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Fate of pharmaceutically active compounds in a pilot-scale A^2O integrated fixed-film activated sludge (IFAS) process treating municipal wastewater

M.J. Gallardo-Altamirano [a,b,*], P. Maza-Márquez [a,c,1], S. Pérez [d], B. Rodelas [a,c], C. Pozo [a,c], F. Osorio [a,b]

[a] Environmental Microbiology Group, Institute of Water Research, University of Granada, Granada, Spain
[b] Department of Civil Engineering, University of Granada, Granada, Spain
[c] Department of Microbiology, University of Granada, Granada, Spain
[d] Water, Environmental and Food Chemistry (ENFOCHEM), Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain

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ABSTRACT

The goal of this research was to study the operation of an integrated fixed-film activated sludge system (IFAS), in anaerobic/anoxic/aerobic (A2O) configuration for the elimination of emerging pollutants, in particular of 27 pharmaceutical active compounds (PhACs) (eight anti-inflammatory and/or analgesic pharmaceuticals (AIAPs), six antibiotics, four b-blockers, two antihypertensives/diuretics, three lipid regulators-bezafibrate and four psychiatric medications). Different operational conditions were analyzed in the biological reactor, controlling at all times both the amount of suspended and fixed biomass present. The A2O-IFAS system has obtained similar or higher removal efficiency (RE) in the elimination of organic matter, and especially of nutrients, nitrogen (N) and phosphorous (P), with respect to the conventional A2O system, working however with very low values of the sludge retention time (SRT) (4.0 days) and mix liquid suspended solid (MLSS) in the reactor (1822 mg L-1). In general, conventional activated sludge (CAS) and IFAS processes show the importance of operating at high SRT and MLSS concentration, that give low food/microorganisms (F/M) ratio, to increase the RE of several PhACs. However, the A^2O-IFAS system, operating with low mixed liquor SRT and MLSS has achieved similar or better RE of PhACs, obtaining the highest average REs values (>80%) for fenofibrate, acetaminophen, ibuprofen, naproxen, clarithromycin and atenolol. According to the results obtained, due to the affordable cost and well-demonstrated performance, IFAS systems become one of the most promising technology for conventional wastewater treatment plants (WWTP) upgrading.

1. Introduction

During the last decades, the production of nutrient-rich wastewaters has been continuously increasing worldwide due to the exponential growth of urbanization, household consumption and industrial production [1]. Accordingly, the continuous discharge of nutrients in water bodies leads to an increasing eutrophication problem. Moreover, the continuous presence of different trace organic contaminants such as personal care products, industrial chemicals, hormones and more specific Pharmaceutical Active Compounds (PhACs) in municipal wastewaters effluents and different environmental compartments is an issue of growing concern worldwide [2]. Nowadays, the conventional wastewater treatment plants (WWTPs) have demonstrated many shortcomings to face more stringent discharge standards in terms of nutrient release and emerging contaminants removal [3,4]. Consequently, the old WWTPs require upgrading (e.g., construction of new aeration tanks and secondary clarifiers) and the implementation of new advanced biological treatment processes. In the last decades, conventional biological nutrient removal (BNR) processes have been well characterized and obtain good performance. However, they need massive reactor volumes and operate at high sludge retention time (SRT), especially in cold weather, which often required high energy and investment costs [5]. Additionally, WWTPs were not designed to remove trace organic contaminants and only a fraction of each PhAC and their metabolites can

* Correspondence to: Department of Civil Engineering, University of Granada, C/ Campus Universitario de Fuentenueva (Edificio Politécnico), CP:18071 Granada, Spain.
E-mail address: manujga@ugr.es (M.J. Gallardo-Altamirano).
[1] Present address: Microbial Ecology/Biogeochemistry Research Laboratory, NASA Ames Research Centre, CA, USA.

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be removed [6,7].

To overcome these problematics, several investigations have been published regarding the fate and removal efficiencies (REs) of PhACs in WWTPs, as well as, to solve the main drawbacks of the conventional BNR configurations [5–7]. Among the novel technologies, the moving bed biofilm reactors (MBBR) and more specifically the hybrid MBBR-based integrated fixed-film activated sludge (IFAS) reactors are shown as promising technologies due to their advantages compared to the conventional activated sludge (CAS) processes. During the last decade, IFAS system has gained acceptance as a cost-effective technology to enhance nitrogen removal, improve process stability, enhance settleability and increase the overall treatment capacity of the conventional WWTPs [8,9]. Furthermore, recent investigations suggest better REs of some PhACs by IFAS process compared to CAS and membrane bioreactor (MBR) systems [10–16]. The main advantage of IFAS processes is the presence of both quick-growth suspended and slow-growth attached microorganisms in the same reactor compartment [9]. Thus, application of attached microorganisms allows to have biomass with higher sludge retention time (SRT) in the biofilm for the nitrification process and lower SRT in the suspended biomass for the biodegradation of organic matter, which the possibility to operate at higher biomass concentration compared to conventional BNR processes [5]. Indeed, this process became a very simple and efficient technology for upgrading overloaded WWTPs or design a new municipal WWTP [8,17].

Despite the aforementioned benefits of IFAS systems, there are scarce studies investigating, in the same pilot-scale plant with real wastewater, the removal efficiency of PhACs in IFAS systems compared to CAS and MBR systems [15]. Falås et al. [18,19] and Jewell et al. [10] demonstrated during bench-scale batch experiments using both activated sludge and suspended biofilm carrier from full-scale WWTPs that attached biomass could contribute significantly to the removal of some PhACs. Similar results were obtained in several pilot-scale assessments in IFAS systems [12,13]. These studies pointed out that biodegradation/biotransformation served as the primary pathway for PhACs removal and the importance of the operating condition such as SRT and biomass concentration (MLSS). However, more research is needed to fully explore the higher removal capacity and the underlying removal mechanisms in IFAS systems, since there is still a lack of information between the RE of PhACs and the impact of operating conditions on the ability of IFAS process to transform PhACs compered to CAS process.

In this study, the removal rate of organic matter and nutrients, as well as, the removal of twenty seven PhACs (eight anti-inflammatory and/or analgesic pharmaceuticals (AIAPs)- acetaminophen, ibuprofen, naproxen, ketoprofen, diclofenac, codeine, indomethacin and propyphenazone-, six antibiotics-clarithromycin, ofloxacin, sulfadiazine, sulfamethazine, sulfamethoxazole and trimethoprim-, four b-blockers -atenolol, metoprolol, propranolol and sotalol-, two antihypertensives/diuretics -furosemide and hydrochlorothiazide-, three lipid regulators-bezafibrate, fenofibrate and gemfibrozil-, and four psychiatric medications -carbamazepine, diazepam, lorazepam and paroxetine) was evaluated in a pilot-scale anaerobic/anoxic/aerobic (A^2O) plant operating as IFAS process with 50% of the aerobic basin filled with the carrier AnoxKaldnes K5. The A^2O-IFAS pilot-scale plant was long-term operated treating real wastewater coming from the pretreatment unit of the full-scale WWTP Murcia Este. The linking between the removal rates of the targeted PhACs, the changes in environmental/operating variables, and the removal rates of organic matter and nutrients was evaluated by means of Spearman's rank correlations coefficients.

