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Reduction of sewage sludge and N_2O emissions by an Oxic Settling Anaerobic (OSA) process: The case study of Corleone (Italy) wastewater treatment plant

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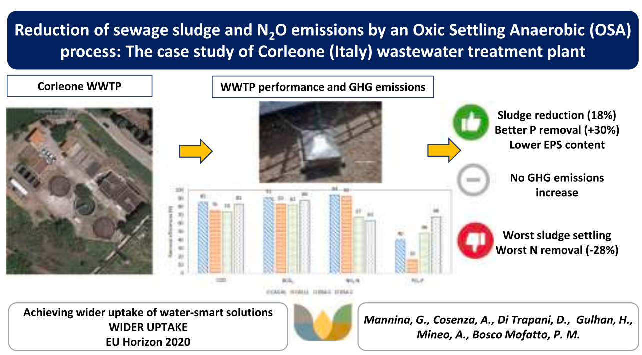
HIGHLIGHTS

- The changes in effluent quality, kinetics, costs, and greenhouse gas emissions were compared.
- The oxic settling anaerobic (OSA) layout enhanced phosphate removal but worsened ammonia removal.
- Decreased extracellular polymeric substances (EPS) in the OSA layout worsened the sludge settling properties.
- The N_2O emission factor was not influenced by the OSA layout.
- OSA layout reduced excess sludge from 9.3 to 7.6 kg/d, resulting in an 18 % cost reduction.

GRAPHICAL ABSTRACT



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ABSTRACT

Biosolid management is becoming one of the most crucial issues for wastewater treatment plant (WWTP) operators. The application of the Oxic Settling Anaerobic (OSA) process allows the minimisation of excess sludge production. This study compares conventional activated sludge (CAS) and OSA layouts in a full-scale WWTP (namely, Corleone - Italy). Extensive monitoring campaigns were conducted to assess treatment performances regarding carbon and nutrient removal, greenhouse gas (GHG) emissions, excess sludge production, and biomass activity (by means of respirometric analysis). Results showed that the effluent quality consistently met the Italian discharge limits. However, with the implementation of the OSA process, there was a decrease in ammonium removal efficiency, which could be attributed to reduced nitrifier activity related to reduced biomass production and extended anaerobic conditions affecting the nitrification process. On the other hand, the OSA configuration significantly increased phosphorus removal, indicating a high phosphorus content in the resulting waste sludge. A worsening of the sludge settling properties was observed with the OSA configuration likely due to decreased EPS concentrations. The sludge production in the OSA configuration decreased by 17.3 % compared to CAS. Nitrous-oxide measurements did not show a variation between CAS and OSA configurations, confirming that the OSA process can be a suitable solution for reducing WWTP's carbon footprint.

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1. Introduction

Biosolid or waste sludge is the end-product of wastewater treatment, whose production is inevitable and will continue to increase with the growing population (Marchuk et al., 2023; Mannina et al., 2022b). Biosolid contains valuable organic matter and nutrients (Kanteraki et al., 2022). Therefore, biosolid recovery and reuse could allow the transition towards the circular economy of the waste sector as recommended by the European Commission (Collivignarelli et al., 2019; EC, 2020; Kanteraki et al., 2022). Energy can be recovered from sewage sludge by pyrolysis (Zaharioiu et al., 2021) or anaerobic digestion (Guven et al., 2019), while valuable organic acids/alcohols can be produced by anaerobic fermentation of sewage sludge (Kleerebezem et al., 2015). In contrast, nutrients can be recovered by composting biosolids (Mulchandani and Westerhoff, 2016). Biosolids can also be adopted for agriculture scope as fertilisers to relieve pressure on non-renewable resources (Chojnacka et al., 2023). However, waste sludge treatment and disposal can reach 60 % of the total plant operational costs (Vitanza et al., 2019). Indeed, since sewage sludge contains pathogens, organic and inorganic pollutants, and micropollutants, it must be subject to further biological treatment before being discharged (Mailler et al., 2017). Furthermore, since sewage sludge is characterised by 95–99 % water content in weight, it must be dewatered before final disposal (Collivignarelli et al., 2021). The final disposal of treated waste sludge is usually represented by landfilling, which is limited by the European Union's waste hierarchy (EU, 2018) with increasing landfilling fees (Kacprzak et al., 2017). In 2020 in Europe, 18.7 thousand tons of dry waste sludge (2.12 kg per capita) (average of 18 countries data) were disposed of in landfills (Eurostat, 2022). Therefore, minimisation of waste sludge disposal is becoming increasingly important. In this light, many efforts have been devoted in the last years towards minimising excess sludge production by investigating several technologies based on physical-chemical, thermal and biological treatments (Morello et al., 2022).

To reduce the sludge production in conventional activated sludge (CAS), sludge disintegration by physical (ultrasonic, thermal, high-pressure homogenisation) or chemical (ozonation, alkaline treatment, chemical uncouplers) methods can be applied to the waste sludge line. However, due to electricity and chemical consumption, applying physical and chemical methods usually requires high investment and operation costs (Wang et al., 2017a, 2017b). Therefore, biological sludge minimisation methods (favouring the following mechanisms such as uncoupling metabolism, maintenance and endogenous decay, selection of slow-growing microorganisms, cell lysis and cryptic growth) in the water line could be more promising than that applied in the sludge line (Semblante et al., 2014; Morello et al., 2022). With maintenance and endogenous decay and uncoupling metabolisms, microorganisms are stimulated to use the acquired energy for surviving (Wang et al., 2017a, 2017b) or restoring adenosine triphosphate (ATP) reserves (Di Capua et al., 2022) rather than for growing. Therefore, the mechanisms above favour the reduction of biomass production. Furthermore, the observed yield - Y_{OBS} decreases under starving conditions since cell lysis is stimulated for cryptic growth, consuming cell content and fragmented extracellular polymeric substances (EPS) (Morello et al., 2022). The oxic-settling-anaerobic (OSA) process includes a sludge holding tank (SHT) to expose the return sludge flow to anoxic/anaerobic conditions under no substrate availability and low oxidation-reduction potential (ORP) to stimulate the reducing mechanisms of sludge production (Morello et al., 2022; Saby et al., 2003). Literature studies suggest that Y_{OBS} in CAS systems is 0.27–0.35 kg total suspended solids (TSS)/kg chemical oxygen demand (COD) removed (Karlikanovaite-Balıkçı and Yagci, 2020; Cantekin et al., 2019; Karlikanovaite-Balikci and Yagci, 2019; Zhou et al., 2015). CAS system can also be modified by inserting an anaerobic side-stream reactor (ASSR) in the return sludge line where the sludge is fed intermittently (Morello et al., 2022). In a previous study, Liu et al. (2021) showed that greenhouse gas (GHG) emissions related to sludge transportation and energy consumption can be decreased by around 23 % by applying OSA and ASSR modifications in CAS systems. However, the alternation between aerobic and anoxic conditions can result in a higher accumulation of nitrous oxide (N_2O), which has a global warming potential 298 times higher compared to carbon dioxide (CO_2) (Massara et al., 2017). Despite the positive results seen at the pilot plant scale (Saby et al., 2003; Romero Pareja et al., 2018; Vitanza et al., 2019), very few information about the upgrade of CAS systems into OSA configuration at full scale exists in the literature (Velho et al., 2016; Ferrentino et al., 2021). This study compares CAS and OSA configurations in a full-scale WWTP located in Corleone, Italy. During the monitoring campaign, carbon and nutrient removal, respirometry analysis, sludge production, extracellular polymeric substances (EPS) production and greenhouse gas emissions have been analysed. The study aims to determine a possible trade-off between effluent quality, treatment cost, and GHG emissions by modifying the original CAS layout with the OSA one.

2. Materials and methods

2.1. Case study: Corleone wastewater treatment plant

Corleone WWTP was designed for treating the domestic wastewater of 12,000 equivalent inhabitants with a daily design flow rate of 3700 m^3/d. The water line consists of preliminary treatments (screening and degritting units), biological treatment with CAS process (2 aerobic tanks, for a total volume of 768 m^3 each equipped with a surface aeration system), secondary sedimentation (3 clarifiers, for a total volume of 800 m^3 – one clarifier not in operation) and tertiary treatment (disinfection). The sludge line is based on aerobic digestion (two tanks, 330 m^3) and sludge dewatering (eight drying beds each with a horizontal surface of 40 m^2). The WWTP was not operating one aeration and one settler since it resulted under loaded compared to the design data. Therefore, the CAS operation is accomplished by only one aerobic reactor (total volume of 384 m^3). Within the EU project Wider-Uptake (Achieving wider-uptake of water-smart solutions) a reduction of the WWTP carbon footprint was planned (Mannina et al., 2022a, 2022b, 2021). In view of the above goal, the following activities and WWTP retrofitting were carried out including the implementation of the OSA process:

i. The vertical surface aerator in the aerobic reactor was replaced with a diffused air-aeration.
ii. One of the two aerobic reactors was equipped with two mixers to guarantee mixing without aeration to be used as SHT when the OSA process was realised.
iii. A pipeline to guarantee the implementation of the OSA process was realised.
iv. Flow meters were installed in each aerobic reactor's influent fluxes (recirculation and wastewater).

