Clofibric acid and gemfibrozil removal in membrane bioreactors

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ABSTRACT

The removal of two blood lipid regulators, clofibric acid (CLA) and gemfibrozil (GFZ), was evaluated using two identical aerobic membrane bioreactors with 6.5 L effective volume each. Polysulfone ultrafiltration hollow fiber membranes were submerged in the reactors. Different operating conditions were tested varying the organic load (F/M), hydraulic residence time (HRT), biomass concentration measured as total suspended solids in the mixed liquor (MLTSS) and the sludge retention time (SRT). Complete GFZ removal was obtained with F/M of 0.21–0.48 kg COD $kgTSS^{-1}$ d^{-1}, HRT of 4–10 hours, SRT of 10–32 d and MLTSS of 6–10 g L^{-1}. The GFZ removal can be attributed to biodegradation and there was no accumulation of the compound in the biomass. The CLA removals improved with the SRT and HRT increase and F/M decrease. Average removals of 78–79% were obtained with SRT 16–32 d, F/M of 0.21–0.34 kgCOD $kgTSS^{-1}$ d^{-1}, HRT of 7–10 hours and MLTSS of 6–10 g L^{-1}. Biodegradation was found to be the main removal pathway.

Key words | lipid regulators, membrane bioreactors, removal

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INTRODUCTION

A large number of pharmaceutical compounds of different varieties are consumed annually worldwide. Some of the more relevant ones in terms of environmental contamination are anti-inflammatory drugs, antibiotics and blood lipid regulators (Santos *et al.* 2010). After their administration, these compounds are partially metabolized, excreted in urine and feces, and subsequently disposed to the wastewater treatment plants (WWTPs). However, conventional WWTPs are not designed to remove hardly biodegradable or highly polar micro-pollutants such as the pharmaceutical compounds (Bendz *et al.* 2005). Consequently, these compounds have been detected in numerous effluents from conventional WWTPs, as well as in surface and groundwater samples (Petrovic & Barcelo 2007). Lipid regulators are frequently used to decrease the concentration of blood-circulating cholesterol and triglycerides. Clofibric acid (CLA) was the first lipid regulator reported in the environment; it is the main metabolite and the pharmacologically active component of the blood lipid-regulating pharmaceuticals clofibrate, etofibrate and etophyllinclofibrate. CLA is a scarcely biodegradable, polar compound, with a high motility, very persistent in the environment, with a half-life of 21 years and water residence time of 1–2 years; it causes endocrine disruption activity through interference with cholesterol synthesis (Buser *et al.* 1998; Pfluger & Dietrich 2001). CLA concentrations of 0.01–15 μg L^{-1} have been detected in WWTP effluents and of 0.07–0.27 μg L^{-1} in surface and ground waters (Herberer *et al.* 1997; Tauxe-Wuersch *et al.* 2005; Barcelo & Lopez 2007; Gagnon *et al.* 2008). Gemfibrozil (GFZ) is a fibric acid derivate. GFZ concentrations of 0.01–5.2 μg L^{-1} have been found in WWTP effluent (Rosal *et al.* 2010; Kosma *et al.* 2010) and of 0.7–1.5 μg L^{-1} in surface waters (Sanderson *et al.* 2003). According to Quinn *et al.* (2008), GFZ could be classified as a toxic compound. Estrogenic and carcinogenic effects had been reported for both CLA and GFZ (Mimeault *et al.* 2005; Urase *et al.* 2005; Radjenovic *et al.* 2009; Gross *et al.* 2010). The CLA and GFZ removals determined in conventional WWTPs with activated sludge systems are relatively low, of 30–60% (Clara *et al.* 2005; Bernhard *et al.* 2006; Radjenovic *et al.* 2009; Jelic *et al.* 2011).

Membrane bioreactors (MBRs) are a relatively new technology currently used for municipal wastewater treatment. This technology offers several advantages over the conventional activated sludge plants, such as operation at high biomass concentration, reduced excess sludge production

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and superior effluent quality. Furthermore, long sludge retention time (SRT) positively affects the overall activity of slow growing micro-organisms acting in nitrification or degradation of specific refractory pollutants. The aim of this work was to assess the removal of CLA and GFZ using MBR with submerged ultrafiltration membranes. The effects of the organic load, SRT and hydraulic residence time (HRT) on the removal effectiveness were investigated.