2. Materials and methods

2.1. Description of the wastewater treatment pilot-scale plant

The pilot-scale A^2O-IFAS plant (Fig. 1) was located at the facilities of the WWTP Murcia Este (EMUASA, Murcia, Spain). The characteristic of the pilot-scale plant has been previously described in full detail by Gallardo-Altamirano et al. [20]. In brief, the pilot-scale plant (volume of bioreactor 1.65 m^3) is designed to treat up to 6 m^3 per day of wastewater coming from the pretreatment unit of the full-scale WWTP Murcia Este. The pilot plant was previously operated and studied as conventional BNR A^2O system during two experimental phases (from May 20th, 2016 to March 31st, 2017) with different operational/environmental conditions [20,21]. After the second phase, the pilot-scale A^2O plant was converted to IFAS system by filling the aerobic basin (1.20 m^3) at 50% with AnoxKaldnes K5 carrier, which correspond to 0.36 filling ratio of the total bioreactor volume. The filling ratio of 0.50 concerning the aerobic basin was recommended by Veolia AnoxKaldnes® in terms of optimal mixing efficiency and optimal nutrient removal efficiency. Table 1 shown the main characteristics of the carrier used with a carrier photograph with and without attached biomass. The start-up and the stabilization phase of IFAS system lasted 92 days (from 2nd May to 2nd August 2017). Since 1st July 2017 the stabilization phase started to operate with the selected working concentration of mixed liquor suspended solids (MLSS) between 1500 and 2000 mg L^{-1}, biofilm suspended solid (BFSS) between 1000 and 2000 mg L^{-1}, mixed liquor sludge retention time (ML-SRT) between 3.5 and 4.5 days, and the mixed liquor food-to-microorganism ratio (ML-F/M) over 0.40 $kgBOD_5$ $kgMLVSS^{-1}$ d^{-1}. The experimental phase started up on 2nd August 2017 after the steady-state was reached (more than threefold SRT) and lasted 105 days. Monthly average values ± standard deviation for operational parameters, and performance rates of the A^2O-IFAS system are shown in Table 2 and the average values ± standard deviation for physico-chemical concentration measured in the decanted influent and effluent (point 2 and 3 respectively, Fig. 1) are shown in the Table 3.

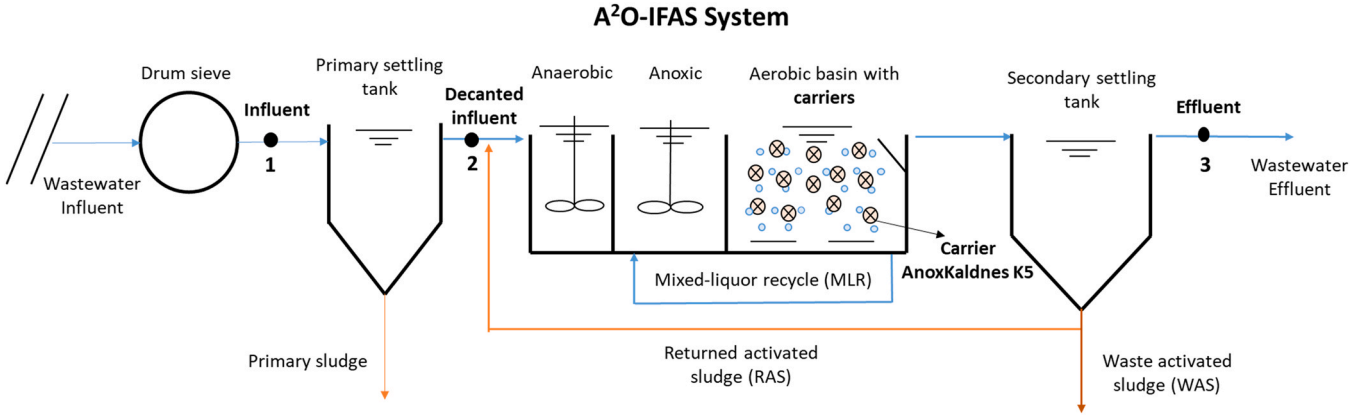


Fig. 1. Schematic diagram of the pilot-scale A^2O-IFAS plant used in the study. Sampling points 1, 2 (influent) and 3 (effluent) are indicated.

Table 1
Main characteristics of AnoxKaldnes K5 carrier.

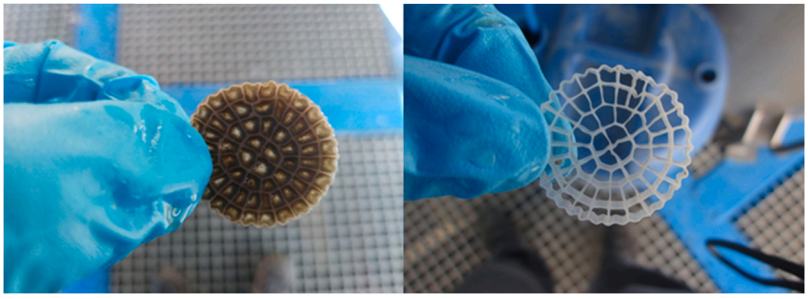
Characteristics	AnoxKaldnes K5	Carrier picture
Material	High-density polyethylene	
Shape	Cylinder	
Density, kg L^{-1}	0.95	
Bulk Density, Kg m^{-3}	118	
Specific surface area, m^2 m^{-3}	800	
Nominal diameter, mm	25	
Nominal thickness, mm	3.5	
Count per m^3	331,000	

Table 2
Monthly average values and global average values (± standard deviation) for operational parameters of the A^2O-IFAS system. Average data marked with an asterisk (*) were significantly different among each month, according to the Kruskal-Wallis test ($p < 0.05$).

Parameter	August 2017	September 2017	October 2017	01–15 November 2017	Average Phase III
Influent flow (L h^{-1})	246 ± 10	253 ± 16	258 ± 4	248 ± 5	251 ± 9
HRT (h)	6.7 ± 0.3	6.6 ± 0.4	6.4 ± 0.1	6.7 ± 0.1	6.6 ± 0.2
RAS (%)	47 ± 2	45 ± 3	42 ± 2.1	43 ± 1	44 ± 2
MLR (%)	318 ± 13	307 ± 20	116 ± 13.2*	224 ± 4*	241 ± 10
ML-SRT (d)	4.5 ± 0.6	3.5 ± 0.6	3.5 ± 0.2	4.4 ± 1.6	4.0 ± 1.0
ML-F/M (kg BOD_5 kg $MLVSS^{-1}$ d^{-1})	0.37 ± 0.18	0.37 ± 0.21	0.40 ± 0.08	0.51 ± 0.11*	0.41 ± 0.14
F/M global (kg BOD_5 kg $biomass^{-1}$ d^{-1})	0.20 ± 0.10	0.21 ± 0.10	0.23 ± 0.09	0.28 ± 0.13	0.23 ± 0.10
SVI (ML g^{-1})	112 ± 10	116 ± 7	108 ± 10.6	121 ± 15	114 ± 10
DO set point (mg L^{-1})	0.5–1.0	0.5–1.0	0.5–1.0	0.5–1.0	0.5–1.0
MLSS (mg L^{-1})	1774 ± 274	1676 ± 155	1935 ± 275	1863 ± 185	1812 ± 217
MLVSS (%)	83 ± 7	85 ± 5	83 ± 7.13	85 ± 22	84 ± 12
BFSS, mg/L	1299 ± 341*	1616 ± 184*	2003 ± 341*	2152 ± 354*	1767 ± 318
BFVSS, %	82 ± 6	90 ± 3	88 ± 6.2	90 ± 5	87 ± 4
OT (°C)	28 ± 2	26 ± 1*	22 ± 2.2 *	19 ± 1*	24 ± 1
COD/TN ratio	6.0 ± 1.0	6.9 ± 0.8*	6.5 ± 0.9	7.1 ± 0.8*	6.6 ± 1.2
OLR (kg BOD_5 m^{-3} d^{-1})	0.564 ± 0.103	0.607 ± 0.249 *	0.771 ± 0.134 *	0.935 ± 0.128 *	0.719 ± 0.154
ORR (%)	93.4 ± 2.0*	95.4 ± 1.4	96.0 ± 2.5	96.6 ± 0.8	95.3 ± 1.4
NLR (Kg TN m^{-3} d^{-1})	0.171 ± 0.028	0.161 ± 0.080	0.216 ± 0.021*	0.240 ± 0.022*	0.197 ± 0.038
NRR (%)	70.4 ± 3.3	80.6 ± 3.1*	70.6 ± 3.7	69.5 ± 4.5	72.8 ± 4.4
PLR (Kg TP m^{-3} d^{-1})	0.020 ± 0.001	0.021 ± 0.005	0.019 ± 0.001	0.020 ± 0.001	0.020 ± 0.002
PRR (%)	47.7 ± 10.7*	82.0 ± 15.3	88.5 ± 10.4	81.9 ± 7.8	75.0 ± 9.1

