to 0.17 gTSS $gCOD^{-1}$ from Period I to Period III. While, $Y_{obs,20}$ decreased from 0.26 to 0.21 gTSS $gCOD^{-1}$ from Period I to Period III (Table 3). The observed yield values are in agreement with the order of magnitude of previous MBR (Wang et al., 2013) and MBR-OSA literature. As an example, Fida et al. (2021), by varying the sludge interchange rate towards the anaerobic reactor, obtained Y_{obs} values ranging between 0.28 and 0.1 gTSS $gCOD^{-1}$.

3.4. EPS and settling properties

Even though in a MBR system, the sludge settleability properties could be considered negligible, SVI values, coupled with EPS could provide useful information on the floc structure and consequently on the effect of operational conditions. Table 4 summarises the average values of EPS and SMP (both proteins and carbohydrates) and SVI for each experimental period. Data summarised in Table 4 show a worsening of the sludge settleability properties from Period I to Period III as suggested by the increase of SVI from 118 to 153 mL $gTSS^{-1}$. This result was likely due to a floc destructuration as suggested by the decrease in EPS values. Indeed, both protein and carbohydrates fractions decreased from Period I to Period III. Specifically, the EPS decreasing trend was mostly due to

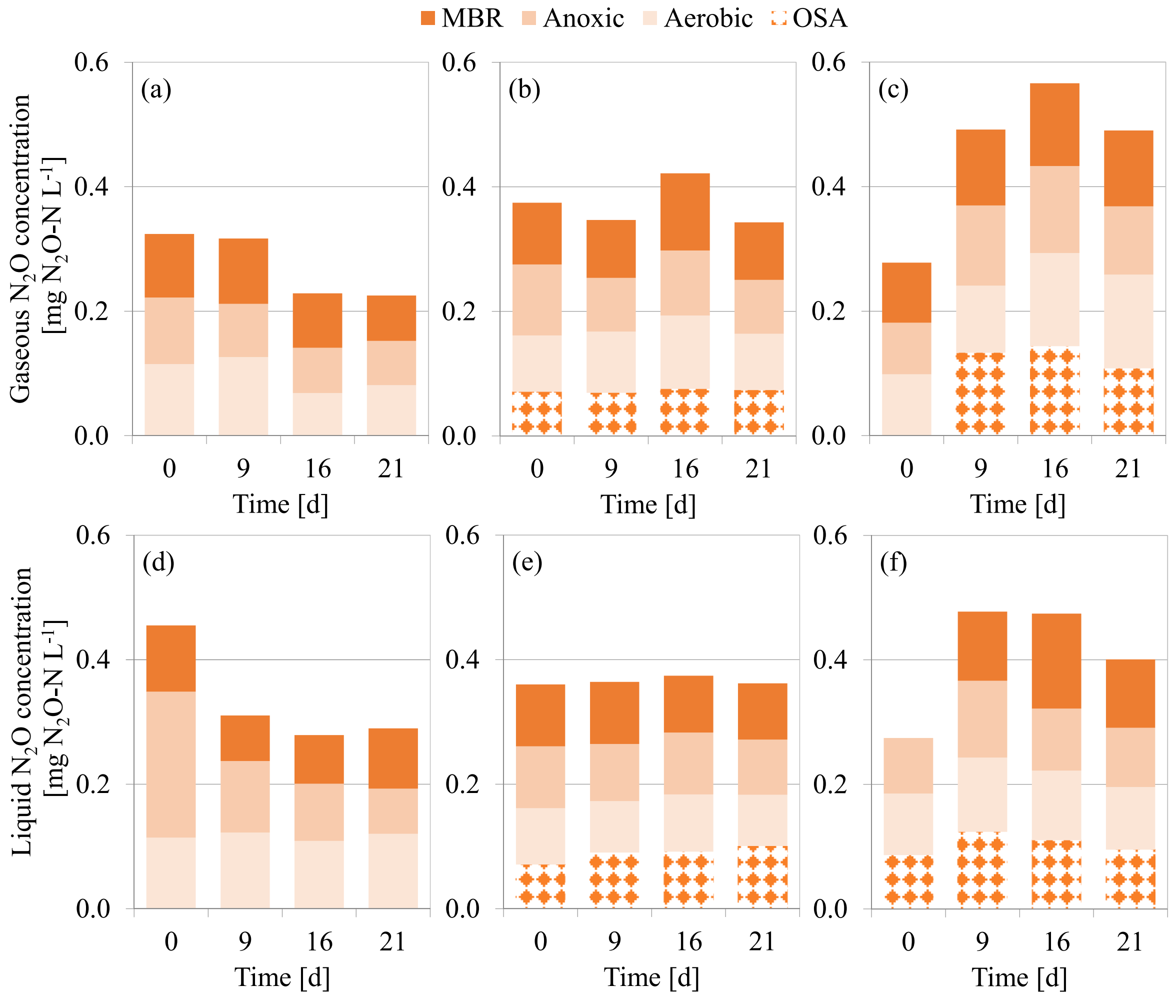

Fig. 5. The pattern of gaseous N_2O–N concentration inside each reactor in Period I (a), Period II (b) and Period (III) (c); pattern of liquid (dissolved) N_2O–N concentration inside each reactor in Period I (d), Period II (e) and Period (III) (f).

the EPS proteins compound (EPS_P) variation (from 104.6 mg $gTSS^{-1}$ in Period I to 78.1 mg $gTSS^{-1}$ in Period III), which represents the glue for the sludge flocs (Huang et al., 2022). Therefore, according to the literature the increase of anaerobic exposure duration (obtained with the addition of HRT within the ASRR) could lead to a floc destructuration (Fida et al., 2021), thus worsening the sludge settleability. Since no substantial variation in total SMP took place, the average R_T values over the experimental periods were comparable. Indeed, the average R_T value was equal to 1.20 10^{13} m^{-1} during Period I, to 1.22 10^{13} m^{-1} during Period II and 1.18 10^{13} m^{-1} during Period III.