MATERIALS AND METHODS

Feed wastewater composition

The experiments were performed using synthetic wastewater (Table 1). Methanol was used as a major carbon source, providing a chemical oxygen demand (COD) of 800–1,000 mg L^{-1} in the synthetic wastewater. CLA and GFZ were added at concentrations of 0.4 and 0.8 μg L^{-1}, respectively, in accordance with reported concentrations in wastewaters for Mexico (Gibson *et al.* 2007; Siemens *et al.* 2008). The synthetic wastewater was complemented with nitrogen, phosphorus and micronutrients; the wastewater pH was adjusted at 7 ± 0.14.

Experimental setup and procedure

Two aerobic MBRs with submerged membranes (6.5 L effective volume) were continuously fed with synthetic wastewater. The schematic diagram of the experimental setup is presented in Figure 1. The MBRs were equipped with ultra-filtration hollow fiber membranes made of polysulfone (General Electric). The membrane had a molecular weight cutoff 100 kDa and total surface area of 0.042 m^2. The reactors were operated under 3 minutes suction and 45 seconds backwashing sequential cycles which were remotely controlled using a control panel and solenoid valves. Level sensors were installed to control the feeding pumps and prevent overflows. Continuous aeration was provided using a blower. The air flow entered the reactor through a stone diffuser placed in the bottom of each reactor, the oxygen concentration was kept between 1 and 2 mg L^{-1}.

The inoculation of the reactors was performed using biomass from the activated sludge reactor of a WWTP. The biomass was acclimated to the substrates in the synthetic wastewater by feeding continuously the reactors during 172 days at an organic load of 0.48 kgCOD $kgTSS^{-1}$ d^{-1} in the first reactor (MBR1) and 0.21 kgCOD $kgTSS^{-1}$ d^{-1} in the second one (MBR2). The mixed liquor total suspended solids (MLTSS) was maintained at 10,000 mg L^{-1} in both reactors. The determination of the trace organic compounds began once the process was completely stabilized (more than 95% COD and NH_4-N removals and a constant biomass growth). The operational parameters are presented in Table 2 for each experimental phase. As can be seen the organic loads (F/M) were between 0.21 and 0.77 kgCOD $kgTSS^{-1}$ d^{-1}, the HRT between 4 and 10 hours, the SRTs between 5 and 32 d. Daily biomass extractions were performed in the reactors to keep the desired MLTSS and SRT in each experimental phase. Two biomass concentrations had been tested: 6,000 and 10,000 mg TSS L^{-1}.

Analysis of the trace organics CLA and GFZ

The gas chromatography-mass spectrometry method (GC-MS) was developed and implemented for CLA and GFZ determination in aqueous and biomass samples, using approaches reported in Patterson *et al.* (2000), Reddersen & Heberer (2003) and Gibson *et al.* (2007). Solid-phase extraction (SPE) was used for extracting and concentrating the compounds from all samples. Chromabond C18 EC

Table 1 | Composition of the synthetic wastewater

Compound	Concentration	Units	Compound	Concentration	Units
Methanol	533–667	mg L^{-1}	$(NH_4)6Mo_7{\cdot}4H_2O$	0.01	mg L^{-1}
CLA	0.4	μg L^{-1}	$CaCl_2{\cdot}2H_2O$	4.4	mg L^{-1}
GFZ	0.8	μg L^{-1}	$MgSO_4{\cdot}7H_2O$	12.2	mg L^{-1}
NH_4Cl	90	mg L^{-1}	$ZnSO_4{\cdot}7H_2O$	0.132	mg L^{-1}
K_2HPO_4	9	mg L^{-1}	$MnSO_4{\cdot}H_2O$	0.04	mg L^{-1}
KH_2PO_4	8.4	mg L^{-1}	$CoCl_2{\cdot}6H_2O$	0.03	mg L^{-1}
$FeSO_4.7H_2O$	17.4	mg L^{-1}			

