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Title: Removal of polar micropollutants from domestic wastewater using a methanogenic - aerobic Moving Bed Biofilm Reactor system

Article Type: Research Paper

Keywords: sewage; biological wastewater treatment; organic loading; anaerobic; biodegradation; attached biomass

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Abstract: A lab-scale system consisting of a methanogenic and an aerobic moving bed biofilm reactor (MBBR) was operated for seven months to investigate major pollutants' and polar organic micropollutants' removal from domestic wastewater under three different organic loading rates (OLRs). For all OLR values tested, the system achieved efficient COD (>84%) and NH4-N removal (>96%); both reactors contributed to the decrease of organic loading, while the aerobic bioreactor was exclusively responsible for the removal of ammonium nitrogen. The increase of OLR from 0.20 ± 0.10 to 2.10 ± 0.20 kg COD m-3 d-1 resulted to increase of the detected volatile fatty acids in the anaerobic reactor and increase of the produced biogas to 172.7 ± 25.3 mL L reactor-1 d-1. All target micropollutants were partially removed during different experimental phases; their removal ranged between 31% (5-methyl-1H-benzotriazole, 5TTR) and 97% (2-hydroxybenzothiazole, OH-BTH). Except benzotriazole (BTR) and OH-BTH, both reactors contributed significantly to the removal of target micropollutants, while the addition of extra aerobic MBBR as a final polishing step enhanced only OH-BTH removal. Use of experimental and literature data showed that the increase of organic loading enhanced the removal of three out of five target micropollutants, while the key factor responsible for this effect was COD concentration in influent wastewater. The modelling of micropollutants' removal efficiency using biodegradation kinetics calculated in batch experiments showed satisfactory correlation between predicted and measured values for most target compounds.

Response to Reviewers: Response to the Reviewers for the manuscript CEJ-D-19-05721R1

Reviewer #1: Authors have made modifications based on the comments and I am now fine with publishing this manuscript in CEJ.

Response
We thank Reviewer 1 for his/her positive comments.

Reviewer #2: General:
The manuscript has improved considerably and is now ready for publication. However, there are some minor recommendations to improve it even further.

Response

We thank Reviewer 2 for all his/her comments. We have improved our manuscript following Reviewer's recommendations.

1. If the authors want to increase impact they could go through the graphical abstract. - I like it very much as it shows the whole story. I am just concerned that considering the space for the Graphical abstract it is so detailed that it will hardly be recognizable..

Response
We simplified the Graphical Abstract removing the chemical formulas from the tanks with influents and effluents.

2. P2 exchange "Excepting" by "except"

Response
Corrected

3. The same holds with the other figures: they tell a good story, but they will be somewhat better received if a little more effort was put into them:
Figure 1: outer frame not needed. Inner frame make stronger avoid greyshades, the differences BA, isobar are difficult to grasp: other colours?
Fig 2/3 frames: see fig 1, align figure colours. Why not use a bit stronger colours
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Fig 6 use stronger colours no structure.

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5. Grey scales:
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Research Data Related to this Submission

Title: Data for: Removal of polar micropollutants from domestic wastewater using a strictly anaerobic - aerobic Moving Bed Biofilm Reactor system
Repository: Mendeley Data
https://data.mendeley.com/datasets/y9mv2hjg8b/draft?a=951d638e-9717-4444-989c-27b2fa502f63

University of the Aegean
Department of Environment

Mytilene, 21 September 2019

Chemical Engineering Journal
Editorial Office
Ms. Ref. No.: CEJ-D-19-05721R1

Dear Editor,

Thank you very much for your correspondence for CEJ-D-19-05721. Attached please find the revised manuscript entitled "Removal of polar micropollutants from domestic wastewater using a methanogenic – aerobic Moving Bed Biofilm Reactor system" by E. Kora, D. Theodorelou, G. Gatidou, M. Fountoulakis and A. Stasinakis, for your consideration for publication in *Chemical Engineering Journal*, as Research Paper.

We have addressed all the minor corrections of the Reviewer and we improved our figures according to his/her recommendations. Additionally, we have uploaded all the figures in colour (for the on line version) as well as in black and white (for the printed version).

Thank you very much again for your consideration, and I look forward to receiving your final decision.

Sincerely yours,

Athanasios Stasinakis
Associate Professor

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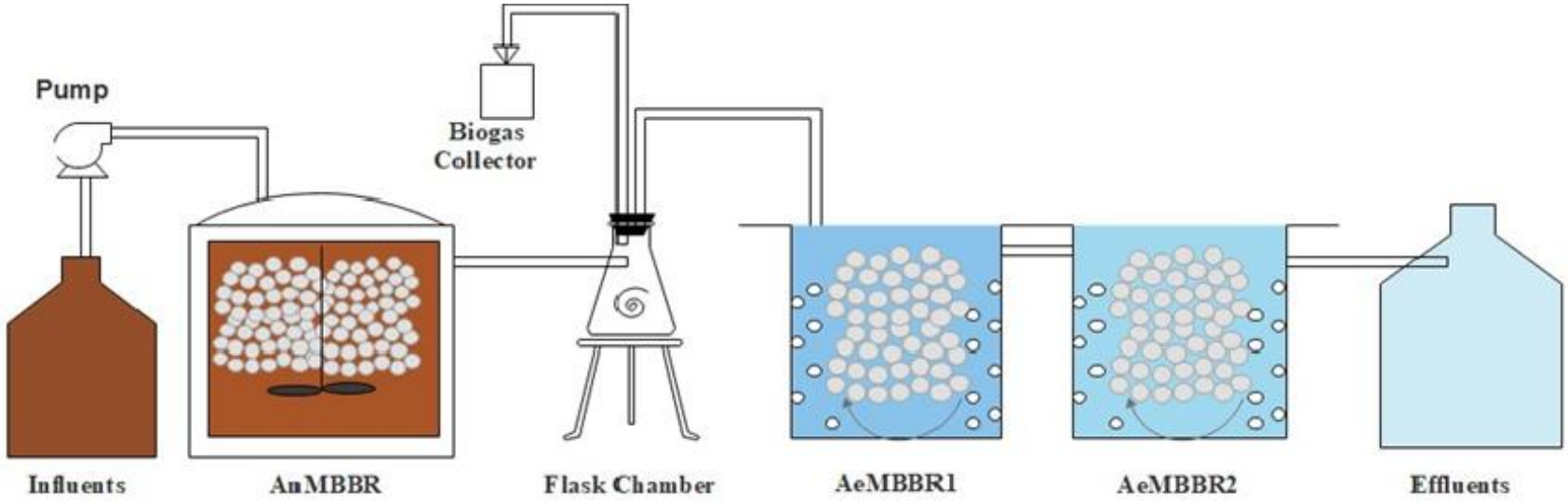
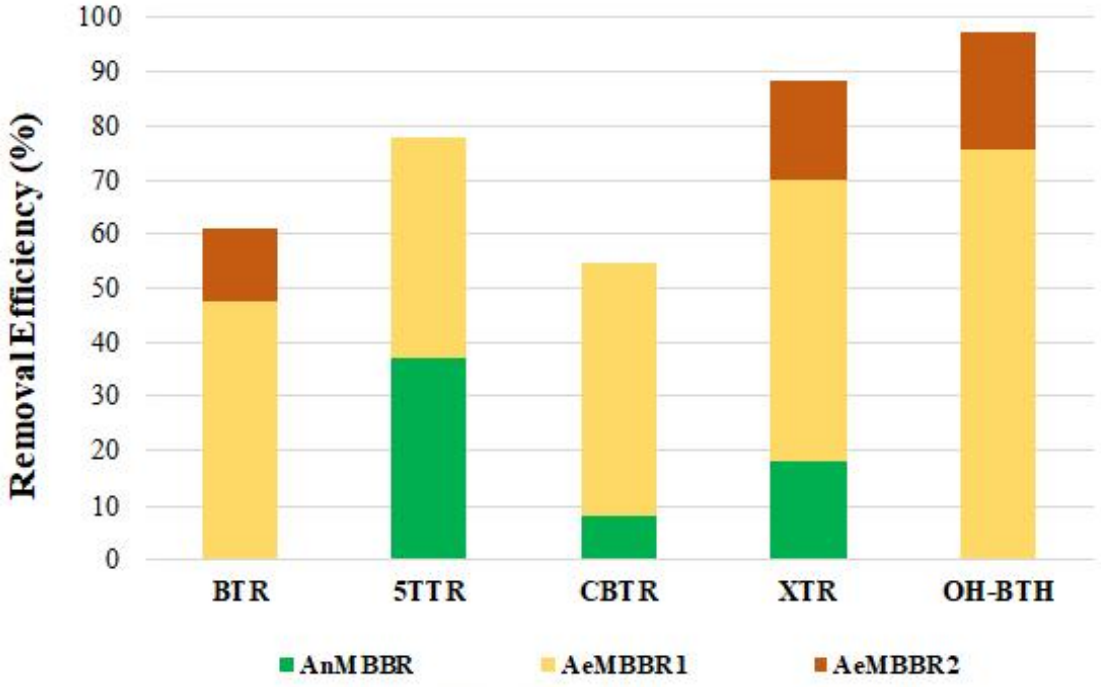
Graphical Abstract



