Odour prevention strategies in wastewater treatment plants: A pilot scale study of activated sludge recycling and oxidized nitrogen recycling

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A B S T R A C T

The potential of activated sludge recycling (ASR) and oxidized nitrogen recycling (ONR) to prevent the emissions of H_2S and acetic acid from the primary settler during domestic wastewater treatment was herein evaluated. The pilot plant consisted of an 8 L primary settler with a 10 L gas-tight headspace coupled to a 11 L nitrification-denitrification activated sludge reactor and an 8 L secondary settler, which were monitored for 175 days. A reduction in the headspace concentrations of H_2S and acetic acid by 95 % and 42 %, respectively, was recorded when combining ASR and ONR. Process operation with ASR and ONR supported stable conditions with average organic matter removals of 96 ± 2 %, NO_2^- concentrations of 24.2 ± 0.4 mg N/L and NO_3^- concentrations of 9.8 ± 0.4 mg N/L in the effluent, and a biological oxidation of S^{2-} higher than 99 % with average SO_4^{2-} concentrations of 52 ± 8 mg/L. Interestingly, the sole recirculation of activated sludge to the primary settler without NO_3^- recycling caused sludge bulking, contributing to increase the concentration of H_2S and acetic acid in the primary settler headspace up to 0.99 ± 0.01 ppm$_v$ and 2.87 ± 0.12 ppm$_v$, respectively. Sludge bulking also resulted in an increase in the effluent soluble total nitrogen concentration from 5.6 ± 0.1 mg N/L to 50.8 ± 0.2 mg N/L and of NH_4^+ from 1.3 ± 0.2 mg N/L to 50.7 ± 0.8 mg N/L due to the loss of nitrification under these operational conditions. Overall, the experimental results indicated that ASR and ONR represent cost-effective strategies for the control of malodorous emissions in wastewater treatment plants.

1. Introduction

The emission of malodours is inherent to wastewater storage, transportation and treatment activities [1]. Odours are generated during wastewater collection and treatment operations depending on the composition of the wastewater, and the environmental and operational conditions. In this context, odorants are mainly emitted through the anaerobic decomposition of organic matter containing sulphur and nitrogen or via sulphate reduction in wastewater [2,3]. A wide variety of volatile organic compounds (VOCs), volatile sulphur compounds (VSCs) and volatile fatty acids (VFAs) can be formed and released from wastewater treatment plants (WWTPs) and sewer networks [4,5]. More specifically, sulphur compounds such as hydrogen sulphide (H_2S) and mercaptans, organic acids like acetic acid, and nitrogen compounds such as ammonia (NH_3), are the main air pollutants emitted by WWTPs [6]. Odorous compounds are typically volatile, corrosive and irritating, even at very low concentrations, and cause odour nuisance due to their low odour thresholds [7]. Therefore, WWTPs should not only target the reduction of pollutants in wastewater and the cost-effective management of the sludge produced during wastewater treatment, but should also minimize the impact of odour pollution on public health and the environment [5].

Today, the strategies to mitigate odour nuisance in WWTPs entail the monitoring, assessment and control of the emission of odorants to the atmosphere. However, this conventional approach implies significant technical and analytical challenges because odours are complex gaseous mixtures at very low concentrations. Odour pollution in WWTPs also exhibit high variability over time, which can be related to weather conditions, effluent load characteristics and variations in operational conditions during wastewater treatment [8]. In this context, odour control strategies are evolving into an integrated approach with wastewater treatment processes [9]. A wide number of physical-chemical and biological odour treatment technologies have been reported in literature to mitigate odours nuisance from WWTPs. Of them, biological methods are preferable due to their effectiveness, low-cost, chemical-free operation and environmentally friendliness [10].

In WWTPs, the principle of prevention of odour formation/emission

prevails over the implementation of costly impact mitigation or treatment measures. Thus, it is more cost-effective to identify the mechanisms of odour formation/emission and implement modifications in process design or operation to prevent odour nuisance, rather than implementing sophisticated end-of-the-pipe treatment technologies. In this context, odour prevention strategies have recently emerged as an economically and environmentally sustainable alternative for the minimization of odour pollution in WWTPs [11]. More specifically, the use of by-products derived from wastewater treatment, such as activated sludge (AS) and oxidized nitrogen, could foster the adsorption and further oxidation of VSCs and VOCs in the raw wastewater. Activated sludge recycling (ASR) is a strategy based on the recirculation of AS from the secondary settler or mixed liquor of the nitrification tank into the inlet of the WWTP headworks in order to prevent the emission of malodorous [9,12,13]. On the other hand, oxidized nitrogen recycling (ONR) consists of the recycling on effluents rich in nitrates (NO_3^-) or nitrites (NO_2^-) (e.g., the nitrified wastewater from the nitrification tank) at the inlet works of the WWTP in order to provide electron acceptors for microorganisms to oxidize the dissolved odorants [13]. In addition, the ONR stream could be obtained from effluents with high ammonia concentration (e.g., centrates from the anaerobic digestion of mixed sludge) subjected to a previous nitrification process [14]. ASR and ONR as odour prevention technologies offer several advantages. Firstly, both strategies present a simple and cost-effective solution for controlling odours at the primary processing units prior to biological treatment. The joint implementation of ASR and ONR does not require the construction of additional dedicated facilities, and only minimal additional pipework or pumps are needed to transfer mixed liquor and oxidized nitrogen to the inlet works. Compared to other conventional odour control technologies such as chemical scrubbers, chemical oxidation, adsorption, among others, ASR and ONR are characterized by their low operational complexity. Additionally, AS typically exhibits high biological diversity, enabling it to adsorb and biologically oxidize a wide range of odorous compounds such as H_2S, mercaptans, aldehydes, amines, indoles, and VFAs [12].

The implementation of ASR and ONR as odour control processes in WWTPs is considered a relatively new technology, and there is limited research available on this specific topic. Zhang [15] conducted a study on the potential of dosing aerated iron-rich waste activated sludge (WAS) to control the soluble sulphide concentration in sewers. The study demonstrated an average reduction in soluble sulphide concentration of up to 99 % when using an iron-rich WAS to sewage ratio (v/v) of 12–16 %. In another comprehensive study conducted at full-scale, it was observed that reintroducing high concentrations of dissolved oxygen and nitrates back to the headworks of the WWTP led to a substantial decrease in odours and H_2S levels. The H_2S levels reached nearly undetectable levels, indicating a significant reduction in sulphide-related odours [16]. However, odour prevention strategies such as ASR and ONR still need to be further investigated to understand the underlying mechanisms of this biological odour prevention methods and elucidate their full potential under continuous operation.