HRT: hydraulic retention time; RAS: return activate sludge rate; MLR: mixed liquor recycle rate; ML-SRT: mixed liquor sludge retention time; ML-F/M: mixed liquor food-to-microorganisms ratio; SVI: sludge volumetric index; DO: dissolved oxygen; MLSS: mixed liquor suspended solids; MLVSS: mixed liquor volatile suspended solids; BFSS: biofilm suspended solid; BFVSS: biofilm volatile suspended solid; OT: operating temperature; OLR: Organic loading rate; NLR: nitrogen loading rate; PLR: phosphorous loading rate; ORR: the corresponding Organic Removal Rate; NRR: Nitrogen Removal Rate; PRR: Phosphorous Removal Rate. HRT, F/M, RAS, MLR, SRT, SVI, OLR, NLP and PLR were calculated as described by Metcalf (2003).

Table 3
Removal and average ±standard deviations of physical-chemical parameters measured in the decanted influent and effluent (point 2 and 3, Fig. 1) water samples during the A^2O-IFAS system. TSS: Total suspended solids; TN: total N; TP: total P.

Parameter	Influent	Effluent	% Removal
COD (mg L^{-1})	376 ± 80	56 ± 13	85.1 ± 2.9
BOD_5 (mg L^{-1})	197 ± 47	8.8 ± 3.0	95.5 ± 1.8
TSS (mg L^{-1})	122 ± 33	15 ± 3.9	87.6 ± 4.7
TN (mg L^{-1})	57 ± 9.1	16 ± 5.2	72.5 ± 6.3
COD/TN (mg COD/mg TN)	6.6 ± 1.2		
$N\text{-}NH_4^+$ (mg L^{-1})	45 ± 8.3	1.1 ± 1.6	97.6 ± 3.7
$N\text{-}NO_3^-$ (mg L^{-1})	0.2 ± 0.3	6.9 ± 2.3	
TP (mg L^{-1})	5.4 ± 0.4	1.3 ± 1.0	76.6 ± 17.0
Turbidity (NTU)	123 ± 30.8	7.2 ± 2.3	
pH	7.4 ± 0.1	7.9 ± 0.1	

2.2. Wastewater sampling collection for the analysis of physico-chemical parameters and PhACs

To determine the physico-chemical parameters shown in the Tables 2 and 3, 24-h composite samples were taken three times per week from the sampling points 2 and 3 (Fig. 1). Chemical oxygen demand (COD), total nitrogen (TN), $N\text{-}NO_3^-$, $N\text{-}NH_4^+$ and total phosphorous (TP) were measured by Merck Spectroquant® kits (Darmstadt, Germany), while MLSS, volatile suspended solids (MLVSS), total suspended solids (TSS), biological oxygen demand (BOD_5), were measured according to standard methods (SM 2540 for MLSS, MLVSS and TSS; SM 5210B for BOD_5) [22]. The biomass concentration attached in the plastic carriers (BFSS) was achieved as follows: twelve representative carriers were removed from the bioreactor, diluted in 50 ML of distillated water with Tween 80 (1/1000 of dilution), sonicated for 15 min and centrifugated for 20 min at 3000 rpm. Once the biomass was separated from the plastic carriers, the BFSS was measured according to the determination of MLSS and assessed through the total number of carriers in a liter of reactor [23,24].

For the analysis of PhACs, twelve influent and effluent wastewater 24-h composite samples were taken from sampling point 1 and 3 of the pilot-scale plant (Fig. 1). The effluent samples were collected according to the constant hydraulic retention time (HRT) (12 h). The first eight samples (influent and effluent) were retrieved at the beginning of the experimental period and after 35 days, both midweek and at the end of the weekend (02/08/2017, 06/08/2017 and 06/09/2017, 10/09/

2017), the last four samples (influent and effluent) were taken at midweek after 35 days until the end of the experimental phase, (11/10/2017 and 15/11/2017). Every samples were taken using 500-ML amber PET bottles, as described previously [20].

2.3. Analytical methods for pharmaceutically active compounds

2.3.1. Chemical and sample treatment

All analytical reference standards were obtained from Sigma-Aldrich (St Louis, MO, USA), while the isotopically labeled compounds used as surrogates were purchased from Cerilliant (Round Rock, TX, USA) or CDN Isotopes (Quebec, Canada), or LGC Promochem (London, UK) or Santa Cruz Biotechnology (Dallas, TX, USA). All the above compounds were prepared individually (100 μg ML^{-1}) from the powder and dissolved in 100% acetonitrile (ACN) methanol (MeOH), or dimethyl sulfoxide (DMSO) according to the solubility of each compound and stored at − 20 °C. Working mixtures (10 μg L^{-1} in MeOH) for calibration and spiking purposes, including all tested compounds or labelled compounds were freshly prepared every three months. Their relevant physico-chemical properties are reported elsewhere Gallardo-Altamirano et al. [20,21]. LC-MS grade solvents (ACN ≥ 99.9%, MeOH ≥ 99.9%, DMSO ≥ 99.9%), and HPLC water were purchased from Merck (Darmstadt, Germany).

2.3.2. On-line extraction and LC-MS/MS analysis

Extraction of PhACs from wastewater samples were performed according to Gros et al. [25] and López-Serna et al. [26] and carefully detailed in Gallardo-Altamirano et al. [20]. Separation of the analytes was achieved on a Purospher STAR RP-18 endcapped column (125 ×2 mm i.d., 5 μm particle size, Merck, Darmstadt, Germany), while analysis was based on selective reaction monitoring (SRM) acquisition performed by a SCEX 4000 QTRAP hybrid triple quadrupole-linear ion trap (QqLIT) mass spectrometer, equipped with a Turbo Ion Spray source (Sciex, Redwood City, CA, U.S.). Quantitative analysis was performed using the Analyst 1.5.2 Software (Sciex, Redwood City, CA, U.S.). Selected SRM transitions for each analyte and for its corresponding surrogate including the optimized parameters as well as any detailed information regarding LC-MS/MS methodology are described elsewhere [20,21].

2.4. Statistical analysis

Analysis of statistical comparison between groups of samples were performed using IBM SPSS Statistics v. 19 (SPSS Inc., IBM, USA). Spearman`s rank correlation coefficients (ρ) were calculated to find significant links between the operational/environmental variables, organic matter and nutrient removal rate and REs of the selected 27 PhACs. Moreover, the Kruskal-Wallis non-parametric test was chosen to determinate significant differences among the different experimental phases and systems (conventional BNR A^2O system vs A^2O-IFAS system) using a 95% significant level ($p < 0.05$) [20].