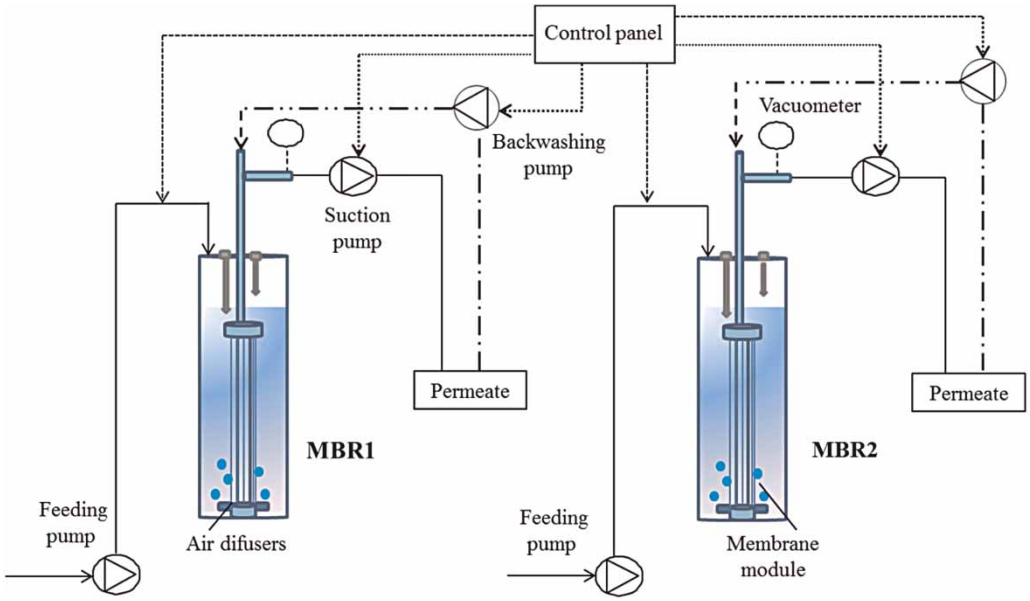

Figure 1 | Experimental setup.

Table 2 | Operational parameters

Experimental phase reactor	Operation days	F/M, kgCOD kgTSS^{-1} d^{-1}		SRT, d		HRT, h		MLTSS, mg L^{-1}	
		MBR1	MBR2	MBR1	MBR2	MBR1	MBR2	MBR1	MBR2
Acclimatization	1–172	0.48	0.21	11	32	4	10	10,000	10,000
1	173–256	0.48	0.21	11	32	4	10	10,000	10,000
2	257–356	0.77	0.34	5	20	4	10	6,000	6,000
3	357–400	0.43	0.26	10	16	7	7	6,000	10,000

cartridges (3 mL, 500 mg octadecyl modified silica as sorbent material) connected to a manifold (Teknokroma) and pumps as vacuum source, were used for the solid phase extraction. Liquid samples of 1 L were collected in amber glass bottles from the MBR influent and effluent. Aqueous samples were filtered through 1.0 μm glass fiber filters and adjusted to pH 2. The samples were then passed through Chromabond C18 EC cartridges which were preconditioned using 6 mL of methanol and 6 mL of acid water. The cartridges were washed with acid water (3 mL) and the target compounds were eluted from the cartridges with organic solvent (4 mL of methanol). The extracts were taken to dryness by a gentle stream of nitrogen gas, and re-dissolved in 1 mL of methanol. The extracts were stored in refrigeration before analysis. Biomass samples were collected from both MBRs. The biomass sample was submitted to the lyophilization process (−70 °C) to obtain 1 g dry weight, afterwards the sample was submitted to three sequential extractions, two with methanol and one with acetone. For each step of said extraction the sample was ultrasonicated (15 minutes) and then centrifuged (4,000 rpm, 15 minutes). The supernatants were taken and the three solvent fractions were combined and then filtered through Teflon filters (Pall Germany) of 0.45 μm. Subsequently, the sample was concentrated with nitrogen gas up to a volume of 4 mL. Before being submitted to SPE, the sample was reconstituted with 500 mL of HPLC water at pH 2.