Phase C



Highlights

- A novel MBBR system combining methanogenic and aerobic conditions was used
- The tested system achieved efficient COD and NH_4-N removal and biogas production
- The removal of micropollutants ranged from 31% (5TTR) to 97% (OH-BTH)
- The methanogenic MBBR contributed to the removal of 3 out of 5 compounds
- COD increase in raw sewage enhanced micropollutants' removal in aerobic MBBR

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Removal of polar micropollutants from domestic wastewater using a methanogenic – aerobic Moving Bed Biofilm Reactor system

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ABSTRACT

A lab-scale system consisting of a methanogenic and an aerobic moving bed biofilm reactor (MBBR) was operated for seven months to investigate major pollutants' and polar organic micropollutants' removal from domestic wastewater under three different organic loading rates (OLRs). For all OLR values tested, the system achieved efficient COD (>84%) and NH_4-N removal (>96%); both reactors contributed to the decrease of organic loading, while the aerobic bioreactor was exclusively responsible for the removal of ammonium nitrogen. The increase of OLR from 0.20 ± 0.10 to 2.10 ± 0.20 kg COD m^{-3} d^{-1} resulted to increase of the detected volatile fatty acids in the anaerobic reactor and increase of the produced biogas to 172.7 ± 25.3 mL L $reactor^{-1}$ d^{-1}. All target micropollutants were partially removed during different experimental phases; their removal ranged between 31% (5-methyl-1H-benzotriazole, 5TTR) and 97% (2-hydroxybenzothiazole, OH-BTH). Except benzotriazole (BTR) and OH-BTH, both reactors contributed significantly to the removal of target micropollutants, while the addition of extra aerobic MBBR as a final polishing step enhanced only OH-BTH removal. Use of experimental and literature data showed that the increase of organic loading enhanced the removal of three out of five target micropollutants, while the key factor responsible for this effect was COD concentration in influent wastewater. The modelling of micropollutants' removal efficiency using biodegradation kinetics calculated in batch experiments showed satisfactory correlation between predicted and measured values for most target compounds.

Keywords: sewage; biological wastewater treatment; organic loading; anaerobic; biodegradation; attached biomass.

1. Introduction

Moving Bed Biofilm Reactor (MBBR) technology uses different types of carriers which flow freely in the bioreactors, under either aerobic or anaerobic conditions, for providing an ideal environment for slow growing bacteria and a diverse biocoenosis [1]. Since 2005, aerobic MBBR have been extensively used for the treatment of domestic and industrial wastewater due to their small footprint, high amount and diversity of biomass per m^3 of reactor and high nitrification rates [2-4]. Strictly anaerobic MBBR have been mainly used for industrial wastewater treatment due to their tolerance in different shock loading conditions, decreased sludge production and biogas recovery [5]. Recently, Gu et al. [6] used a novel MBBR system, combining anaerobic and aerobic conditions in series, for municipal wastewater treatment and reported that the studied system achieved efficient removal of major pollutants, energy recovery and reduced sludge production. The aforementioned characteristics were very encouraging for the future use of such a system on domestic wastewater treatment; however, information was missing for the ability of the system to remove organic micropollutants.

On the other side, the occurrence and the removal of organic micropollutants from domestic wastewater is one of the major challenges that meet nowadays scientists and engineers involved on wastewater management. Numerous organic micropollutants have been detected in municipal wastewater during the last two decades, worldwide; most of them are polar compounds and they are also found in the aquatic environment [7,8]. The monitoring and their efficient removal during domestic wastewater treatment have already been regulated in Switzerland [9], while recommended measures have been reported in other countries [10]. So far, there are several papers

investigating the removal efficiency of various micropollutants, such as pharmaceuticals, endocrine disrupting compounds and benzotriazoles in aerobic MBBR systems [11-18]. Concerning the use of anaerobic MBBR, Torresi et al. [19] studied the removal of specific micropollutants in an anaerobic-aerobic MBBR system operated in sequencing-batch mode for achieving biological phosphorus removal, while Derakhshan et al. [20] operated a strictly AnMBBR under mesophilic conditions and reported important atrazine removal. To the best of our knowledge, no information is available for the ability of combined methanogenic-aerobic MBBR systems to remove organic micropollutants from domestic wastewater as well as for the contribution of different bioreactors on their elimination. Additionally, beside the well-known role of organic loading rate (OLR) on the removal of major pollutants in MBBR systems [21, 22], limited information is available for the role of this parameter on micropollutants' elimination.

Based on the above, the main objective of this study was to examine the ability of a novel lab-scale system, consisting of a methanogenic and an aerobic MBBR, to remove polar organic micropollutants under different organic loading conditions. As target compounds, four benzotriazoles (BTRs) and one benzothiazole were selected (Table S1). These compounds are characteristic polar micropollutants commonly detected in raw and treated sewage as well as in the aquatic environment at concentrations between few ng L^{-1} and some μg L^{-1} [7, 8, 23]. BTRs consist of a benzene ring fused with a triazole ring, while in the case of benzothiazoles a benzene ring is fused with a thiazole ring. They are partially removed in conventional sewage treatment plants, mainly due to biotransformation during activated sludge process [24, 25]. The studied system operated under three OLR values and it was monitored for a total period of seven (7) months for major pollutants' removal, biogas production and

micropollutants' elimination. At the last part of the study, the addition of an extra aerobic MBBR, as a polishing step, was also evaluated, while batch experiments were conducted and the estimated biodegradation constants were used to predict the removal of target compounds in applied system. Experimental data from the current study and two previous articles were also used to study the role of OLR and COD concentration on the removal efficiency of target micropollutants in MBBR systems.

2. Materials and methods

2.1. Analytical standards and reagents

Analytical standards of xylytriazole (XTR) and 5-chlorobenzotriazole (CBTR) were supplied by Sigma-Aldrich (USA). 2-hydroxybenzothiazole (OH-BTH) was supplied by Alfa Aesar (USA), 5-methyl-1H-benzotriazole (5TTR) by Acros Organics (Belgium) and 1H-benzotriazole (BTR) by Merck (Germany). For every compound, a stock solution in methanol was prepared at 1000 mg L^{-1} and stored at -18 $^{\circ}$C. A mixed solution of target compounds (440 mg L^{-1}) was prepared for spiking purposes, when needed, and it was kept at -18 $^{\circ}$C. Methanol (MeOH; HPLC-MS grade) and ultra-pure HCl (32%) was purchased by Merck (Germany), while acetonitrile (ACN; HPLC grade) by Fisher (USA). The cartridges used for solid phase extraction were supplied by Phenomenex (USA). A MilliQ/MilliRO Millipore system (USA) was used for the production of HPLC grade water.