The final Corleone WWTP flow diagram with the above upgrades is given in Fig. 1. The average wastewater flow rate fed to the aerobic reactor was 126 m^3/h. The mixed liquor from the aerobic reactor is divided into two radial settlers (diameter: 12 m each). The return activated sludge (RAS) flow rate from the settlers is 74 m^3/h to the aerobic reactor. Under OSA configuration, the fraction of RAS pumped to SHT is defined as the internal ratio (IR). Two IRs were tested 50 % and 100 %, OSA-1 and OSA-2, respectively. The operators manually adjusted the DO set-point daily to avoid oxygen limitation and keep the average at 2 mg/L. The supernatant from the settlers flows to the disinfection unit and is then discharged to the river.

2.2. Sampling campaigns

The sampling campaigns were divided into: (i) long-term and (ii) intensive. The long-term campaign was characterised by a sampling frequency of twice samples per week. In contrast, 24 samples (one per hour in 1 day) were collected daily via autosamplers during the intensive sampling campaign. The long-term sampling campaign aimed to monitor the treatment performance and sludge production comparatively with and without the OSA configuration. During the long-term sampling campaign, grab samples were collected twice per week from the influent of the aerobic reactor, the effluent of sedimentation tanks, the aerobic reactor, and the RAS line. Effluent samples were collected 6.5 h after the influent sample according to the hydraulic retention time (HRT) between the influent section and the effluent one. The long-term sampling campaign monitoring the CAS period (without OSA) lasted 148 days, the OSA-1 period with a 50 % IR ratio lasted 28 days, and the OSA-2 period with a 100 % IR ratio lasted 26 days. During the long-term monitoring period, seven intensive sampling campaigns were conducted. Influent and effluent samples were collected every hour by two autosamplers. The autosampler that collects samples from the effluent of the settlers was started 6.5 h after the influent auto-sampler, according to the HRT as previously mentioned. During intensive sampling campaigns, three grab samples were collected from the aerobic tank, and one grab sample was collected from the RAS line. To measure N_2O emissions from the aerobic tank, gas and liquid samples (6 grab samples) were collected hourly between 10:00–16:00. A gas sampling hood (cross-sectional area: 1.0 m × 0.9 m) was placed on the surface of the aerobic tank and OSA tank and gas samples were collected in 0.5 L of gas bags (Tedlar, USA) via an air pump (Sensidyne, USA). The air flow rate from the surface of the aerobic tank was measured by an anemometer (Extech, USA) according to Caniani et al. (2019). N_2O concentrations in the liquid were monitored by a micro-sensor (Unisense Environment A/S, Denmark) per minute during gas sampling (Table 1).

2.3. Analytical methods

COD, BOD_5, NH_4-N, nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), orthophosphate (PO_4-P), TSS and volatile suspended solids (VSS) concentrations were measured according to Standard Methods (APHA, 1999). The removal efficiencies of COD, BOD_5, NH_4-N, and PO_4-P were calculated by subtracting the effluent from the influent concentration and dividing the result by the influent concentration.

The sludge settling performance was assessed by the sludge volume index (SVI). EPS and soluble microbial products (SMP) were extracted according to the method given by Le-Clech et al. (2006). Proteins and carbohydrates were measured according to Lowry et al. (1951) and DuBois et al. (1956), respectively. The Y_{obs} was evaluated by dividing the TSS produced by the COD removed, in terms of cumulated mass (Gardoni et al., 2011) (Eq. (1)). COD_{in} and COD_{out} are the inlet and outlet total COD concentrations, Q_i is the daily influent flow rate, and

Table 1
Details of sampling campaigns.

Sampling campaigns		Period	Duration (days)	Samples collected
Long-term sampling campaigns	CAS	14.09.2022–09.02.2023	148	Influent Effluent Sludge (aerobic tank and RAS)
	OSA-1	14.02.2023–14.03.2023	28	
	OSA-2	16.03.2023–20.04.2023	26	
Intensive sampling campaigns	I-1	6.12.2022 (CAS)	1	Influent Effluent Sludge (aerobic tank and RAS) Gas (tank)
	I-2	12.12.2022 (CAS)		
	I-3	19.12.2022 (CAS)		
	I-4	01.03.2023 (OSA-50 % IR)		
	I-5	14.03.2023 (OSA-50 % IR)		
	I-6	21.03.2023 (OSA-100 % IR)		
	I-7	26.04.2023 (OSA-100 % IR)		

CAS: Conventional activated sludge; OSA: Oxic-settling-anaerobic; RAS: Return activated sludge; IR: Internal ratio.

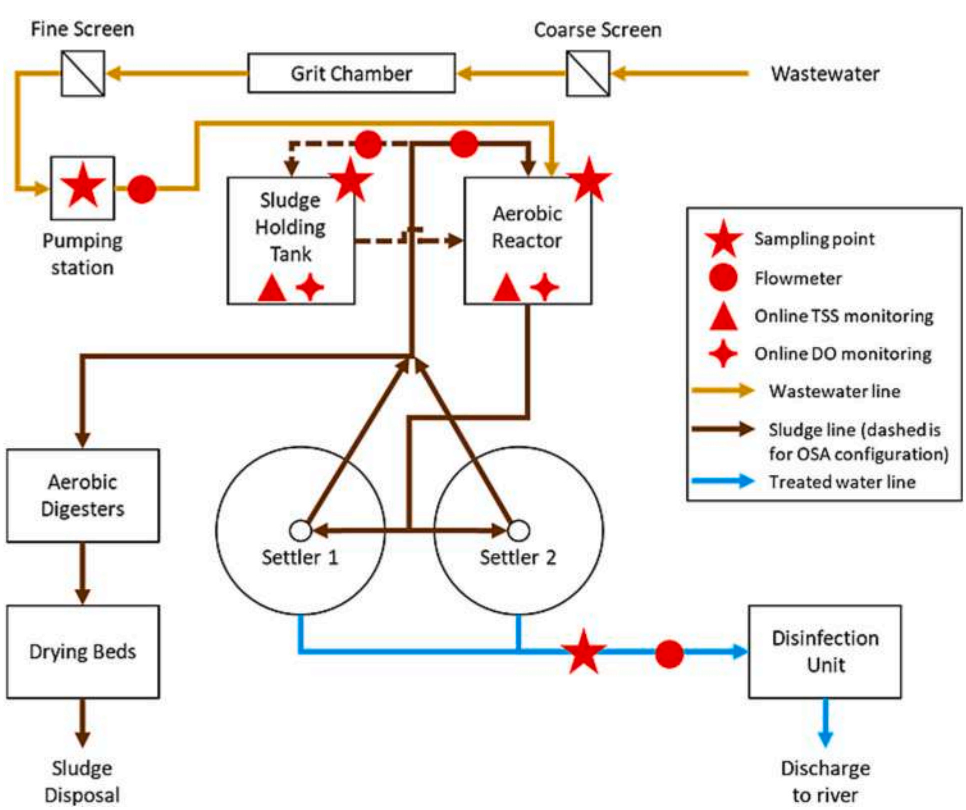

Fig. 1. Flow diagram of Corleone WWTP.

ΔX is the excess sludge produced daily.

$$Y_{obs} = \frac{\Delta X}{Q_i \bullet (TCOD_{in} - TCOD_{out})}\ (\text{g TSS/g COD}) \quad (1)$$

Dissolved and gaseous N_2O concentrations were evaluated by the procedure reported by Mannina et al. (2018) by using a Gas Chromatograph (GC) (Agilent 8860) with an Electron Capture Detector (ECD). The N_2O emission factor (EF_{N2O}) was calculated according to the literature (Mannina et al., 2016; Tsuneda et al., 2005) (Eq. (2)):

$$EF_{N2O} = \frac{\frac{N_2O-N_g}{HRT_{hs}} + \frac{N_2O-N_d}{HRT}}{TN} \quad (2)$$

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

Periodic respirometric batch tests were carried out to assess biomass kinetic and stoichiometric parameters. 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}) were assessed according to literature (Di Trapani et al., 2018). The respirometric batch tests were carried out by measuring the Oxygen Uptake Rate (OUR) for consuming a readily biodegradable substrate spiked during the test (sodium acetate in the present study).

Nitrogen mass balance has been performed according to Ekama (2009) (Eq. (3)).

$$TN_{in} = NH_4 - N_{out} + NO_3 - N_{out} + NO_2 - N_{out} + N_2O - N_{accumulated} + N_{denitrified} + N_{metabolic} \quad (3)$$

where TN_{in} is the total nitrogen influent concentration, NH_4-N_{out} is the effluent ammonia concentration, $N_{metabolic}$ represents the nitrogen consumed due to the metabolic processes, $N_{denitrified}$ is the concentration of nitrogen consumed for denitrification, NO_3-N_{out} is the effluent concentration of nitrates, NO_2-N_{out} is the effluent concentration of nitrites and N_2O-$N_{accumulated}$ is the accumulated concentration of nitrous oxide.