This study evaluated the performance of ASR and ONR in a pilot plant treating domestic wastewater in terms of their potential to minimize the emission of H_2S and acetic acid from the primary settler. In addition, the influence of these innovative odour prevention strategies on the performance of wastewater treatment was systematically assessed.

2. Materials and methods

2.1. Synthetic wastewater

A synthetic wastewater (SWW) was prepared with the physicochemical characteristics and composition of a model urban wastewater to maintain stable operating conditions. The preparation of the SWW was carried out according to Bajaj [17] as follows (mg/L in tap water): 250 of glucose, 110 of meat extract, 160 of casein peptone, 30 of NH_2COH_2, 7 of NaCl, 4 of $CaCl_2 \cdot 2H_2O$, 2 of $MgSO_4 \cdot 7H_2O$, 112 of $K_2HPO_4 \cdot 3H_2O$, 0.5 of $CuCl_2 \cdot 2H_2O$, and 1100 of $NaHCO_3$. A volume of 140L was prepared weekly, divided into three batches for continuous operation of the pilot WWTP. Each fresh batch of SWW was stored at 4ºC to avoid changes in its composition. The physicochemical characterization of the SWW was carried out according to Standard Methods for the Examination of Water and Wastewater [18].

2.2. Experimental set-up

The pilot plant consisted of a primary settler, a denitrification-nitrification activated sludge biological reactor and a secondary settler (Fig. 1). The SWW was supplemented with sodium sulphide (Na_2S) and acetic acid using a syringe pump (Fusion 100) in order to mimic septic wastewater. Sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$) ACS reagent manufactured by Sigma-Aldrich Co. (U.S.A.) with a purity ≥98 % was used as a source of H_2S. On the other hand, acetic acid, with a purity level > 99.9 %, was purchased from Panreac Applichem (Spain). More specifically, 6.21 g of Na_2S and 3.42 mL of acetic acid were diluted in 50 mL volumetric flasks with distilled water and then, 20 mL syringes were used to inject both contaminants into the system by a syringe pump with a flow rate of 0.21 mL/h. Thus, the concentration of Na_2S and acetic acid injected into the system considering the inlet flow of SWW accounted for 36 mg/L and 21 mg/L, respectively. Table 1 shows the main characteristics of each volatile compound according to its chemical formula, odour perception, Henry solubility (H^{cc}), molecular weight and structure [19–21]. The synthetic septic wastewater passed through a 1.5 m loop pipe to ensure complete mixing of the Na_2S and CH_3COOH with the SWW. The primary treatment consisted of an air-tight primary settler made of polyvinylchloride (PVC) with 8 L of total liquid volume and an additional 10 L headspace built in transparent PVC, where malodorous samples were drawn. The activated sludge biological reactor was built in polypropylene with a working volume of 11 L and separated into two chambers (anoxic and aerobic). The anoxic chamber was closed with a lid and continuously stirred at 200 rpm with a Rushton turbine in order to favour anoxic conditions. An air blower with three fine bubble diffusers installed at the bottom of the aerated tank and a rotameter provided the oxygen required to maintain the dissolved oxygen concentration at 1.5–3.0 mg O_2/L [13]. The biological reactor was equipped with an internal recirculation of the mixed liquor from the aerobic chamber to the anoxic chamber. Finally, the activated sludge produced in the biological reactor was settled in a secondary settler built in PVC with 8 L of working volume and provided with a rotating scrapper responsible of dragging the settled sludge to the bottom. A fraction of the settled activated sludge was continuously recirculated to the biological reactor and the excess sludge produced was eliminated by wasting or recirculated to the headworks of the plant in order to prevent the generation of odours during the implementation of the ASR process. The clarified effluent was discharged into a treated water tank. Fresh AS collected from the AS external recirculation line of the denitrification-nitrification WWTP of Valladolid (Spain) was used as inoculum. The volatile suspended solids (VSS) concentration of the AS inoculum was 6000 mg VSS/L, which is within typical mean values in secondary settlers (4000–12,000 mg VSS/L) [12]. In addition, the air atmosphere in the headspace of the primary settler was initially flushed with helium in order to provide anaerobic conditions during the start-up of the process, and the O_2 headspace composition was quantified by gas chromatography (GC-TCD) to ensure oxygen contents lower than 1 % during the whole experimental period.

2.3. Operational conditions

The present study was carried out over a period of 175 days in order to guarantee the stability of the pilot WWTP under different operational conditions during the implementation of ASR and ONR. The process was
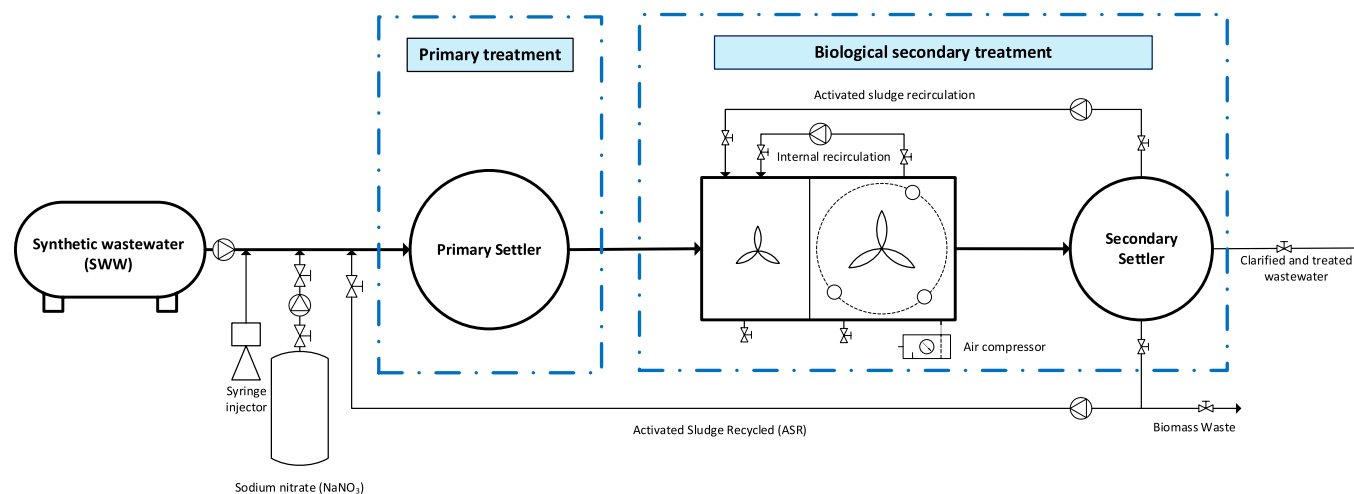
Fig. 1. Schematic diagram of the wastewater treatment pilot plant with ASR and ONR.