3. Results and discussion

3.1. Operational parameters and evolution of biomass and physico-chemical parameters of the pilot-scale A^2O-IFAS bioreactor

Fig. 2 shows the evolution of suspended biomass (MLSS), attached biomass concentration (BFSS) and sludge volumetric index (SVI) during the start-up phase, stabilization phase and experimental phase of the A^2O-IFAS bioreactor. During the start-up phase (60 days) the MLSS was decreasing from 3500 mg L^{-1} to 1500 mg L^{-1} and the BFSS was increasing progressively from 0 mg L^{-1} to 1000 mg L^{-1} until the steady state was reached. The progressive decrease of MLSS was done manually during the start-up phase to reach the MLSS concentration required (around 1500 mg L^{-1}) for the optimal operation of the A^2O-IFAS bioreactor that left sufficient substrate for the growth of the attached biomass in the carrier and improves the sedimentation process [27]. The SVI was very high at the beginning (210–270 ML g^{-1}) producing several sedimentation problem episodes in the secondary settling tank. It was due to the introduction of the plastic carriers that disrupted the activate sludge flocs. Afterward, the sedimentation problems disappeared at the end of the start-up phase when the SVI decreased to 120 ML g^{-1} due to the restoration of the activated sludge flocs and the progressively decrease of MLSS concentration and ML-SRT (from 12 to 4 days); similar results were referenced by Kim et al. [27]. Subsequently, the stabilization phase started when steady-state conditions were achieved and the bioreactor operated at constant MLSS concentration (1500 ± 200 mg L^{-1}), constant BFSS (1000 ± 100 mg L^{-1}), constant SVI (120 ± 10 ML g^{-1}) and constant ML-SRT (4.0 ± 0.6 days) during 32

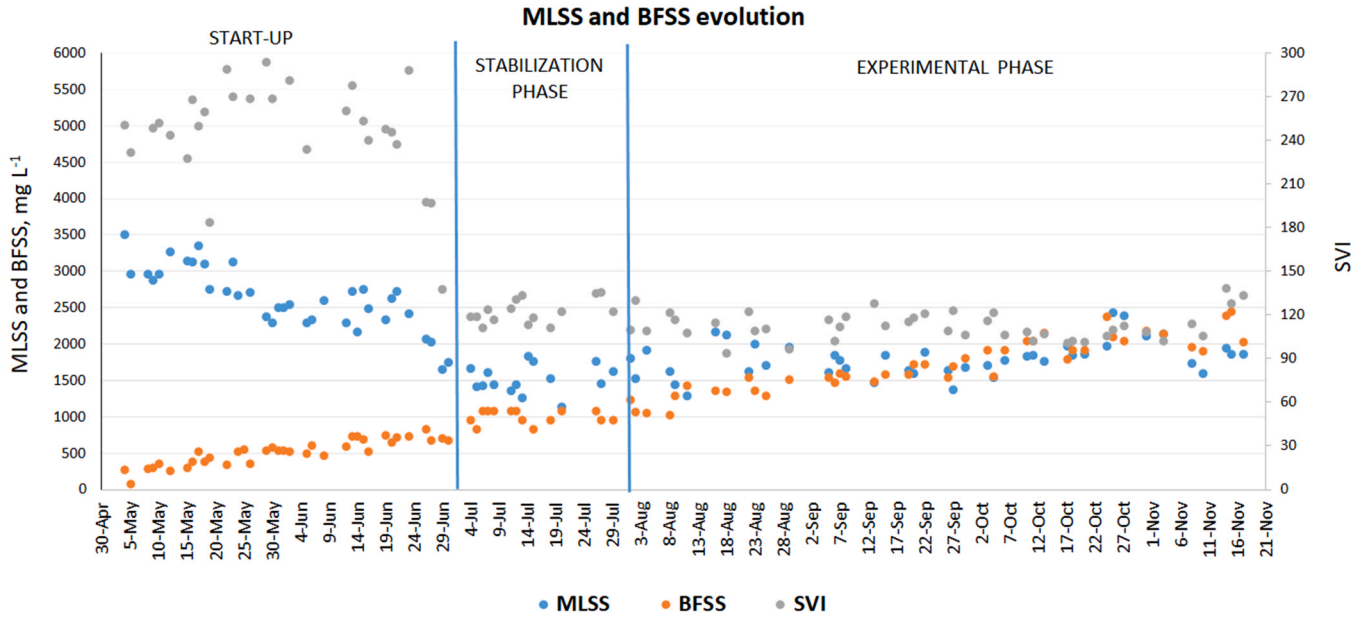


Fig. 2. Evolution of the biofilm fixed suspended solid (BFSS), the mixed liquor suspended solids (MLSS) and sludge volumetric index (SVI) during the start-up, stabilization phase and experimental phase of the pilot-scaler A^2O-IFAS bioreactor.

days. Finally, the experimental phase started at 2nd August with the first analysis of PhACs.

Table 2 shows the monthly average values for each operational parameter in the experimental phase. To find significant operational and performance advantages of the A^2O-IFAS system, the pilot-scale plant was overloaded, operating at peak hydraulic influent flowrate (251 L h^{-1} and 6.6 h of HRT), instead of the design average hydraulic flowrate (167 L h^{-1}) and design HRT (10 h). The F/M global ratio (MLSS+BFSS), % return activated sludge (RAS), HRT, ML-SRT and MLSS concentration values were kept constant during the experimental phase, whereas the mixed liquor recycle (MLR) was changed from very high (310%) to low (116%) and medium (224%) during the experimental phase to observe the relationship with the TN removal rate (NRR) and N-NO_3^- concentration effluent. The attached biomass concentration (BFSS) increases progressively from 1299 ± 341 mg L^{-1} in August 2017–2152 ± 354 mg L^{-1} in November 2017 during the experimental phase (see Fig. 2 and Table 2), in accordance with the constant increase of the organic loading rate (OLR) and nitrogen loading rate (NLR) (Table 2). The continuous increase of OLR with constant MLSS concentration created a gradually increment of the ML-F/M ratio (from 0.37 to 0.51 kg BOD_5 kg MLVSS $^{-1}$ d^{-1}) that gave more assimilable organic matter for the attached biomass. The operative temperature (OT) was decreasing from August (28 °C) to November (19 °C) while the dissolve oxygen was kept stable (0.5–1.0 mg L^{-1}). Slightly significant lower organic removal rate (ORR) (93.4 ± 2.0) and considerably significant lower TP removal rate (PRR) (47.7 ± 10.7%) were obtained in August compared with the following months. This is possible due to the lower influent OLR (0.564 ± 0.103 kg BOD_5 m^{-3} d^{-1}), lower COD/TN ratio (6.0 ± 1.0) and lower BOD_5/TP ratio (28.2 ± 3.1) reported in August compared with the next months. These low values provide low easily biodegradable organic matter (BOD_5) for phosphorous accumulating microorganisms (PAOs) and denitrifying microorganisms. Additionally, some N-NO_3^- can be recycled by the RAS into the anaerobic zone and consume the (BOD_5) necessary for an efficient biological phosphorous removal process [28–30]. The NRR was similar during the all-experimental phase except for September; the high value detected in this month was possibly due to the combination of lower NLR and higher OLR that produce higher COD/TN (6.9) and higher NRR (80.6 ± 3.1%). In this sense, higher TN removal efficiencies have been reported at higher COD/TN ratio [29,31,32]. Interestingly, similar NRR was obtained in August, October and November independently of the MLR rate (N-NO_3^- recycle ratio, responsible for the denitrification process) despite big variation was done during the experimental phase for each month. Therefore, independently of the MLR, a high percentage of TN removal occurred by simultaneous nitrification-denitrification process in the aerobic basin due to the combination of low oxygen dissolved (0.5–1.0 mg L^{-1}) and the presence of attached biomass that produces different redox conditions into the biofilm layer [5,33].