Because of their high polarity and low volatility, the analytes required chemical derivatization prior to GC-MS analysis. Trimethylsilyldiazomethane (TSDM) was used as the derivatizing agent to form methylester derivatives. The samples were derivatizated adding 50 μL of TSDM and 100 μL of methanol, at environmental temperature for 2 hours. Chemical analysis was carried out by GC-MS

(Varian CP-3800) with an ion-trap tandem mass spectrometer (Varian Saturn 2200). Standards and sample concentrates were injected using a Varian CP-8400 (Switzerland) automatic sample injector. A 30 m × 0.25 mm ID column coated with 0.25 μm chemically bonded phase VF-5 ms, 5% phenyl +95% dimethylpolysiloxane was used (Variant Technologies). Injector temperature was kept at 280 °C, under split/splitless mode. For the analysis of all compounds the oven program was as follows: 65 °C for 2 minutes, 30 °C min^{-1} to 180 °C, 15 °C min^{-1} to 230 °C for 1 minutes, 15 °C min^{-1} to 300 °C for 2 minutes. The detector was used predominantly in selected ion mode. The molecular ions [M]+ with m/z 128 and 143 correspond to compound derivatives of CLA and GFZ, respectively. The electron impact ion source temperature was 220 °C with electron energy of 70 eV. All quantitative results were calculated by integration of the peak areas obtained by monitoring the respective ion fragments using selective ion monitoring mode. The analytical methods for CLA and GFZ determination were validated using standard solutions in liquid and solid feces. Calibration curves, response linearity, sensitivity, limit of detection and quantification, recovery and precision of the analytical procedure were calculated. The results summarized in Table 3 indicated that the methods allow accurate, precise and reliable determination of both compounds.

All the target compounds as well as the internal standard 4,4′-dichlorobiphenyl and the derivatization agent TSDM (trimethylsilyl diazomethane, 2.0 mol/L solution in hexane) were purchased from Sigma–Aldrich (Gillingham, UK) with >97% purity grade. The solvents and water were HPLC grade, provided by Burdik & Jackson (Morristown NJ, USA).

Other analyses

To assess the process performance in the MBR, in addition to the CLA and GFZ analysis, COD, NH_4-N and P_{total} had been determined twice a week in the influent and in the MBR effluents, as well as NO_2-N, NO_3-N in the effluents. TSS was determined once a week in the reactors. These parameters were determined according to *Standard Methods* (APHA 2012). Dissolved oxygen and temperature were measured daily.

RESULTS AND DISCUSSION

Process performance

During the acclimatization period, COD removals higher than 95% were reached in almost 1 week and a constant biomass growth was obtained in 30 days; however, the NH_4-N removal increased slowly and 95% was reached at day 119 after the startup. The determination of the CLA and GFZ concentrations was started at day 174, the day when the Phase 1 evaluation began. The results obtained during the three experimental phases are presented in Figure 2. As can be seen, the CLA concentration decreased slightly over time and the average removals for days 209–256 were 65 ± 10% and 78 ± 2% in MBR1 and MBR2, respectively. The higher removal obtained in MBR2 can be attributed to the higher SRT and lower F/M applied in this reactor. Meanwhile the GFZ could be completely removed in both reactors despite the different operation conditions. During the first experimental stage the average COD and NH_4-N removals were between 98 and 99%, average NO_3-N concentrations were 6 and 8 mg L^{-1} in the effluents of MBR1 and MBR2, respectively. These results indicated a good process performance in both reactors.

The F/M increase performed on day 257 caused an increase of the CLA and GFZ concentrations in the effluents of both reactors. The CLA concentrations increased in MBR1 while they decreased gradually over time in MBR2. The F/M increase to 0.77 kgCOD $kgTSS^{-1}$ d^{-1} and the SRT decrease to 5 d affected the CLA removal in MBR1 during the second experimental phase. The average CLA removal was 67 ± 8% in MBR1. Almost 50 days were required to reach again the CLA concentrations already

Table 3 | Detection limits, accuracy and precision of the analytical method

Compound	Phase	LD	LQ	RSD (%)	Recovery (%)
CLA	Water	0.0026 μg L^{-1}	0.0053 μg L^{-1}	0.25	89 ± 2
	Biomass	0.0014 μg g^{-1}	0.0026 μg g^{-1}	1.8	90 ± 2
GFZ	Water	0.0033 μg L^{-1}	0.0071 μg L^{-1}	0.21	106 ± 4
	Biomass	0.0025 μg g^{-1}	0.0033 μg g^{-1}	1.5	86 ± 4

LD – limit of detection; LQ – limit of quantification; RSD – relative standard deviation = (standard deviation/mean value) 100.