Table 1
Main characteristics of the model odorous compounds evaluated.

Compound	Chemical formula	Odour perception	Odour threshold value (ppm, v/v) [19]	H^{cc}	Molecular weight (g/mole)	Molecular structure
Hydrogen sulfide	H_2S	Rotten egg	0.00041	0.00091 [20]	34.10	
Acetic acid	$C_2H_4O_2$	Vinegar	0.0060	14.00 [21]	60.05	

operated at 15 h of hydraulic retention time (HRT), with a return activated sludge (RAS) ratio of 0.25 and an internal recirculation ratio of 2. The sludge retention time (SRT) was set at 10 days by daily wasting 450 mL of settled sludge, which represented a daily biomass productivity of 4.6 g VSS (Supplementary material). The experimental period was divided into 6 operational phases (I, II, III, IV, V, VI), which were maintained until steady state was reached and lasted for 22, 23, 28, 26, 42 and 34 days, respectively.

During phase I, the system was inoculated with fresh activated sludge and fed with SWW at a HRT of 15 h in the absence of external chemical odorants injection. In phase II, the chemical odorants were injected at the inlet of the primary settler mimicking process operation with a septic wastewater. Phase III was characterized by the recirculation of the waste activated sludge from the bottom of the secondary settler to the head of the pilot plant at a flowrate of 450 mL/day in order to evaluate the ability of activated sludge to prevent odorant emission in the primary settler. Phase IV implemented the addition of nitrates (NO_3^-) to the primary settler jointly with the recirculation of activated sludge. $NaNO_3$ at a concentration of 600 mg N/L was injected in the inlet SWW at a flow rate of 0.2 mL/min, which represented an inlet concentration of 10 mg N/L. Phase V focused on recovering the nitrifying bacteria in the biological reactor, which was affected during Phase III. For this purpose, 700 mL of the mixed liquor were withdrawn from the reactor and 700 mL of fresh activated sludge was inoculated. Phase V operated with ASR and ONR as previously described. During phase VI, the concentration of nitrates in the inlet SWW was increased to 20 mg N/L by increasing the $NaNO_3$ stock solution flowrate to 0.4 mL/min. Table 2 shows the different sampling points in the experimental set-up and the frequency of the physicochemical analyses carried out to monitor wastewater treatment performance. Gaseous samples were collected from the headspace of the primary settler by means of two valves installed in the lid. A Tedlar bag with 1 L of helium was connected to the first valve and a modified gas sampling container BD GasPak™ EZ manufactured by Fischer Scientific S.L. (Spain) was used as a vacuum gas sampler box with an empty Tedlar bag inside, which was connected

to the second valve. A two-way air compressor (ElectroAD) was connected to a port of the sampling box to collect a 1 L odour sample from the primary settler headspace by suction.

2.4. Analytical techniques

The activated sludge VSS concentration, pH, soluble total carbon (TC), soluble total organic carbon (TOC), soluble inorganic carbon (IC), soluble total nitrogen (TN_S) and chemical oxygen demand (COD) were analysed following the Standard Methods for the Examination of Water and Wastewater [18]. Nitrate (NO_3^-), nitrite (NO_2^-) and sulphate (SO_4^{2-}) concentrations were measured by HPLC-IC according to García [20]. The dissolved oxygen concentration (DO) in the activated sludge reactor was measured using a portable oxygen probe Oxi 3310 SET 1 WTW™, which was daily calibrated prior to each analysis. The ammonium concentration was determined by Nessler method with a Spectrostra Nano spectrophotometer at a wavelength of 425 nm [23,24]. Finally, sulphide concentration (S^{2-}) was analysed using a sulphide analysis kit (Spectroquant®, Merck) by spectrophometry with a Spectrostra Nano spectrophotometer at a wavelength of 665 nm. In addition, the concentration of O_2 in the headspace of the primary settler was periodically measured by GC-TCD as described by Posadas [22].

The measurements of H_2S and acetic acid in the headspace of the primary settler was conducted using a Hiden QGA mass spectrometer (Hiden Analytical, United Kingdom). This spectrometer consists of a simple quadrupole with an APSI ionization source, an internal dual secondary electron multiplier (SEM), a Faraday detector and a precision quartz inlet heated capillary (QIC) sampling interface. The injection of the odour samples was conducted at a sweep rate of 50 mL/min through a QIC capillary inlet that can operate at pressures ranging from 100 mbar to 2 bar and high temperatures (200 °C), providing fast response times of less than 300 ms for most common gases and vapours, including water and organic vapours. QGA boasts a standard mass range of 1–200 at. mass units (amu) with detection over an extremely high dynamic range up to 100 %, detecting concentration above 0.1 ppm_v. The

Table 2

Physicochemical and odour monitoring: sampling points and frequency.

Parameter	Sampling points					
	IN	Headspace	PS	R	SS	OUT
COD*	X					X
pH	X		X			X
DO				X		
VSS	X			X	X	
C_{TOC}	X					X
C_{TC}	X					X
C_{IC}	X					X
TN_S	X					X
NH_4^+	X					X
NO_2^-	X		X			X
NO_3^-	X		X			X
S^{2-}	X					X
SO_4^{2-}	X		X			X
H_2S		X				
$C_2H_4O_2$		X				

IN, Inlet wastewater; OUT, Outlet wastewater; PS, Primary settler outlet; R, Activated sludge reactor; SS, Secondary settler. *COD was analysed at the beginning of the experiment to determine the expected production of microorganisms in the system.   Daily   Three times per week

Faraday and SEM detectors were previously calibrated using the MAS-soft Professional software and atmospheric air. Moreover, the QGA mass spectrometer was also calibrated from a pressurized bottle with helium containing H_2S and acetic acid in the gas phase at 586 and 582 ppm$_v$, respectively. For this purpose, 8 mL of gaseous H_2S standard (22 % in N_2) and 4 μL of acetic acid were injected into an empty glass bottle of 2.1 L containing helium and allowed to volatilize. Then, the bottle was pressurized with helium at 500 mbar prior to analysis in order to fulfill the pressure requirements of the mass spectrometer.