3.2. Performance of the A^2O-IFAS pilot-scale plant

Table 3 shows the mean concentration of COD, BOD_5, TSS, TN, N-NH_4^+, N-NO_3^-, TP, turbidity and pH in the bioreactor's influent and effluent (point 2 and 3 respectively, Fig. 1) during the experimental phase of the pilot-scale A^2O-IFAS plant. The mean effluent values were below the discharge limit value of the WWTP Murcia Este. Similar and better performance in terms of ORR, NRR and PRR was obtained in the present study compared with those studies that operated with analogous operational conditions [27,31,32,34,35], despite that the present A^2O-IFAS bioreactor was generally operated at lower HRT (6.6 ± 0.2 h), higher NLR (0.197 ± 0.038 Kg TN m^{-3} d^{-1}) and lower COD/TN ratio (6.6 ± 1.2); the HRT of Kim et al. [27], Di Trapani et al. [34] and Araujo Junior et al. [35] was 6.4, 7.4 and 48 h, respectively and the COD/TN ratio of Kim et al. [27], Regmi et al. [31], Mannina et al. [32], Di Trapani et al. [34], and Araujo Junior et al. [35] was 13.5, 7.0, 10–5, 15.7 and 14.1 mgCOD $mgTN^{-1}$, respectively. On the other hand, several authors obtained better organic and nutrients removal performance operating with IFAS-systems. In this sense, Xiao et al. [36] obtained higher TN and TP removal (86% and 97%, respectively) in a full-scale A^2O-IFAS plant, and Ashrafi et al. [5] obtained higher TN and TP removal (92.4% and 96.5%, respectively) in a pilot-scale IFAS five-stage Bardenpho plant. However, these bioreactors operated at considerable higher COD/TN ratio (10.33 and 14.2), higher HRT (11 and 8.4 h, respectively) and lower NLR (0.096 and 0.100 Kg TN m^{-3} d^{-1}, respectively) compared with the present study (Tables 2 and 3); in this sense, higher NRR and PRR have been related with bioreactor operated with higher anoxic and anaerobic HRT, higher COD/TN ratio and lower NLR [29,31,32,37,38].

Numerous comparisons have been done between IFAS-system and CAS processes to find the best performance of organic matter and nutrients removal with the lower operational cost. Generally, the IFAS-systems can operate with lower volume bioreactor, lower SRT and lower temperature than CAS systems to achieve a better nitrification-denitrification process. Di Trapani et al. [39] demonstrated that high nitrification process was possible at low SRT and low temperatures. Accordingly, Araujo Junior et al. [35] found that the addition of carriers (18% filling ratio) in a CAS process improved the NRR over 14% with a reduction of sludge waste production. Similarly, Güneş et al. [3] found in a comparison among CAS WWTP and a pilot-scale IFAS plant that higher NRR and PRR was possible in the IFAS-system with lower operational and investment costs (50%) and with a considerably lower orthophosphate effluent level (0.7 mg L^{-1} vs 4.2 mg L^{-1}). Moreover, Bashar et al. [14] concluded in a study about the cost-effectiveness of TP removal efficiency that A^2O-IFAS system was one of the most cost-effective process ($42.22/lb-P removed) among six different process configurations for nutrient removal.

Tables S1 and S2 compare the environmental/operational parameters, physico-chemical parameters and removal efficiency values achieved by the A^2O-IFAS bioreactor (phase III) with the values previously achieved by the A^2O bioreactor [20] in the same pilot-scale plant. Significant lower effluent concentration of BOD_5, TN, TP and N-NO_3^- were obtained in this study (phase III, A^2O-IFAS system) compared with phase I (A^2O system), while significant lower effluent concentration of total suspended solid (TSS), N-NO_3^-, and turbidity were obtained in phase III (A^2O-IFAS system) compared with phase II (A^2O system) (Table S2). Consequently, significantly higher NRR (72.8 ± 4.4%) and PRR (75.0 ± 9.1%) were obtained in phase III compared with both phases operated with A^2O system (Table S1), while lower ORR were obtained only in phase I among phase II and III (Table S1). Interestingly, despite A^2O-IFAS bioreactor was operated at significant lower COD/TN rate compared with the A^2O bioreactor in phase II (6.6 ± 1.2 vs 7.8 ± 1.9, Table S2), higher nutrients removal efficiencies were achieved in the A^2O-IFAS bioreactor. Generally, higher biological nutrients removal efficiencies have been reported at higher COD/TN ratio [29,31,32]. Therefore, this fact highlights the capacity of the attached biomass to enhance the TN and TP removal processes. Moreover, better performances were obtained by the A^2O-IFAS bioreactor compared to the A^2O bioreactor (phase I and II) despite the A^2O-IFAS system operated with significant lower RAS, ML-SRT, MLSS and HRT, as well as, significant higher ML-F/M ratio, NLR and PLR (Table S1) that normally decrease the organic and nutrients removal performance [37,41]. Operating with lower RAS and MLR, lower MLSS and lower HRT decrease the operational and investment cost of the WWTP [3,40]. The main operational parameters that reduce the operational cost between the A^2O system (Phase I and II) and the A^2O-IFAS system (Phase III) were the energy consumption needed for the RAS and MLR. The average energy consumption of the two peristaltic pumps was 1.03 kWh m^{-3}. Assuming that the A^2O-IFAS bioreactor can operate at 116% of MLR in Phase III, the A^2O-IFAS system could save 3.42 kWh d^{-1} compared to Phase I and 7.05 kWh d^{-1} compared to Phase II (A^2O system). Additionally, operating with lower ML-SRT produces waste activated sludge with higher volatile biomass percentage (MLVSS of 84% vs 79%, Table S1) which increase the potential biodegradability and biogas production of the

waste activated sludge in the further anaerobic digestion process [42–44]. In addition, ML-SRT below 5 days is preferred for faster-growing PAOs and other heterotrophs such as denitrifiers that improve the biological phosphorous and nitrogen removal capacity [45]. Regarding settleability of the activated sludge, the A^2O-IFAS system shows significant higher SVI compared with phase I (114 ± 10 vs 84 ± 24 ML g^{-1}), while similar SVI were found with phase II. Despite this difference among phase I, the settling process was considered acceptable during the all-experimental phase. Accordingly, Kim et al. [27] demonstrated worse settling in A^2O-IFAS system than the control conventional A^2O system, although these differences were small and settling process was considered acceptable. On the other hand, Di Trapani et al. [34] concluded that IFAS-systems improve the activated sludge settling process concerning CAS process.

Similar to the aforementioned authors, the present study demonstrated that IFAS-systems improve the CAS performance at lower operational and investment costs. Consequently, this process became a very simple and efficient technology for upgrading overloaded WWTPs or design a new municipal WWTP [8,17].

3.3. Occurrence of PhACs in the influent and effluent wastewater samples

Table 4 display the range, mean, median, relative standard deviation (RSD) and frequency of the 27 targeted PhACs in the influent and effluent wastewater samples of the A^2O-IFAS system during the experimental phase. Only 3 compounds (sulfadiazine, sulfamethazine and paroxetine) had a frequency of detection < 100% in the influent wastewater, while 7 compounds (acetaminophen, clarithromycin, sulfadiazine, sulfamethazine, metoprolol, fenofibrate, diazepam, and paroxetine) presented frequency of detection < 100% in the effluent wastewater, in which all of them obtained very low mean concentration (<20 ng L^{-1}) included the AIAPs indomethacin and propyphenazone. Ibuprofen, acetaminophen and naproxen had the highest mean concentration (8716, 8667 and 5252 ng L^{-1}, respectively) in the influent wastewater samples, while the highest mean concentration in the effluent wastewater samples correspond for the antibiotic ofloxacin (2495 ng L^{-1}) and the diuretics hydrochlorothiazide (1748 ng L^{-1}) and furosemide (1034 ng L^{-1}). To our best knowledge, scarce studies exist about the PhACs concentration in the influents and effluents from pilot-scale or full-scale IFAS-system plants treating municipal wastewaters compared with CAS or MBR systems [12,15,46]. Interestingly, Shreve and Brennan, (2019) reported the influent and effluent of 22 PhACs in 6 full-scale IFAS WWTPs, in which the average influent and effluent concentration of the targeted PhACs in the present study were in the range of data reported by these authors. Generally, the range concentrations of all targeted PhACs in both influent and effluent wastewater was consistent with data from the reviewed studies [6,47,48] that reported data mainly from CAS and MBR systems. However, the average concentration in the influent and effluent of the majority selected PhACs were below to the average influent and effluent concentrations reported by the review study Verlicchi et al. [6].

