



# The effect of aeration mode (intermittent vs. continuous) on nutrient removal and greenhouse gas emissions in the wastewater treatment plant of Corleone (Italy)

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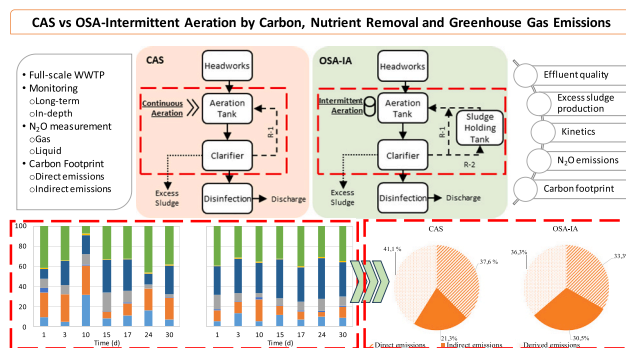
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## HIGHLIGHTS

- Both configurations adhered to the legal requirements for effluent quality.
- OSA-IA reduced sludge production but settleability worsened (SVI up to 180 mL/g).
- OSA-IA did not affect nitrification ability with similar average efficiency of 85 %.
- Similar N<sub>2</sub>O values in gas-liquid samples of both configurations (0.5 and 0.22 mg/L).
- OSA-IA reduced CF (0.36 kgCO<sub>2</sub>/m<sup>3</sup>) with higher proportion of indirect emissions.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

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## ABSTRACT

The paper reports the results of an experimental study aimed at comparing two configurations of a full-scale wastewater treatment plant (WWTP): conventional activated sludge (CAS) and oxic-settling-anaerobic process (OSA) with intermittent aeration (IA). A comprehensive monitoring campaign was carried out to assess multiple parameters for comparing the two configurations: carbon and nutrient removal, greenhouse gas emissions, respirometric analysis, and sludge production. A holistic approach has been adopted in the study with the novelty of including the carbon footprint (CF) contribution (as direct, indirect and derivative emissions) in comparing the two configurations. Results showed that the OSA-IA configuration performed better in total chemical oxygen demand (TCOD) and ortho-phosphate (PO<sub>4</sub>-P) removal. CAS performed better for Total Suspended Solids (TSS) removal showing a worsening of settling properties for OSA-IA. The heterotrophic yield coefficient and maximum growth rate decreased, suggesting a shift to sludge reduction metabolism in the OSA-IA configuration. Autotrophic biomass showed a reduced yield coefficient and maximum growth yield due to the negative effects of the sludge holding tank in the OSA-IA configuration on nitrification. The OSA-IA configuration had higher indirect emissions (30.5 % vs 21.3 % in CAS) from additional energy consumption due to additional

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mixers and sludge recirculation pumps. The CF value was lower for OSA-IA than for CAS configuration (0.36 kgCO<sub>2</sub>/m<sup>3</sup> vs 0.39 kgCO<sub>2</sub>/m<sup>3</sup> in CAS).

## 1. Introduction

The global population growth and industrialisation have strained wastewater treatment systems, resulting in challenges related to energy consumption, sludge production and carbon footprint (CF) (Garrido-Baserba et al., 2015; Campos et al., 2016; Kumar et al., 2021). Greenhouse gas (GHG) emissions in wastewater treatment originate directly from biological processes and indirectly from electricity consumption, transportation and use of chemicals (Mannina et al., 2020). The direct emissions arise from biomass growth producing carbon dioxide (CO<sub>2</sub>), from the reactions of nitrogenous substances, producing nitrous oxide (N<sub>2</sub>O), and from anaerobic digestion and sludge disposal, generating methane (CH<sub>4</sub>). These two latter with a higher global warming potential than CO<sub>2</sub> (IPCC, 2014). While efforts to reduce indirect emissions through electricity consumption minimisation seem promising, the trade-off between treatment performance and energy savings in wastewater treatment plants (WWTP) remains a significant challenge (Feng et al., 2018; Lu et al., 2023). The management practices and disposal of waste activated sludge (WAS) also require substantial energy consumption and chemical usage, leading to notable rises in CF and resource utilisation (Wang et al., 2023). Implementing sludge reduction strategies is an alternative approach in view of WAS disposal, but such a strategy must adhere to environmentally friendly practices to achieve sustainable wastewater treatment.

Several technologies/strategies for reducing sludge production in WWTPs have been adopted worldwide (Foladori et al., 2010). In particular, chemical (e.g., ozonation or chlorination), biological (e.g., biological predation), physical (increasing biomass retention membrane and granular sludge bioreactors), thermal (e.g., combustion, pyrolysis) and electrochemical processes (e.g., electro-osmosis) have been applied (Mannina et al., 2022a, 2022b). The reduced amounts of sludge to be treated in the sludge line will decrease the related operational cost and GHG emissions (Ferrentino et al., 2023). Among the processes that can be applied in the water line, adopting the oxic-settling-anaerobic (OSA) process strongly favours the reduction of sludge production (Mannina et al., 2023). Specifically, the OSA process modifies the conventional activated sludge (CAS) by inserting a sludge holding tank in the return activated sludge (RAS) line between the secondary settler and the aerobic bioreactor (Chudoba et al., 1992). Retaining the recirculated sludge under anaerobic conditions favours biological processes of sludge reduction (Ferrentino et al., 2023).

In traditional WWTPs, energy consumption may be responsible for approximately 25–40 % of the operational costs (Descoins et al., 2012; Campo et al., 2023). Moreover, energy consumption-related indirect emissions may represent 30 % of the total CF of WWTPs (Wang et al., 2023). Therefore, it is important to find a trade-off between CF reduction while maintaining high effluent quality and low operational costs

**Table 1**

Basic characteristics of the influent wastewater (average values ± standard deviations).

Parameter	Units	CAS	OSA-IA
COD	mg O <sub>2</sub> /L	173 (±47.5)	186 (±40.7)
BOD <sub>5</sub>	mg O <sub>2</sub> /L	100 (±43.4)	78 (±26)
Total nitrogen (TN)	mg N/L	42 (±12)	31 (±6.9)
Ammonium nitrogen (NH <sub>4</sub> <sup>+</sup> -N)	mg N/L	25 (±4.5)	23 (±2.4)
Phosphate (PO <sub>4</sub> <sup>3-</sup> -P)	mg P/L	7 (±2.6)	5 (±2.2)
Food to Microorganisms ratio (F/M)	kgBOD/(kgTSS d)	0.24 (±0.15)	0.21 (±0.10)

(Wang et al., 2023). Therefore, it is of great importance to introduce measures that may optimise processes in WWTPs with energy efficiency as a key issue. This aspect is particularly relevant considering the European Union (EU) energy dependency and the need to minimise GHG emissions (Elías-Maxil et al., 2014). In this light, introducing new strategies and/or advanced wastewater treatment technologies could represent a possible solution. Among the latest techniques, intermittent aeration (IA) can be an optimal solution (Di Bella and Mannina, 2020). More specifically, IA reduces the aeration time in a bioreactor by introducing non-aerated periods to enhance denitrification, while reducing energy consumption (Pang et al., 2020; Xu et al., 2020; Huang et al., 2021). Karlikanovaite-Balikci and Yagci (2019) tested IA in a laboratory scale OSA configuration (sequencing-batch reactor - SBR) and achieved TN removal efficiencies of over 85 %. Recently, Luan et al. (2022) showed the effectiveness of applying IA in an advanced pilot-scale moving bed biofilm reactor given guaranteeing nitrogen removal with low energy consumption from a low C/N influent wastewater.