The plant SRT (day) was calculated according to Eq. (4).

$$SRT = \frac{V_{AER}*MLSS_{AER} + V_{SHT}*MLSS_{SHT}}{Q_{out}*TSS_{out} + Q_{WAS}*TSS_{RAS}} \quad (4)$$

where V_{AER} (m^3) and V_{SHT} (m^3) are the aerobic and SHT reactor volume, respectively. $MLSS_{AER}$ (kg/m^3) and $MLSS_{SHT}$ (kg/m^3) are the mixed liquor suspended solid concentration inside the aerobic and SHT reactor, respectively. Q_{out} (m^3/d) is the treated effluent flow rate; Q_{WAS} (m^3/d) is the waste sludge flow rate. Finally, TSS_{out} (kg/m^3) and TSS_{RAS} (kg/m^3) are the total suspended solid concentration in the effluent and the RAS, respectively.

During CAS operation the contribution due to the SHT was null in Eq. (4).

3. Results and discussion

3.1. Long-term sampling campaign results

3.1.1. Influent wastewater characteristics

Table 2 summarises the influent wastewater characteristics. COD, biological oxygen demand (BOD_5), and TSS average concentrations in the influent wastewater were higher in the first 85 days than in the last days. Consequently, the CAS period was divided into CAS-High load (HL) and CAS-Low load (LL) period that has influent features in terms of COD, BOD_5, and ammonium nitrogen (NH_4-N) similar compared to OSA-1 and OSA-2 periods.

3.1.2. Treatment performances

Fig. 2 reports the results of long-term monitoring for COD, BOD_5, NH_4-N, and PO_4-P. The average removal efficiencies for COD, BOD_5, NH_4-N and PO_4-P for each experimental period are reported in Fig. 3. The average COD and BOD_5 concentrations in the effluent wastewater were always below 125 mg COD/L and 25 mg BOD_5/L in the different experimental periods, which represented the Italian discharge limits (Legislative Decree n. 152/2006) (Fig. 2a and b).

As noticeable from Fig. 2a and b, the COD and BOD_5 removal efficiencies were very high in the CAS-HL period (on average, 85 % and 91 % for COD and BOD_5, respectively). Only a moderate decrease was observed in the CAS-LL period, likely due to the decrease of the influent COD and BOD_5 concentrations (Fig. 3). During monitoring, OSA periods had lower strength due to natural variations in wastewater. The period when wastewater had low strength in the CAS layout was selected to compare OSA vs CAS layouts. Based on the findings, the OSA layout may have been more successful than the CAS layout in a situation with low wastewater strength.

During the study, very few temperature excursions took place from one period to another, thus allowing to neglect of any temperature influence in the obtained results. In particular, during CAS periods the average temperature inside the aerobic reactor was equal to 25 °C and 23 °C for HL and LL, respectively. During OSA-1 and OSA-2, the average temperature inside the aerobic reactor was equal to 22 °C and 25 °C, respectively. The temperature inside the anaerobic reactor was equal to 23 °C and 25.3 °C during OSA-1 and OSA-2, respectively.

When the plant layout was switched from CAS-LL to OSA process configuration, it was noticed similar behaviour in terms of organic matter removal, with comparable average removal efficiencies (76 and 74 % for COD and 83 and 82 % for BOD_5 in CAS-LL and OSA-1,

Table 2
Characteristics of the influent wastewater.

Parameter	Units	CAS-HL		CAS-LL		OSA-1		OSA-2	
		Average	SD	Average	SD	Average	SD	Average	SD
COD	mg/L	197	77	107	24	91	11	111	32
BOD_5	mg/L	106	40	49	6	51	14	58	23
Total nitrogen (TN)	mg/L	37	11	28	5	26	11	34	9
Ammonium nitrogen (NH_4-N)	mg/L	24	7	18	3	17	3	22	6
Phosphate (PO_4-P)	mg/L	8.5	4.3	7.7	2.4	7.2	1.2	6.2	2.9
TSS	mg/L	219	184	164	77	143	73	140	49
Number of data	–	27		6		9		112	

SD = Standard deviation.

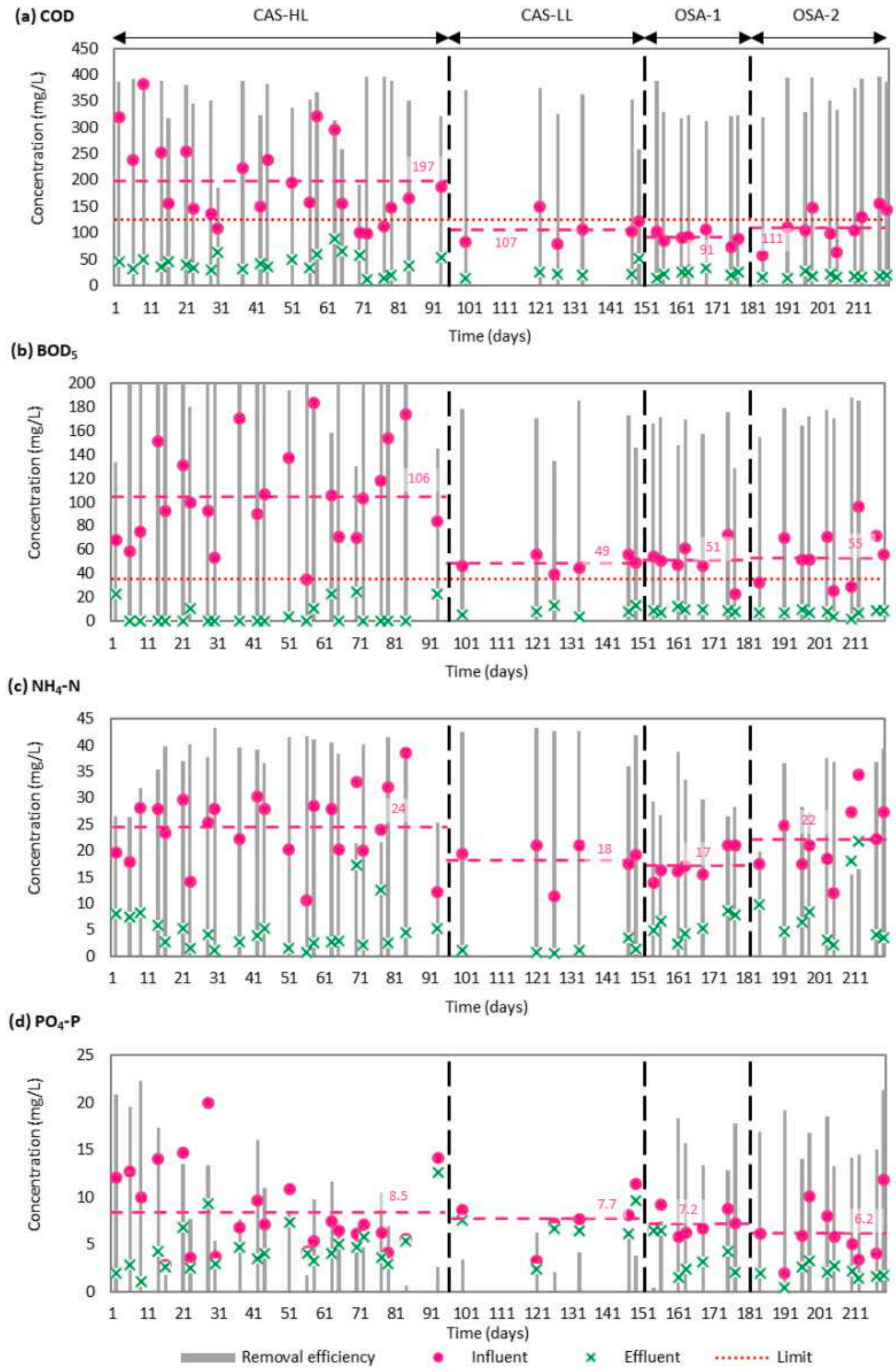

Fig. 2. Influent and effluent concentrations and removal efficiencies of COD (a); BOD_5 (b); NH_4-N (c); and PO_4-P (d).

respectively). Indeed, from Figs. 2a–b and 3 can be noted that COD and BOD_5 removals were not significantly affected by the OSA configuration.