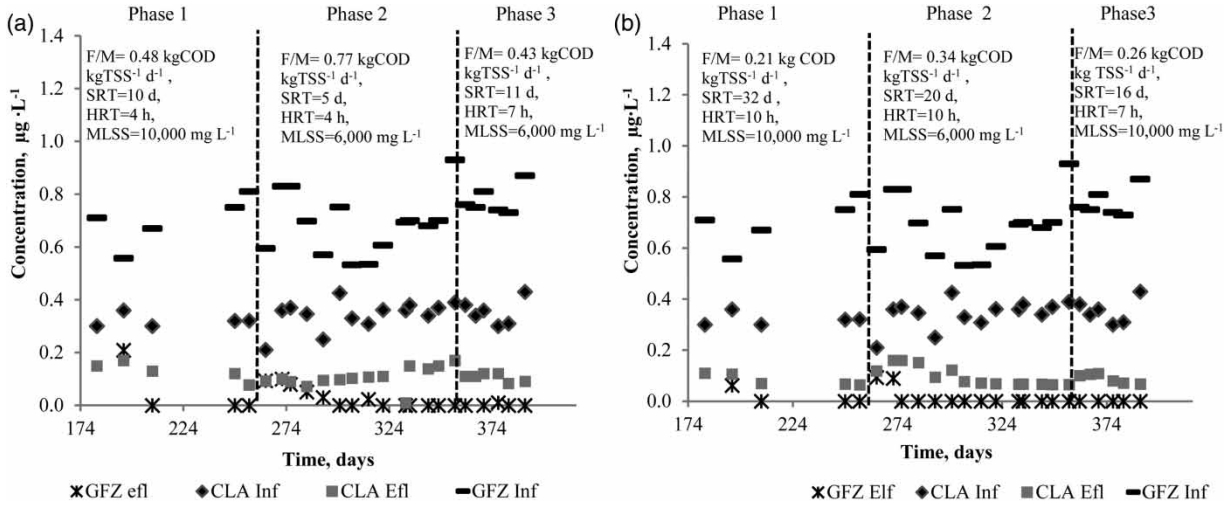

Figure 2 | CLA and GFZ concentrations during the MBR operation: (a) MBR1; (b) MBR2.

obtained in MBR2 effluent during the first phase. The average CLA removal for days 300–356 was 79 ± 7% in MBR2, similar to the one obtained during the first phase. The highest CLA removal of 83% was reached on day 356. The results suggest that the F/M increase to 0.34 kgCOD $kgTSS^{-1}$ d^{-1} and the SRT decrease to 20 d did not affect the CLA removal, as only a period of time was required for the acclimation to the new operational conditions. GFZ concentrations decreased gradually in both reactors over time and 100% removal was reached at day 300 and day 276 in MBR1 and MBR2, respectively. This means that 36 and 12 days were required to reach the removal capacity already obtained in MBR1 and MBR2. The average GFZ removals were 99 ± 1% (for days 292–256) in MBR1 and 100% (for days 276–356) in MBR2. The operation conditions slightly affected the GFZ removal in MBR1 during the second phase, while they did not affect the removal in MBR2. During the second experimental stage the average COD and NH_4-N removals were between 99.3 and 99.9%, average NO_3-N concentrations were 5 and 6 mg L^{-1} in the effluents of MBR1 and MBR2, respectively. The biological process was stable and a good performance was determined in both reactors.

The organic loads were reduced at the beginning of the third experimental phase. The SRT was increased in MBR1 and decreased in MBR2. HRT was increased in MBR1 and it was decreased in MBR2. The new operational conditions enhanced the CLA removal in MBR1, while a slight concentration increase was observed in the effluent from MBR2, which can be attributed to the SRT and HRT decreases. After 30 days of operation an 84% removal was reached in MBR2. The average CLA removals were calculated of 71 ± 6% and 78 ± 6% (days 377–390) in MBR1 and MBR2, respectively. The GFZ was completely removed in both reactors despite the different operation conditions. During the third experimental stage the average COD and NH_4-N removals were between 99.5 and 99.9%, average NO_3-N concentrations were 5 ± 1 mg L^{-1} in the effluents of both reactors; so the process performance was also good during the third experimental stage.