In this light, the present study explored a modified configuration of the OSA system, referred to as OSA-IA that implements IA in the main aerobic bioreactor. As the authors are aware, the OSA-IA configuration has never been adopted at full scale in the literature. The treatment performance of the OSA-IA configuration, including carbon, nitrogen, and phosphorus removal efficiencies, was compared with the CAS configuration in a full-scale WWTP located in Corleone, Italy. The process kinetics, EPS production, settleability, and sludge production were regularly monitored and quantified throughout the study. This study offers a novel aspect of conducting a full-scale comparison of the two configurations investigated with a holistic approach, including GHG emissions and CF.

## 2. Materials and methods

### 2.1. Study site

The WWTP in Corleone has a capacity of 12,000 population equivalents (PE) and employs a CAS configuration; the WWTP layout is characterised by two identical aeration tanks followed by three identical settling tanks. Since the Corleone WWTP is underloaded compared to the design values, only one aeration tank followed by two secondary clarifiers are under operation (Mannina et al., 2021a, 2021b, 2023). The wastewater is transported to the plant by a combined sewer system. The average influent flow rate to the aeration tank is given in Table 1.

The aeration tank has a volume of 384 m<sup>3</sup>, while each clarifier has a diameter of 12 m. The RAS flow rate from the clarifiers to the aeration tank is 74 m<sup>3</sup>/h. In the CAS configuration, RAS is pumped back to the aeration reactor, and this flow rate is referred to as R-1.

To modify the Corleone WWTP to the OSA configuration, a sludge holding tank (SHT), piping and instruments have been implemented using the existing empty aeration tank (Fig. 1). Additionally, the blower supplying air to the aeration tank is controlled in an on/off mode to achieve IA with 40 min of aeration and 20 min of mixing. Under the OSA-IA configuration, the same amount of sludge is recirculated from the clarifiers to the aeration tank. However, the flow rate is equally split between the aeration tank (referred to as R-1) and the SHT (referred to as R-2). Excess sludge is pumped from the bottom of the clarifiers to aerobic digesters. The digested sludge is then thickened in drying beds before disposal. The secondary effluent is directed to the disinfection unit and discharged into a nearby river.

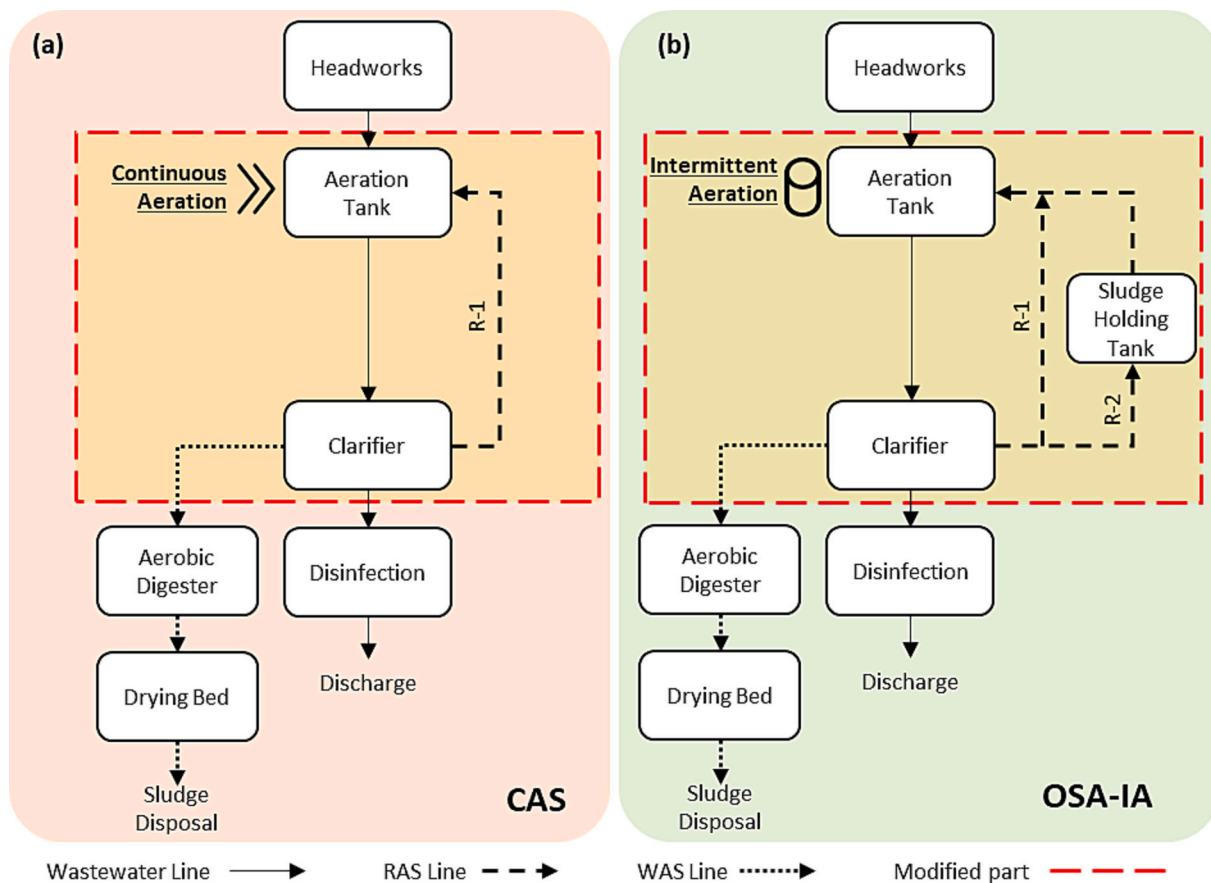


Fig. 1. Layout of the CAS (a) and OSA-IA (b) systems.