In contrast, during the OSA-2 period, a slight increase in the COD and BOD_5 removal efficiencies was observed, reaching values very close to that observed during the CAS-HL period (83 and 89 %, respectively for COD and BOD_5) (Fig. 3). Therefore, in the present study, the IR increase positively affected the removal of COD and BOD_5. The results were generally consistent with those reported in previous literature studies, where no significant negative effects on organic matter removal were observed when the OSA process was implemented (Vitanza et al., 2019; Fida et al., 2021). Concerning ammonia removal, high nitrification performances were observed in both CAS-HL and CAS-LL periods, with average efficiencies respectively equal to 94 and 92 % and effluent NH_4-N concentrations lower than 2 mg/L (Figs. 2c and 3). With the implementation of the OSA configuration, a significant decrease in the ammonium removal efficiency was observed, with an average value of

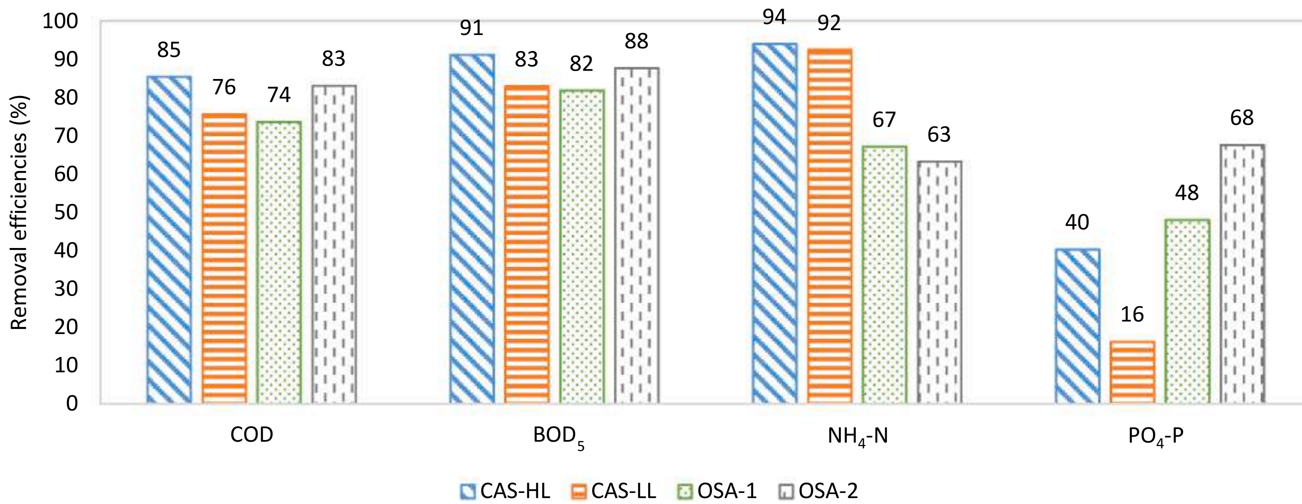

Fig. 3. COD, BOD_5, NH_4-N, and PO_4-P removal efficiencies.

67 % in the OSA-1 period, which further decreased to 63 % during the OSA-2 period (Fig. 3). One reason could be the lower biomass production in the OSA periods, which implied a lower NH_4-N metabolic assimilation. On the other hand, the exposure of nitrifying microorganisms to prolonged anaerobic conditions in the SHT could have promoted autotrophic decay, thus leading to a significant worsening of nitrification. This behaviour was slightly emphasised when the IR was increased to 100 %. Zhou et al. (2015) also observed a decrease in nitrification efficiency after introducing an SHT and attributed this decrease to the anaerobic decay of nitrifying microorganisms. Another reason for the decrease in the nitrification efficiency could be the decreased aerobic sludge age that limits nitrifying activity (Kemmou et al., 2021).

Concerning phosphorus, it is worth noting that the original CAS layout was not conceived for its removal; therefore, P removal during CAS periods could be ascribable to metabolic consumption only. The average P removal in the CAS-HL period (40 %) was significantly higher compared to CAS-LL one (16 %), mainly due to the decrease of the influent COD and BOD_5 concentrations which limited the heterotrophic biomass growth and consequently the metabolic phosphorus consumption (Fig. 2d and 3). When the OSA process was implemented, a significant increase in PO_4-P removal was observed, which reached an average value of 48 % in the OSA-1 period (Fig. 3). During the OSA-2 period, PO_4-P removal increased, reaching an average value of 68 % (Fig. 3). These results were consistent with previous studies, where an increase in P removal was achieved in plants implementing the OSA

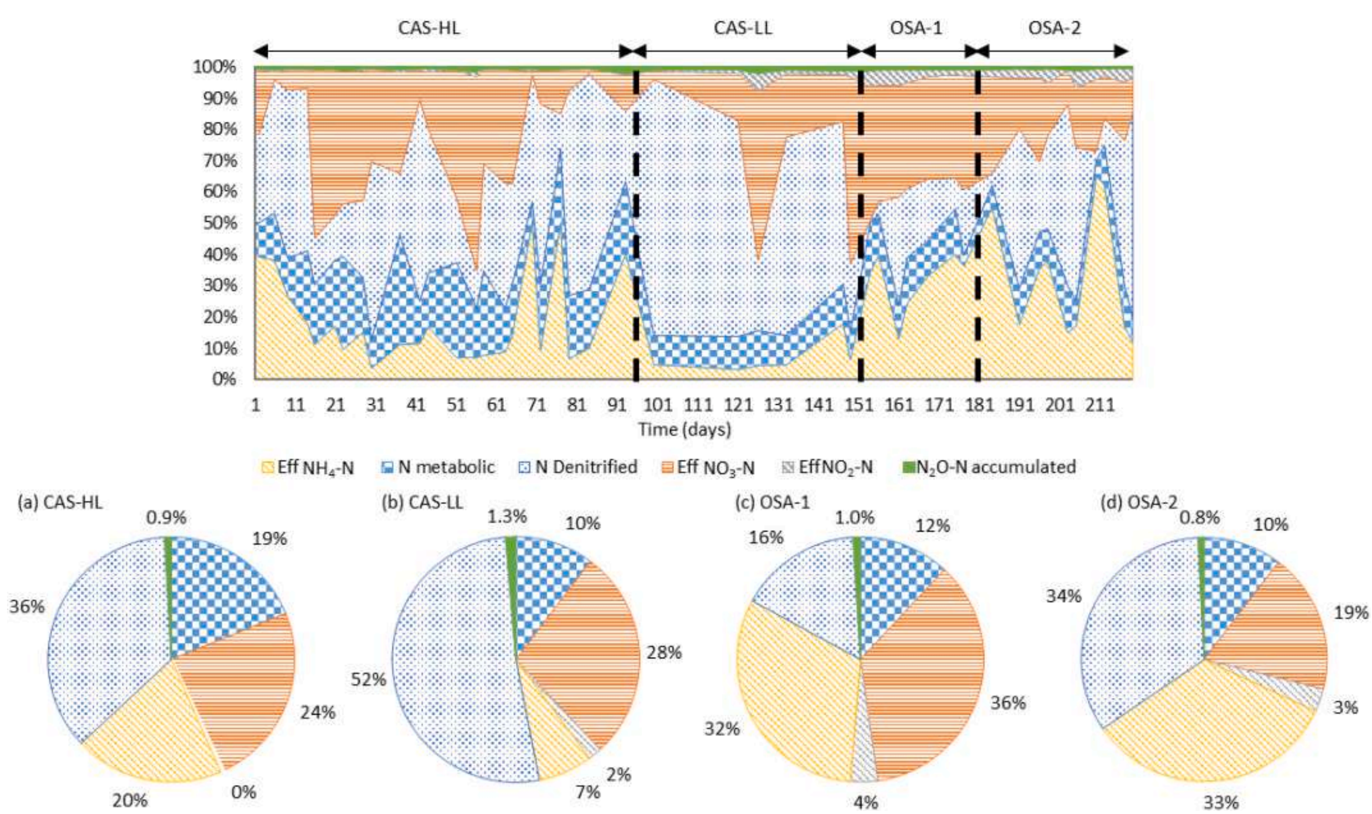

Fig. 4. Effluent nitrogen compounds fractions.

process (Martins et al., 2020). Indeed, previous literature suggested 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), thus contributing to biological P removal. Moreover, the disruption of the EPS structure, discussed in the section below, could have provided the carbon source for PAO or DPAO organisms, thus supporting the biological mechanism of P removal.

3.1.3. Nitrogen removal and mass balance

Fig. 4 shows the fractions of nitrogen pathways calculated from nitrogen mass balance. N metabolic represents the ammonia consumption during biomass growth. The N metabolic fraction was the highest in the CAS-HL period and decreased in the CAS-LL period due to the low substrate loading rate in the influent wastewater that slowed down the metabolic activities in the bioreactor. The N metabolic fractions showed similar values in CAS-LL, OSA-1, and OSA-2. The effluent ammonia fraction decreased from 20 % to 7 % in the CAS-LL period compared to the CAS-HL period (Fig. 4). An increase in the effluent ammonia fraction has been observed in the OSA periods, although the influent ammonia concentrations were similar to the CAS-LL period. Such an increase might be due to the lowered biomass activity, especially for the autotrophic one, also affecting the NH_4-N metabolic consumption. Indeed, the increased anaerobic volume due to the SHT likely reduced the autotrophic biomass growth and consequently the nitrification efficiency. The lower N denitrified fractions in the OSA periods also resulted from worsened nitrification since oxidised nitrogen forms were scarce compared to CAS periods. During the OSA-1 period the effluent NO_3-N fraction (36 %) was higher than that of the OSA-2 period (18 %) (Fig. 4c and d). The effluent NO_3-N concentrations in CAS-HL, CAS-LL, OSA-1, and OSA-2 were 5.8 ± 4.1 mg/L, 5.0 ± 3.6 mg/L, 6.3 ± 0.8 mg/L, and 4.1 ± 1.2 mg/L, respectively. The effluent NO_2-N concentrations were below 0.8 mg/L in all periods because the increase of the IR ratio in the OSA-2 period decreased the hydraulic retention time and the duration of anaerobic exposure of biomass.

