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E-mail: ambi.agua@gmail.com

Removal of 17α-ethinylestradiol and total phosphorus in a sequencing batch reactor under two different sludge retention-time conditions

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Lícia Murito de Paula[1]; Eline Simões Gonçalves[2]; Larissa Coelho Auto Gomes[1]; José Carlos Rodrigues de Moura Júnior[3]; Jaime Lopes da Mota Oliveira[1*]

[1]Departamento de Saneamento e Saúde Ambiental. Escola Nacional de Saúde Pública. Fundação Oswaldo Cruz (ENSP/FIOCRUZ), Rua Leopoldo Bulhões, n° 1480, CEP: 21041-210, Manguinhos, RJ, Brazil.
E-mail: liciamurito@gmail.com, larissa.coelho.auto@gmail.com
[2]Instituto de Química. Departamento de Geoquímica Ambiental. Universidade Federal Fluminense (UFF), Outeiro São João Batista, s/n, CEP: 24020-141, Niterói, RJ, Brazil. E-mail: elinesg@gmail.com
[3]Departamento de Engenharia Química e de Materiais. Pontifícia Universidade Católica do Rio de Janeiro (PUC-Rio), Rua Marquês de São Vicente, n° 124, CEP: 22451-040, Gávea, RJ, Brazil.
E-mail: carlos.bjn@gmail.com
[*]Corresponding author. E-mail: jaimelmoliveira@gmail.com

ABSTRACT

Sewage treatment systems can prevent the direct discharge of endocrine disruptors, such as 17α-ethinylestradiol (EE2), into the environment. Treatment systems capable of promoting total phosphorus (TP) removal, such as sequencing batch reactors (SBRs), are promising in this regard. Two lab-scale SBRs with different sludge retention times (SRTs) were assessed for their EE2 and TP removal rates. Anaerobic/aerobic/anoxic phases with cycles of 6 h were used to treat sewage containing EE2 at a concentration of 5 μg L^{-1}. The removal rates of chemical oxygen demand and TP were approximately 80% for both the SBRs. Partial nitrification was observed in the SBRs. Initially, concentrations of EE2 above 1.0 μg L^{-1} in the treated sewage were measured. These concentrations were smaller in SBR 1, which used lower SRTs; EE2 was removed by sludge sorption. After the 56th cycle, the concentrations of EE2 in the treated sewage were below 0.1 μg L^{-1} in both the SBRs, indicating that its removal may have occurred by biodegradation due to acclimation to the process. Therefore, both TP removal and nitrification seem to play an important role in EE2 removal by SBRs.

Keywords: A2O SBR, EE2 and TP removal, sewage treatments.

Remoção de 17α-etinilestradiol e fósforo total por um reator em batelada sequencial sobre dois diferentes tempos de retenção de sólidos

RESUMO

Sistemas de tratamento de esgotos podem representar barreiras no descarte de desreguladores endócrinos como 17α-etinilestradiol (EE2) no meio ambiente. Processos capazes de promover a remoção de fósforo total (FT) como reatores em batelada sequenciais (RBSs) podem ser promissores para esta proposta. Dois RBSs em escala de laboratório aplicando diferentes tempos de retenção de sólidos (TRS) foram avaliados para a remoção de

EE2 e FT. As fases anaeróbia/aeróbia/anóxica com ciclos de 6 h foram usadas para tratar esgoto contendo 5 μg L^{-1} de EE2. As taxas de remoção de demanda química de oxigênio e de FT foram acima de 80% nos RBSs. A nitrificação parcial foi observada nos dois RBSs. Inicialmente valores acima de 1,0 μg L^{-1} de EE2 no esgoto tratado foram medidos. Estas concentrações foram menores no RBS 1 que usou um menor TRS; logo, provavelmente o EE2 foi removido por sorção no lodo. Após o 56º ciclo, as concentrações de EE2 no esgoto tratado foram abaixo de 0,1 μg L^{-1} nos dois RBSs mostrando que sua remoção pode ter ocorrido por biodegradação devido a aclimatação do processo. Portanto, tanto a remoção de FT quanto a nitrificação parecem ter um papel importante na remoção de EE2 por RBS.

Palavras-chave: RBS A2O, remoção de EE2 e FT, tratamento de esgotos.

1. INTRODUCTION

The presence of endocrine-disrupter micropollutants, such as 17α-ethinylestradiol (EE2), has become an increasingly serious problem (Zhou *et al.*, 2019). EE2 is a synthetic hormone primarily used in contraceptives and hormone replacement therapy or prophylaxis (Cogliano *et al.*, 2005). Their contamination in the environment occurs mainly through sanitary sewage discharge. Although their concentrations are usually low (µg L^{-1} to ng L^{-1}) (Chimchirian *et al.*, 2007; Froehner *et al.*, 2011; Martín *et al.*, 2012; Queiroz *et al.*, 2012; Pessoa *et al.*, 2014; Marcantonio *et al.*, 2020; Khasawneh and Palaniandy, 2021), EE2 exposure can negatively impact living organisms (Jorgensen and Halling-Sorensen, 2000; Briciu *et al.*, 2009; Robinson and Hellou, 2009), leading to vitellogenin level alterations in male fish and causing feminization, reproduction declines, gonad development inhibition, and hermaphroditism (Birnbaum, 2013; Aris *et al.*, 2014; Garmhausen *et al.*, 2015). These effects have been described in several taxa, including fish, amphibians, crustaceans, and gastropods (Metcalfe *et al.*, 2001; Silva *et al.*, 2013; Giusti *et al.*, 2014; Luna *et al.*, 2015; Azizi-Lalabadi and Pìrsaheb, 2021). In humans, EE2 exposure can lead to neuroendocrine function alterations along with breast, testicular, and prostate cancers (Cogliano *et al.*, 2005; Gore, 2010).