## 2.2. Sampling campaigns

Two sampling strategies were implemented, each characterised by a different sampling frequency: (i) in-depth analysis during 24 h and (ii) long-term monitoring. In the first strategy, 24 samples were collected daily using refrigerated autosamplers (one sample per hour in one day of operation). In the second strategy, two weekly grab samples were collected to get information on the long-term plant behaviour. In more detail, the long-term monitoring campaign aimed to gain insights into the treatment performance and WAS production in the CAS and the OSA-IA layout. In the long-term campaign, the samples were collected at the inlet to the biological reactor, the outlet from the final clarifiers, points inside the bioreactor and in the RAS line. Samples at the outlet of the clarifiers were collected 6.5 h after the inlet samples, to account for the HRT of the system. The duration of the long-term campaign was 30 days for both configurations. In two in-depth sampling campaigns, influent (after pre-treatment) and effluent (at the outlet of final clarifiers) were collected hourly for one day by refrigerated auto-samplers. To account for the plant's HRT, the autosampler placed at the outlet of clarifiers was started 6.5 h after that in the inlet. In addition, three grab samples were collected from the bioreactor and one from the RAS line to monitor the MLSS concentration.  $N_2O$  emissions from the bioreactors were monitored between 11:00 and 14:00 h and comprised four samples per hour. A gas sampling hood (cross-sectional area: 1.0 m × 0.9 m) was placed on the surface of the bioreactors (CAS) and the aerobic and anaerobic tanks (OSA-IA configuration). Gas samples were collected in 0.5 L Tedlar bags (Tedlar, USA) through an air pump (Sensidyne, USA). The airflow rate from the surface of the aerobic tank was measured by an anemometer (Extech, USA) according to [Caniani et al. \(2019\)](#). In the liquid phase,  $N_2O$  concentrations were measured by a micro-sensor (Unisense Environment A/S, Denmark) per minute

parallel to the gas sampling.

[Table 1](#) presents basic characteristics (average values ± standard deviations) of the influent wastewater, including chemical oxygen demand (COD), 5-day biochemical oxygen demand ( $BOD_5$ ), total and ammonium nitrogen, orthophosphate and TSS concentrations.

## 2.3. Analytical methods

The concentrations of 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) were determined using the Standard Methods ([APHA, 2012](#)). The sludge settling performance was evaluated using the sludge volume index (SVI). To extract extracellular polymeric substances (EPS) and soluble microbial products (SMP), the method described in [Le-Clech et al. \(2006\)](#) was followed. Proteins were quantified based on the procedure outlined by [Lowry et al. \(1951\)](#), while carbohydrates were measured according to the method given by [DuBois et al. \(1956\)](#). Respirometric batch tests were carried out according to literature ([Di Trapani et al., 2018](#)) and were aimed at measuring the Oxygen Uptake Rate (OUR) for consuming a readily biodegradable substrate spiked during the test (sodium acetate for heterotrophs and ammonium chloride for autotrophs, respectively). The dissolved and gaseous  $N_2O$  concentrations were measured using the methodology described by [Mannina et al. \(2018\)](#). They were conducted using a Gas Chromatograph (GC) equipped with an Electron Capture Detector (ECD) (Agilent 8860). The  $N_2O$  emission factor ( $EF_{N_2O}$ ) was determined using the calculation method outlined by [Tsuneda et al. \(2005\)](#) (Eq. (1)):

$$EF_{N_2O} = \frac{\frac{N_2O-N_g}{HRT_{bs}} + \frac{N_2O-N_d}{HRT}}{TN} \quad (1)$$

where  $N_2O-N_g$  represents the concentration of gaseous  $N_2O$ , while  $N_2O-N_d$

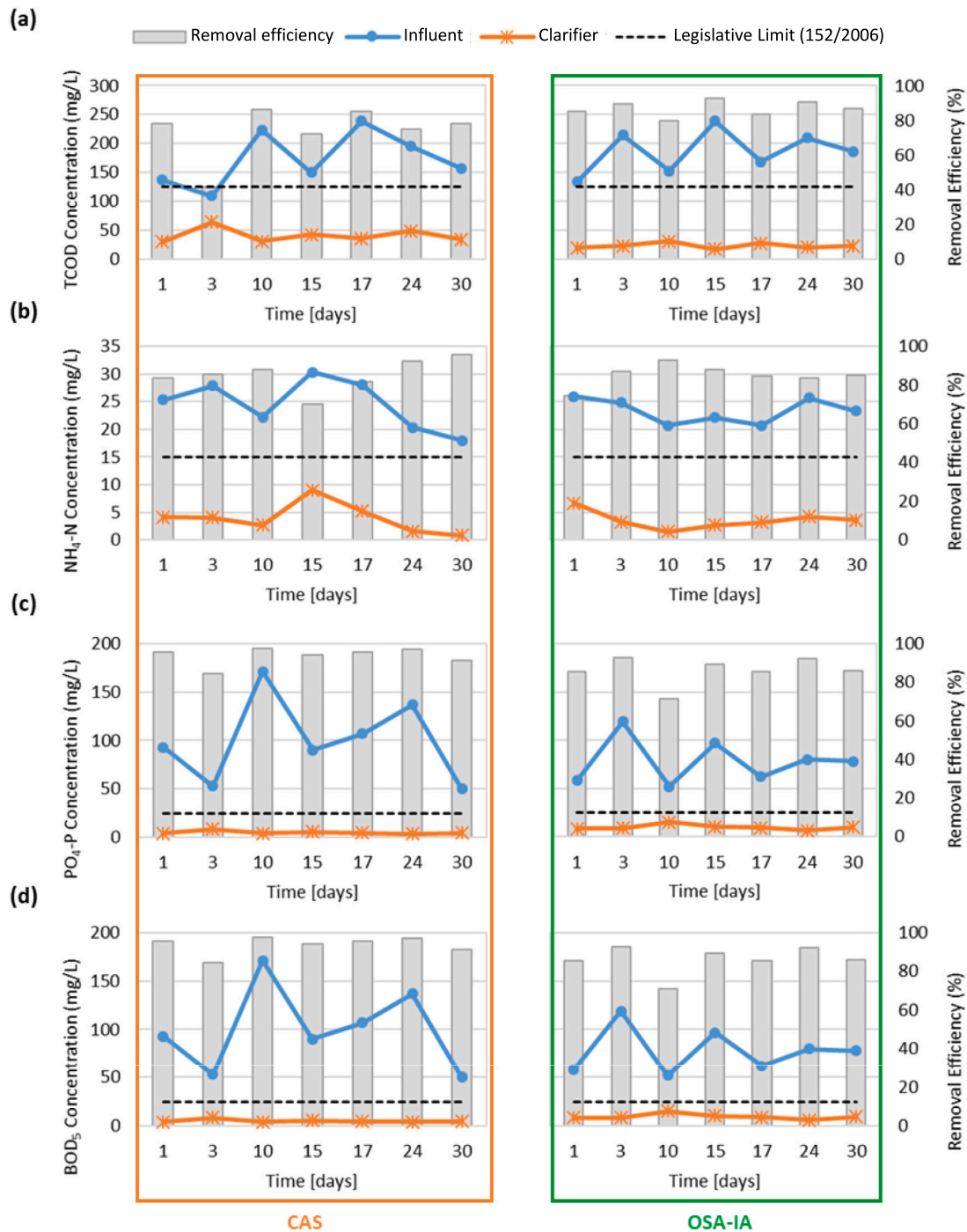


Fig. 2. Treatment performances and legislative limits for CAS and OSA-IA for (a) TCOD, (b) NH<sub>4</sub>-N, (c) PO<sub>4</sub>-P, and (d) BOD<sub>5</sub>.