2.2. Set-up of the continuous flow system

A lab-scale continuous flow system was installed by combing an anaerobic moving bed biofilm reactor (AnMBBR) with an aerobic moving bed biofilm reactor (AeMBBR1) for micropollutants' removal and wastewater treatment with parallel biogas production (Figure S1a). The AnMBBR had a working volume of 3 L, the hydraulic residence time (HRT) was set at 10 h and the filling ratio was 40% (v/v). Sponge cubic form carriers (Nisshinbo Chemical Inc.) were used with density ranging between 52~64 kg m^{-3} (at dry stage), while 1 L of anaerobically digested sludge was initially added to seed the system with anaerobic microorganisms. The AnMBBR was airtight sealed and it had double walls connected to a water bath for operating at mesophilic conditions (32-34 °C). Nitrogen gas was initially introduced to achieve air displacement and anaerobic conditions. The AnMBBR runoff was achieved through natural flow because of the increasing pressure that biogas production caused and it was ended up to a flask chamber connected with a bag for biogas collection and with AeMBBR1 for further wastewater treatment. The AeMBBR1 had a working volume of 3.8 L, it was filled by K3 type biocarriers (AnoxKaldnes) at a filling ratio of 33% (v/v), while the HRT was 13 h. These biocarriers had been used during the previous 4 years in an aerobic MBBR system operated at University of the Aegean for the treatment of municipal wastewater originated from the University Campus and a mature biofilm had been developed on them [13, 26]. At the last part of the study, an extra aerobic reactor (AeMBBR2) was added in series as a polishing step, filled with K3 type biocarriers and HRT of 13 h (Figure S1b). Air-stone diffusers were used for the maintenance of aerobic conditions and biomass mixing in the aerobic MBBRs, the dissolved oxygen concentration in these reactors was higher than 4 mg L^{-1}, while mixing in the AnMBBR was achieved through mechanical stirring. The aerobic

systems operated under constant room temperature controlled by central air-conditioning system.

2.3. Operation of the lab-scale continuous-flow system

The studied system operated for a period of seven (7) months receiving municipal wastewater from the University Campus (Table S2). The experiments were divided in three Phases where different organic loading conditions were applied (Phase A: 0.82 ± 0.40 kg COD m^{-3} d^{-1}; Phase B: 0.20 ± 0.10 kg COD m^{-3} d^{-1} and Phase C: 2.10 ± 0.20 kg COD m^{-3} d^{-1}) keeping stable the HRT value and increasing the concentrations of influent COD. For system's monitoring, samples were taken twice a week from the influents and effluents of each reactor for COD, NH_4-N, Total Phosphorus (TP), Total Suspended Solids (TSS) and alkalinity. Samples for the analysis of Volatile Fatty Acids (VFAs) were also taken twice a week from the AnMBBR. The temperature and pH were monitored on a daily basis, while biogas volume was continuously collected and measured once a week (as a weekly average). At the last part of each Phase, an aliquot of target micropollutants in methanol (0.5 mL) was added to the influents for seven consecutive days in order to achieve initial concentrations of approximately 20 μg L^{-1} [13, 26]. Samples were taken from different points of the system taking into account the applied HRT and were analyzed for BTRs and OH-BTH according to the method described below.

2.4. Effect of OLR on removal of micropollutants in aerobic MBBRs

For studying the effect of OLR on the removal of target micropollutants in aerobic MBBRs, data was used from the operation of AeMBBR1 and AeMBBR2 as well as from two previous studies where pure MBBR [13] and HMBBR systems [26] had been used for investigating the removal of target micropollutants from municipal wastewater. The pure MBBR system consisted of two aerobic bioreactors connected in series, with a working volume of 4.5 L each [13]. It operated at two experimental phases where OLR values equal to 0.25 kg m^{-3} d^{-1} and 0.60 kg m^{-3} d^{-1} were applied at the first bioreactor and 0.05 kg m^{-3} d^{-1} and 0.17 kg m^{-3} d^{-1} at the second one. The HMBBR system consisted of two aerobic bioreactors of 3 L in series and a settling tank [26]. The OLR values that were applied in these bioreactors were equal to 0.64 kg m^{-3} d^{-1} and 0.11 kg m^{-3} d^{-1}, respectively. Further information for the operating conditions of both systems is presented in Table S3.

2.5. Batch biodegradation experiments

After the termination of Phase C, batch experiments were conducted in all reactors to determine biodegradation kinetics of the target compounds and to predict their removal rate using mass balances. For this reason, the flow stopped and a mixture of the target compounds was spiked in each reactor simultaneously at a final concentration of 25 μg L^{-1}. Samples (50 mL) were collected at different time intervals (0, 2, 5, 8, 10 and 24 hours) and the concentrations of the target compounds were determined as described in Session 2.6.

2.6. Analytical methods

COD, TSS, NH_4-N, TP, and alkalinity were measured according to the Standard Methods [27]. Temperature and pH were measured using portable instruments. The volume of the produced biogas was determined by water displacement method, while biogas composition was determined with the use of a GA3000 gas analyzer (Geotech). The concentration of biofilm solids was measured at the end of each experimental phase after removing the biofilm from biocarriers and measuring the dried weight difference [11].

For the determination of the VFAs, 1 mL of the sample was collected, diluted, acidified with 2N HNO_3 in order to shift the pH to approximately 2 and stored at 4 ºC up to analysis. Chromatographic separation and quantification of VFAs was based on a previously developed method with some modifications [28], while a Shimatzu LC20-AD prominence liquid chromatographer associated with a SPD-M20A prominence diode array detector (LC-DAD) and a SIL-20AC auto sampler was used for the analysis. The column was Zorbax SB-C18 4.6 mm × 15 cm (5 μm) connected with a Zorbax SB–C18 pre-column (Hewlett Packard, USA). Column and pre-column were heated at 35 ^{o}C with a CTO-20AC column oven (Shimatzu-Japan). The mobile phase consisted of 10 mM KH_2PO_4, pH 2.4 with phosphoric acid (solvent A) and methanol (MeOH, solvent B). Gradient elution was performed as follows: from 20% MeOH to 60% in 10 min, hold in 60% for 2 min and then decreased to 20% in 0.1 min and hold there until 20 min. Flow-rate was 1 mL min^{-1}. The diode array detector (DAD) was set at 210 nm. The VFAs concentration were converted to COD-VFA (mg L^{-1}) by using conversion factors of 1.82 for butyric (BA) and isobutyric acid (Iso-BA), 2.04 for valeric (VA) and isovaleric acid (Iso-VA), 1.07 for acetic acid (AA) and 1.51 for propionic acid (PA) [29].

Regarding micropollutants' analysis, due to the high polarity of these compounds, the contribution of sorption during wastewater treatment was considered of minor importance [30]. Therefore, their concentrations were measured only in the dissolved phase based on the method developed by Mazioti et al. [30] (Figure S2). In brief, samples were initially filtered (LLG-Filter paper) and the filtrates were stored at 4 ^{o}C after acidification (pH 3.0 ± 0.1). For the analysis, solid phase extraction (SPE) was followed, while chromatographic separation was performed using HPLC-DAD (Shimatzu, Japan). The detection limits of studied micropollutants ranged between 0.17 ng L^{-1} (BTR) to 125 ng L^{-1} (CBTR).

2.7. Equations

The removal of target micropollutants at the lab-scale system was calculated according to Eq. (1):

$$Removal = \left(1 - \frac{Cout}{Cin}\right) \times 100 \quad (1)$$

Where, C_{in} is the concentration in influent wastewater (μg L^{-1}) and C_{out} is the concentration in treated wastewater of each examined reactor (μg L^{-1}).