The higher N denitrified fraction in the OSA-2 period might be related to two aspects: on one hand, the increased loading rate of organic carbon observed in the last experimental days; on the other hand, the SHT could contribute to the release of additional carbon source resulting from bacterial cell lysis, providing more organic carbon to sustain denitrification; the increased IR in OSA-2 carried more sludge passing through the SHT, thus influencing denitrification. The result is in good agreement with previous literature studies (Cheng et al., 2017; Romero-Pareja et al., 2017).

3.1.4. Respirometric analysis

Table 3 summarises the average values of the heterotrophic and autotrophic kinetic parameters obtained during experiments. From the observation of data reported in Table 3, it can be noticed that the heterotrophic biomass yield showed a slight decrease, from 0.44 gVSS g^{-1}COD to 0.41, when WWTP configuration was changed from CAS to OSA with IR of 50 %. This result highlighted that the OSA configuration can effectively promote the reduction of biological sludge. Concerning the maximum growth rate, a reduction was observed when the OSA configuration was implemented, suggesting that the latter enhanced the establishment of the maintenance metabolism. This result was also corroborated by the simultaneous increase of the endogenous decay b_H, from 0.47 to 0.56 d^{-1}. The net growth rate also decreased during the OSA-1 period, highlighting that the operational and metabolic conditions imposed by the OSA configuration were unfavourable for bacterial growth. When the IR was increased to 100 % (OSA-2), a further decrease of the maximum yield $Y_{H \text{ was not observed}}$, which remained stable, similar to the heterotrophic active fraction; in contrast, an increase in the maximum growth yield $\mu_{H \text{ was observed}}$. This unexpected result could be related to the increase in the organic loading rate observed in the last period, with a slight increase in the influent COD concentrations. This variation could have counterbalanced the effect of OSA configuration in the sludge reduction.

Table 3
Summary of the main heterotrophic kinetic and stoichiometric parameters as average values (in brackets the standard deviation).

Parameter	Symbol	Units	Heterotrophic		
			CAS-LL	OSA-1	OSA-2
Max. growth yield	Y_H	gVSS/g COD	0.44 (±0.04)	0.41 (± 0.06)	0.41 (± 0.02)
Decay rate	b_H	1/d	0.47 (± 0.09)	0.56 (± 0.21)	0.92 (± 0.04)
Max. growth rate	μ_H	1/d	1.64 (± 0.39)	1.39 (± 0.34)	1.84 (± 0.87)
Max. removal rate	ν_H	1/d	3.90 (± 0.45)	3.89 (± 0.26)	4.48 (± 1.72)
Net growth rate	μ_H- b_H	1/d	1.16 (± 0.22)	0.83 (± 0.37)	0.92 (± 0.43)
Active fraction	f_X	%	14.20 (± 1.32	10.33 (± 4.35)	10.36 (± 3.67)

Parameter	Symbol	Units	Autotrophic	
			Literature	OSA-2
Max. growth yield	Y_A	g VSS/g NH_4-N	0.19–0.26 (Ramirez-Vargas et al., 2013)	0.21 (± 0.01)
Max. growth rate	μ_A	1/d	0.26–0.38 (Ramirez-Vargas et al., 2013)	0.21 (± 0.09)
Max. removal rate	ν_A	1/d	1.39–1.48 (Ramirez-Vargas et al., 2013)	1.27 (± 0.61)
Nitrification rate	N_R	mg NH_4/L/h	N.A.	4.28 (± 2.73)

Concerning autotrophic species, since in the CAS-LL period the biokinetic parameters of heterotrophic bacteria were in good agreement with literature data from Table 3, and due to the very high nitrification performance, it was supposed that the same conclusions could be drawn for autotrophic species. Therefore, it was decided not to perform any respirometric batch test on nitrifying species. However, due to the worsening of ammonia removal observed with the implementation of OSA configuration, especially in period OSA-2, it was decided to perform respirometric tests also for autotrophic species. The results achieved in the OSA-2 period (Table 3) show a slight decrease in kinetic/stoichiometric parameters compared to the literature data. Therefore, based on measured data, it can be concluded that implementing the anaerobic reactor can negatively impact the activity of nitrifying species, thus reducing the nitrification efficiency of the system.

3.2. Intensive sampling campaign results

The intensive sampling campaign was conducted to evaluate how OSA configuration affects the N_2O emissions originating from the WWTP. From Figs. 5 and 6, the hourly fluctuations of pollutant concentrations can be seen. The COD, BOD_5, TSS, TN and NH_4-N influent concentrations were similar in all intensive sampling days except for campaign I-7 (Figs. 5 and 6). The variation of PO_4-P concentrations in the influent wastewater was also high (changing between 0 and 17 mg/L). It is worth mentioning that although the influent COD and BOD_5 concentrations increased, the OSA configuration enabled to meet the discharge standards. During the I-4 sampling campaign, the average MLSS concentration in the aerobic reactor was 5570 mg/L, while the average of all intensive sampling campaigns was 3990 mg/L. So, the sludge's high MLSS concentration probably reduced the settler's solid-liquid separation efficiency (Morello et al., 2022).

The N_2O concentrations measured in the liquid did not fluctuate for the first six intensive sampling campaigns (the average was 0.2 mg/L) (Fig. 7). However, during the I-7 sampling campaign, it decreased to 0.06 mg/L. Nitrifiers can produce N_2O during not completed

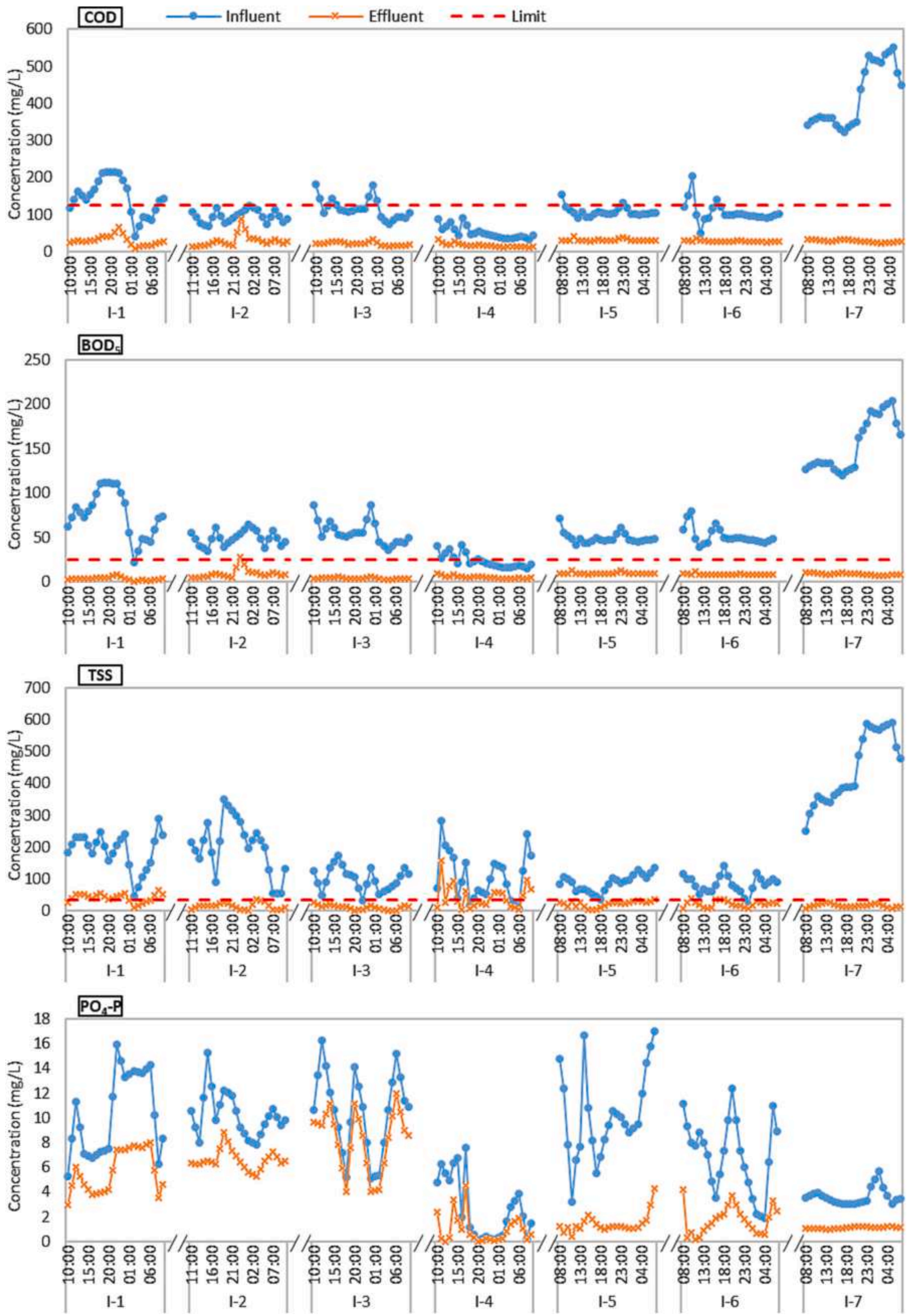

Fig. 5. Intensive sampling campaign: Influent and effluent COD, BOD_5, TSS, and PO_4-P concentrations.