N<sub>d</sub> refers to the concentration of dissolved N<sub>2</sub>O. The HRT stands for the hydraulic retention time in the plant, while HRT<sub>hs</sub> represents the retention time in the tank headspace. TN represents the influent concentration of total nitrogen in the influent flow.

The observed yield coefficient (Y<sub>obs</sub>) was calculated by dividing the cumulative mass of biomass produced by the COD removed (Mannina et al., 2023) and is represented by Eq. (2):

$$Y_{obs} \left( \frac{gTSS}{gCOD} \right) = \frac{\Delta X}{Q_i \times (TCOD_{inf} - TCOD_{eff})} \quad (2)$$

where COD<sub>inf</sub> and COD<sub>eff</sub> represent the total COD concentrations at the influent and effluent, respectively. Q<sub>i</sub> denotes the daily influent flow

rate, and ΔX represents the daily WAS produced.

#### 2.4. Carbon footprint quantification

To assess the CF of the WWTP under study, the GHG emissions (direct, indirect and derived) were quantified and summed. The direct emissions (DE) were quantified by considering the emitted CO<sub>2</sub> due to organic carbon oxidation (CO<sub>2,OrgOx</sub>), endogenous respiration (CO<sub>2,Endog.</sub>) and the equivalent CO<sub>2</sub> due to N<sub>2</sub>O emission (CO<sub>2eq,N2O</sub>). CO<sub>2,OrgOx</sub> (Eq. (3)) and CO<sub>2,Endog</sub> (Eq. (5)) were quantified according to Boiocchi et al. (2023):

$$CO_{2,OrgOx} = FC_s \bullet r_{o2} \quad [kgCO_2/d] \quad (3)$$

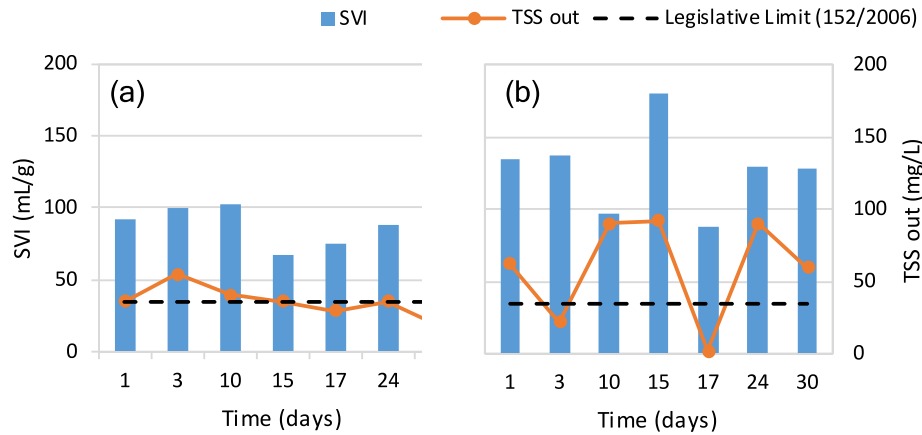


Fig. 3. Comparison of the SVI values end effluent TSS concentrations in (a) CAS and (b) OSA-IA.

Table 2

Summary of the main heterotrophic kinetic and stoichiometric parameters as the average values ( $\pm$  the standard deviation).

Parameter	Symbol	Units	Heterotrophic	
			CAS	OSA-IA
Yield	$Y_H$	[gVSS/gCOD]	0.44 ( $\pm 0.04$ )	0.39 ( $\pm 0.07$ )
Decay rate	$b_H$	[1/d]	0.47 ( $\pm 0.09$ )	0.46 ( $\pm 0.17$ )
Max. growth rate	$\mu_H$	[1/d]	1.64 ( $\pm 0.39$ )	0.93 ( $\pm 0.68$ )
Max. removal rate	$\nu_H$	[1/d]	3.90 ( $\pm 0.45$ )	2.24 ( $\pm 1.31$ )
Net growth rate	$\mu_H - b_H$	[1/d]	1.16 ( $\pm 0.22$ )	0.47 ( $\pm 0.52$ )
Active fraction	$f_x$	[%]	14.2 ( $\pm 1.32$ )	18.1 ( $\pm 1.06$ )

Parameter	Symbol	Units	Autotrophic	
			Literature	OSA-IA
Yield	$Y_A$	[gVSS/gNH <sub>4</sub> -N]	0.19–0.26 (Ramirez-Vargas et al., 2013)	0.15 ( $\pm 0.09$ )
Max. growth rate	$\mu_A$	[1/d]	0.26–0.38 (Ramirez-Vargas et al., 2013)	0.19 ( $\pm 0.06$ )
Max. removal rate	$\nu_A$	[1/d]	1.39–1.48 (Ramirez-Vargas et al., 2013)	2.154 ( $\pm 1.79$ )
Nitrification rate	$N_R$	[mgNH <sub>4</sub> /L h]	na	3.77 ( $\pm 2.66$ )

Table 3

Operational parameters of Corleone WWTP during the monitoring campaign (in brackets the standard deviation).

Parameter	Units	CAS	OSA-IA
$Y_{obs}$	[gTSS/gCOD]	0.45 ( $\pm 0.08$ )	0.34 ( $\pm 0.08$ )
F/M	[kgCOD/(kgTSS d)]	0.24 ( $\pm 0.19$ )	0.21 ( $\pm 0.10$ )
SRT	[d]	20 ( $\pm 4.5$ )	59.71 ( $\pm 7.56$ )

where FCS is the conversion factor describing the amount of CO<sub>2</sub> emitted per kg of consumed O<sub>2</sub> (equal to 1.1 kg CO<sub>2</sub>/kg O<sub>2</sub> according to Boiocchi et al. (2023)) and ro<sub>2</sub> is the amount of oxygen consumed per day [kg O<sub>2</sub>/d] calculated according to Eq. (4):

$$r_{o_2} = V_{rs} \times 1/f - 1.42 \times Y \quad (4)$$

where  $V_{rs}$  is the removed BOD<sub>5</sub> [kgBOD<sub>5</sub>/d] calculated based on the measured data, f equals 0.68 and Y is the yield coefficient [kgVSS/kgBOD<sub>5</sub>]. In this study, the average measured  $Y_{obs}$  value was adopted as Y.

$$CO_{2,Endog} = FC_{End} \cdot m_{VSS} \quad [kgCO_2/d] \quad (5)$$

where FC<sub>End</sub> is the conversion factor describing the amount of CO<sub>2</sub> emitted per kg of produced VSS (equal to 1.947 kg CO<sub>2</sub>/kgVSS according to Boiocchi et al., 2023) and  $m_{VSS}$  is the mass of VSS evaluated based on the mass balance.