3. Results and discussion

3.1. Wastewater treatment performance

The extent of carbon and nitrogen removal in activated sludge processes depends on many factors such as pH, dissolved oxygen, solid retention time, sludge settleability and organic load to be treated, among others [25].

3.1.1. pH and dissolved oxygen

The DO in the aeration tank supports the aerobic bacterial respiration and consumption of organic matter, sulphur and nutrients. When DO is present in excess (> 2–3 mg O_2/L) in the mixed liquor of the aeration tank, organic matter, sulphur and NH_4^+ become the rate limiting

substrates for heterotrophic and autotrophic bacterial metabolism [26]. Oxygen mass transfer from the gas phase to the mixed liquor and microbial oxygen consumption are critical for effectively removing COD, TOC, TN_S, S^{2-} and NH_4^+ [27]. Conversely, low DO concentrations are often responsible for operational problems like sludge bulking caused by the selection and proliferation of highly competitive filamentous bacteria in activated sludge flocs, which entail a poor sludge settling [25]. Indeed, filamentous bacteria are especially prone to grow when DO concentrations remain below 0.5 mg O_2/L.

During phase I (devoted to system stabilization), fluctuations due to the instability of the system and the amount of air supplied to the reactor were observed, reaching minimum and maximum oxygen concentrations of 0.7 and 3.3 mg O_2/L, respectively (Fig. 2). By day 27, a stabilization of the DO concentration in the aerated tank was achieved. In fact, 98.18 % of the data measured from phase II to phase VI ranged from 1.7 to 3.0 mg O_2/L, with an average value of 2.09 ± 0.1 mg O_2/L, which confirmed that DO in the pilot plant activated sludge consistently supported the oxidation of organic matter, sulphur and ammonia [28]. Overall, the DO concentration decreased with increasing activated sludge concentrations over time as a result of the increased oxygen demand for bacterial respiration. It should be noted that on experimental day 12, a sharp decrease in DO concentration was observed below 1.0 mg O_2/L due to the temporary disconnection of the airline for few hours, which led to a drastic decrease in oxygen concentration inside the
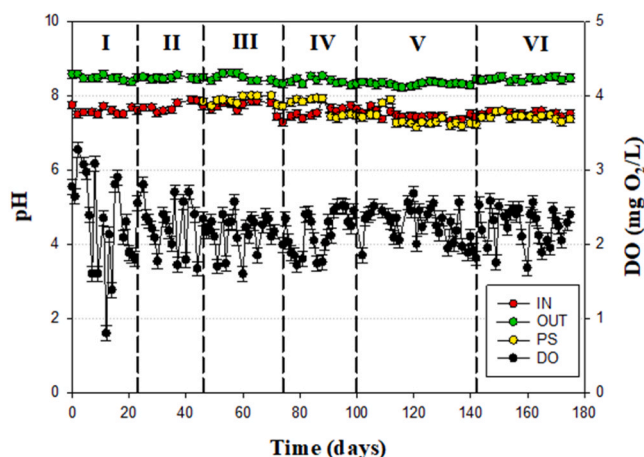
Fig. 2. Time course of the dissolved oxygen concentration (DO) in the aerated tank and pH in the influent (red), effluent (green) and outlet of the primary settler (yellow).

Fig. 3. Time course of VSS concentration in the aerated reactor (red), primary clarified inlet (yellow) and settled sludge (green).

biological reactor.

On the other hand, pH is widely recognized as one of the most influential control parameter governing the overall efficiency and effectiveness of a biological treatment process. Variations of pH in the wastewater can cause adverse effects in the activated sludge process, including the nitrification process and the sedimentation of the activated sludge in the secondary settler [29]. The optimal pH for wastewater treatment in biological reactors ranges between 6.5 and 8.5, where nitrifying bacteria are metabolically more active [30]. Moreover, cations such as sodium, calcium and magnesium constitute a significant fraction of activated sludge flocs and extreme acidity (pH < 4 or lower) can dissolve calcium solids from the floc structure, reducing the ability of bacteria to flocculate and settle [31].

pH at the different sampling points remained relatively constant (Fig. 2) [32]. The inlet pH value during phase I average 7.75 ± 0.10 and 7.64 ± 0.50 during phases II-VI, reaching maximum pH values of 8.01 ± 0.1 and minimum values of 7.28 ± 0.10. The maximum value of pH in the effluent was 8.61 ± 0.10 and the minimum value was 8.31 ± 0.10, with an average of 8.46 ± 0.20. These pH values support maximum nitrification rates, since pH values below 7 lead to a decrease in the nitrification process [33]. The implementation of ASR and ONR strategies did not modify the pH in the treated wastewater (8.47 ± 0.10), which complied with the discharge limit values established in the European Union Directive 98/15/CE [34]. Interestingly, a slight increase can be seen in the pH value at the inlet during phase III, which could be caused by the recirculation of the activated sludge at the headwork of the system, since the pH values in the treated and clarified water were higher than those recorded in the influent. The pH at the outlet of the primary settler averaged 7.8 ± 0.1, which confirmed that the bioreactions occurring in this unit for ~ 2 h did not mediate significant changes in this parameter.