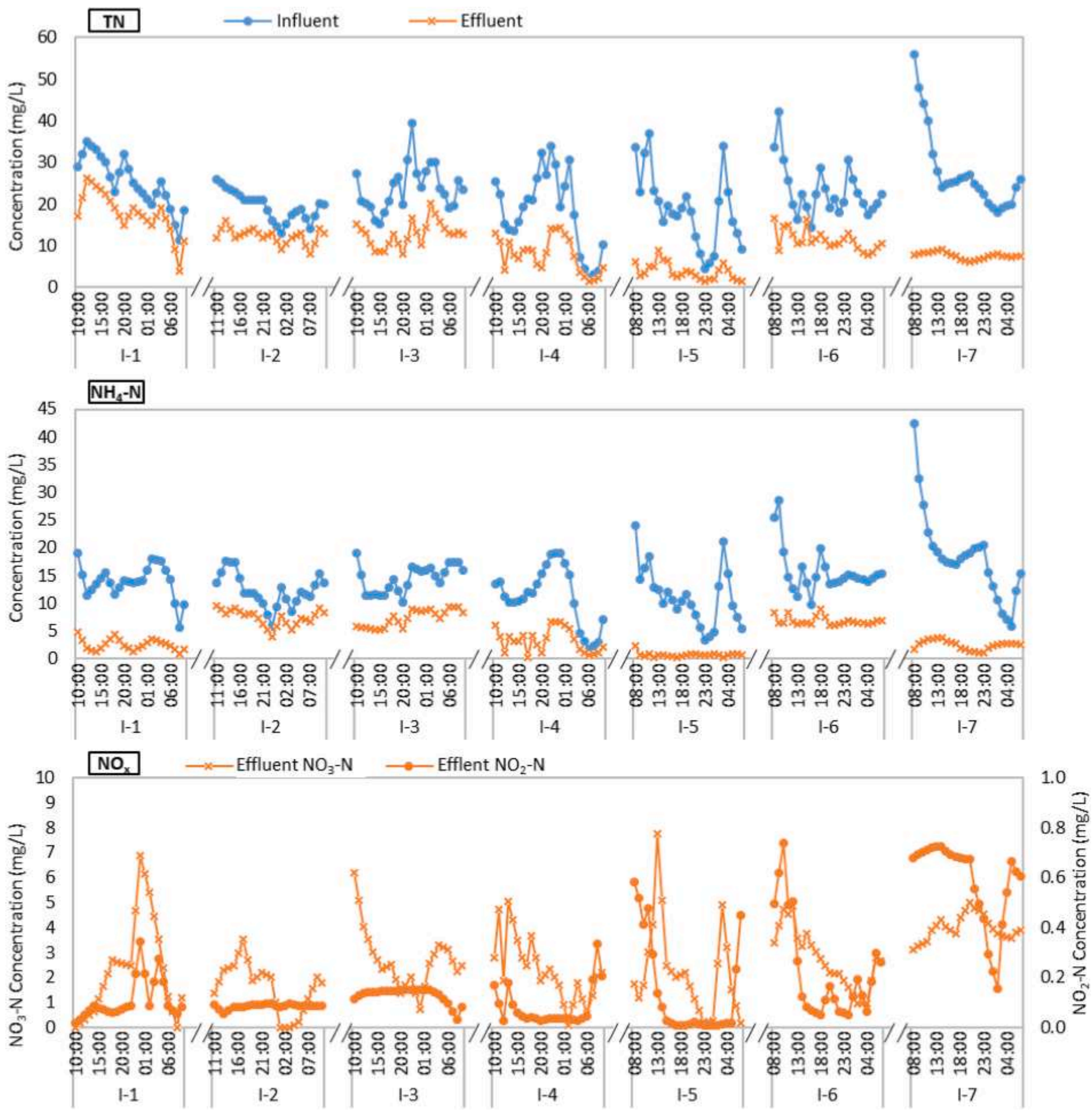

Fig. 6. Intensive sampling campaign: Influent and effluent concentrations of TN, NH_4-N, NO_3-N and NO_2-N.

nitrification. Heterotrophs produce N_2O due to nitrite oxidation in the third step of denitrification. So, if the fourth step of denitrification (N_2O reduction to N_2) cannot be completed, N_2O accumulates (Ni and Yuan, 2015). The higher NO_2-N concentrations in the effluent than NO_3-N concentrations suggest that nitrification was not completed. However, incomplete nitrification should increase N_2O accumulation. So, the decrease in N_2O concentration in the liquid can be only justified by the reduced biomass activity due to SHT (Campos et al., 2016). It is also worth mentioning that the N_2O concentrations in the SHT were lower than in the aeration tank. The anoxic/anaerobic heterotrophic activity in SHT might be the reason for lower N_2O concentrations.

N_2O flux from the aeration tank was 3.2 mg/m^2/h on average in the first six intensive sampling campaigns and decreased to 0.6 mg/m^2/h at I-7. Since there was no aeration in SHT, the N_2O flux was very low. Since N_2O concentrations were similar in the gas samples, N_2O flux variations depended on the airflow velocities measured during the sampling campaign (Hwang et al., 2016).

The ratio between N_2O concentration and influent nitrogen is generally used in benchmarking. The N_2O emission (liquid+gas) was 1.9 % of the influent nitrogen for the first six intensive sampling campaigns. Massara et al. (2017) compiled data from the literature on N_2O emission from biological nutrient removal systems. In their literature review, N_2O emission to influent nitrogen range was 0.13–2.69 %, which agrees with the N_2O results in this study.

Operational parameters and sludge reduction (Table 4) summarise the average and standard deviation (SD) values of Y_{obs}, F/M ratio, SRT and SVI for each experimental period. Data from Table 4 shows that, on average, a reduction in sewage sludge production occurred through the monitoring periods. Indeed, the average Y_{obs} value decreased from 0.46 g TSS/g COD (period CAS–HL) to 0.28 (period OSA-2) during the monitoring periods. The suggestion is that the insertion of the OSA reactor has had an average positive role in sewage sludge reduction, especially in the last three periods. Indeed, in periods CAS-LL, OSA-1 and OSA-2, a comparable F/M value occurred (0.13, 0.16 and 0.19 g BOD_5/g TSS/d for CAS-LL, OSA-1 and OSA-2 periods, respectively). Further, the increase of the IR from 50 % to 100 % (from period OSA-1 to

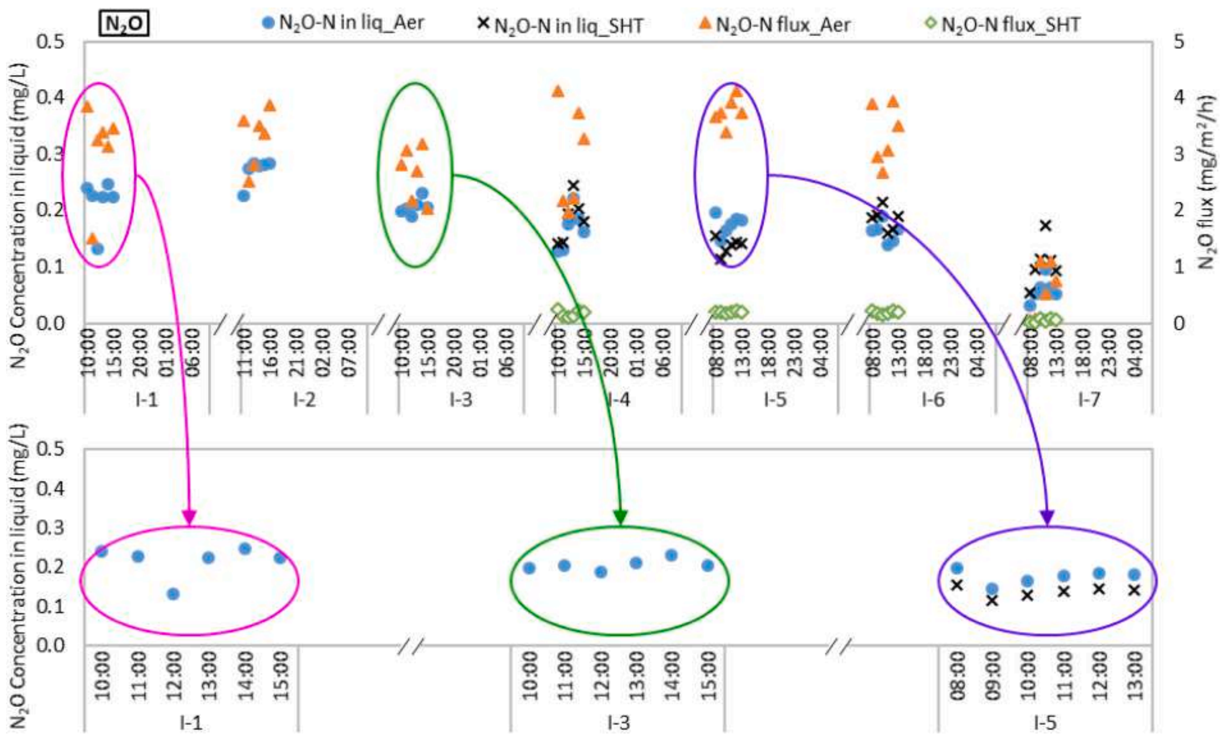

Fig. 7. Intensive sampling campaign: N_2O-N concentrations in the liquid and gas samples and N_2O-N fluxes from the aeration tank and SHT.