The CO<sub>2eq,N2O</sub> was quantified based on the measured data according to Eq. (6):

$$CO_{2,N2O} = Q_g \cdot C_{g,N2O} \cdot GWP_{N2O} \quad [kgCO_{2eq}/d] \quad (6)$$

where  $Q_g$  [m<sup>3</sup>/d] is the average gas flow rate,  $C_{g,N2O}$  [kgN<sub>2</sub>O/m<sup>3</sup>] is the average gaseous measured N<sub>2</sub>O concentration emitted and GWP<sub>N2O</sub> [kgCO<sub>2eq</sub>/kgN<sub>2</sub>O] is the N<sub>2</sub>O global warming potential (equal to 298 according to IPCC, 2022).

The indirect emissions (IE) count the equivalent CO<sub>2</sub> due to energy consumption (CO<sub>2eq,En</sub>) (Eq. (7)) and to the sludge treatment, transportation and landfill disposal (CO<sub>2eq,Sludge</sub>) (Eq. (8)).

$$CO_{2eq,En} = E_n \cdot FC_{En} \quad [kgCO_{2eq}/d] \quad (7)$$

where  $E_n$  [kWh/d] is the total energy consumption of the water line and FC<sub>En</sub> [kgCO<sub>2eq</sub>/kWh] is the conversion factor of the energy (equal to 0.252 kgCO<sub>2eq</sub>/kWh according to EEA, 2016).

$$CO_{2eq,Sludge} = M_{sludge} \cdot FC_{Sludge} \quad [kgCO_{2eq}/d] \quad (8)$$

where  $M_{sludge}$  [ton/day] is the mass of wasted sludge per day and FC<sub>Sludge</sub> [kgCO<sub>2eq</sub>/ton] is the emission factor due to the sludge treatment, transportation and landfill disposal (equal to 714.74 kgCO<sub>2eq</sub>/ton according to Zhao et al. (2023)).

Finally, the derivative emissions (DerE) were quantified as those originated from the pollutants discharged into receiving water bodies (Eq. (9)):

$$DerE = CO_{2eq,effBOD} + CO_{2eq,effN2O} \quad [kgCO_{2eq}/d] \quad (9)$$

Specifically, the contribution of BOD (CO<sub>2eq,effBOD</sub>) and dissolved N<sub>2</sub>O (CO<sub>2eq,effN2O</sub>) was calculated according to Eqs. (10) and (11), respectively.

$$CO_{2eq,effBOD} = M_{BOD} \cdot FC_{BOD} \quad [kgCO_{2eq}/d] \quad (10)$$

where  $M_{BOD}$  [kgBOD/d] is the mass of discharged BOD per day and FC<sub>BOD</sub> [kgCO<sub>2eq</sub>/kgBOD] is the conversion factor due to the BOD discharge (equal to 0.96 kgCO<sub>2eq</sub>/kgBOD according to Boiocchi et al. (2023)).

$$CO_{2eq,effN2O} = Q_w \cdot C_{1,N2O} \cdot GWP_{N2O} \quad [kgCO_{2eq}/d] \quad (11)$$

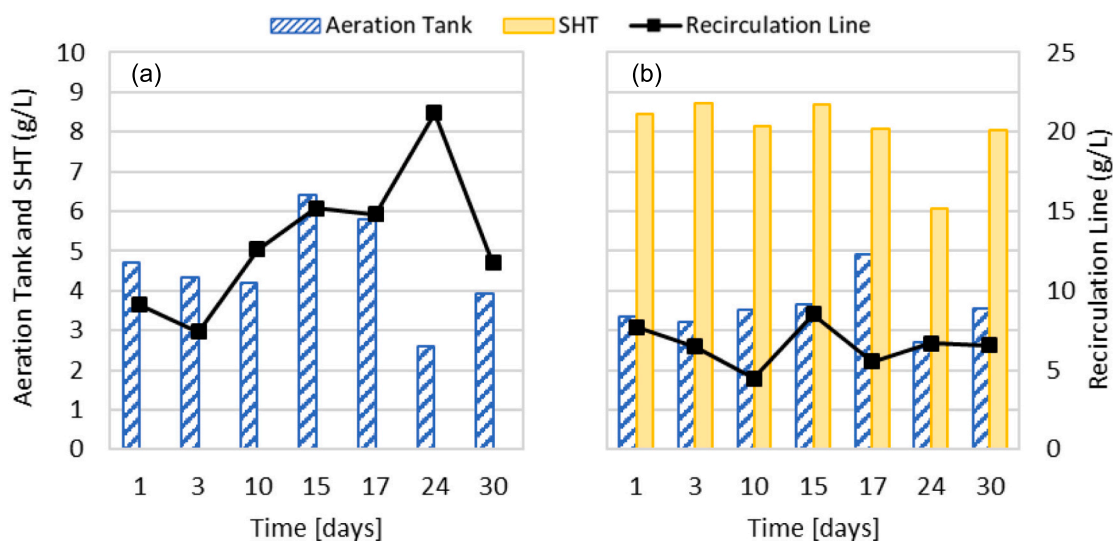


Fig. 4. TSS concentrations in the aeration tank, SHT, and RAS for (a) CAS and (b) OSA-IA.

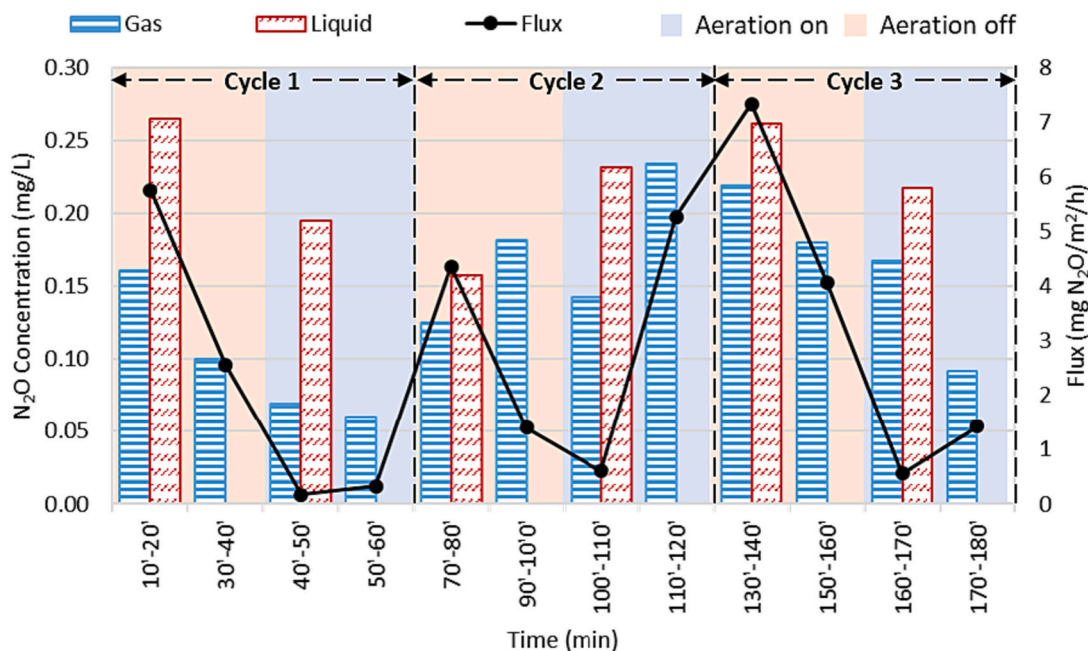


Fig. 5. Variations in  $N_2O$  concentrations in the gas and liquid samples and the  $N_2O$  flux during the three-phase cycle.

where  $Q_w$  [ $m^3/d$ ] is the average effluent flow rate,  $Cl_{N_2O}$  [ $kgN_2O/m^3$ ] is the average liquid measured  $N_2O$  concentration discharged into the water body and  $GWPN_2O$  [ $kgCO_{2eq}/kgN_2O$ ] is the  $N_2O$  global warming potential (equal to 298 according to IPCC, 2022).