3.1.2. Biomass concentration

The biodegradation performance of activated sludge processes depends on the biomass concentration in the bioreactors, which itself is affected by sludge bulking, rising sludge or floating sludge [35]. Therefore, the influence of ASR and ONR implementation on the concentration of microorganisms and the settling characteristics of the sludge needs to evaluated. During the acclimation stage (Phase I), a slight fluctuation in VSS concentration was observed with an average value of 3.04 ± 0.07 g VSS/L and 7.05 ± 1.11 g VSS/L in the reactor and bottom of the secondary settler, respectively (Fig. 3). VSS concentration in phase II increased significantly when chemical odorants (Na$_2$S and acetic acid) were injected into the system up to 4.45 ± 0.07 g VSS/L in the bioreactor and 9.64 ± 0.09 g VSS/L in the secondary settler.
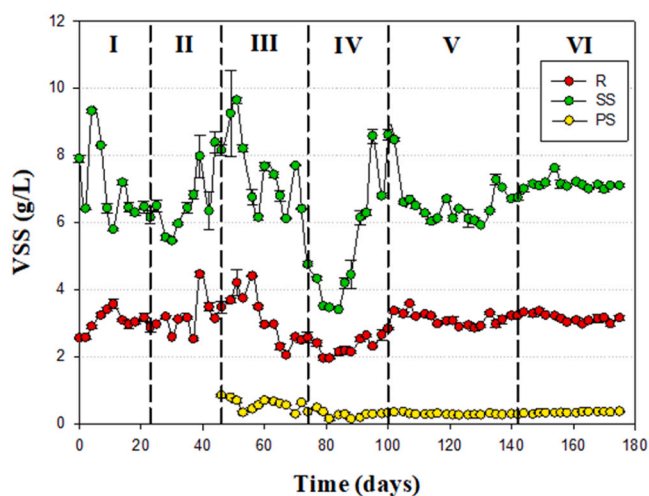
Interestingly, the implementation of ASR in phase III caused a sharp decrease in the VSS concentration of both units, where concentrations of 1.95 ± 0.01 g VSS/L in the bioreactor and 3.46 ± 0.03 g VSS/L in the settler were recorded at the beginning of phase IV as a result of the occurrence of sludge bulking in the secondary settler. This empirical finding suggested that the recirculation of activated sludge at the headwork of the pilot plant without electron acceptors (i.e., NO$_3^-$/NO$_2^-$ via implementation of ONR) promoted the growth of filamentous organisms, with the associated poor settling characteristics and poor compactability of the sludge. Phase IV was operated with the combined recirculation of activated sludge and NO$_3^-$, and the re-inoculation of the biological reactor with fresh activated sludge. A marked reduction in VSS concentration in the inlet of the primary clarifier from 0.78 ± 0.01 g VSS/L to 0.14 ± 0.01 g VSS/L was consequently observed in phases III and IV. Process operation with ASR and ONR in phases V and VI supported constant VSS concentrations of 2.97 ± 0.09 g VSS/L in the aerated tank and 7.52 ± 0.20 g VSS/L in the secondary settler. Similarly, the concentration of biomass at the primary settler inlet stabilized at 0.32 ± 0.03 g VSS/L during the last two operational stages.

3.1.3. Carbon fate

Activated sludge treatment involves two major steps: degradation of organic pollutants in the anoxic and aeration tank, followed by the separation of the bacterial sludge and treated water by settling in the secondary clarifier. The organic pollution of domestic wastewater present in soluble, colloidal and particulate forms is converted by heterotrophic microorganisms into biomass, carbon dioxide, water, cell debris and soluble extracellular metabolites [36]. In this study, SWW was used to guarantee stable conditions in terms of organic loading rate and accurate determination of the carbonaceous compounds. The system showed a good stability and efficiency during the degradation of organic matter, with slight fluctuations in phase I attributed to the gradual acclimation of the microbial community. The global removal efficiency of organic matter (measured as TOC) upon the biological treatment and secondary settling was 96 ± 2 % along the entire experiment (Fig. 4). However, when ASR and ONR were applied in phases III and IV, an accumulation of inorganic species (e.g., carbonates, bicarbonates and carbon dioxide) was observed in the treated effluent (Fig. 4B). This fact could be related to bulking sludge effect (see Section 3.1.2) and the loss of nitrifying organisms (see Section 3.1.4), which consumed inorganic carbon as a carbon source for ammonia nitrification [37].

3.1.4. Nitrogen fate

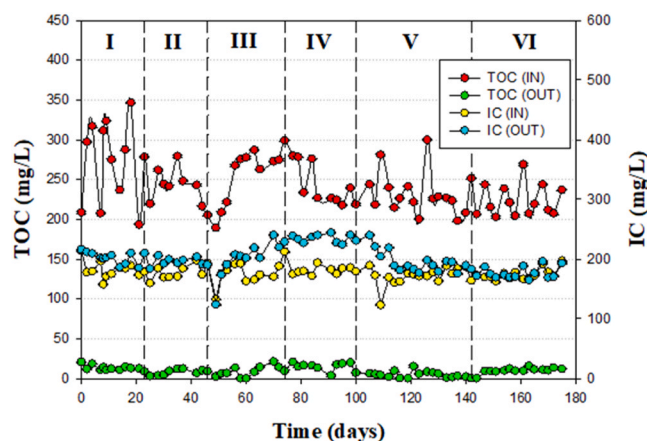During ammonia nitrification, a sequential aerobic oxidation of

Fig. 4. Time course of the concentration of TOC and IC throughout the operational phases.

ammonium to nitrite is conducted by ammonium oxidizing bacteria (AOB) (i.e., *Nitrosomonas*) and then to nitrate by nitrite oxidizing bacteria (NOB) (i.e., *Nitrobacter*). Subsequently, nitrite and nitrate ions are transformed into gaseous N_2 or N_2O by denitrifying bacteria (i.e., *Achromobacter*, *Aerobacter*, among others) [38]. The nitrification/denitrification process in wastewater treatment is critical due to the low growth rate of nitrifying bacteria and their high sensitivity to toxicity

and operational perturbations. The impact of ASR and ONR on the nitrogen cycles during domestic wastewater treatment has been poorly explored.