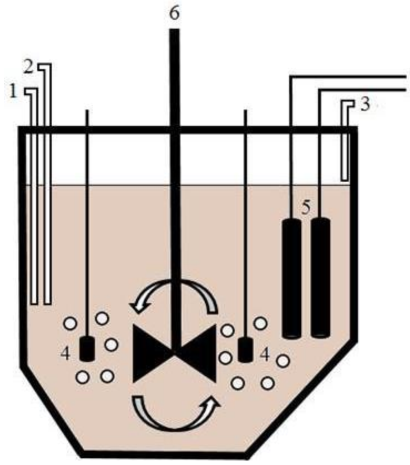
Sanitary sewage treatment systems can prevent the disposal of EE2 in the environment. Sequencing batch reactors (SBRs) are promising in this regard because of their greater metabolic flexibility. These types of reactors can alternately promote aerobic and anoxic reactions in the same tank, thus favoring simultaneous nitrification and denitrification and nitrogen removal (Ahn, 2006). In addition, alternating anaerobic and aerobic steps can also be employed in SBRs for biological total phosphorus (TP) removal (Bunce *et al.*, 2018). Some authors have also reported EE2 removal from sewage through nitrification processes (Maeng *et al.*, 2013; Kassotaki *et al.*, 2019), which were associated with ammonium oxidase (AmoA), an enzyme that oxidizes ammonia to nitrite, thus promoting EE2 co-metabolism (Fernandez-Fontaina *et al.*, 2016). In addition to biodegradation, sludge sorption has also been applied in EE2 removal studies (Kassotaki *et al.*, 2019). However, according to a literature review, little is known about the behavior of EE2 during biological TP removal (Gomes *et al.*, 2021b).

In this context, our study evaluated the EE2 removal potential of two SBRs, adopting anaerobic, aerobic, and anoxic cycles to achieve partial nitrification and removal of TP. Further, a lower cellular retention time was applied to SBR 1 (10 d) compared to that applied to SBR 2 (30 d) to assess the potential differential EE2 sorption and/or biodegradation effects.

2. MATERIAL AND METHODS

2.1. Experimental setup

Two reactors with a working volume of 6 L were used as SBRs: SBR 1 and SBR 2, and operated electronically and on 6-h cycles (Figure 1), which comprised filling (0.05 h), an anaerobic phase (0.5 h), an aerobic or oxic phase (2 h), an anoxic phase (2 h), sedimentation (1.4 h), and emptying (0.05 h). Dissolved oxygen (DO) and redox potential (ORP) probes were placed in the SBRs to monitor the different metabolic phases. Peristaltic pumps were used for sewage feeding and discharge of treated effluent and mixed liquor. Mixed liquor discharges were performed in the final anoxic phase prior to sedimentation. The mixed liquor volume discharged from each reactor was fixed to maintain a sludge retention time (SRT) of 10 d in SBR 1 and 30 d in SBR 2. Both the SBRs were fed synthetic sewage (raw synthetic sewage) composed of casein peptone (320 mg L^{-1}), beef extract (220 mg L^{-1}), urea (60 mg L^{-1}), dibasic potassium phosphate (56 mg L^{-1}), sodium chloride (14 mg L^{-1}), calcium chloride dehydrate (8 mg L^{-1}), and magnesium sulfate dehydrate (4 mg L^{-1}), according to Holler and Trösh (2001). The characteristics of raw synthetic sewage are presented in Table 1. The biological sludge used in the experiment was obtained from the activated sludge process of a sewage treatment plant. Each SBR cycle used a mixture of 3 L of synthetic sewage and 3 L of biological sludge.



Cycles of the SRBs

Phase	Operational condition	Time
Feed	Inlet raw sewage – feed peristaltic pump turn on	5 min.
Anaerobic phase	Mixed turn on	60 min.
Aerobic phase	Mixed and aeration system turn on	120 min.
Anoxic phase	Only mixed turn on	120 min.
Settled	All equipments turn off	55 min.
Discharge	Outlet treated sewage – release peristaltic pump turn on	5 min.
	Total time	360 min.

Figure 1. Scheme of the employed SBRs and their components: 1) Treated effluent outlet; 2) mixed liquor outlet; 3) sewage inlet; 4) oxygen diffusers; 5) Dissolved oxygen (DO), pH, and redox potential (ORP) probe; and 6) mechanical agitator.

Table 1. Raw synthetic sewage characteristics used in the employed SBRs.

	COD	TP	TN
Average	387 mg L^{-1}	22 mg L^{-1}	71 mg L^{-1}
Minimum	178 mg L^{-1}	8 mg L^{-1}	46 mg L^{-1}
Maximum	707 mg L^{-1}	34 mg L^{-1}	105 mg L^{-1}

Notes: COD – Chemical Oxygen Demand; TP – Total Phosphorus; TN – Total Nitrogen.