The biodegradation rate constants (k) in batch experiments were estimated using first order kinetics (Eq. 2) for the three reactors used in Phase C.

$$C_t = C_o e^{-k_i t} \quad (2)$$

Where C_t and C_0 are the target compounds' concentrations in batch experiments at time t and $t = 0$, respectively, (μg L^{-1}), k_i is the rate constant for each reactor (d^{-1}) and i the relevant reactor.

For the calculation of the predicted removal in Phase C, the first-order biodegradation rate constants (*k*), calculated in the batch experiments, were used in Eq. (3) [13]:

$$Predicted\ Removal = 1 - \left(\frac{1}{(1+k_1\tau)(1+k_2\tau)+(1+k3\tau)}\right) \quad (3)$$

Where τ is the hydraulic retention time for each reactor (d) and k_1, k_2 and k_3 the biodegradation rate constants (d^{-1}) at AnMBBR, AeMBBR1 and AeMBBR2, respectively.

2.8. Statistical analysis

One-way ANOVA was used for comparing major pollutants and target compounds' removal during different experimental Phases. Tukey–Kramer's post-test was used for the determination of the significant differences between groups.

3. Results and Discussion

3.1 Operation of the system and removal of major pollutants

As reported in Session 2.3, different OLR values were applied during different experimental Phases and an extra aerobic reactor (AeMBBR2) was added in the system during Phase C. The experimental conditions in the reactors used in the current study and their performance regarding COD, NH_4-N removal and biogas production are presented in Table 1.

According to the results, the concentrations of the biofilm solids in AeMBBR1 were similar in Phases A and B (885 mg L^{-1} and 951 mg L^{-1}, respectively), while the application of a higher OLR resulted to a thicker biofilm and a concentration of 3007 mg L^{-1} during Phase C. Alkalinity and pH values were reduced in aerobic reactors comparing to AnMBBR due to the nitrification process occurring in AeMBBR1 [31]. The increase of OLR resulted to an important increase of biogas production in the AnMBBR (Table 1, Figure S3). Specifically, the daily produced biogas was 24.3 ± 5.6 mL L $reactor^{-1}$ d^{-1} under conditions of lower OLR (Phase B), while it was increased to 53.3 ± 20.9 mL L $reactor^{-1}$ d^{-1} (Phase A) and 172.7 ± 25.3 mL L $reactor^{-1}$ d^{-1} (end of Phase C). The findings of the current study are consistent with those of Chatterjee et al. [31] and Sun et al. [32] that reported that the increase of OLR contributed to the increase of the daily biogas production. Biogas composition was determined twice in each Phase and the methane content ranged from 59% to 64%.

Analysis of different VFAs using HPLC and expression of their concentrations as COD-VFA (mg L^{-1}) showed a positive correlation between increase of ORL and increase of COD-VFA (Table 1, Figure 1). Regarding the different types of analyzed VFAs, isovaleric acid (Iso-VA) was not detected in any Experimental Phase. Under conditions of higher OLR (Phase C), all other VFAs were found, while propionic acid (PA) was the dominant. On the other side, acetic acid (AA) and butyric acid (BA) were the VFA components detected mainly when the moderate and lower OLRs were applied (Phase A and B, respectively) (Figure 1). It is known that an increase in the loading rate tends to increase the VFA production [33]. In addition, it should be mentioned that acetic acid (AA) and butyric acid (BA) reported to be the most favorable components for methane production, while contribution of acetic acid (AA) is more than 70% [34]. In contrast, propionic acid (PA) is the most difficult

compound to convert to other intermediates as its degradation is thermodynamically less favorable [35]. As a result, propionic acid (PA) is often considered as the most toxic VFA found in anaerobic digesters [36]. During this study, propionic acid (PA) concentrations between 100-150 mg L^{-1} were found during higher OLR. However, no negative effect on biogas production rate was observed. Previous studies with typical continuous stirred tank reactors (CSTRs) shown that methanogenic bacteria could be inhibited at propionic acid (PA) concentrations more than 1-2 g L^{-1}, while they could tolerate acetic acid (AA) and butyric acid (BA) concentrations more than 10 g L^{-1} [37]. On the other hand, to our knowledge, there is no available data about the toxic concentrations of VFAs in AnMBBRs treating low strength wastewater.

Regarding the appearance of different VFAs during different experimental stages and the fact that acetic acid (AA) and valeric acid (VA) were detected only in the early stage of Phases A and B, respectively, while butyric acid (BA) was detected in late stage of Phase A, it should be mentioned that due to the low HRT applied (10 h) in the anaerobic bioreactor the microbial population at the first stage of the operation was quite low. The use of biocarriers increased sludge residence time (SRT) and favored gradually the presence of slow-growing microorganisms and the establishment of different microbial species [38, 39]. Specifically, in the early stage of Phase A the relative fast-growing acetogens produced acetic acid (AA) but the population of slow-growing methanogens was still low. In the late stage of Phase A, the population of methanogens increased and as a result the concentration of acetic acid (AA) decreased. In addition, the increase of the population of hydrolytic bacteria and butyrate-producing bacteria resulted to the occurrence of butyric acid (BA) in the reactor. Similarly, in the early stage and late stage of Phase B, valerate-producing and valerate-degrading bacteria were gradually established, respectively.

Concerning the removal of major pollutants, the system was able to remove efficiently COD (>84%) and NH_4-N (>96%), while TP removal was negligible (Table 1). No statistically significant differences on NH_4-N removal were noticed during different Experimental Phases, while the higher COD removal (96%) was observed when the higher OLR was applied. Concerning the contribution of different reactors on the removal of major pollutants, in Phase A, both AnMBBR and AeMBBR1 contributed equally to COD removal, while in Phases B and C the greatest part of COD was removed in AnMBBR (Figure S4). On the other side, as expected due to the lack of aerobic conditions, the AnMBBR was unable to remove NH_4-N and as a result all ammonium nitrogen was removed via nitrification in AeMBBR1. The addition of AeMBBR2 in Phase C increased NH_4-N removal by 5%. Calculation of the nitrification rates showed that their values in Phase C for AeMBBR1 (48.6 ± 14.0 mg g^{-1} d^{-1}) and AeMBBR2 (12.9 ± 16.4 mg g^{-1} d^{-1}) were statistically lower than those calculated for Phase A (128.8 ± 27.0 mg g^{-1} d^{-1}) and B (78.6 ± 8.5 mg g^{-1} d^{-1}).

3.2. Removal of target micropollutants in continuous-flow systems: role of different reactors

All target micropollutants were partially removed during different Experimental Phases in the lab-scale system consisting of AnMBBR and AeMBBR1 in series (Figure 2). As these micropollutants are not degraded abiotically under the conditions found in such bioreactors and the role of sorption on attached biomass is considered of minor importance, biotransformation is considered the main mechanism responsible for their elimination [13, 26]. Among target compounds, the lowest total removal efficiencies were observed for 5TTR (31% in Phase B), BTR (33% in Phase

B), and CBTR (37% in Phase B) while the highest for OH-BTH (93% in Phase B). Use of ANOVA test showed the existence of statistically significant differences between Experimental Phases. For three of the target compounds (5TTR, CBTR, XTR), higher removal efficiency was noticed at Phase C, while for OH-BTH at Phase B. Comparing the removal efficiencies achieved in the current system with those reported in the literature, CBTR, XTR and OH-BTH eliminated at similar percentages to previously reported for lab-scale activated sludge, pure MBBR or HMBBR systems, whereas higher removal efficiency was achieved for 5TTR [13, 26].