Slight fluctuations in concentration of the different forms of nitrogen evaluated at the inlet and outlet of the system were observed during phases I and II, when TN_S and NH_4^+ showed high nitrogen removal efficiencies of 87 ± 2 % and 98 ± 1 %, respectively, by assimilation, nitrification and denitrification. Steady state nitrate and nitrite concentrations of 22.5 ± 0.4 mg N/L and 7.6 ± 0.1 mg N/L were recorded in the effluent in phase I, and 18.6 ± 2.6 mg N/L and 7.9 ± 0.1 mg N/L in phase II. Interestingly, the microbiology involved in nitrogen removal was negatively influenced by ASR in phase III, which caused an increase in the outlet concentration of TN_S from 5.62 ± 0.1 mg N/L to 50.8 ± 0.2 mg N/L and of NH_4^+ from 1.4 ± 0.2 mg N/L to 50.7 ± 0.8 mg N/L due to the loss of nitrification (Fig. 5A and B). In this context, the recirculation of activated sludge without electron acceptors negatively influenced the growth of NOB with a concomitant decrease in NO_3^- concentration to 0 mg N/L by the end of phase III, while NO_2^- concentrations remained stable at 7.2 ± 0.2 mg N/L (Fig. 5C). In fact, the presence of reduced sulphur compounds (i.e., sulphur injected during phase II) combined with the inhibition of the nitrification process favored sludge bulking caused by the growth of filamentous bacteria under these operational conditions [39]. The injection of NO_3^- via ONR, along with ASR in phase IV and the inoculation of fresh activated sludge in phase V, recovered the population nitrifying bacteria with a progressive increase in the effluent NO_3^- and NO_2^- concentrations (Fig. 5C
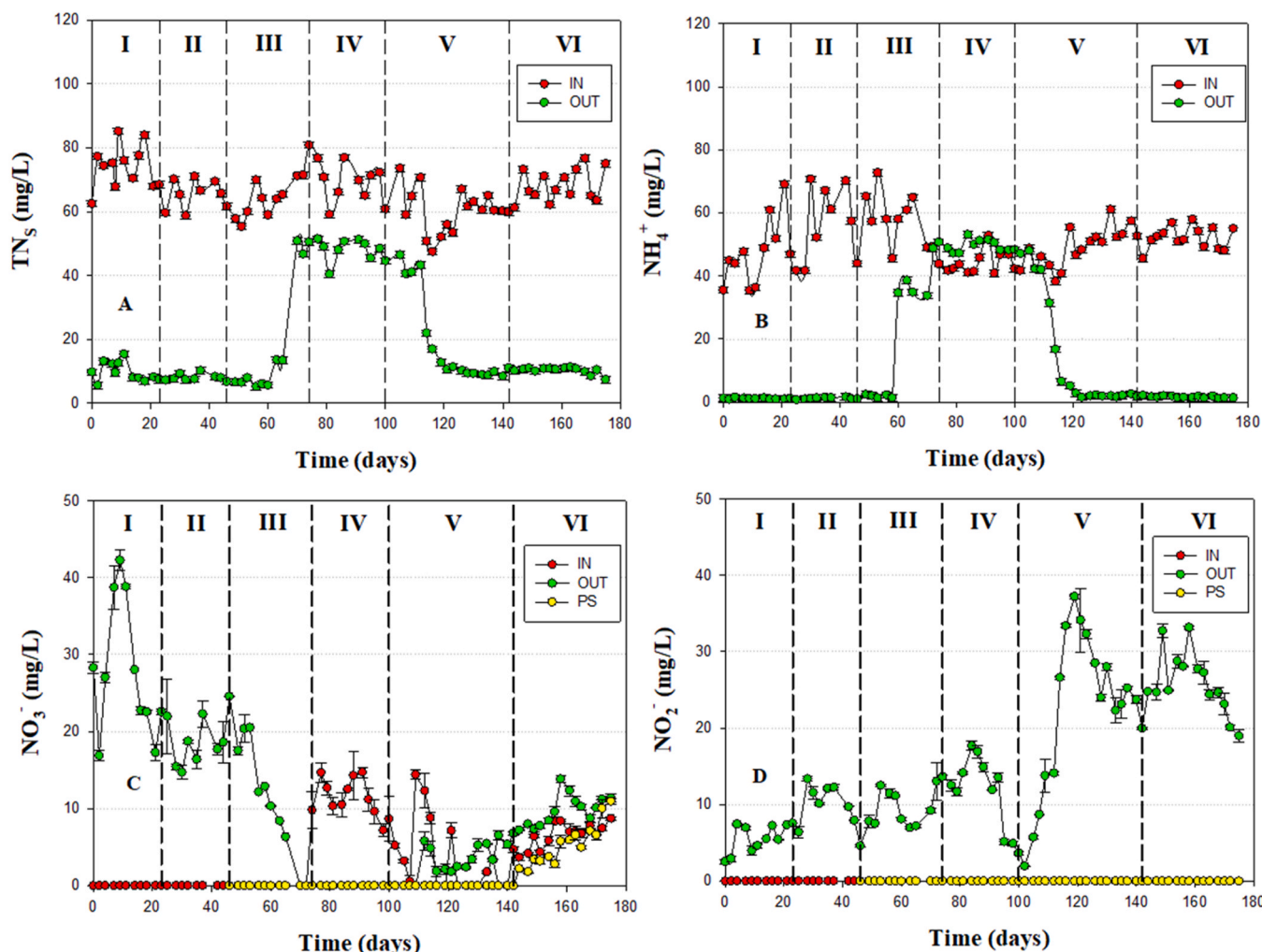

Fig. 5. Time course of the concentration of TN_S (A), NH_4^+ (B), NO_3^- (C) and NO_2^- (D) at the inlet (red), outlet (green) and primary settler outlet (yellow).

and D), which brought about average TN_S and NH_4^+ of 10.5 $\pm$ 0.2 mg N/L and 2.7 $\pm$ 0.4 mg N/L in phase V, and 7.4 $\pm$ 0.2 mg N/L and 1.2 $\pm$ 0.2 mg N/L in phase VI (Figs. 5A and 5B). Interestingly, average concentrations of NO_2^- of 23.7 $\pm$ 0.9 mg N/L and 24.7 $\pm$ 0.7 mg N/L were achieved in phase V and VI, respectively, which were higher than the corresponding NO_3^- concentrations (7.8 $\pm$ 0.3 mg N/L and 11.2 $\pm$ 0.4 mg N/L, respectively).

3.2. Odorant fate

One of the main concerns associated with WWTPs is the emission of unpleasant odours, where H_2S is typically considered to be a dominant contributor of these emissions [40]. The increase in S^{2-} concentration from phase I to phase II was mediated by the injection of Na_2S at the inlet of the pilot plant (Fig. 6A). The biological oxidation of S^{2-} to SO_4^{2-} by sulphur oxidizing bacteria (SOB) was complete during phase I and II (Fig. 6B). SOB play an important role in the oxidation of sulphide, thiosulphate and elemental sulphur [41]. Indeed, sulphate concentration in the effluent increased from average values of 28.9 $\pm$ 1.9 mg SO_4^{2-}/L at the end of phase I up to 59.5 $\pm$ 7.5 mg SO_4^{2-}/L at the end of phase II. Some SOB are filamentous microorganisms, which is central in wastewater treatment because they are responsible for bulking in activated sludge cultures [42]. The implementation of ASR and ONR in phase IV, V and VI induced a slight decrease in S^{2-} concentration to 14.7 $\pm$ 0.9, 10.7 $\pm$ 0.7 and 13.1 $\pm$ 0.8 mg S^{2-}/L, respectively. During phase V and VI, the recovery of the sludge settleability and nitrification activity improved the stability of SOB, which supported a biological oxidation of S^{2-} higher than 99 % with average SO_4^{2-} concentrations of 52 $\pm$ 8 mg/L.