The effects of the operation parameters on the CLA and GFZ removals are summarized in Table 4. The CLA removal decreased with the organic load increase from 0.34 to values in the range of 0.43–0.77 kgCOD $kgTSS^{-1}$ d^{-1}. The average CLA removals were 65–71% with SRT of 5–11 d and 78–79% with SRT of 16–32 d. Thus, the SRT increase enhanced the CLA removal. These results were consistent with Kimura *et al.* (2005), as they reported 80% of CLA removal in a MBR with SRT of 65 d and lower removal (50%) when the STR decreased to 15 d. Other authors such as Quinn *et al.* (2008), Radjenovic *et al.* (2009) and Sipma *et al.* (2010) had also observed that better removals of pharmaceutical

Table 4 | Effects of the operation parameters on CLA and GFZ removals in MBR

SRT, d	F/M, kgCOD $kgTSS^{-1}$ d^{-1}	HRT, h	MLTSS, mg L^{-1}	CLA removal (%)	GFZ removal (%)
5	0.77	4	6,000	67 ± 8	99 ± 1
10	0.48	4	10,000	65 ± 10	100
11	0.43	7	6,000	71 ± 6	100
16	0.26	7	10,000	78 ± 6	100
20	0.34	10	6,000	79 ± 7	100
32	0.21	10	10,000	78 ± 2	100

compounds could be obtained with higher SRT. The HRT increase favored the CLA removal; removals of 78–79% were obtained with HRT of 10 hours. The lowest removals of CLA and GFZ (65% and 99%, respectively) were obtained with HRT of 4 hours. Kosjek *et al.* (2007) indicated that the increase of HRT enhanced the biodegradation, results that agree with this study. Nevertheless they obtained CLA removal of only 29% using pilot scale MBR with an HRT of 48 hours and SRT of 20 d.

GFZ removal of 99% was only obtained with SRT of 5 d and F/M of 0.77 kgCOD $kgTSS^{-1}$ d^{-1}. The rest of the SRT values (10–32 d) with F/M of 0.21–0.48 kgCOD $kgTSS^{-1}$ d^{-1} allowed for a complete GFZ removal. Regarding the effect of HRT it was observed that complete GFZ removal was obtained with HRT of 7 and 10 hours. Complete GFZ removal was also obtained with HRT of 4 hours when the SRT was 10 d, F/M of 0.48 kgCOD $kgTSS^{-1}$ d^{-1} and MLTSS of 10,000 mg L^{-1}. The GFZ removal was 99% applying HRT of 4 hours when the SRT was 5 d, with F/M of 0.77 kgCOD $kgTSS^{-1}$ d^{-1} and MLTSS of 6,000 mg L^{-1}. These results are better than the ones reported by Radjenovic *et al.* (2009) for MBR, of 30–40% with HRT of 7–15 hours and mixed liquor volatile suspended solids (MLVSS) of 1,400–8,400 mg L^{-1}. GFZ removals of 20–60% had been determined by Gross *et al.* (2010) and Jelic *et al.* (2011) in conventional activated sludge systems.

CLA and GFZ concentrations in biomass samples

Because of the low values of the Henry coefficients of the studied compounds, the major removal mechanisms during the treatment are biodegradation and sorption onto the biomass. The determinations of the CLA and GFZ concentrations in biomass samples from both MBRs were, performed to elucidate the sorption contribution to the removal, started at day 129. The results obtained are presented in Figure 3. As can be seen, the GFZ content in the biomass decreased over time, reaching values lower than the detection limits at day 209 after the start up. The slow decrease (209 days) can be explained by the adjustment of the biomass consortia and metabolism to the biodegradation of the micro-pollutants, which is limited by their low concentrations and difficult biodegradability.

The decrease of the GFZ concentration was slightly faster in the biomass of MBR2, which can be attributed to the higher SRT applied in this reactor during the acclimatization and the first evaluation phase. At day 209 the GFZ concentrations in the effluents from both MBR were also lower than the detection limit. During the experimental phases 2 and 3, the GFZ content in the biomass of both MBRs remained below the detection limits, which indicated that after the acclimatization, the major mechanism of the GFZ removal was the biodegradation.