Concerning the role of different reactors on micropollutants' removal, excepting BTR and OH-BTH, both reactors contributed significantly to the removal of target compounds (Figure 2). Important contribution of the AnMBBR on the biodegradation of target compounds was noticed for 5TTR (Phase A, C), CBTR (Phase A, B) and XTR (Phase A, B). To the best of our knowledge, this is the first study reporting the removal of BTRs in strictly anaerobic biological wastewater treatment systems. Limited relevant information is available in the literature mainly from experiments with aquifer material. Specifically, Liu et al. [40] investigated the biodegradation of three BTRs in aquifer material under anaerobic conditions and reported half-life values of 57, 59 and 44 d for BTR, 5TTR and CBTR, respectively, while Alotaibi et al. [41] reported half-lives of 29 and 26 d for BTR and 5TTR in anaerobic column studies inoculated with aquifer sediment. Herzog et al. [42] conducted batch anaerobic experiments with activated sludge and digested sludge as inoculum and reported no biodegradation of BTR and 5TTR during the 50 d of the experiment. However, it is worth mentioning that in those experiments the target compounds were added at ppm levels.

On the other side, the existence of AeMBBR1 resulted to important increase of target compounds removal in all Experimental Phases, whereas it was exclusively responsible for the removal of OH-BTH and BTR (Figure 2). To investigate whether the addition of an extra aerobic step could enhance target micropollutants' removal, AeMBBR2 was added in Phase C. According to the results presented in Figure 3, excepting OH-BTH where an additional removal efficiency of 30% was achieved, the addition of AeMBBR2 did not decrease significantly the concentrations of target compounds in treated wastewater.

3.3. Effect of organic loading on the removal of target micropollutants in aerobic MBBR

Contradictory results have been reported in the literature for the role of organic loading on micropollutants' removal during biological wastewater treatment with MBBR systems [13, 15, 18]. To study the role of OLR and related parameters, we decided to use data from the operation of AeMBBR1 and AeMBBR2 from the current study as well as from two previous studies that investigated target compounds removal in aerobic pure MBBR and HMBBR systems and operated at different OLR values [13, 26]. The experimental conditions applied in ten (10) different experiments (type of the system, applied OLR, influents' COD concentration, HRT value) and the observed removal of target compounds are summarized in Table S3. It must be mentioned that results from six (6) experiments were used for OH-BTH due to its high removal efficiency during the first aerobic stage of tested systems (>70%) and the low remaining concentrations at the influents of the second stage (Table S3).

As it can be seen in Figure 4, the increase of applied organic loading enhanced the removal efficiency of three of target micropollutants for the range of OLR values applied in these studies (0.05 to 0.64 kg COD m^{-3} d^{-1}). The correlation was stronger for CBTR and XTR and weaker for BTR. No correlation was observed for OH-BTH and 5TTR. OH-BTH is an easily biodegradable compound that is removed significantly during biological wastewater treatment [43]. As a result, its removal seems to be independent of the applied OLR. On the other side, the removal of 5TTR seems not to be affected by the OLR but from other factors that were not studied in the current article (e.g. characteristics of biomass). Previous articles have also reported the slow and partial removal of 5TTR under different experimental conditions and the positive effect of biomass acclimatization on its biodegradation [40, 42, 13].

As OLR value is affected by COD concentration in influent wastewater as well as by HRT value, the role of influent COD on CBTR, XTR and BTR removal was studied separately using data from 7 out of 10 systems where similar HRT values had been applied (10.8 to 13 hours, see also Table S3). According to the results shown in Figure 5, the increase of COD concentration in influent wastewater enhanced these micropollutants' removal. The positive role of organic substrate on several micropollutants' removal has also been reported in MBBR and other biological wastewater treatment systems and is due to co-metabolic phenomena [44, 12, 15, 16, 18].

According to Dalton and Stirling [45], co-metabolism is defined as "the transformation of a non-growth substrate in the obligate presence of a growth substrate or another transformable compound". Non-growth substrate refers to compounds that cannot support cell replication due to their low concentrations (e.g.

micropollutants in wastewater). Cometabolic processes depend on the nature and the amount of the growth substrate and it is considered that the higher the substrate concentration, the faster the degradation of the micropollutant. Casas et al. [12] investigated the removal of different pharmaceuticals in a staged MBBR system and reported that their biodegradation occurred in parallel with the removal of COD and nitrogen. Tang et al. [16] observed that the biodegradable pharmaceuticals degraded faster in the presence of higher humic acid concentrations and the average increase of their first-order degradation rate was 5% per mg DOC. Torresi et al. [19] reported the cometabolic activity of phosphorus accumulating organisms towards the removal of several micropollutants that stopped when the primary substrate (phosphorus) and/or the internal stored polyhydroxyalkanoates were no longer available. Concerning the compounds investigated in the current study, previous batch experiments with activated sludge and attached biomass have also shown that the biodegradation kinetics of BTR, CBTR and XTR were increased with increase of organic substrate [13].

3.4. Biodegradation kinetics and prediction of the removal

As described in Session 2.5, batch experiments were conducted at the end of Phase C to estimate first-order biodegradation rate constants (*k*) in different reactors and to calculate predicted removal using Equation 3. Biodegradation rate constants values reported in Table S4 indicate that the attached biomass developed in both aerobic MBBR had the ability to biodegrade all target compounds. In aerobic reactors the lowest *k* values were estimated for 5TTR (AeMBBR1: 0.491 d^{-1}) and the highest for OH-BTH (AeMBBR1: 8.254 d^{-1}). These values are similar (for BTR, CBTR, OH-

BTH) or higher (for CBTR, XTR) than those reported in a previous study for pure aerobic MBBR systems [13] (Table S4). For the AnMBBR, *k* values ranged between 0.068 d^{-1} (BTR) and 1.104 d^{-1} (5TTR). It should be mentioned that it is the first time that biodegradation kinetics are estimated for the target compounds in a strictly anaerobic, methanogenic biological wastewater treatment system.

According to Figure 6, the predicted removal in the system including a methanogenic and two aerobic MBBR was close to the measured removal for 4 out of the 5 target compounds (BTR, CBTR, XTR and OH-BTH). On the other side, the model seems to underestimate system's performance for 5TTR.

4. Conclusions

The tested lab-scale system was able to biodegrade target polar micropollutants under all OLR values applied. Total removal efficiencies ranged from 33 to 60% for BTR, 31 to 86% for 5TTR, 37 to 56% for CBTR, 43 to 91% for XTR and 80 to 97% for OH-BTH. The contribution of the strictly anaerobic, methanogenic bioreactor was important for 5TTR, CBTR, XTR and COD, while the use of the first aerobic bioreactor resulted to important increase of target micropollutants' removal and it was exclusively responsible for the removal of OH-BTH, BTR and NH_4-N. The experimental results show that for municipal wastewater containing target micropollutants, there is no important benefit from the addition of a second aerobic step in anaerobic-aerobic MBBR system. The increase of OLR and COD concentrations in influent wastewater enhanced biodegradation of most target microcontaminants, indicating the important role of co-metabolism on their elimination.

In summary, the operation of the current attached growth biomass wastewater treatment system achieved efficient removal COD and NH_4-N removal, negligible P removal, biogas production and partial polar micropollutants' elimination. These results encourage the future use of this system as the required biological treatment step for the production of recovered water capable for agricultural irrigation with simultaneously energy recovery and low biomass production. Further research is needed for identifying the biotransformation products of polar micropollutants in such systems as well as for studying their performance when an anoxic step has been added for full nitrogen removal.