2.2. Process monitoring

The SBRS were first acclimated for 3 months and then monitored for 80 d (320 cycles) in three phases. During the first 40 d, the SBRs were fed only synthetic sewage. Then, 5 µg L^{-1} of EE2 was added to the synthetic sewage formulation and monitored for 30 d (120 cycles). Finally, the SBRs were fed synthetic sewage without EE2 for the last 6 d (24 cycles).

Throughout the monitoring period, raw synthetic sewage and treated effluent samples were collected to evaluate the removal efficiencies of chemical oxygen demand (COD) and TP, and to determine the concentrations of nitrite ($N\text{-}NO_2^-$) and nitrate ($N\text{-}NO_3^-$). The mixed liquor discharged during the process was used to determine the concentrations of volatile suspended solids (VSSs) in each SBR.

In the second and third phases of the experiments (with and without EE2, respectively), the treated effluent samples were also used to determine the concentrations of EE2 residues. Raw synthetic sewage samples prepared with EE2 were analyzed to determine the actual EE2 concentration entering each SBR.

The concentrations of COD, TP, VSSs, $N\text{-}NO_2^-$, and $N\text{-}NO_3^-$ parameters were determined according to APHA *et al.* (2017) protocols. The COD and TP removal rates from the two SBRs were compared using the Shapiro-Wilk statistical normality test using the Past Program, with $p > 0.05$.

2.3. EE2 residue analysis

A standard 1 mg L^{-1} EE2 solution (Sigma-Aldrich) was prepared using only 1 mL of high-performance liquid chromatography (HPLC)-grade methanol and purified water. This solution was employed to determine the method limits of detection and quantification and the sewage EE2 recovery percentages.

The 0.5-L treated effluent samples were filtered through 0.7 and 0.45 μm membranes, which were then subjected to an extraction process three times using 10 mL of HPLC-grade methanol in an ultrasonic bath for 20 min. The extracts were then dried, and the pellets were resuspended in purified water and mixed with filtered samples. The filtered samples were then percolated through pre-conditioned Strata X SPE cartridges (500 mg/3 mL, Phenomenex) at a flow rate of 3 mL min^{-1}. Cartridge pre-conditioning was performed using 5 mL of methanol, 5 mL of acetonitrile, and 7 mL of purified water. After sample percolation, the cartridges were cleaned with 10 mL of an acetonitrile/water solution (30:70 v/v) and dried under vacuum for 20 min. The EE2 residues were recovered from the cartridges using 12 mL of acetonitrile to obtain EE2 extracts, which were then dried under a gentle nitrogen flow and resuspended in a 0.5 mL acetonitrile/water solution (50:50). Thus, the treated effluent EE2 residues were concentrated 1,000-fold. The purified samples were analyzed using the HPLC-fluorescence method (HPLC-FL).

2.4. Chromatography Setup

The EE2 residues were analyzed using an Agilent 1200 Series HPLC-FL (Agilent Technologies) coupled with an Agilent Eclipse Plus C18 separation column model containing 5 μm particles, 250 mm in length and 4.6 mm in internal diameter. An isocratic flow set at 1.0 mL min^{-1} was applied, comprising an acetonitrile and water (50:50 v/v) solution. The detector was set at 280 nm excitation and 306 nm emission wavelengths. Under these conditions, the EE2 retention time was 8.2 min. The method limits of quantification and detection were 5.61 and 17.01 ng L^{-1}, respectively, and the recovery percentage from the treated wastewater was 101%.

3. RESULTS AND DISCUSSION

3.1. SBR performance

The average influent and effluent COD and TP concentrations from each SBR and their respective removal rates are shown in Figure 2. In the 84^{th} cycle, something unexpected was observed, probably due to a switch in one of the reagents brands used in the formulation of synthetic sewage, but it has not provoked a significant effect in the efficiency of the processes. Although the two SBRs employed different SRTs, similar performances were observed in both ($p>0.05$). No profile variations in COD and TP concentrations in the treated effluent were noted, even after the addition of EE2. The average COD and TP removal rates were 84 and 82%, respectively, in SBR 1 and 86 and 89%, respectively, in SBR 2.