3.5. Heterotrophic and autotrophic biomass kinetics

Table 5 summarises the average values of the heterotrophic and autotrophic kinetic parameters obtained in the different experimental periods.

In Period I the average values of heterotrophic kinetic and stoichiometric parameters were well in line with the literature value for MBR systems conceived for nutrients removal (Di Trapani et al., 2018). Concerning the maximum growth yield of heterotrophic bacteria, Y_H, did not observe a decrease from Period I through Period 2 and 3, respectively; suggesting that the implementation of the anaerobic reactor did not affect the biomass growth rate, likely because only a fraction of the return activated sludge from the MBR compartment was subject to anaerobic conditions. In contrast, an increase of the endogenous decay rate b_H was observed in Period II and Period III, respectively, with a decrease of the maximum growth rate μ_H in Period II and III compared to Period I, thus promoting a reduction of the net growth rate (μ_H- b_H). This result suggests that in Period II and Period III, the main mechanism for sludge reduction could be the endogenous metabolism and bacterial decay, likely enhanced by prolonged exposure to anaerobic conditions under substrate scarcity, which represents a stressful condition for bacterial community, as highlighted in previous studies (Martins et al., 2020). Moreover, the endogenous decay rate decrease could be enhanced by the low ORP, which reached an average value of −137 mV in the ASSR tank in Period III, thus promoting sludge decay as the main mechanism. This result is in line with previous observations (Chen et al., 2003).

Regarding autotrophic species, respirometric batch tests in Period I revealed an excellent development of nitrifiers, with experimental values well in line with literature data (Di Trapani et al., 2018). With the implementation of the MBR-OSA configuration in Periods II and III, excepting the first days after anaerobic reactor start-up characterised by a decrease in the autotrophic respiration rates, no significant stress effect on nitrifying bacteria was observed throughout experiments. Indeed, a significant development of autotrophic activity was noticed, with respirogram charts highlighting an increase in respiration rates.

Fig. 4 shows an example of a respirogram chart in Period I (Fig. 4a), Period II (Fig. 4b) and Period III (Fig. 4c) for autotrophic bacteria, where it is highlighted the different behaviour after substrate spiking in the two periods, suggesting an increase of autotrophic activity.

3.6. Greenhouse gases

Fig. 5 reports the pattern of gaseous and dissolved N_2O–N concentration for each reactor during Periods I, II and III. Data reported in Fig. 5 show that for each period the highest gaseous N_2O–N concentration occurred in the aerated reactors (aerobic and MBR). Specifically, the average value of the N_2O–N concentration in the aerobic and MBR off-gas was 0.09, 0.1 and 0.12 mg N_2O–N L^{-1} in Periods I, II and III respectively. However, with the increase of anaerobic HRT the amount of N_2O–N produced inside the non-aerated reactors increases. This result suggests, as corroborated by the kinetic results, that with the increase of HRT under anaerobic conditions the denitrification process is inhibited (likely due to the low carbon availability) thus producing a more significant amount of N_2O–N. The emission factor results also confirm this result.

Indeed, the N_2O–N emission factor (concerning the total influent nitrogen) obtained here was equal to 1.2, 1.3 and 2.17% of total influent nitrogen for Period I, Period II and Period III, respectively. Excepting for Period III, during which the worsening of TN removal took place the EF values obtained here are almost in line with the literature that suggests a value of 1.1% ± 0.16% (de Haas and Andrews, 2022).

The results discussed above have strong relevance and suggest that there is a need for a trade-off between the reduction of sewage sludge production and environmental protection (even including the GHG emissions).

4. Conclusions

The results achieved in the present study demonstrated that the implementation of the OSA configuration in the original MBR scheme enabled a decrease in the production of biological excess sludge of 75% underlying some novel results concerning the current literature. In particular, the OSA reactor influenced the Total Nitrogen (TN) removal efficiency of the system. When OSA was introduced, TN removal efficiency decreased from 82 to 73%. However, an increase in HRT of the OSA reactor (from 4 h to 6 h) did not significantly affect TN removal (73–74%). Moreover, the HRT increase in the anaerobic reactor promoted a N_2O–N increase inside the unaerated reactors, highlighting the need for a trade-off between sludge minimisation and GHG emission. The findings of this work can provide useful insights into sludge reduction in MBR-OSA systems, in view of full-scale application. Nevertheless, future results are needed to investigate the role of other parameters, as well as plant configuration.

CRediT authorship contribution statement

Alida Cosenza: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Daniele Di Trapani:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Paulo Marcelo Bosco Mofatto:** Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Giorgio Mannina:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Supervision, Funding acquisition.

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.

Data availability

The authors do not have permission to share data.

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