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Removal of polar micropollutants from domestic wastewater using a methanogenic – aerobic Moving Bed Biofilm Reactor system

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ABSTRACT

A lab-scale system consisting of a methanogenic and an aerobic moving bed biofilm reactor (MBBR) was operated for seven months to investigate major pollutants' and polar organic micropollutants' removal from domestic wastewater under three different organic loading rates (OLRs). For all OLR values tested, the system achieved efficient COD (>84%) and NH_4-N removal (>96%); both reactors contributed to the decrease of organic loading, while the aerobic bioreactor was exclusively responsible for the removal of ammonium nitrogen. The increase of OLR from 0.20 ± 0.10 to 2.10 ± 0.20 kg COD m^{-3} d^{-1} resulted to increase of the detected volatile fatty acids in the anaerobic reactor and increase of the produced biogas to 172.7 ± 25.3 mL L $reactor^{-1}$ d^{-1}. All target micropollutants were partially removed during different experimental phases; their removal ranged between 31% (5-methyl-1H-benzotriazole, 5TTR) and 97% (2-hydroxybenzothiazole, OH-BTH). Except benzotriazole (BTR) and OH-BTH, both reactors contributed significantly to the removal of target micropollutants, while the addition of extra aerobic MBBR as a final polishing step enhanced only OH-BTH removal. Use of experimental and literature data showed that the increase of organic loading enhanced the removal of three out of five target micropollutants, while the key factor responsible for this effect was COD concentration in influent wastewater. The modelling of micropollutants' removal efficiency using biodegradation kinetics calculated in batch experiments showed satisfactory correlation between predicted and measured values for most target compounds.

Keywords: sewage; biological wastewater treatment; organic loading; anaerobic; biodegradation; attached biomass.

1. Introduction

Moving Bed Biofilm Reactor (MBBR) technology uses different types of carriers which flow freely in the bioreactors, under either aerobic or anaerobic conditions, for providing an ideal environment for slow growing bacteria and a diverse biocoenosis [1]. Since 2005, aerobic MBBR have been extensively used for the treatment of domestic and industrial wastewater due to their small footprint, high amount and diversity of biomass per m^3 of reactor and high nitrification rates [2-4]. Strictly anaerobic MBBR have been mainly used for industrial wastewater treatment due to their tolerance in different shock loading conditions, decreased sludge production and biogas recovery [5]. Recently, Gu et al. [6] used a novel MBBR system, combining anaerobic and aerobic conditions in series, for municipal wastewater treatment and reported that the studied system achieved efficient removal of major pollutants, energy recovery and reduced sludge production. The aforementioned characteristics were very encouraging for the future use of such a system on domestic wastewater treatment; however, information was missing for the ability of the system to remove organic micropollutants.

On the other side, the occurrence and the removal of organic micropollutants from domestic wastewater is one of the major challenges that meet nowadays scientists and engineers involved on wastewater management. Numerous organic micropollutants have been detected in municipal wastewater during the last two decades, worldwide; most of them are polar compounds and they are also found in the aquatic environment [7,8]. The monitoring and their efficient removal during domestic wastewater treatment have already been regulated in Switzerland [9], while recommended measures have been reported in other countries [10]. So far, there are several papers

investigating the removal efficiency of various micropollutants, such as pharmaceuticals, endocrine disrupting compounds and benzotriazoles in aerobic MBBR systems [11-18]. Concerning the use of anaerobic MBBR, Torresi et al. [19] studied the removal of specific micropollutants in an anaerobic-aerobic MBBR system operated in sequencing-batch mode for achieving biological phosphorus removal, while Derakhshan et al. [20] operated a strictly AnMBBR under mesophilic conditions and reported important atrazine removal. To the best of our knowledge, no information is available for the ability of combined methanogenic-aerobic MBBR systems to remove organic micropollutants from domestic wastewater as well as for the contribution of different bioreactors on their elimination. Additionally, beside the well-known role of organic loading rate (OLR) on the removal of major pollutants in MBBR systems [21, 22], limited information is available for the role of this parameter on micropollutants' elimination.

Based on the above, the main objective of this study was to examine the ability of a novel lab-scale system, consisting of a methanogenic and an aerobic MBBR, to remove polar organic micropollutants under different organic loading conditions. As target compounds, four benzotriazoles (BTRs) and one benzothiazole were selected (Table S1). These compounds are characteristic polar micropollutants commonly detected in raw and treated sewage as well as in the aquatic environment at concentrations between few ng L^{-1} and some μg L^{-1} [7, 8, 23]. BTRs consist of a benzene ring fused with a triazole ring, while in the case of benzothiazoles a benzene ring is fused with a thiazole ring. They are partially removed in conventional sewage treatment plants, mainly due to biotransformation during activated sludge process [24, 25]. The studied system operated under three OLR values and it was monitored for a total period of seven (7) months for major pollutants' removal, biogas production and

micropollutants' elimination. At the last part of the study, the addition of an extra aerobic MBBR, as a polishing step, was also evaluated, while batch experiments were conducted and the estimated biodegradation constants were used to predict the removal of target compounds in applied system. Experimental data from the current study and two previous articles were also used to study the role of OLR and COD concentration on the removal efficiency of target micropollutants in MBBR systems.

2. Materials and methods

2.1. Analytical standards and reagents

Analytical standards of xylytriazole (XTR) and 5-chlorobenzotriazole (CBTR) were supplied by Sigma-Aldrich (USA). 2-hydroxybenzothiazole (OH-BTH) was supplied by Alfa Aesar (USA), 5-methyl-1H-benzotriazole (5TTR) by Acros Organics (Belgium) and 1H-benzotriazole (BTR) by Merck (Germany). For every compound, a stock solution in methanol was prepared at 1000 mg L^{-1} and stored at -18 $^{\circ}$C. A mixed solution of target compounds (440 mg L^{-1}) was prepared for spiking purposes, when needed, and it was kept at -18 $^{\circ}$C. Methanol (MeOH; HPLC-MS grade) and ultra-pure HCl (32%) was purchased by Merck (Germany), while acetonitrile (ACN; HPLC grade) by Fisher (USA). The cartridges used for solid phase extraction were supplied by Phenomenex (USA). A MilliQ/MilliRO Millipore system (USA) was used for the production of HPLC grade water.

2.2. Set-up of the continuous flow system

A lab-scale continuous flow system was installed by combing an anaerobic moving bed biofilm reactor (AnMBBR) with an aerobic moving bed biofilm reactor (AeMBBR1) for micropollutants' removal and wastewater treatment with parallel biogas production (Figure S1a). The AnMBBR had a working volume of 3 L, the hydraulic residence time (HRT) was set at 10 h and the filling ratio was 40% (v/v). Sponge cubic form carriers (Nisshinbo Chemical Inc.) were used with density ranging between 52~64 kg m^{-3} (at dry stage), while 1 L of anaerobically digested sludge was initially added to seed the system with anaerobic microorganisms. The AnMBBR was airtight sealed and it had double walls connected to a water bath for operating at mesophilic conditions (32-34 °C). Nitrogen gas was initially introduced to achieve air displacement and anaerobic conditions. The AnMBBR runoff was achieved through natural flow because of the increasing pressure that biogas production caused and it was ended up to a flask chamber connected with a bag for biogas collection and with AeMBBR1 for further wastewater treatment. The AeMBBR1 had a working volume of 3.8 L, it was filled by K3 type biocarriers (AnoxKaldnes) at a filling ratio of 33% (v/v), while the HRT was 13 h. These biocarriers had been used during the previous 4 years in an aerobic MBBR system operated at University of the Aegean for the treatment of municipal wastewater originated from the University Campus and a mature biofilm had been developed on them [13, 26]. At the last part of the study, an extra aerobic reactor (AeMBBR2) was added in series as a polishing step, filled with K3 type biocarriers and HRT of 13 h (Figure S1b). Air-stone diffusers were used for the maintenance of aerobic conditions and biomass mixing in the aerobic MBBRs, the dissolved oxygen concentration in these reactors was higher than 4 mg L^{-1}, while mixing in the AnMBBR was achieved through mechanical stirring. The aerobic

systems operated under constant room temperature controlled by central air-conditioning system.