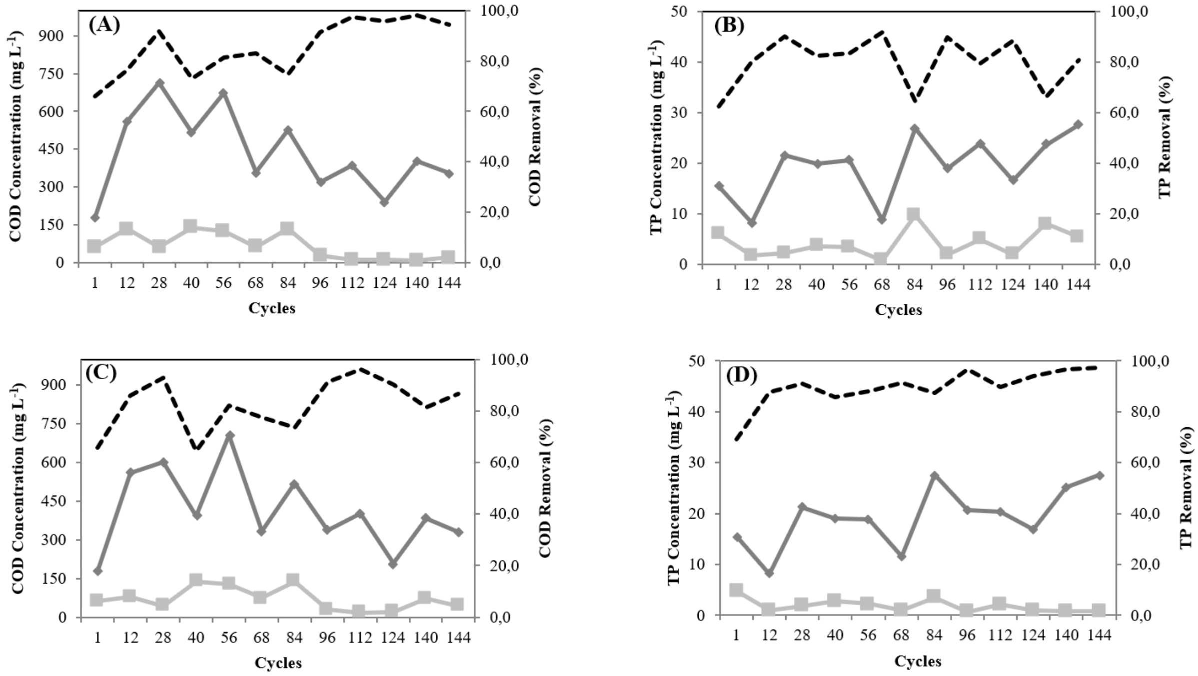


Figure 2. Influent and effluent concentrations and COD and TP removal rates in the employed SBRs. (A) and (B) indicate COD and TP behaviors in SBR 1, respectively; (C) and (D) indicate COD and TP behaviors in SBR 2, respectively.

Notes: ( ) influent, ( ) effluent and ( ) % removal.

Li *et al.* (2014) evaluated an SBR employing aerobic, anoxic, and extended-aeration steps and obtained COD and TP removal rates of 87% and 95%, respectively. Liu *et al.* (2020) used an anaerobic/aerobic/anoxic SBR and reported removal rates of > 90% for both COD and TP. Jia *et al.* (2012) also reported a TP removal rate of > 90% in an SBR performing simultaneous nitrification and denitrification and operating at a high aeration rate, whereas Li *et al.* (2020) obtained a TP removal rate of > 90% in an anaerobic/aerobic SBR.

The lower TP removal efficiency achieved by the two SBRs employed herein may be due to the longer anaerobic phase time (1.5–3 h) compared to that achieved by the aforementioned studies (Li *et al.*, 2014; 2020; Liu *et al.*, 2020). During anaerobiosis, phosphorus-accumulating organisms degrade organic matter present in the sewage and store it in the form of polyhydroxybutyrate (PHB), promoting environmental phosphate release (Li *et al.*, 2020). In addition, the lower the ORP, the greater the phosphate release (Akin and Ugurlu, 2005). During the aerobic stage, these organisms produce energy using stored PHB and reabsorb phosphate from the medium, resulting in phosphate removal. Therefore, the 0.5-h period employed in the anaerobic phase of the two SBRs may have been the determining factor for the lower TP removal efficiency achieved herein.

Phosphorus is discarded along with the sludge; therefore, the employed SRT is important for its removal. Li *et al.* (2014) reported a TP removal rate of > 90% applying an 8-d SRT, while Jia *et al.* (2012) reported a TP removal rate of > 90% with a 15-d SRT, and Liu *et al.* (2020) reported > 90% variations between 12 and 25 d-old sludge. The two SBRs employed two different SRTs (10 and 30 d) herein with no significant difference in TP removal efficiency ($p>0,05$).

The nitrate (NO_3^-) effluent concentrations in both the SBRs were below 1 mg L^{-1}, while that of nitrite (NO_2^-) reached 20 mg L^{-1} in SBR 1 and 14 mgL^{-1} in SBR 2 (Figure 3). The obtained $N\text{-}NH_x$ ($NH_3 + NH_4^+$) concentrations were less than 10 mg L^{-1}. The DO concentrations throughout the process in both the SBRs were always above 1 mg L^{-1}, in addition to a positive ORP value, which may have affected the anoxic phase, resulting in a reduced total nitrogen

(TN) removal rate of <50%. It is noteworthy that partial nitrification occurred in both the SBRs, whereas denitrification may not have been favored. Gomes *et al.* (2021a) indicated that DO control is essential for total nitrification and denitrification and promoting TN removal, and Soliman *et al.* (2016) demonstrated the importance of DO control in achieving partial nitrification. Ammonia nitrogen oxidation and nitrite accumulation were observed in both the SBRs. This metabolic step is normally carried out by a group of aerobic autotrophic microorganisms, termed ammonia-oxidizing bacteria (AOB), capable of oxidizing ammonia. To achieve this, they synthesize AmoA, which can promote estrogenic hormone co-metabolism, including EE2 (Forrez *et al.*, 2009; Maeng *et al.*, 2013). Thus, the two SBRs, even when employing different SRTs, could promote partial nitrification and TP removal.