Table S3 and S4 compare the mean concentration values in the influent and effluent wastewater samples of the A^2O-IFAS bioreactor (phase III) with the values previously achieved by the A^2O bioreactor [20,21] in the same pilot-scale plant. As shown in Table S3, 7 compounds (acetaminophen, diclofenac, ibuprofen, furosemide, hydrochlorothiazide, gemfibrozil, and carbamazepine) obtained significant lower influent concentration in phase III (A^2O-IFAS system) compared with phase I and II, while only diazepam and trimethoprim obtained higher significant influent concentration in phase III compared with both phases. Correspondingly, 11 compounds obtained significant lower effluent concentration in phase III compared with phase I and II (Table S4), while only sotalol and diazepam obtained significant higher effluent concentration in phase III compared with phase I and II. The significant lower effluent concentration for many compounds in phase III (A^2O-IFAS system) was due to the significant lower influent concentration for several compounds in phase III, as well as, for the significant higher REs observed in A^2O-IFAS system, as it will be further discussed in Section 3.4.

In order to identify which compound could pose a risk for aquatic ecosystems, the lowest predict non-effect concentration (PNEC) values described in the recent literature [6,48,49] for all tested PhACs are

Table 4
Concentration range, mean concentration, median concentration, relative standard deviation (RSD) and detection frequencies of pharmaceutically active compounds (PhACs) in the influent wastewater samples of the A^2O-IFAS system. BLD: Below detection limit.

Therapeutic groups	Compounds	Influent (n = 6)					Effluent (n = 6)				
		Range	Mean	Median	RSD (%)	Freq. (%)	Range	Mean	Median	RSD (%)	Freq. (%)
AIAPs	**Acetaminophen**	3174–14310	8667	9078	45	100	BLD −116	19	0	245	17
AIAPs	**Codeine**	111–253	210	232	25	100	87–179	137	153	29	100
AIAPs	**Diclofenac**	508–646	591	589	9	100	423–632	538	569	16	100
AIAPs	**Ibuprofen**	6600–11450	8716	8755	19	100	37–111	62	53	45	100
AIAPs	**Indomethacin**	11–18	14	14	19	100	10–19	14	13	27	100
AIAPs	**Ketoprofen**	910–1710	1236	1211	26	100	317–981	624	649	39	100
AIAPs	**Naproxen**	3663–6610	5252	5318	23	100	72–351	191	140	67	100
AIAPs	**Propyphenazone**	4.9–6.5	5.5	5.4	11	100	6–9	6.9	6.5	16	100
Antibiotics	**Clarithromycin**	197–529	321	268	44	100	BLD-23	10	10	103	68
Antibiotics	**Ofloxacin**	1448–3171	2467	2756	32	100	1679–3903	2495	2200	35	100
Antibiotics	**Sulfadiazine**	BLD	–	–	–	–	BLD	–	–	–	–
Antibiotics	**Sulfamethazine**	BLD-14	3.5	0.0	169	33	BLD-5	0.8	0.0	245	17
Antibiotics	**Sulfamethoxazole**	186–750	469	440	53	100	45–274	162	154	52	100
Antibiotics	**Trimethoprim**	102–398	219	203	51	100	6–97	50	52	83	100
Beta-blocker	**Atenolol**	581–953	754	750	18	100	14–261	118	103	71	100
Beta-blocker	**Metoprolol**	24–108	49	35	65	100	BLD-69	18	12	141	68
Beta-blocker	**Propranolol**	15–117	52	40	79	100	16–36	23	23	31	100
Beta-blocker	**Sotalol**	39–109	62	49	45	100	34–107	56	50	48	100
Diuretics	**Furosemide**	1134–1806	1475	1468	16	100	582–1401	1034	1038	28	100
Diuretics	**Hydrochlorothiazide**	1226–1917	1560	1547	15	100	1305–2169	1748	1795	19	100
Lipid-regulators	**Bezafibrate**	82–142	104	99	23	100	22–40	31	31	22	100
Lipid-regulators	**Fenofibrate**	7–74	48	54	56	100	BLD	–	–	–	–
Lipid-regulators	**Gemfibrozil**	651–1075	854	882	19	100	90–411	268	278	46	100
Psychiatrics	**Carbamazepine**	70–156	98	87	32	100	82–186	117	100	33	100
Psychiatrics	**Diazepam**	5–18	8.0	6.6	60	100	BLD-9	6.2	7.0	51	83
Psychiatrics	**Lorazepam**	101–305	158	142	48	100	125–320	188	172	36	100
Psychiatrics	**Paroxetine**	BLD −28	8.1	2.7	140	50	BLD −2	0.3	0.0	245	17

shown in Table S4. Out of the 27 targeted PhACs, seven compounds had mean effluent concentration higher than their PNEC (diclofenac, ibuprofen, ofloxacin, sulfamethoxazole, trimethoprim, furosemide and gemfibrozil) in the A^2O-IFAS system. Therefore, these compounds could pose a risk for the aquatic ecosystems [6,48]. However, the number of compounds with higher mean effluent concentration than their PNEC were almost double (12) in phase I and II (A^2O system). Consequently, the application of the IFAS system to the A^2O bioreactor could reduce considerably the risk for the aquatic ecosystem (from 12 to 7 compounds). However, implementation of tertiary treatments (membrane processes, activated carbon adsorption, advanced oxidaton processes) is desirable to prevent the continuous discharge of these PhACs into the receiving water bodies [47].

3.4. Removal efficiency of PhACs and links with the operational/performance variables: A^2O-IFAS system vs conventional A^2O system

Table 5 shows the REs (range, mean, median and RSD) of the targeted PhACs throughout the experimental phase in the A^2O-IFAS system. Those pharmaceuticals with mean influent concentration < 20 ng L^{-1} and influent frequency detection $< 50\%$ (indomethacin, propyphenazone, sulfadiazine, sulfamethazine, diazepam and paroxetine) were omitted from the calculations, since conclusions concerning its REs could not be accurately drawn.

The term *removal* of PhACs in the pilot-scale plant accounts for all the losses of a parent compound produced by different physico-chemical and biological mechanisms (sorption to solid matter, volatilisation and biodegradation/biotransformation). The removal by volatilisation is considered residual due to the low vapour pressures ranging from 1.0E-15–1.0E-7 (Table S7). The PhACs removal extent during wastewater treatment process is influenced by many factors such as physico-chemical and biological properties of the compound, operational parameters, treatment technology used, and biomass characteristics. For this reason, a firm conclusion about the RE of each compound cannot be easily drawn, as high variation in RE rates is generally found in different WWTPs [7,47].

The RE by preliminary and primary treatments is generally considered quite poor according to many authors [6,50–53], and in some cases, parent compounds may even be increased in the water phase during the process, probably caused for transformation/deconjugation of undetected PhACs into the parent compounds [53–56].

In general, many authors concluded through a complete mass balance calculation (aqueous and suspended phase) that the removal of the majority PhACs are mainly attributed to the biodegradation/biotransformation process in the secondary biological treatment [6,51,53,56–60]. However, other authors highlight the importance of sorption removal for several PhACs such as some antibiotics and fluoroquinolones [51,61–64] as well as, fenofibrate, diazepam, clarithromycin and hydrochlorothiazide [65]. In this sense, removal by sorption onto activated sludge flocs in water line could be a significant removal pathway for compounds with high hydrophobicity represented by the coefficient octanol-water (K_{ow}) and more specific by the sorption potential indicated by the experimental solid-water distribution coefficient (Kd) (Table S7). To date, a simple rule has been widely accepted, if the compound has a high sorption potential ($K_d > 500$ L Kg^{-1} or log $K_{ow}>2.5$) the PhACs tend to adsorb onto sludge and particles, being a candidate to be removed via excess sludge [6,47,48,66]. Accordingly, from the 21 PhACs reported in Table 5, only the ofloxacin, propranolol, fenofibrate and lorazepam could be a candidate to be removed via excess sludge due to the high lipophilicity properties indicated by the high sorption potential ($K_d>500$ L Kg^{-1} and $K_{ow}>2.5$, Table S7).