2.3. Operation of the lab-scale continuous-flow system

The studied system operated for a period of seven (7) months receiving municipal wastewater from the University Campus (Table S2). The experiments were divided in three Phases where different organic loading conditions were applied (Phase A: 0.82 ± 0.40 kg COD m^{-3} d^{-1}; Phase B: 0.20 ± 0.10 kg COD m^{-3} d^{-1} and Phase C: 2.10 ± 0.20 kg COD m^{-3} d^{-1}) keeping stable the HRT value and increasing the concentrations of influent COD. For system's monitoring, samples were taken twice a week from the influents and effluents of each reactor for COD, NH_4-N, Total Phosphorus (TP), Total Suspended Solids (TSS) and alkalinity. Samples for the analysis of Volatile Fatty Acids (VFAs) were also taken twice a week from the AnMBBR. The temperature and pH were monitored on a daily basis, while biogas volume was continuously collected and measured once a week (as a weekly average). At the last part of each Phase, an aliquot of target micropollutants in methanol (0.5 mL) was added to the influents for seven consecutive days in order to achieve initial concentrations of approximately 20 μg L^{-1} [13, 26]. Samples were taken from different points of the system taking into account the applied HRT and were analyzed for BTRs and OH-BTH according to the method described below.

2.4. Effect of OLR on removal of micropollutants in aerobic MBBRs

For studying the effect of OLR on the removal of target micropollutants in aerobic MBBRs, data was used from the operation of AeMBBR1 and AeMBBR2 as well as from two previous studies where pure MBBR [13] and HMBBR systems [26] had been used for investigating the removal of target micropollutants from municipal wastewater. The pure MBBR system consisted of two aerobic bioreactors connected in series, with a working volume of 4.5 L each [13]. It operated at two experimental phases where OLR values equal to 0.25 kg m^{-3} d^{-1} and 0.60 kg m^{-3} d^{-1} were applied at the first bioreactor and 0.05 kg m^{-3} d^{-1} and 0.17 kg m^{-3} d^{-1} at the second one. The HMBBR system consisted of two aerobic bioreactors of 3 L in series and a settling tank [26]. The OLR values that were applied in these bioreactors were equal to 0.64 kg m^{-3} d^{-1} and 0.11 kg m^{-3} d^{-1}, respectively. Further information for the operating conditions of both systems is presented in Table S3.

2.5. Batch biodegradation experiments

After the termination of Phase C, batch experiments were conducted in all reactors to determine biodegradation kinetics of the target compounds and to predict their removal rate using mass balances. For this reason, the flow stopped and a mixture of the target compounds was spiked in each reactor simultaneously at a final concentration of 25 μg L^{-1}. Samples (50 mL) were collected at different time intervals (0, 2, 5, 8, 10 and 24 hours) and the concentrations of the target compounds were determined as described in Session 2.6.

2.6. Analytical methods

COD, TSS, NH_4-N, TP, and alkalinity were measured according to the Standard Methods [27]. Temperature and pH were measured using portable instruments. The volume of the produced biogas was determined by water displacement method, while biogas composition was determined with the use of a GA3000 gas analyzer (Geotech). The concentration of biofilm solids was measured at the end of each experimental phase after removing the biofilm from biocarriers and measuring the dried weight difference [11].

For the determination of the VFAs, 1 mL of the sample was collected, diluted, acidified with 2N HNO_3 in order to shift the pH to approximately 2 and stored at 4 ºC up to analysis. Chromatographic separation and quantification of VFAs was based on a previously developed method with some modifications [28], while a Shimatzu LC20-AD prominence liquid chromatographer associated with a SPD-M20A prominence diode array detector (LC-DAD) and a SIL-20AC auto sampler was used for the analysis. The column was Zorbax SB-C18 4.6 mm × 15 cm (5 μm) connected with a Zorbax SB–C18 pre-column (Hewlett Packard, USA). Column and pre-column were heated at 35 ^{o}C with a CTO-20AC column oven (Shimatzu-Japan). The mobile phase consisted of 10 mM KH_2PO_4, pH 2.4 with phosphoric acid (solvent A) and methanol (MeOH, solvent B). Gradient elution was performed as follows: from 20% MeOH to 60% in 10 min, hold in 60% for 2 min and then decreased to 20% in 0.1 min and hold there until 20 min. Flow-rate was 1 mL min^{-1}. The diode array detector (DAD) was set at 210 nm. The VFAs concentration were converted to COD-VFA (mg L^{-1}) by using conversion factors of 1.82 for butyric (BA) and isobutyric acid (Iso-BA), 2.04 for valeric (VA) and isovaleric acid (Iso-VA), 1.07 for acetic acid (AA) and 1.51 for propionic acid (PA) [29].

Regarding micropollutants' analysis, due to the high polarity of these compounds, the contribution of sorption during wastewater treatment was considered of minor importance [30]. Therefore, their concentrations were measured only in the dissolved phase based on the method developed by Mazioti et al. [30] (Figure S2). In brief, samples were initially filtered (LLG-Filter paper) and the filtrates were stored at 4 ^{o}C after acidification (pH 3.0 ± 0.1). For the analysis, solid phase extraction (SPE) was followed, while chromatographic separation was performed using HPLC-DAD (Shimatzu, Japan). The detection limits of studied micropollutants ranged between 0.17 ng L^{-1} (BTR) to 125 ng L^{-1} (CBTR).

2.7. Equations

The removal of target micropollutants at the lab-scale system was calculated according to Eq. (1):

$$Removal = \left(1 - \frac{Cout}{Cin}\right) \times 100 \quad (1)$$

Where, C_{in} is the concentration in influent wastewater (μg L^{-1}) and C_{out} is the concentration in treated wastewater of each examined reactor (μg L^{-1}).

The biodegradation rate constants (k) in batch experiments were estimated using first order kinetics (Eq. 2) for the three reactors used in Phase C.

$$C_t = C_o e^{-k_i t} \quad (2)$$

Where C_t and C_0 are the target compounds' concentrations in batch experiments at time t and $t = 0$, respectively, (μg L^{-1}), k_i is the rate constant for each reactor (d^{-1}) and i the relevant reactor.

For the calculation of the predicted removal in Phase C, the first-order biodegradation rate constants (*k*), calculated in the batch experiments, were used in Eq. (3) [13]:

$$Predicted\ Removal = 1 - \left(\frac{1}{(1+k_1\tau)(1+k_2\tau)+(1+k3\tau)}\right) \quad (3)$$

Where τ is the hydraulic retention time for each reactor (d) and k_1, k_2 and k_3 the biodegradation rate constants (d^{-1}) at AnMBBR, AeMBBR1 and AeMBBR2, respectively.

2.8. Statistical analysis

One-way ANOVA was used for comparing major pollutants and target compounds' removal during different experimental Phases. Tukey–Kramer's post-test was used for the determination of the significant differences between groups.

3. Results and Discussion

3.1 Operation of the system and removal of major pollutants

As reported in Session 2.3, different OLR values were applied during different experimental Phases and an extra aerobic reactor (AeMBBR2) was added in the system during Phase C. The experimental conditions in the reactors used in the current study and their performance regarding COD, NH_4-N removal and biogas production are presented in Table 1.