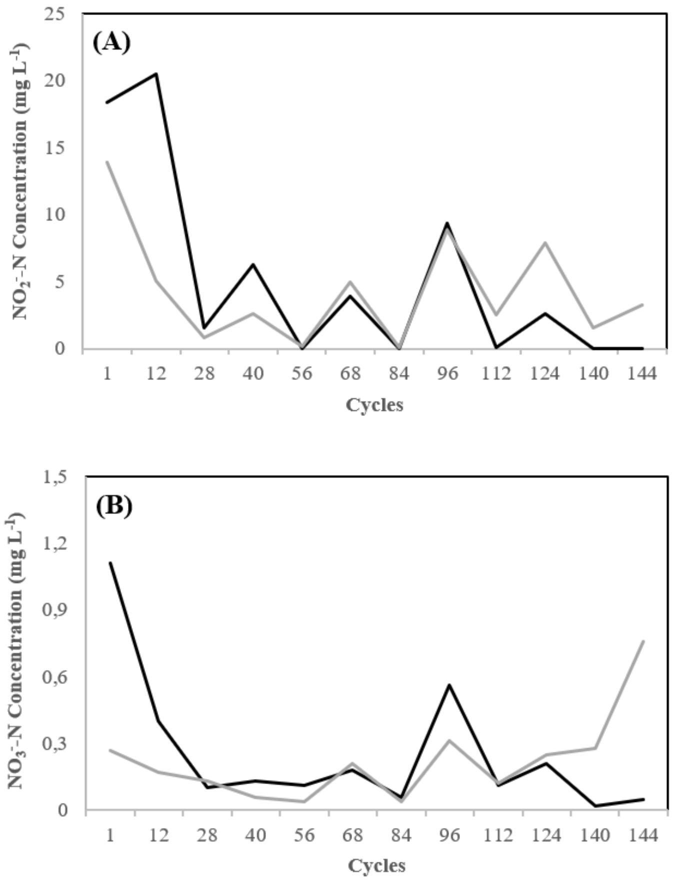


Figure 3. NO_2^--N (A) and NO_3^--N (B) concentrations in the two employed SBR.
Notes: (——) SBR 1 and (——) SBR 2.

3.2. EE2 removal

The EE2 concentrations supplied to both the SBRs throughout the experiment were 4.88 µg L^{-1} on average. After the first cycle, the treated effluent from SBR 1 and SBR 2 contained 1.20 and 2.33 µg L^{-1} of EE2, respectively (Figure 4). Until the 56th cycle, the concentration of EE2 in the treated sewage from SBR 1 was lower than that of SBR 2. The lower SRT used in SBR 1 may have favored a higher removal rate of EE2 from sewage by sorption and subsequent disposal with sludge. From the 68th cycle (equivalent to over 15 d of processes), the EE2 concentrations in the treated effluent were below 0.10 µg L^{-1}, indicating over 90% removal and probable microbial consortium acclimation to this compound. The overall EE2 removal efficiencies of both the SBRs were similar.

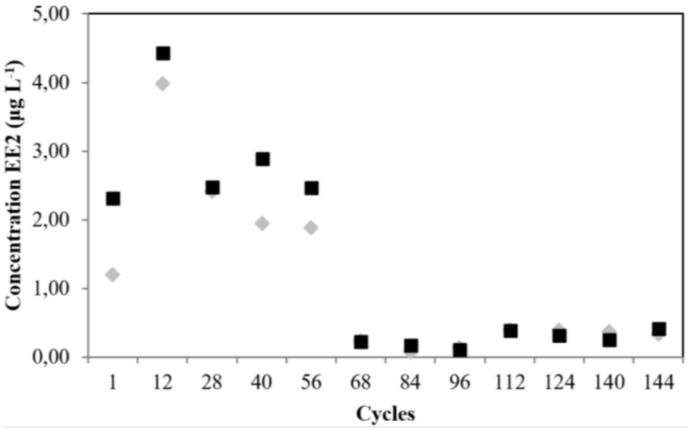


Figure 4. EE2 concentrations in treated SBRs effluent during the monitored 6-h cycles
Notes: (◆) SBR 1 and (■) SBR 2.

Maeng *et al.* (2013) suggested that SBRs operating with higher SRTs exhibit greater hormone removal capacity due to biomass acclimation to contaminants. Amim *et al.* (2018) observed the same behavior in Moving Bed Biofilm Reactors (MBBR) systems. Both the SBRs that received EE2 continuously could develop mechanisms for hormone removal. Notably, the potential removal mechanism in the first 56 cycles of SBRs may have been sorption to the sedimented sludge, as reported by other authors (Kassotaki *et al.*, 2019). The two SBRs displayed a suitable TP removal efficiency owing to anaerobic/aerobic metabolic alternation and sludge disposal. Although Chen *et al.* (2018) observed low hormone removal rate in a system with a high phosphorus removal rate, the present study demonstrated a probable hormone removal mechanism by sludge sorption following TP removal. Therefore, the TP removal efficiency of the SBRs may be associated with EE2 removal.

As indicated above, the employed SBRs exhibited a modest performance in TN removal but promoted nitrification and consequent nitrite accumulation. This metabolic activity is mainly promoted by AOB via the AmoA enzyme, which some authors have suggested has the potential to metabolize NH_x and EE2. Vader *et al.* (2000) observed that nitrifying sludge displays the ability to fully degrade EE2, suggesting that nitrification activity probably results in EE2 hydroxylation; *i.e.*, the conversion of EE2 into hydrophilic products. Suárez *et al.* (2010) suggested that the aerobic removal of EE2 in their processes occurred predominantly by biodegradation through co-metabolism during the nitrification process. Servos *et al.* (2005) stated that nitrifying processes are efficient in EE2 removal; thus, they require a greater SRT. Forrez *et al.* (2009) and Silva *et al.* (2012) also observed that ammonia oxidation activity is associated with EE2 degradation. Maeng *et al.* (2013) also reported that AOB enhanced the EE2 removal rate, indicating the importance of nitrification in hormone degradation. In contrast, Kassotaki *et al.* (2019) found no correlations between hormone removal and increased nitrification rates. The authors also obtained non-significant results concerning AOB, suggesting that sorption is a potential hormone elimination route. These studies reinforce the hypothesis that EE2 removal by sorption at the beginning of the process (up to the 56th cycle) was promoted mainly in SBR 1 employed herein and that EE2 degradation took place as a function of nitrifying activity with increasing process time (above 15 d).