Table 4
Operational parameters of Corleone WWTP.

Parameter	Units	CAS-HL		CAS-LL		OSA-1		OSA-2	
		Average	SD	Average	SD	Average	SD	Average	SD
Y_{obs}	g TSS/g COD	0.45	0.05	0.44	0.07	0.36	0.14	0.28	0.12
F/M ratio	g BOD_5/g TSS/d	0.20	0.10	0.13	0.03	0.16	0.05	0.19	0.08
SRT	d	33	18	27	11	51	4	56	4
SRT_{AER}	d	33	18	27	11	28.6	2	28.2	2

period OSA-2) demonstrated a further reduction of sewage sludge production with the decrease of the average Y_{obs} value from 0.36 to 0.28 g TSS/g COD (Table 4). The Y_{obs} values obtained in this study during OSA-1 and OSA-2 periods are almost in line with literature data with synthetic wastewater. For example, Ferrentino et al. (2018) under a 100 % recirculation ratio obtained a Y_{obs} value of 0.12 g TSS/g COD. However, Ferrentino et al. (2018) obtained a higher sludge reduction, accounting for 66 %; nevertheless, this result was likely related to the higher SRT established here. While, for real wastewater Ferrentino et al. (2021) obtained a Y_{obs} value of 0.38 g TSS/g COD after implementing the OSA process in a full-scale plant. The average Y_{obs} value is slightly lower than the other full-scale results obtained in the literature, likely due to the low F/M value of the Corleone WWTP.

In view of a better understanding of the key mechanisms that have caused the sewage sludge reduction, a detailed discussion on some measured or quantified compounds will be provided below.

The measurement of SVI values enabled the monitor of the sludge settling properties throughout experiments. In detail, the best performance was achieved during the CAS-HL period, which showed excellent sludge settling properties, with an average value of 94 mL/g TSS. In the CAS-LL period, a moderate worsening of settling properties was observed, with an average value of 135 mL/g TSS, mainly related to the variation of the influent wastewater features and the decrease of the MLSS in the aerobic reactor occurring in this period. Nevertheless, the sludge-settling properties remained quite good. In contrast, after the implementation of the OSA configuration, a significant worsening of sludge settling properties was observed, and an average value of 205 mL/g TSS was observed in the OSA-1 period. The worsening of sludge settling properties could be related to the significant decrease of EPS observed during experiments; indeed, the EPS decrease, connected to bacterial substrate consumption under fasting conditions, likely promoted a destructuration of activated sludge floc, thus causing a worsening of settling properties. Only a moderate improvement was observed in the OSA-2 period, with an average SVI value of 189 mL/g TSS. This result could be related to the establishment of feasting/fasting conditions that promoted the selection of microorganisms with storage ability similarly to selector-like systems in the anaerobic reactor. This result aligns with previous literature that emphasised the deterioration of sludge settleability with the implementation of an anaerobic reactor (Sun et al., 2020).

Fig. 8a shows the trend of average MLSS concentration in the aerobic reactor and the waste sludge flow rate (Q_{WAS}). Fig. 8b shows the average values of the MLSS concentration inside the aerobic reactor, the Q_{WAS} and the mass of wasted sludge for each period. Data from Fig. 8a–b show that the average MLSS concentration in the aerobic reactor during period CAS-HL was around 4400 mg/L; this value decreased by approximately 1000 mg/L in periods CAS-LL, OSA-1, and OSA-2. The decrease in MLSS concentration was attributed to the different F/M ratios by comparing CAS-HL and CAS-LL. Indeed, since the average Q_{WAS} values of periods CAS-HL and CAS-LL were very similar (3.5 and 3.4 m^3/d), we can affirm that the high organic loading rate in period CAS-HL leads to the increase of biomass production (and consequently

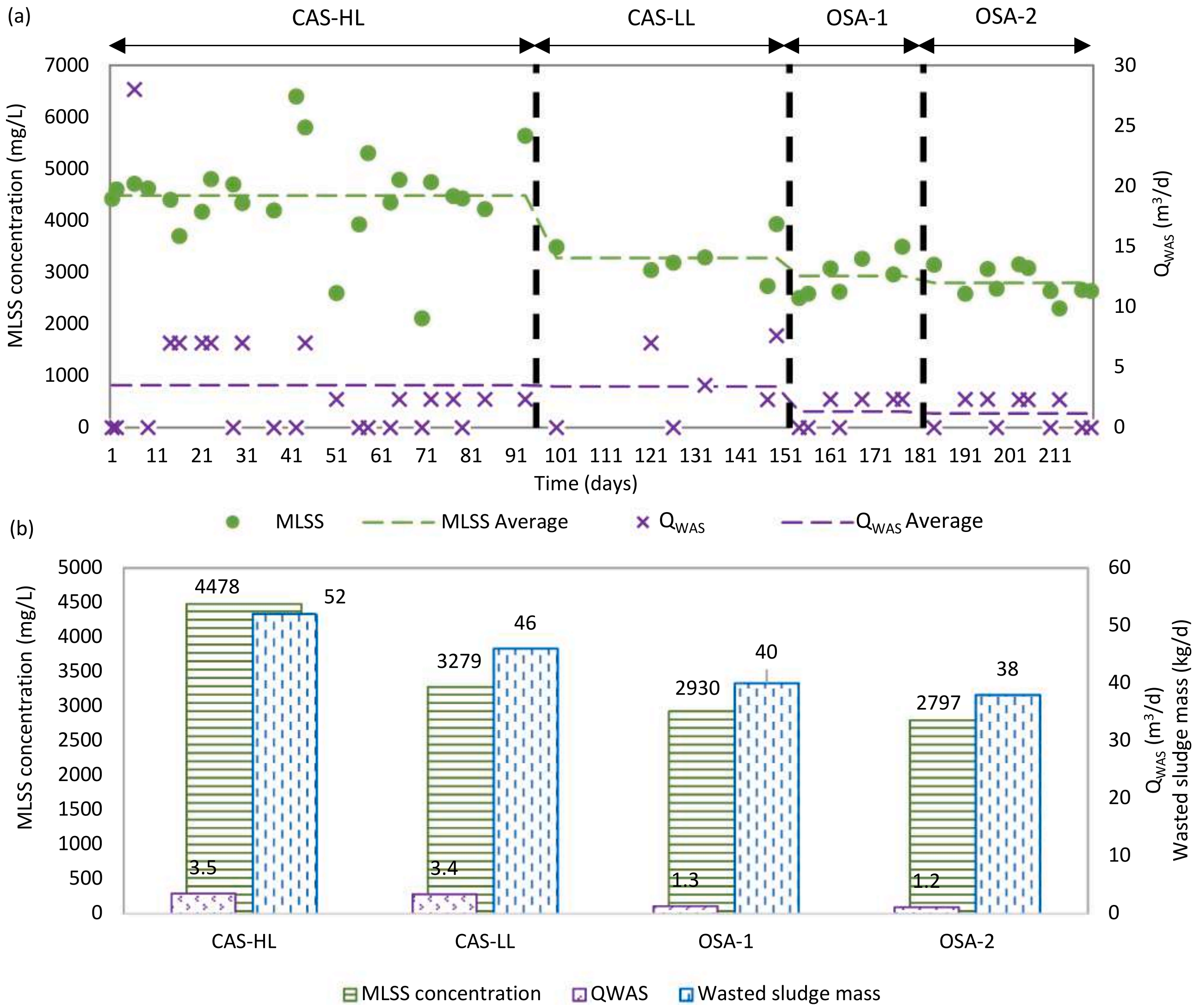

Fig. 8. MLSS concentration in the aerobic reactor and Q_{WAS} through monitoring period (a) and the averages of MLSS concentration, Q_{WAS}, and wasted sludge mass (b).

of the MLSS concentration inside the aerobic reactor) (Fig. 8). Therefore, the decrease of MLSS concentration inside the aerobic reactor during periods OSA-1 and OSA-2 can be due to the insertion of the anaerobic reactor. Further, during periods OSA-1 and OSA-2 a substantial reduction of the average Q_{WAS} (1.3–1.2 m^3/d) value occurred compared to the CAS-LL period, even maintaining the same MLSS concentration inside the aerobic reactor (Fig. 8a–b).