The CLA content in the biomass also decreased over time (slightly faster in MBR2), however concentrations lower than the detection limit were reached at day 256. During the first evaluation stage, the average CLA concentration in the biomass was 0.05 mg kg^{-1} in both MBRs. During the next experimental phases, the CLA concentrations were lower than the detection limit most of the time, except days 296 and 385 when concentrations of 0.03 and 0.15 mg kg^{-1} had been found in MBR2 and day

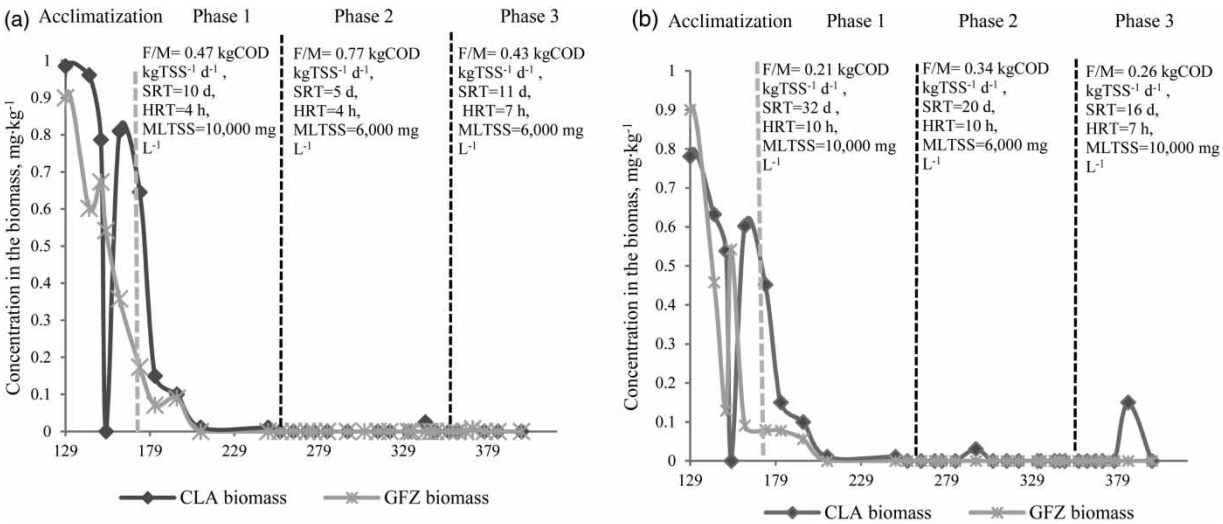

Figure 3 | CLA and GFZ concentrations in the biomass of MBR1 (a) and MBR2 (b).

342 when a concentration of 0.02 had been determined in MBR1. The mass balance performed for CLA indicated that the removal due to the extractions of biomass with sorbed CLA was only 1.0–2.5% when the best operational conditions were applied (HRT of 10 hours and STR of 20–32 d) and the CLA removals were 78–79%. The contribution of the sorption onto the biomass was 1.5–3.6% when low HRT and SRT were applied (4 hours and 5–11 d, respectively) and CLA removals were 65–71%. This means that the major mechanism of the CLA removal was also the biodegradation. The CLA was however harder to biodegrade compared with the GFZ and small CLA can remain sorbed onto the biomass even operating with HRT of 10 hours and SRT of 32 days.

CONCLUSIONS

The MBR with submerged ultrafiltration membranes are able to remove the blood lipid regulator compound GFZ present in wastewaters. SRT of 10 d and HRT of 4 hours would be sufficient for complete GFZ removal, under organic load of 0.48 kg COD $kgTSS^{-1}$ d^{-1} and MLTSS of 10,000 mg L^{-1}. Slightly greater SRT and HRT, of 11 d and 7 hours, respectively, would be required when MLTSS is 6,000 mg L^{-1}. The gemfibrozil removal can be attributed to biodegradation and there is no accumulation of the compound in the biomass. The scarcely biodegradable pharmaceutical compound CLA could be removed up to 84% using MBR with submerged ultrafiltration membranes. The SRT and HRT increases enhance the CLA removal. Average removals of 78–79% were obtained with SRT 16–32 d, F/M of 0.21–0.34 kgCOD $kgTSS^{-1}$ d^{-1}, HRT of 7–10 hours and MLTSS of 6,000–10,000 mg L^{-1}. Based on the CLA content determined in the biomass, biodegradation was found to be the main removal pathway, as the contribution of the sorption was estimated to be of only 1.0–2.5%.

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