The headspace of the primary settler during phase I showed an H_2S concentration of 0.15 $\pm$ 0.04 ppm$_v$ (Figs. 7A) and 2.89 $\pm$ 0.64 ppm$_v$ of acetic acid (Fig. 7B) according to the SWW characteristics. The injection of chemical odorants during phase II resulted in a significant increase in the headspace concentration of both pollutants, reaching a stable H_2S concentrations of 0.40 $\pm$ 0.01 ppm$_v$ and a maximum concentration of acetic acid of 5.90 ppm$_v$, which was rapidly stabilized at 3.21 $\pm$ 0.17 ppm$_v$. During ASR implementation (Phase III), there was an initial sharp decrease in H_2S and acetic acid headspace concentrations due to the AS-mediated biological oxidization of most biogenic dissolved odorous compounds (e.g., sulphide, volatile fatty acids) in raw wastewater [13,14]. This resulted in a reduction in the headspace concentration by 75 % and 40 % for H_2S and acetic acid, respectively. However, the poor sludge settleability during phase III and IV entailed a low recirculation of AS at the inlet of the system, contributing to increase the concentration of H_2S and acetic acid in the primary settler headspace to

0.99 $\pm$ 0.01 ppm$_v$ and 2.87 $\pm$ 0.12 ppm$_v$, respectively, at the end of phase III and phase IV. In this context, sulphur reducing bacteria (SRB) played an important role in these stages, being responsible for the metabolic processes in microbial sulphur reduction. Thus, SO_4^{2-} can serve as an electron acceptor of SRB under anaerobic conditions (i.e., the operational conditions prevailing in the primary settler), reducing oxidized sulphur compounds into H_2S [43]. The reduction of sulphate reaction by SRB can be described by Eq. (1) [44]:

$$SO_4^{2-} + 10H^+ + 8e^- \xrightarrow{SRB} H_2S + 4H_2O \tag{1}$$
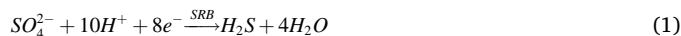
This fact could explain the fastest depletion in SO_4^{2-} concentration during phase III in the primary settler (Fig. 6B) and the concomitant increase in H_2S concentration (Fig. 7A). The treatment of H_2S laden malodorous emissions in activated sludge processes has been associated to the development of filamentous bacteria, but this hypothesis has not been consistently demonstrated in literature [45,46]. Finally, the injection of electron acceptors by ONR and ASR during phases V and VI, along with the enhancement in sludge settleability and nitrification activity, effectively prevented the emission of odorous compounds in the primary settler, achieving reductions of 95 % and 42 % for H_2S and acetic acid, respectively. The combined implementation of ASR and ONR favored the microbiological oxidation of H_2S by SOB using the recirculated NO_3^- or NO_2^- as electron donor, being the main mechanism governing the minimization of H_2S. Indeed, all NO_3^- introduced in the inlet wastewater was depleted in the primary settler (Fig. 5C). The anoxic oxidation of H_2S by SOB can be described by Eq. (2) [47]:

$$5H_2S + 2NO_3^- + 2H^+ \xrightarrow{SOB} 5S^0 + N_2 + 6H_2O \tag{2}$$
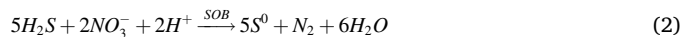
The use of SOB in wastewater remediation has been extensively studied [48]. While chemotrophic SOB are mainly aerobic microorganisms, certain species within the genera *Thiobacillus*, *Thiomicrospira*, and *Thiosphaera* exhibit distinct metabolic characteristics. These bacteria can grow under anoxic conditions by utilizing NO_3^- or NO_2^- as the final electron acceptor, a process commonly referred to as autotrophic denitrification [49]. In this context, the ability of SOB to oxidize sulphur and sulphide using nitrate as an electron acceptor under anoxic conditions has been previously reported, with *Thiobacillus* being the predominant bacteria governing the process [50]. Reactions involving simultaneous sulphur or sulphide oxidation and denitrification can be represented by Eqs. (3) and (4) [51]:
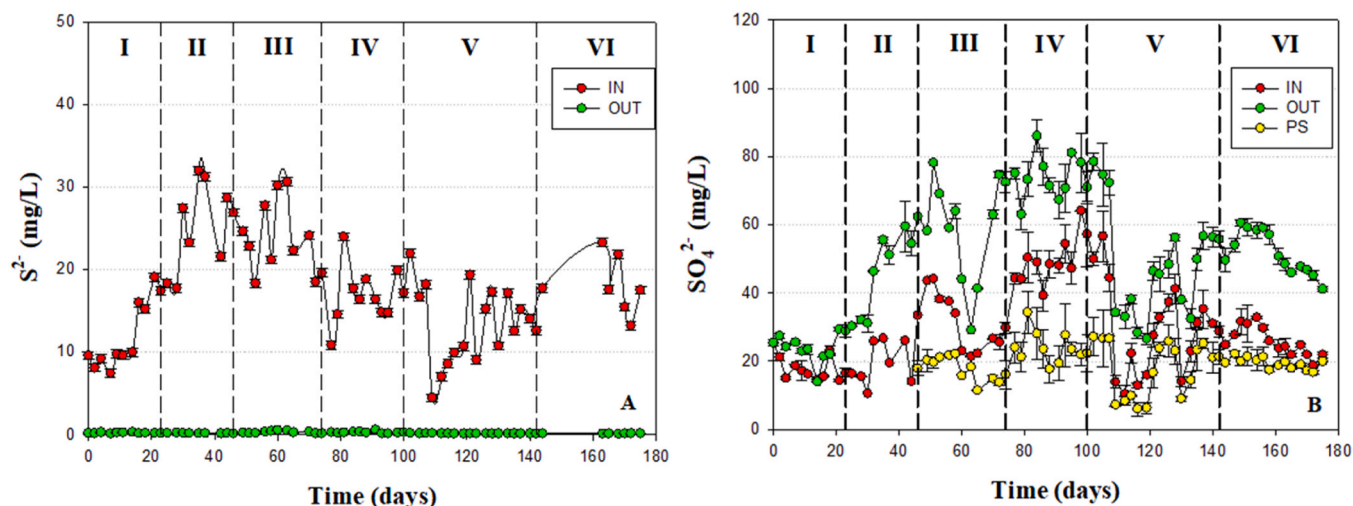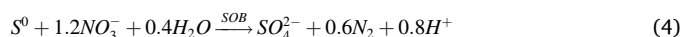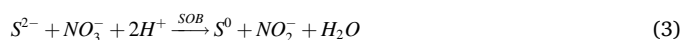
$$S^{2-} + NO_3^- + 2H^+ \xrightarrow{SOB} S^0 + NO_2^- + H_2O \tag{3}$$

$$S^0 + 1.2NO_3^- + 0.4H_2O \xrightarrow{SOB} SO_4^{2-} + 0.6N_2 + 0.8H^+ \tag{4}$$