## 2.5. Comparison criteria

The study compared the treatment performance of the CAS and OSA-IA configurations by measuring carbon, nitrogen, and phosphorus removal efficiencies. Further, the carbon footprint was assessed as a further criterion of the comparison.

## 3. Results and discussion

### 3.1. Comparison of the treatment performances

Fig. 2 presents the performances for COD,  $NH_4-N$ ,  $PO_4-P$ , and  $BOD_5$  for both CAS and OSA-IA configurations along with the corresponding legislative limits. The CAS configuration exhibited a COD removal efficiency of  $74 \pm 14\%$ , while the OSA-IA configuration achieved a higher COD removal efficiency of  $87 \pm 4\%$ . For comparison, Vitanza et al. (2019) reported an 85% COD removal efficiency in a pilot-scale OSA plant over an extended period, which aligns with this study's findings regarding the OSA-IA configuration. That configuration outperformed the CAS configuration regarding the  $PO_4-P$  removal efficiency, with values of  $55 \pm 27\%$  vs.  $35 \pm 17\%$ . These results are in line with the results of earlier studies, which also reported enhanced biological

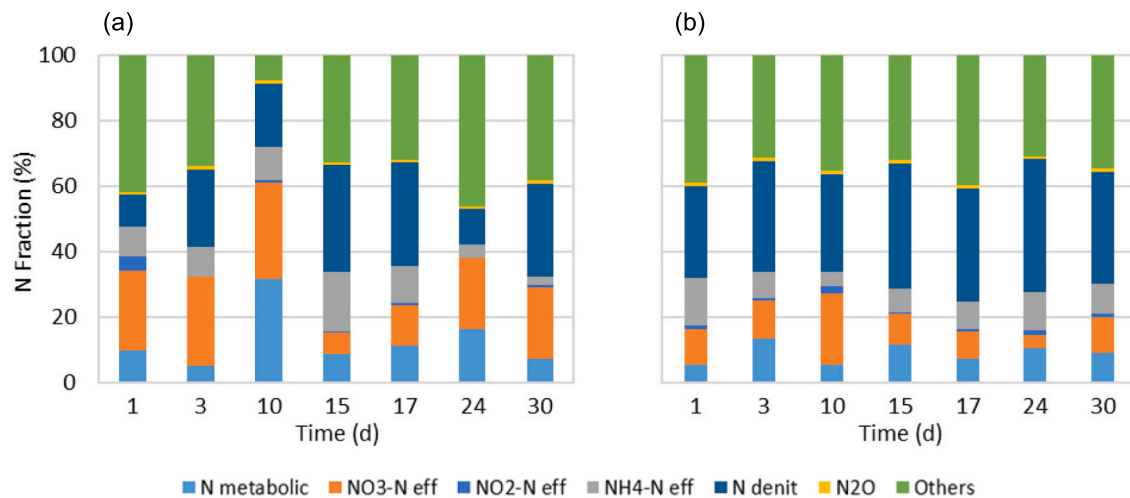


Fig. 6. Comparison of N fractions in (a) CAS and (b) OSA-IA.

phosphorus removal (EBPR) in WWTPs employing the OSA process. Cosenza et al. (2023) emphasised that altering between aerobic/anoxic and anaerobic conditions in the OSA configuration could foster the development of phosphate-accumulating organisms (PAOs) or denitrifying phosphate-accumulating organisms (DPAOs). Although the OSA configuration enhances phosphorus removal by promoting PAO or DPAOs with increased anoxic/anaerobic conditions, the OSA-IA configuration in this study did not negatively affect the  $\text{NH}_4\text{-N}$  removal efficiency. The CAS and OSA-IA configurations demonstrated similar efficiencies, with the values of  $85 \pm 8\%$  vs.  $85 \pm 6\%$ , respectively. However, regarding the  $\text{BOD}_5$  removal efficiency, the CAS configuration had a higher performance, achieving  $94 \pm 4\%$  compared to  $86 \pm 7\%$  for the OSA-IA configuration. Similarly, in terms of TSS removal, the CAS configuration outperformed the OSA-IA configuration, achieving a removal efficiency of  $89 \pm 6\%$  compared to  $59 \pm 22\%$ . Mannina et al. (2023) compared the CAS and OSA configurations, demonstrating that the OSA configuration did not influence COD removal. This suggests that intermittent aeration compromised settling performance in the OSA-IA configuration. Singh et al. (2018) also observed a worsening of the sludge settleability with intermittent aeration. Nevertheless, both configurations had an effluent quality that complies with the legal Italian limits.

Fig. 3 shows the trend of SVI and the effluent TSS concentrations in the CAS (Fig. 3a) and the OSA-IA (Fig. 3b) periods. The worsened sludge settleability and TSS removal efficiency during the OSA-IA configuration were correlated with the huge decrease in EPS content observed in the OSA-IA configuration. The sum of bound EPS and SMP decreased from 1812 mg/g TSS to 44 mg/g TSS. This reduction in the EPS content may be related to the consumption of stored substrates under fasting conditions. Such conditions promoted the destruction of the activated sludge flocs, thus causing a worsening of settling properties. Indeed, alternating aerobic/anoxic conditions may enhance the reductive conditions and promote hydrolysis (Singh et al., 2018).

### 3.2. Comparison of kinetic and stoichiometric coefficients

Table 2 summarises the kinetic/stoichiometric parameters achieved during experiments using respirometric batch tests. The data reported in Table 2 suggest that the shift from the CAS to OSA-IA configuration significantly impacted the biomass activity. Concerning the heterotrophs, the yield coefficient showed a slight decrease from 0.44 to 0.39  $\text{gVSS g}^{-1}\text{COD}$ , thus highlighting that the new plant layout might effectively promote the reduction of biological sludge production. This result was corroborated by the maximum specific growth rate, which showed a significant reduction when the new OSA-IA configuration was

implemented. This suggests the establishment of the maintenance metabolism responsible for the reduced sludge production. The net growth rate also showed a significant decrease, in agreement with the previous results.

When the plant configuration was changed to OSA-IA, respirometric batch tests were also performed on autotrophs. The observed results showed a slight decrease in both the yield coefficient and the maximum specific growth rate, thus corroborating the findings of Sun et al. (2020). In that study, the implementation of the anaerobic reactor resulted in a reduction of the nitrification activity (Sun et al., 2020).