Data from Fig. 8b corroborate the above discussion and show that the average mass of waste sludge for period CAS-LL was 46 kg/d. The average wasted sludge mass decreased to 40 kg/d and 38 kg/d during periods OSA-1 and OSA-2, respectively. The total reduction of wasted sludge mass compared to CAS-LL of 13 % and 17.3 % occurred during the OSA-1 and OSA-2 periods, respectively. This result could have relevant implications regarding operating costs reduction, reducing the costs related to sludge disposal.

For the sake of completeness, in Fig. 9, the trend of daily and average Y_{obs} and F/M values for each monitoring period are shown. From the observation of data reported in Fig. 9, noticeable fluctuations of Y_{obs} value can be seen, referring in particular to CAS-LL, OSA 1 and OSA 2 periods. The reason could be found in the fact that the target was to keep almost constant MLSS concentration in the system and it was challenging for two reasons. First, adding an anaerobic reactor in the RAS line made it more difficult to maintain a constant MLSS concentration. Second, sludge waste operations were not carried out regularly by the plant operator, thus making it challenging to meet the target MLSS value and influence Y_{obs} values, even if an average decrease was observed under OSA operation.

In Fig. 10, the trends of EPS concentration (divided into protein – pEPS, and carbohydrate, cEPS) are shown over the monitoring periods. In terms of total EPS (sum of pEPS and cEPS), a progressive reduction occurred from CAS-LL to OSA-1 and OSA-2 on average. Specifically, the total amount of EPS was, on average equal to 191 mg/g TSS, 188 mg/g TSS and 138 mg/g TSS for CAS-LL, OSA-1 and OSA-2 period, respectively. This result suggests that with biomass staying under anaerobic conditions (from CAS-LL period to OSA-1 and OSA-2 periods) the EPS destruction took place due to the cell lysis under anaerobic conditions and this behaviour was emphasised with the increase of retention time under anaerobic conditions (Cheng et al., 2021).

From the discussion reported above, it is possible to conclude that a major mechanism of sludge reduction during periods OSA-1 and OSA-2 compared to period CAS-LL might be related to anaerobic cell lysis and cryptic growth. Such a result was demonstrated by the decrease in EPS concentration.

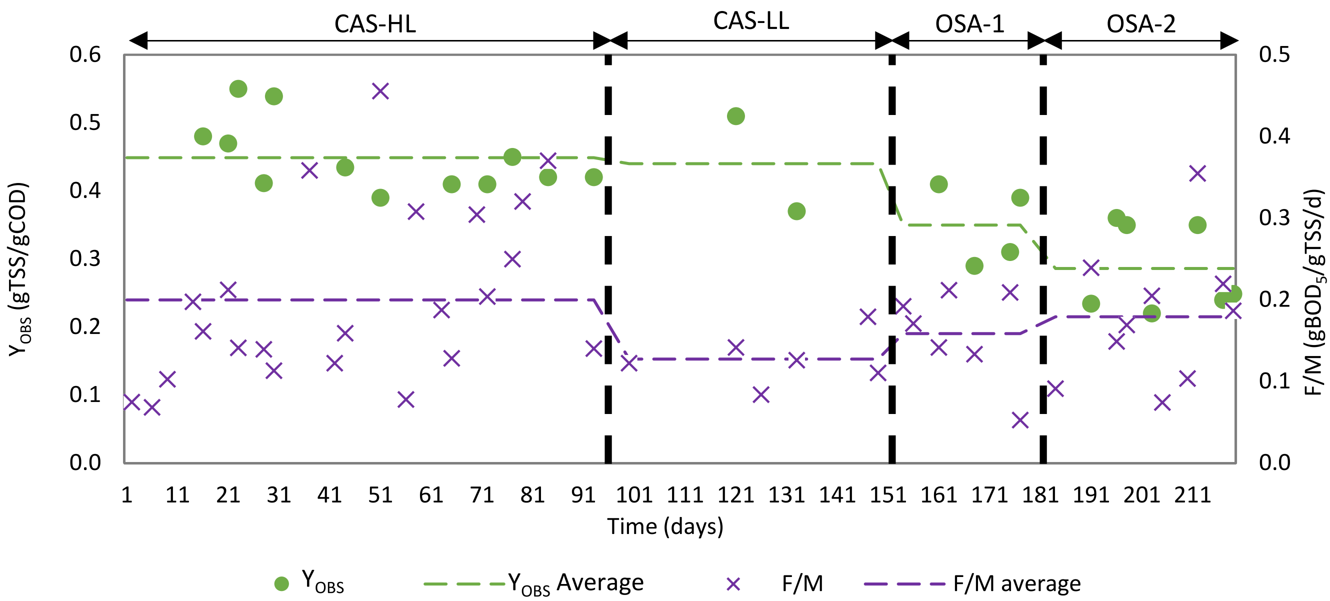

Fig. 9. Y_{OBS} and F/M values and averages of the monitoring periods.

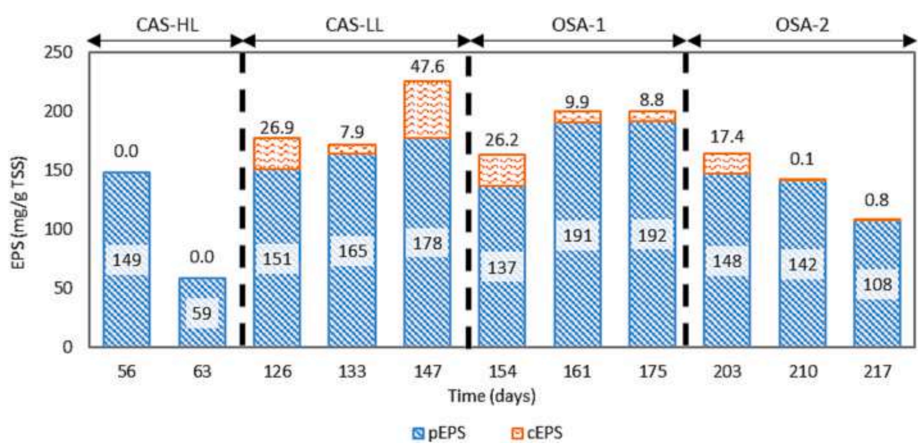

Fig. 10. pEPS and cEPS concentrations from different monitoring periods.

4. Conclusions

This study examined how modifying the CAS process with OSA affects effluent quality, bacteria kinetics, treatment cost, and GHG emissions.

- The effluent quality consistently met the Italian discharge limits, but there was a decrease in ammonium removal efficiency (CAS-HL: 94 %; CAS-LL: 92 %; OSA-1: 67 %; OSA-2: 63 %), potentially due to reduced biomass production and prolonged anaerobic conditions impacting nitrification.
- The OSA configuration significantly increased PO_4-P removal (CAS-HL: 40 %; CAS-LL: 16 %; OSA-1: 48 %; OSA-2: 68 %), indicating a higher phosphorus content in the wasted sludge.
- Heterotrophic biomass yield slightly decreased, indicating reduced biological sludge production, while endogenous decay increased, suggesting an increase in maintenance metabolism.
- The CAS-HL period had the best settling properties, while the OSA configuration resulted in a deterioration of sludge settling properties, likely due to decreased EPS and disruption of activated sludge floc under anaerobic conditions.
- Implementing the OSA configuration did not affect N_2O emissions from the aerobic reactor (the average was 0.2 mg/L in liquid), with lower N_2O concentrations observed in the SHT.
- By implementing OSA, there was a reduction in daily wasted sludge mass from 46 kg/d (CAS-LL) to 40 kg/d (OSA-1) and 38 kg/d (OSA-2), leading to 17.3 % potential cost savings associated with sludge disposal for OSA-2.
- Based on the findings, the OSA layout may have been more successful than the CAS layout in a situation with low wastewater strength. Although the findings of this study can be useful for other WWTPs with low wastewater strength, it would be beneficial to compare the effectiveness of CAS and OSA layouts during periods of higher wastewater strength to broaden the study's scope.

CRediT authorship contribution statement

Giorgio Mannina: Conceptualization, Resources, Writing – review & editing, Supervision, Project administration. **Alida Cosenza:** Investigation, Data curation, Writing – original draft, Writing – review & editing. **Daniele Di Trapani:** Investigation, Data curation, Writing – original draft, Writing – review & editing. **Hazal Gulhan:** Formal analysis, Data curation, Writing – original draft, Writing – review &

editing. **Antonio Mineo:** Formal analysis, Writing – original draft. **Paulo Marcelo Bosco Mofatto:** Formal analysis, Data curation, Writing – original draft.

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 data that has been used is confidential.

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