Fig. 6. Time course of the concentration of S^{2-} (A) and SO_4^{2-} (B) at the inlet (red), outlet (green) and primary settler outlet (yellow).
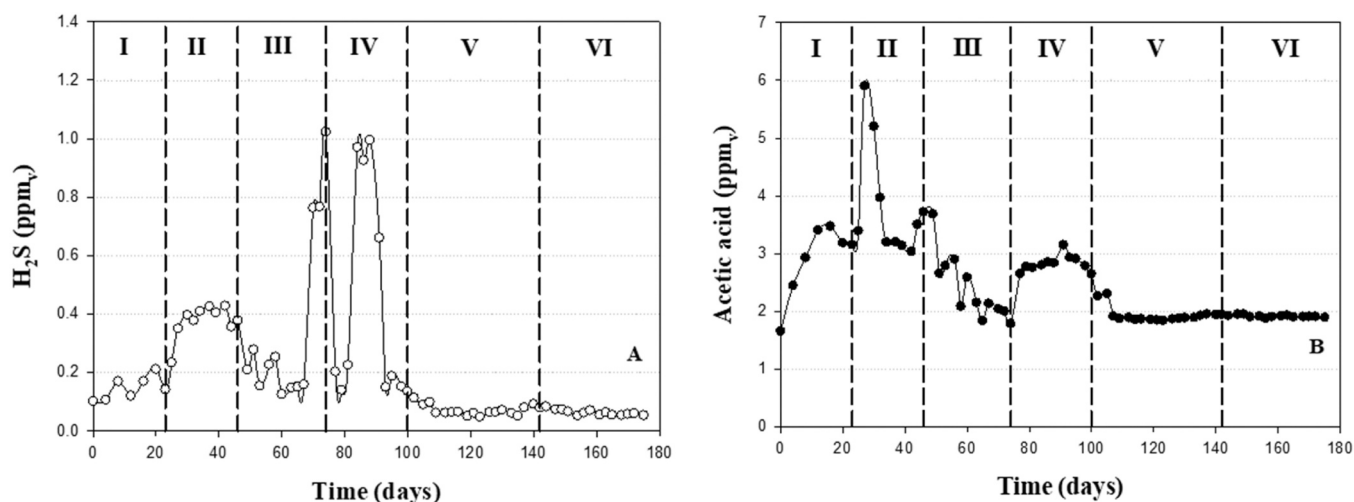
Fig. 7. Time course of H_2S (A) and acetic acid (B) headspace concentration.

Finally, the increase in the concentration of NO_3^- injected into the system in phase VI, from 10 mg/L to 20 mg/L, had no significant impact on odorant reduction in the headspace of the settler. In fact, NO_3^- was detected at the outlet of the primary settler (PS) (Fig. 5C) during this stage, which demonstrated that NO_3^- concentrations higher than 10 mg/L are not required to biodegrade the most biogenic dissolved odorous compounds. In addition, most microorganisms present in activated sludge can use NO_3^- as electron acceptor to metabolize the most readily biodegradable compounds such as VFAs. In this context, acetic acid is a substrate required in AS processes for biological phosphorus and nitrogen removal, supporting enhanced elimination of nitrogen but limiting enhanced biological phosphorous removal [52]. The biological oxidation of acetic acid in wastewater by AS involves the conversion of acetic acid into carbon dioxide and water through microbial activity. In this context, acetic acid is dissociated into acetate anion and hydrogen proton in wastewater. AS is capable of efficiently biodegrading the acetate anion during the implementation of ASR, in which microorganisms in the sludge, particularly aerobic bacteria, utilize acetate as a carbon and energy source for their growth and metabolism [53]. More specifically, inside the microbial cells present in the activated sludge, acetate is metabolized and fully oxidized to carbon dioxide as the end product of biodegradation under enzymatic reactions including oxidation, decarboxylation and further metabolic transformations, resulting in the production of energy-rich compounds such as adenosine triphosphate. The energy and carbon derived from acetate are utilized by microorganisms for their growth, leading to an increase in the biomass concentration in the AS. The biodegradation of acetic acid by AS contributes to the removal of organic carbon from wastewater and reduces its odour potential in wastewater with the consequent environmental benefit.

4. Conclusions

ASR and ONR demonstrated to be effective odour prevention strategies that can be easily implemented into WWTPs with significant environmental and economic benefits. In terms of odorant fate, the emission of odorous compounds in the primary settler was reduced by 95 % for H_2S and 42 % for acetic acid due to their anoxic biodegradation. Stable process operation in terms of wastewater treatment performance was achieved when ASR and ONR were combined (Phases V-VI). Interestingly, the sole recirculation of activated sludge at the headwork of the pilot plant without electron acceptors (Phase III) promoted sludge bulking and the loss of nitrifying microorganisms. This fact ultimately reduced the activated sludge recirculation at the inlet of the system from 0.78 ± 0.01 g VSS/L to 0.14 ± 0.01 g VSS/L, contributing to significantly increase the concentration of odorants in the

primary settler. The results herein obtained are of great importance to improve the understanding of ASR and ONR as odour prevention strategies and potentially expand their application at industrial scale. In conclusion, while ASR and ONR processes have demonstrated multiple advantages in odour control, their successful implementation may require careful optimization procedures. This includes determining the optimal recycling rates, oxygen and nutrient dosing, and hydraulic control. By fine-tuning these parameters, the full potential of ASR and ONR can be realized, ensuring efficient and effective wastewater treatment processes while minimizing odour emissions.

CRediT authorship contribution statement

Toledo Manuel: Conceptualization, Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision. **Muñoz Raúl:** Methodology, Writing - Review & Editing, Visualization, Supervision, Project administration, 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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2023.110366.

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