The two SBRs employed in this study could remove EE2 over time while maintaining their TP removal efficiency and nitrifying activity. An important point to be investigated is metabolite formation in the treated sewage as well as the identification of EE2 residues in the settled sludge. Although a higher EE2 removal efficiency by A2O SBRs was observed herein, the remaining residues in the treated effluent may still affect wildlife following environmental discharge.

4. CONCLUSION

The anaerobic/aerobic/anoxic SBRs using different SRTs (10 and 30 d) employed herein removed >80% of COD and TP from sewage. They were also able to remove >90% EE2, resulting in a concentration of less than 0.1 μg L^{-1} in the treated effluent The removal mechanism is believed to be initial sludge sorption until the 56^{th} cycle, owing to TP removal, followed by biodegradation due to nitrification activity and further process acclimation.

5. REFERENCES

AHN, Y.H. Sustainable nitrogen elimination biotechnologies: a review. **Process Biochemistry**, v. 41, n. 8, p. 1709-1721, 2006. https://doi.org/10.1016/j.procbio.2006.03.033

AKIN, B. S.; URGULU, A. Monitoring and control of biological nutrient removal in a Sequencing Batch Reactor. **Process Biochemistry**, v. 40, n. 8, p. 2873-2878, 2005. https://doi.org/10.1016/j.procbio.2005.01.001

AMIN, M. M.; BINA, B.; EBRAHIM, K.; YAVARI, Z.; MOHAMMADI, F. Biodegradation of natural and synthetic estrogens in moving bed bioreactor. **Chinese Journal of Chemical Engineering**, v. 26, n. 2, p. 393-399, 2018. https://doi.org/10.1016/j.cjche.2017.06.006

APHA; AWWA; WEF. **Standard Methods for the examination of water and wastewater**. 23nd ed. Washington, 2017.

ARIS, A. Z.; SHAMSUDDIN, A. S.; PRAVEENA, S. M. Occurrence of 17alpha-ethynylestradiol (EE2) in the environment and effect on exposed biota: a review. **Environment International**, v. 69, p. 104-119, 2014. https://doi.org/10.1016/j.envint.2014.04.011

AZIZI-LAKABADI, M.; PIRSAHEB, M. Investigation of steroid hormone residues in fish: A systematic review. **Process Safety and Environmental Protection**, v. 152, p. 14-24, 2021. https://doi.org/10.1016/j.psep.2021.05.020

BIRNBAUM, L. S. State of the Science of Endocrine Disruptors. **Environmental Health Perspectives**, v. 121, p. 107, 2013. https://doi.org/10.1289/ehp.1306695

BRICIU, R. D.; KOT-WASIK, A.; NAMIESNIK, J. Analytical challenges and recent advances in the determination of estrogens in water environments. **Journal of Chromatographic Science**, v. 47, n. 2, p. 127-139, 2009. https://doi.org/10.1093/chromsci/47.2.127

BUNCE, J. T.; NDAM, E.; OFITERU, I. D.; MOORE A.; DAVID, W.; GRAHAM, D. W. A Review of Phosphorus Removal Technologies and Their Applicability to Small-Scale Domestic Wastewater Treatment Systems. **Frontiers in Environmental Science**, v. 6, p. 1-15, 2018. https://doi.org/10.3389/fenvs.2018.00008

COGLIANO, V.; GROSSE, Y.; BAAN, R.; STRAIF, K.; SECRETAN, B.; GHISSASSI, F. E. Carcinogenicity of combined estrogen-progestagen contraceptives and menopausal treatment. **The Lancet. Oncology**, v. 6, n. 8, p. 552-553, 2005. https://doi.org/10.1016/S1470-2045(05)70273-4

CHIMCHIRIAN, R.F.; SURI, R. P. S.; FU, H. Free synthetic and natural estrogen hormones in influent and effluent of three municipal wastewater treatment plants. **Water Environment Research**, v. 79, n. 9, p. 969-74, 2007. https://doi.org/10.2175/106143007X175843

CHEN, Q.; LI, Z.; HUA, X. Fate of estrogens in a pilot-scale step-feed anoxic/oxic wastewater treatment system controlling by nitrogen and phosphorus removal. **Environmental Science and Pollution Research International**, v. 25, p. 12981-12991, 2018. https://doi.org/10.1007/s11356-018-1584-3

FERNANDEZ-FONTAINA, E.; GOMES, I. B.; AGA, D. S.; LEMA, J. M.; CARBALLA, M. Biotransformation of pharmaceuticals under nitrification, nitration and heterotrophic conditions. **The Science of the Total Environment**, v. 541, p. 1439-1447, 2016. https://doi.org/10.1016/j.scitotenv.2015.10.010