### 3.3. Comparison of sludge production in the two configurations

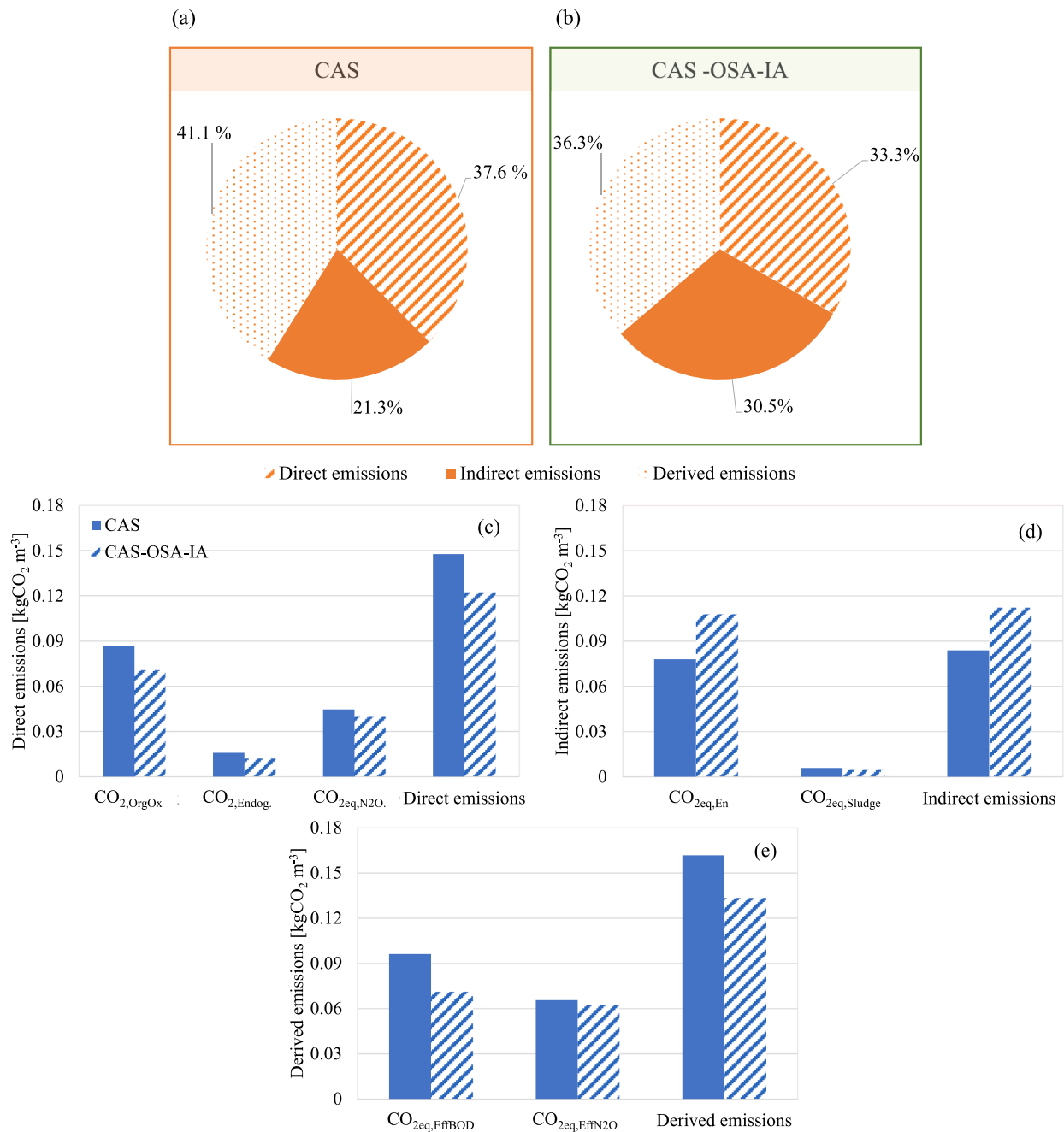
Table 3 reports the average values of  $Y_{\text{obs}}$ , F/M and SRT. It can be noticed that the average  $Y_{\text{obs}}$  value decreased from 0.45  $\text{gTSS/gCOD}$  (period CAS) to 0.34 in the OSA-IA period, respectively. The modification of the WWTP layout (insertion of the anaerobic reactor in the RAS line) coupled with changing the aeration strategy (from continuous to intermittent) had a positive role in reducing WAS production. Indeed, sludge reduction could be improved by the intermittent aeration strategy, which generally might reduce sludge production compared to a conventional system with continuous aeration (Jung et al., 2006). Fig. 4 shows the trend of TSS concentrations in the aeration tank, SHT, and recirculation line for the CAS and OSA-IA configuration.

### 3.4. Comparison of $\text{N}_2\text{O}$ emissions

Liquid and gas samples were collected at specific time intervals to assess the concentrations of  $\text{N}_2\text{O}$ . Fig. 5 shows three cycles of IA with alternating on/off periods. During the blower off periods, the concentrations of  $\text{N}_2\text{O}$  decreased in both gas and liquid samples and the  $\text{N}_2\text{O}$  fluxes were also reduced.

When the blower was on, the average  $\text{N}_2\text{O}$  concentrations in the gas and liquid samples were measured at  $0.16 \pm 0.04 \text{ mgN}_2\text{O/L}$  and  $0.23 \pm 0.05 \text{ mgN}_2\text{O/L}$ , respectively. When the blower was off, the average  $\text{N}_2\text{O}$  concentration decreased to  $0.13 \pm 0.06 \text{ mg/L}$  in the gas samples and  $0.21 \pm 0.02 \text{ mg/L}$  in the liquid samples. This decrease in  $\text{N}_2\text{O}$  concentrations led to a significant 67% reduction in the  $\text{N}_2\text{O}$  flux, which decreased from  $4.24 \pm 1.95 \text{ mg N}_2\text{O/m}^2/\text{h}$  to  $1.39 \pm 1.78 \text{ mg N}_2\text{O/m}^2/\text{h}$ . The  $\text{N}_2\text{O}$  concentrations in the gas and liquid samples collected from the SHT were  $0.17 \text{ mg/L}$  and  $0.27 \text{ mg/L}$ , respectively, and the calculated  $\text{N}_2\text{O}$  flux from the SHT was  $1.18 \text{ mg N}_2\text{O/m}^2/\text{h}$ .