During the biological secondary treatment, the microorganisms of the activated sludge can biodegrade the organic compounds by anabolic or co-metabolic mechanisms. According to some authors, biodegradation of PhACs is mainly attributed to co-metabolic mechanisms due to the low concentration to support substantial biomass growth [7,67–69]. Many studies have emphasized that biodegradation processes are correlated to the concentration, composition and characteristic of biomass (i.e. microbial community composition), which in turn is related to the configuration plant and operational/environmental parameters of the WWTP (such as, SRT, HRT, F/M ratio, temperature, etc.) [6,7,69].

In the present study, the highest average REs values (>80%) correspond for fenofibrate, acetaminophen, ibuprofen, naproxen, clarithromycin and atenolol, highlighting that those PhACs obtained the lowest RSD percentages values (from 0% to 14%). The removal of the majority targeted PhACs are mainly attributed to the biodegradation/biotransformation process in the secondary biological treatment [6,7,53,56,57,60]. However, the high RE of fenofibrate was attributed to sorption mechanisms due to the high lipophilicity properties indicated by the high K_{ow} (Table S7). On the other hand, very low and negative average REs values (<30%) were obtained for lorazepam, carbamazepine, ofloxacin, hydrochlorothiazide, sotalol, diclofenac, propranolol and metoprolol, highlighting that those PhACs obtained the highest

Table 5
Removal efficiencies (REs, %) of PhACs and relative standard deviations (RSD) calculated for each compound measured in the A^2O-IFAS system. The compounds with average influent concentration values < 20 ng/L and an influent frequency detection < 50% are not shown.

Therapeutic groups	Compounds	Range	Mean	Median	RSD (%)	Freq. (%)
AIAPs	**Acetaminophen**	99–100	99.8	100	0.41	100
AIAPs	**Codeine**	18–63	33	29	50	100
AIAPs	**Diclofenac**	(−10)−23	9.2	8.5	122	100
AIAPs	**Ibuprofen**	99–100	99.5	100	1	100
AIAPs	**Ketoprofen**	39–65	51	50	19	100
AIAPs	**Naproxen**	94–98	97	98	2	100
Antibiotics	**Clarithromycin**	90–100	96	97	5	100
Antibiotics	**Ofloxacin**	(−170)−39	-17	11	-468	100
Antibiotics	**Sulfamethoxazole**	29–77	63	69	30	100
Antibiotics	**Trimethoprim**	37–98	69	71	39	100
Beta-blocker	**Atenolol**	63–98	84	86	14	100
Beta-blocker	**Metoprolol**	(−193)−100	29	64	381	100
Beta-blocker	**Propranolol**	(−43)−79	22	30	261	100
Beta-blocker	**Sotalol**	(−5)−30	8.8	2.5	156	100
Diuretics	**Furosemide**	8–58	30	28	58	100
Diuretics	**Hydrochlorothiazide**	(−28)−9	-12	-15	-122	100
Lipid-regulators	**Bezafibrate**	55–76	69	72	11	100
Lipid-regulators	**Fenofibrate**	100	100.0	100	0	100
Lipid-regulators	**Gemfibrozil**	56–86	70	71	16	100
Psychiatrics	**Carbamazepine**	(−30)–(−9)	-19	-18	-37	100
Psychiatrics	**Lorazepam**	(−71)–(−5)	-25	-16	-103	100

RSD. Negative REs of the aforementioned PhACs are commonly observed and reported in wastewater treatment plants operated with different treatment process (CAS, MBR, MBBR and IFAS systems) and are likely due to the release of molecules enclosed/absorbed in suspended particles or due to the microbially-mediated reversion of influent metabolites conjugate forms into the parent compounds [6,7,15,47,53]. Therefore, to prevent negative RE of these PhACs is important to analyze the molecules that was enclosed/absorbed into the suspended solid particles and the conjugated analytes to close the mass balance sheet. The remaining PhACs obtained medium RE values (between 30% and 70%) with medium RSD percentage values (between 11% and 58%). In general, the average RE values obtained in the A^2O-IFAS system for the selected PhACs are in the range of REs reported by different studies operating with MBBR or IFAS systems, except for diclofenac that was lower compared with the REs values reported in recent studies [12–15] and for clarithromycin and atenolol for which it was higher [14,18,70,71]. In accordance to the best of author's knowledge, no REs data in literature for continuous MBBR or IFAS process have been reported for ofloxacin, sotalol, furosemide and lorazepam.

During the last decades, many studies have compared the REs of PhACs by the most common technology for wastewater treatment, CAS and MBR [72–76]. However, few studies have compared the REs of PhACs by these conventional technologies versus MBBR or IFAS systems [12,15]. In this sense, De La Torre et al. [12] compared the REs of several PhACs in a full-scale CAS plant with a semi-real plant operated with different configuration (MBR, IFAS-MBR, pure MBBR) that treated the same urban wastewater. They concluded that the IFAS-MBR system exhibited similar or better REs for most of the studied PhACs, in which the operating conditions (SRT, MLSS, HRT and F/M ratio) were proved to be important because lower removal rates were obtained at lower SRT and lower MLSS concentration. A similar comparison between technologies was done in bench-scale experiments; for instance, Murray et al. [77] found in parallel bench-scale sequencing batch reactors fed with real municipal wastewater higher REs of atenolol and trimethoprim in the IFAS reactor compared to the control CAS reactor. Likewise, Falås et al. [18,19] and Jewell et al. [10] compared the IFAS system with the CAS system through bench-scale batch experiments using both activated sludge and suspended biofilm carrier from full-scale IFAS WWTPs. Their results proved that attached biomass contributed significantly to the removal of some PhACs in the IFAS processes. However, more research is needed to explore the higher RE capacity of these technologies and links to the operational/performance variables.

Table S5 shows the mean ± standard deviation of the REs values obtained in the A^2O-IFAS bioreactor (phase III) with the REs values obtained in the conventional A^2O bioreactor previously studied in two experimental phases (phase I and II) by Gallardo-Altamirano et al. [20,21]. Additionally, in order to find significant links among the RE obtained in the three phases with its corresponding operational/performance parameters, the Spearman's rank correlation coefficient (ρ) was calculated in Table S6. The SRT, MLSS and BFSS operational parameters was no included in the test because the A^2O-IFAS systems operate with two types of biomass (suspended activated sludge and attached biomass) where the attached-biomass SRT is considered higher than the ML-SRT. In this sense, those variables are not equally comparable among the two different technologies (A^2O vs A^2O-IFAS system) because the microbial diversity and function of the biomass is different and enhanced in the A^2O-IFAS system [15].