FORREZ, I.; CARBALLA, M.; NOPPE, H.; DE BRABANDER, H.; BOON, N.; VERSTRAETE, W. Influence of manganese and ammonium oxidation on the removal of 17 α-ethinylestradiol (EE2). **Water Research**, v. 43, n. 1, p. 77-86, 2009. https://doi.org/10.1016/j.watres.2008.10.006

FROEHNER, S.; PICCIONI, W.; MACHADO, K. S.; AISSE, M. M. Removal capacity of caffeine, hormones, and bisphenol by aerobic and anaerobic sewage treatment. **Water, Air, & Soil Pollution**, v. 216, p. 463-471, 2011. https://doi.org/10.1007/s11270-010-0545-3

GARMSHAUSEN, J.; KLOAS, W.; HOFFMANN, F. 17α-Ethinylestradiol can disrupt hemoglobin catabolism in amphibians. **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology**, v. 171, p. 34-40, 2015. https://doi.org/10.1016/j.cbpc.2015.03.004

GIUSTI, A.; LAGADIC, L.; BARSI, A.; THOMÉ, J.-P.; JOAQUIM-JUSTO, C.; DUCROT, V. Investigating apical adverse effects of four endocrine active substances in the freshwater gastropod Lymnaea stagnalis. **Science of The Total Environment**, v. 493, p. 147-155, 2014. https://doi.org/10.1016/j.scitotenv.2014.05.130

GOMES, L. C. A.; PEREIRA, B. C.; RIBEIRO, R. P.; OLIVEIRA, J. L. M. Nitrite build-up effect on nitrous oxide emissions in a laboratory-scale anaerobic/aerobic/anoxic/aerobic sequencing batch reactor. **Revista Ambiente & Água**, v. 16, p. 1-11, 2021a. https://doi.org/10.4136/ambi-agua.2634

GOMES, J. P.; TIMO, G. O.; PAULA, L. M.; COSTA, T. V. L.; ROCHA-JÚNIOR, P. R.; HOMEM-DE-MELLO, M. *et al.* Occurrence of 17α-ethinylestradiol in Paranoá Lake watershed (Brasília, Brazil): sewage, freshwater and treated water. **Revista Ambiente & Água**, v.16, p. 1-15, 2021b. https://doi.org/10.4136/ambi-agua.2686

GORE, A. Neuroendocrine targets of endocrine disruptors. **International Journal of Endocrinology and Metabolism**, v. 9, n. 1, p. 16-27, 2010. https://dx.doi.org/10.14310/horm.2002.1249

HOLLER, S.; TRÖSCH, W. Treatment of urban wastewater in a membrane bioreactor at high organic loading rates. **Journal of Biotechnology**, v. 92, n. 2, p. 95-101, 2001. https://doi.org/10.1016/S0168-1656(01)00351-0

JIA, W.; ZHANG, J.; XIE, H.; YAN, Y.; WANG, J.; ZHAO, Y. *et al*. Effect of PHB and oxygen uptake rate on nitrous oxide emission during simultaneous nitrification denitrification process. **Bioresource Technology**, v. 113, p. 232-238, 2012. https://doi.org/10.1016/j.biortech.2011.10.095

JORGENSEN, S. E.; HALLING-SORENSEN, B. Drugs in the environment. **Chemosphere**, v. 40, n. 7, p. 691-699, 2000. https://doi.org/10.1016/S0045-6535(99)00438-5

KASSOTAKI, E.; PIJUAN, M.; RODRIGUEZ-RODA, I.; BUTTIGLIERI, G. Comparative assessment of endocrine disrupting compounds removal in heterotrophic and enriched nitrifying biomass. **Chemosphere,** v. 217, p. 659-668, 2019. https://doi.org/10.1016/j.chemosphere.2018.11.012

KHASAWNEH, O. F. S.; PALANIANDY, P. Occurrence and removal of pharmaceuticals in wastewater treatment plants. **Process Safety and Environmental Protection**, v. 150, p. 532-556, 2021. https://doi.org/10.1016/j.psep.2021.04.045

LI, H.; ZHONG, Y.; HUANG, H.; TAN, Z.; SUN, Y.; LIU, H. Simultaneous nitrogen and phosphorus removal by interactions between phosphate accumulating organisms (PAOs) and denitrifying phosphate accumulating organisms (DPAOs) in a sequencing batch reactor. **Science of The Total Environment**, v. 744, p. 140852, 2020. https://doi.org/10.1016/j.scitotenv.2020.140852

LI, X. M.; CHEN, H.B.; YANG, Q.; WANG, D. B.; LUO, K.; ZENG, G. M. Biological nutrient removal in a sequencing batch reactor operated as oxic/anoxic/extended-idle regime. **Chemosphere**, v. 105, p. 75-81, 2014. https://doi.org/10.1016/j.chemosphere.2013.12.043

LIU, S.; DAIGGER, G. T.; LIU, B.; ZHAO, W.; LIU, J. Enhanced performance of simultaneous carbon, nitrogen and phosphorus removal from municipal wastewater in an anaerobic-aerobic-anoxic sequencing batch reactor (AOA-SBR) system by alternating the cycle times. **Bioresource Technology**, v. 301, p. 122750, 2020. https://doi.org/10.1016/j.biortech.2020.122750