The average  $\text{N}_2\text{O}$  concentrations in the gas and liquid samples collected in an IA cycle were  $0.15 \pm 0.05 \text{ mg/L}$  and  $0.22 \pm 0.04 \text{ mg/L}$ , respectively. The flux from the OSA-IA configuration was  $3.29 \pm 2.35$



**Fig. 7.** Contributions of emission compounds for the CAS (a) and OSA-IA (b) configuration; the direct (c), indirect (d) and derived (e) emission contributions for the CAS and OSA-IA configurations.

mg N<sub>2</sub>O/m<sup>2</sup>/h. For comparison, the CAS configuration had the same average N<sub>2</sub>O concentrations in the gas and liquid samples ( $0.15 \pm 0.07$  mg/L and  $0.22 \pm 0.02$  mg/L, respectively). However, the N<sub>2</sub>O flux calculated from the CAS configuration was  $2.25 \pm 1.06$  mg N<sub>2</sub>O/m<sup>2</sup>/h. The increased N<sub>2</sub>O flux observed in the OSA-IA configuration resulted from a higher air flow rate measured over the aeration tank surface, likely due to the lower MLSS concentration in this layout. The average MLSS concentrations were  $4.6 \pm 1.2$  g/L and  $3.6 \pm 0.6$  g/L for the CAS and OSA-IA configurations, respectively. It is worth noting that the reported N<sub>2</sub>O concentrations in the liquid and gas phase represent the average value of three replicates, with standard deviation values one order of magnitude lower compared to the mean, thus highlighting good data reliability.

### 3.5. Nitrogen mass balance

Fig. 6 illustrates the N mass balance, which includes various nitrogenous components, such as N consumed for metabolic activities, the effluent N in the forms of NO<sub>3</sub>-N, NO<sub>2</sub>-N, NH<sub>4</sub>-N, and N<sub>2</sub>O-N, as well as N denitrified and others referring to organic N.

In the CAS configuration,  $13 \pm 8$  % of the influent N load was consumed for metabolic activities. However, this percentage decreased to  $9 \pm 3$  % in the OSA-IA configuration, indicating a reduced biomass activity. The decrease in the percentage of N load consumed for metabolic activities during OSA-IA configuration was debited to the reduction during this period of the influent BOD<sub>5</sub>. Indeed, the average BOD<sub>5</sub> concentration decreased from  $100 (\pm 43.4)$  mg/L during the CAS configuration period to  $78 (\pm 26)$  mg/L of OSA-IA one. The effluent



fractions of  $\text{NH}_4\text{-N}$  ( $9 \pm 5\%$  for CAS and  $9 \pm 3\%$  for OSA-IA) and  $\text{NO}_2\text{-N}$  ( $0.9 \pm 1.4\%$  for CAS and  $1.1 \pm 0.6\%$  for OSA-IA) were similar for both configurations. However, notable differences were observed in the effluent  $\text{NO}_3\text{-N}$  and N denitrified fractions. The OSA-IA configuration exhibited higher denitrification with an N denitrified fraction of  $34 \pm 4\%$ , compared to  $22 \pm 9\%$  for the CAS configuration with an N denitrified fraction. Consequently, the effluent fraction of  $\text{NO}_3\text{-N}$  was lower in the OSA-IA configuration ( $11 \pm 5\%$ ) than in the CAS configuration ( $21 \pm 8\%$ ). This difference could be attributed to the anoxic exposure of biomass in the SHT (Vitanza et al., 2019) and the off-period of IA cycles (Miao et al., 2022).

### 3.6. Carbon footprint

The CF value obtained for the CAS and OSA-IA configuration was equal to  $0.39 \text{ kgCO}_2/\text{m}^3$  and  $0.36 \text{ kgCO}_2/\text{m}^3$ , respectively, showing a slight CF reduction for the OSA-IA configuration. These values are lower than those reported in the literature for CAS systems treating real wastewater (Hu et al., 2019). The contributions of the direct, indirect and derived emissions for both CAS and OSA-IA configurations are shown in Fig. 7. For the CAS configuration, the most outstanding CF contribution was due to the derived emissions (41.1%), followed by the direct (37.6%) and indirect emissions (21.3%) (Fig. 7a). For the OSA-IA configuration, the contributions were different with the values of 36.3%, 33.3% and 30.5%, respectively, for the direct, indirect and derived emissions (Fig. 7b).

The indirect emission contribution in the OSA-IA configuration (30.5%) strongly increased compared to CAS (21.3%). In Fig. 7c–e, the direct, indirect and derived emissions compounds are reported for both CAS and OSA-IA configurations to illustrate better which emission compound greatly contributes to the total CF. By analysing the data reported in Fig. 7c–e, it can be seen that except for the combinations of the indirect emissions, all the emission contributions related to the OSA-IA configurations are lower than that of CAS. Indeed, the direct emissions of OSA-IA reduced to  $0.12 \text{ kgCO}_2/\text{m}^3$  from  $0.14 \text{ kgCO}_2/\text{m}^3$  of CAS (Fig. 7c). The derived emissions of OSA-IA were also reduced to  $0.13 \text{ kgCO}_2/\text{m}^3$  from  $0.16 \text{ kgCO}_2/\text{m}^3$  of CAS (Fig. 7e). In contrast, the contribution due to the energy consumption of OSA-IA ( $0.1 \text{ kgCO}_2/\text{m}^3$ ) was higher than that of the CAS configuration ( $0.07 \text{ kgCO}_2/\text{m}^3$ ) (Fig. 7d). This result is mainly related to the additional energy consumption due to the mixer and RAS pumps installed in the plant to set up the OSA-IA configuration. However, despite the increased energy consumption, the total CF of the OSA-IA configuration was lower than that of CAS.

## 4. Conclusions

This study has the novelty of comparing CAS and OSA-IA configuration at full-scale using a holistic approach including GHG emissions and CF. Both configurations enabled the meeting of regulation limits for effluent quality. The OSA-IA configuration performed better than CAS in COD and  $\text{PO}_4\text{-P}$  removal, but had lower efficiencies in  $\text{BOD}_5$  and TSS removal, suggesting a compromised settling performance. However, the addition of the SHT negatively affected nitrifiers activity and the overall nitrification performance.

The OSA-IA configuration produced less sewage sludge than CAS as  $Y_{\text{obs}}$  was  $0.45 \text{ gTSS/gCOD}$  for CAS vs.  $0.34 \text{ gTSS/gCOD}$  for OSA-IA. Regarding the CF, both configurations had similar  $\text{N}_2\text{O}$  concentrations in gas and liquid samples, but the  $\text{N}_2\text{O}$  flux in the OSA-IA configuration was higher. Furthermore, the OSA-IA setup had a higher contribution from the indirect emissions (21.3% in CAS vs. 30.5% in OSA-IA) due to the additional energy consumption from mixers and recycled sludge pumps. Despite this increase, the total CF of OSA-IA ( $0.36 \text{ kgCO}_2/\text{m}^3$ ) was slightly lower than that of CAS ( $0.39 \text{ kgCO}_2/\text{m}^3$ ).

## CRedit authorship contribution statement

**Giorgio Mannina:** Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Paulo Marcelo Bosco Mofatto:** Conceptualization. **Alida Cosenza:** Data curation, Conceptualization, Investigation, Formal analysis, writing. **Daniele Di Trapani:** Data curation, Conceptualization. **Hazal Gulhan:** Data curation, Conceptualization, Investigation, Formal analysis, writing. **Antonio Mineo:** Data curation. **Jacek Makinia:** Data curation, Conceptualization.

## 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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