According to the Kruskal-Wallis test, statistically significant higher RE was observed for phase III (A^2O-IFAS system) compared with phase I and II (A^2O system) for ibuprofen, naproxen and trimethoprim (Table S5). The REs of the AIAPs ibuprofen and naproxen were low in phase I (39% and 41%, respectively), high in phase II (88% and 87%) and very high in phase III (99.5% and 97%). The increase of the RE between phase I and II was favored by the increase of MLSS and the decrease of F/M ratio according to Gallardo-Altamirano et al. [20]. Similarly, the significant increase of RE in phase III compared with phase I and phase II was favored by the decrease of the global F/M ratio for ibuprofen ($\rho = -0.62$, Table S6) and naproxen ($\rho = -0.52$, Table S6), as well as the increase of the operating temperature (OT, $\rho = 0.53$) and NRR ($\rho = 0.53$) for naproxen. Concerning to the antibiotic trimethoprim, no significant RE were found between phase I and II [21], while significant higher RE (Table S5) were obtained in phase III with the presence of attached biomass and favored by higher OT ($\rho = 0.56$). Similarly, Murray et al. [77] demonstrated improved RE of trimethoprim under all conditions in the IFAS bioreactors as compared to the CAS control bioreactors, also correlated with higher OT. In this sense, Falås et al. [18] also reported considerable higher removal rate of trimethoprim in a bath experiment using suspended biofilm carrier compared with activated sludge. The attached biomass systems can lead to different redox conditions at different thicknesses of the biofilm layer where the substrates are transported into the biofilm via diffusion mechanisms. These different redox conditions enable the simultaneous N and P removal, and can enhance the biotransformation of several micropollutants by co-metabolisms [7,33,78]. In this sense, several authors have reported high REs of naproxen and trimethoprim in anaerobic conditions [79–82]. Therefore, higher RE of trimethoprim and naproxen in the A^2O-IFAS system may be related also to the higher anaerobic conditions produce by the attached biomass.

Furthermore, statistically significant higher RE was observed for phase III (A^2O-IFAS system) and phase II compared with phase I (A^2O system) for ketoprofen, gemfibrozil, atenolol, clarithromycin and bezafibrate (Table S5). Interestingly, these higher RE in phase II and III was favored for the decrease of global F/M ratio (Table S6). Accordingly, several authors [12,13,19] found in parallel studies considerable higher RE of ketoprofen and gemfibrozil in IFAS process compared to CAS and MBR systems. They concluded that the IFAS process shown higher RE for most of the studied PhACs, highlighting the importance of operate at high SRT and MLSS concentration that give low F/M ratio to increase the RE of several PhACs [12]. Similarly, Ooi et al. [14] found high RE of atenolol (79%) and clarithromycin (78%) in a pilot-scale staged anoxic/aerobic MBBR system. The nitrifying basin of the pilot-scale plant obtained higher biodegradation rate per gram of biomass for atenolol, while the denitrifying basin obtained higher biodegradation rate for clarithromycin, it pointed out the importance of the denitrification process (shown in the NRR value) for the biodegradation/transformation of clarithromycin. Equally, a strong positive correlation of the RE values with NRR and influent N-NH_4^+ concentration for clarithromycin ($\rho = 0.62$ and $\rho = 0.55$, respectively) was found in our study. Moreover, Murray et al. [77] also found higher RE of atenolol in IFAS process compared with the control CAS. Finally, similar to our study, Falås et al. [18] also found higher removal rate per gram of biomass for bezafibrate in the suspended biofilm carriers compared to suspended activated sludge; interestingly, the higher RE of bezafibrate in IFAS system was positivity correlated in our study (Table S6) with the N-NH_4^+ influent concentration, ORR, NRR, and PRR ($\rho = 0.67$, $\rho = 0.59$, $\rho = 0.59$, $\rho = 0.44$, respectively).

Lastly, the beta-blocker sotalol and the AIAPs diclofenac showed significant higher RE in phase III compared with phase II but the RE was very low (8.8% and 9.2% respectively). Correspondingly, low RE (<40%) was also reported for sotalol in bath experiment for IFAS or MBBR systems by several studies [14,70,71]. However, contrary to the present study, higher RE (>30%) was normally obtained for diclofenac in IFAS or MBBR system in the literature [10,12–15,19] The results of Jewell et al. [10], suggest that reductive dechlorination associated with the IFAS biofilms could be a mechanism for differential removal in IFAS and CAS systems. On the other hand, only the diuretic hydrochlorothiazide obtained significant lower mean RE in phase III compared with phase I (−12% vs 33%) (Table S5). Similarly, low RE values (<2%) was obtained in full-scale IFAS WWTP and batch experiment with attached biomass and activated sludge by Falås et al. [18].

To conclude, the aforementioned studies determined that IFAS or MBBR systems reach similar or better RE of PhACs compared to other

technologies, where the biodegradation generally occurred in parallel to the removal of organic matter, nitrogen and phosphorous indicating co-metabolism [7,14,70] as it occurred with naproxen, clarithromycin and bezafibrate in the present study. Additionally, the combination of suspended activated sludge and attached-growth biomass in the A^2O-IFAS bioreactor gives a combination of slow-growing microorganisms in the carrier media and quick-growing microorganisms in the activated sludge that increases the total biomass concentration (MLSS + BFSS) and the global SRT. Therefore, the bioreactor operates at lower global F/M ratio with a high range of possible active strains capable of improve the biodegradation/biotransformation rate of several PhACs and organic micropollutant [7,9,15,18,70,71,83].

4. Conclusion

The A^2O-IFAS system showed similar or better performance in terms of ORR, NRR and PRR compared with the conventional A^2O systems. In particular, significantly higher NRR ($72.8 \pm 4.4\%$) and PRR ($75.0 \pm 9.1\%$) were obtained compared with the A^2O system. Despite A^2O-IFAS bioreactor was operated at significant lower COD/TN rate compared with the A^2O bioreactor (6.6 ± 1.2 vs 7.8 ± 1.9). This fact highlights the capacity of the attached biomass to enhance the TN and TP removal processes. Besides, the A^2O-IFAS system operated with significant lower RAS, ML-SRT, MLSS and HRT, as well as, significant higher ML-F/M ratio, NLR and PLR, that normally decrease the organic and nutrients removal performance. All these aspects imply lower operational and investment costs. For instance, the A^2O-IFAS system could save 3.42 and 7.05 kWh d^{-1} of energy cost compared to the conventional A^2O system (Phase I and II, respectively).

In relation to PhACs removal, previous studies showed that IFAS process achieves higher RE for most of the studied PhACs compared to CAS process, highlighting the importance of operate at high SRT and MLSS concentration that give low F/M ratio to increase the RE of several PhACs. In the A^2O-IFAS system, the combination of suspended activated sludge and attached-growth biomass gives a higher total biomass concentration (MLSS + BFSS). This implies a similar or better RE of PhACs, operating at very low values of ML-SRT (4.0 days) and MLSS (1822 mg L^{-1}). For instance, from the 19 PhACs that was evaluated base on their REs, the A^2O-IFAS system obtained significant higher REs for 8 PhACs (ibuprofen, ketoprofen, naproxen, clarithromycin, trimethoprim, atenolol, bezafibrate and gemfibrozil) compared to the A^2O system operated in Phase I, and 5 PhACs (diclofenac, ibuprofen, naproxen, trimethoprim, and sotalol) compared to the A^2O system operated in Phase II. Additionally, the operational/performance parameters that most influenced the significant improvement of the REs were mainly the higher biomass concentration (MLSS+BFSS) and the lower value of the F/M ratio for the PhACs ibuprofen, naproxen, ketoprofen, gemfibrozil, atenolol, clarithromycin, and bezafibrate. Likewise, high influent concentration of N-NH_4^+, with high removal efficiencies of total nitrogen, organic matter and phosphorus (NRR, ORR and PRR) increased the REs of naproxen, clarithromycin and bezafibrate.

According to these results, due to the affordable cost and well-demonstrated high efficiency in organic matter, nutrients and organic micropollutants removal, IFAS systems become one of the most promising technologies for conventional WWTP upgrading.

CRediT authorship contribution statement

M.J. Gallardo-Altamirano: Investigation, Formal analysis, Writing - original draft, Visualization. **P. Maza-Márquez:** Software, Formal analysis. **S. Perez:** Methodology. **B. Rodelas**: Conceptualization, Writing - review & editing. **C. Pozo:** Supervision, Conceptualization, Writing - review & editing. **F. Osorio:** Supervision, Conceptualization, Project administration.

Declaration of Competing Interest

All the authors of the present manuscript have no conflict of interest to declare.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2021.105398.

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