LUNA, T. O.; PLAUTZ, S. C.; SALICE, C. J. Chronic Effects of 17α-Ethinylestradiol, Fluoxetine, and the Mixture on Individual and Population-Level End Points in Daphnia magna. **Archives of Environmental Contamination and Toxicology**, v. 68, p. 603-611, 2015. https://doi.org/10.1007/s00244-014-0119-2

MAENG, S. K.; CHOI, B. G.; LEE, K. T.; SONG, K. G. Influences of solid retention time, nitrification and microbial activity on the attenuation of pharmaceuticals and estrogens in membrane bioreactors. **Water Research**, v. 47, n. 9, p. 3151-3162, 2013. https://doi.org/10.1016/j.watres.2013.03.014

MARCANTONIO, C. D.; CHIAVOLA, A.; DOSSI, S.; CECCHINI, G.; LEONI, S.; FRUGIS, A. *et al*. Occurrence, seasonal variations and removal of Organic Micropollutants in 76 Wastewater Treatment Plants. **Process Safety and Environmental Protection**, v. 141, p. 61-72, 2020. https://doi.org/10.1016/j.psep.2020.05.032

MARTÍN, J.; CAMACHO-MUÑOZ, D.; SANTOS, J. L.; APARAICIO, I.; ALONSO, E. Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: removal and ecotoxicological impact of wastewater discharges and sludge disposal. **Journal of Hazardous Materials**, v. 239–240, p. 40-41, 2012. https://doi.org/10.1016/j.jhazmat.2012.04.068

METCALFE, C. D.; METCALFE, T. L.; KIPARISSIS, Y.; KOENIG, B. G.; KHAN, C.; HUGHES, R. J. *et al.* Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka (Oryzias latipes). **Environmental Toxicology and Chemistry**, v. 20, n. 2, p. 297-308, 2001. https://doi.org/10.1002/etc.5620200210

PESSOA, G. P.; SOUZA, N. C.; VIDAL, C. B.; ALVES, J. A. C.; FIRMINO, P. I. M.; NASCIMENTO, R. F. *et al.* Occurrence and removal of estrogens in Brazilian wastewater treatment plants. **Science of The Total Environment**, v. 490, p. 288-295, 2014. https://doi.org/10.1016/j.scitotenv.2014.05.008

QUEIROZ, F. B.; BRANDT, E. M. F.; AQUINO, S. F.; CHERNICHARO, C. A. L.; AFONSO, R. J. C. F. Occurrence of pharmaceuticals and endocrine disruptors in raw sewage and their behavior in UASB reactors operated at different hydraulic retention times. **Water Science & Technology**, v. 66, n. 12, p. 2562-2569, 2012. https://doi.org/10.2166/wst.2012.482

ROBINSON, B. J.; HELLOU, J. Biodegradation of endocrine disrupting compounds in harbour seawater and sediments. **Science of The Total Environment**, v. 407, n. 21, p. 5713-5718, 2009. https://doi.org/10.1016/j.scitotenv.2009.07.003

SERVOS, M. R.; BENNIE, D. T.; BURNISON, B. K.; JURKOVIC, A.; MCINNIS, R.; NEHELI, T. *et al.* Distribution of estrogens, 17 β-estradiol and estrone, in Canadian municipal wastewater treatment plants. **Science of The Total Environment**, v. 336, p. 155-170, 2005. https://doi.org/10.1016/j.scitotenv.2004.05.025

SILVA, P. S.; OTERO, M.; ESTEVES, V. Processes for the elimination of estrogenic steroid hormones from water: A review. **Environmental Pollution**, v. 165, p. 38-58, 2012. https://doi.org/10.1016/j.envpol.2012.02.002

SILVA, P. S.; LIMA, D. L. D.; SCHNEIDER, R. J.; OTERO, M.; ESTEVES, V. I. Development of ELISA methodologies for the direct determination of 17 β-estradiol and 17 α-ethinylestradiol in complex aqueous matrices. **Journal of Environmental Management**, v. 124, p. 121-127, 2013. https://doi.org/10.1016/j.jenvman.2013.03.041

SOLIMAN, M.; ELDYASTI, A. Development of partial nitrification as first step of nitrite shunt process in a sequential batch reactor (SBR) using ammonium oxidizing bacteria (AOB) controlled by mixing regime. **Bioresource Technology**, v. 221, p. 85-95, 2016. https://doi.org/10.1016/j.biortech.2016.09.023

SUÁREZ, S.; LEMA, J.; OMIL, F. Removal of pharmaceutical and personal care products (PPCPs) under nitrifying and denitrifying conditions. **Water Research**, v. 44, n. 10, p. 3214-3224, 2010. https://doi.org/10.1016/j.watres.2010.02.040

VADER, J. S.; VAN GINKEL, C. G.; SPERLING, F. M. G. M.; JONG, J.; BOER, W.; GRAAF, J. S. *et al.* Degradation of ethinylestradiol by nitrifying activated sludge. **Chemosphere**, v. 41, n. 8, p. 1239-1243, 2000. https://doi.org/10.1016/S0045-6535(99)00556-1

ZHOU, S.; DI PAOLO, C.; WU, X.; SHAO, Y.; SEILER, T.; HOLLERT, H. Optimization of screening-level risk assessment and priority selection of emerging pollutants - the case of pharmaceuticals in European surface waters. **Environment International**, v. 128, p. 1-10, 2019. https://doi.org/10.1016/j.envint.2019.04.034