According to the results, the concentrations of the biofilm solids in AeMBBR1 were similar in Phases A and B (885 mg L^{-1} and 951 mg L^{-1}, respectively), while the application of a higher OLR resulted to a thicker biofilm and a concentration of 3007 mg L^{-1} during Phase C. Alkalinity and pH values were reduced in aerobic reactors comparing to AnMBBR due to the nitrification process occurring in AeMBBR1 [31]. The increase of OLR resulted to an important increase of biogas production in the AnMBBR (Table 1, Figure S3). Specifically, the daily produced biogas was 24.3 ± 5.6 mL L $reactor^{-1}$ d^{-1} under conditions of lower OLR (Phase B), while it was increased to 53.3 ± 20.9 mL L $reactor^{-1}$ d^{-1} (Phase A) and 172.7 ± 25.3 mL L $reactor^{-1}$ d^{-1} (end of Phase C). The findings of the current study are consistent with those of Chatterjee et al. [31] and Sun et al. [32] that reported that the increase of OLR contributed to the increase of the daily biogas production. Biogas composition was determined twice in each Phase and the methane content ranged from 59% to 64%.

Analysis of different VFAs using HPLC and expression of their concentrations as COD-VFA (mg L^{-1}) showed a positive correlation between increase of ORL and increase of COD-VFA (Table 1, Figure 1). Regarding the different types of analyzed VFAs, isovaleric acid (Iso-VA) was not detected in any Experimental Phase. Under conditions of higher OLR (Phase C), all other VFAs were found, while propionic acid (PA) was the dominant. On the other side, acetic acid (AA) and butyric acid (BA) were the VFA components detected mainly when the moderate and lower OLRs were applied (Phase A and B, respectively) (Figure 1). It is known that an increase in the loading rate tends to increase the VFA production [33]. In addition, it should be mentioned that acetic acid (AA) and butyric acid (BA) reported to be the most favorable components for methane production, while contribution of acetic acid (AA) is more than 70% [34]. In contrast, propionic acid (PA) is the most difficult

compound to convert to other intermediates as its degradation is thermodynamically less favorable [35]. As a result, propionic acid (PA) is often considered as the most toxic VFA found in anaerobic digesters [36]. During this study, propionic acid (PA) concentrations between 100-150 mg L^{-1} were found during higher OLR. However, no negative effect on biogas production rate was observed. Previous studies with typical continuous stirred tank reactors (CSTRs) shown that methanogenic bacteria could be inhibited at propionic acid (PA) concentrations more than 1-2 g L^{-1}, while they could tolerate acetic acid (AA) and butyric acid (BA) concentrations more than 10 g L^{-1} [37]. On the other hand, to our knowledge, there is no available data about the toxic concentrations of VFAs in AnMBBRs treating low strength wastewater.

Regarding the appearance of different VFAs during different experimental stages and the fact that acetic acid (AA) and valeric acid (VA) were detected only in the early stage of Phases A and B, respectively, while butyric acid (BA) was detected in late stage of Phase A, it should be mentioned that due to the low HRT applied (10 h) in the anaerobic bioreactor the microbial population at the first stage of the operation was quite low. The use of biocarriers increased sludge residence time (SRT) and favored gradually the presence of slow-growing microorganisms and the establishment of different microbial species [38, 39]. Specifically, in the early stage of Phase A the relative fast-growing acetogens produced acetic acid (AA) but the population of slow-growing methanogens was still low. In the late stage of Phase A, the population of methanogens increased and as a result the concentration of acetic acid (AA) decreased. In addition, the increase of the population of hydrolytic bacteria and butyrate-producing bacteria resulted to the occurrence of butyric acid (BA) in the reactor. Similarly, in the early stage and late stage of Phase B, valerate-producing and valerate-degrading bacteria were gradually established, respectively.

Concerning the removal of major pollutants, the system was able to remove efficiently COD (>84%) and NH_4-N (>96%), while TP removal was negligible (Table 1). No statistically significant differences on NH_4-N removal were noticed during different Experimental Phases, while the higher COD removal (96%) was observed when the higher OLR was applied. Concerning the contribution of different reactors on the removal of major pollutants, in Phase A, both AnMBBR and AeMBBR1 contributed equally to COD removal, while in Phases B and C the greatest part of COD was removed in AnMBBR (Figure S4). On the other side, as expected due to the lack of aerobic conditions, the AnMBBR was unable to remove NH_4-N and as a result all ammonium nitrogen was removed via nitrification in AeMBBR1. The addition of AeMBBR2 in Phase C increased NH_4-N removal by 5%. Calculation of the nitrification rates showed that their values in Phase C for AeMBBR1 (48.6 ± 14.0 mg g^{-1} d^{-1}) and AeMBBR2 (12.9 ± 16.4 mg g^{-1} d^{-1}) were statistically lower than those calculated for Phase A (128.8 ± 27.0 mg g^{-1} d^{-1}) and B (78.6 ± 8.5 mg g^{-1} d^{-1}).

3.2. Removal of target micropollutants in continuous-flow systems: role of different reactors

All target micropollutants were partially removed during different Experimental Phases in the lab-scale system consisting of AnMBBR and AeMBBR1 in series (Figure 2). As these micropollutants are not degraded abiotically under the conditions found in such bioreactors and the role of sorption on attached biomass is considered of minor importance, biotransformation is considered the main mechanism responsible for their elimination [13, 26]. Among target compounds, the lowest total removal efficiencies were observed for 5TTR (31% in Phase B), BTR (33% in Phase

B), and CBTR (37% in Phase B) while the highest for OH-BTH (93% in Phase B). Use of ANOVA test showed the existence of statistically significant differences between Experimental Phases. For three of the target compounds (5TTR, CBTR, XTR), higher removal efficiency was noticed at Phase C, while for OH-BTH at Phase B. Comparing the removal efficiencies achieved in the current system with those reported in the literature, CBTR, XTR and OH-BTH eliminated at similar percentages to previously reported for lab-scale activated sludge, pure MBBR or HMBBR systems, whereas higher removal efficiency was achieved for 5TTR [13, 26].

Concerning the role of different reactors on micropollutants' removal, excepting BTR and OH-BTH, both reactors contributed significantly to the removal of target compounds (Figure 2). Important contribution of the AnMBBR on the biodegradation of target compounds was noticed for 5TTR (Phase A, C), CBTR (Phase A, B) and XTR (Phase A, B). To the best of our knowledge, this is the first study reporting the removal of BTRs in strictly anaerobic biological wastewater treatment systems. Limited relevant information is available in the literature mainly from experiments with aquifer material. Specifically, Liu et al. [40] investigated the biodegradation of three BTRs in aquifer material under anaerobic conditions and reported half-life values of 57, 59 and 44 d for BTR, 5TTR and CBTR, respectively, while Alotaibi et al. [41] reported half-lives of 29 and 26 d for BTR and 5TTR in anaerobic column studies inoculated with aquifer sediment. Herzog et al. [42] conducted batch anaerobic experiments with activated sludge and digested sludge as inoculum and reported no biodegradation of BTR and 5TTR during the 50 d of the experiment. However, it is worth mentioning that in those experiments the target compounds were added at ppm levels.

On the other side, the existence of AeMBBR1 resulted to important increase of target compounds removal in all Experimental Phases, whereas it was exclusively responsible for the removal of OH-BTH and BTR (Figure 2). To investigate whether the addition of an extra aerobic step could enhance target micropollutants' removal, AeMBBR2 was added in Phase C. According to the results presented in Figure 3, excepting OH-BTH where an additional removal efficiency of 30% was achieved, the addition of AeMBBR2 did not decrease significantly the concentrations of target compounds in treated wastewater.

3.3. Effect of organic loading on the removal of target micropollutants in aerobic MBBR

Contradictory results have been reported in the literature for the role of organic loading on micropollutants' removal during biological wastewater treatment with MBBR systems [13, 15, 18]. To study the role of OLR and related parameters, we decided to use data from the operation of AeMBBR1 and AeMBBR2 from the current study as well as from two previous studies that investigated target compounds removal in aerobic pure MBBR and HMBBR systems and operated at different OLR values [13, 26]. The experimental conditions applied in ten (10) different experiments (type of the system, applied OLR, influents' COD concentration, HRT value) and the observed removal of target compounds are summarized in Table S3. It must be mentioned that results from six (6) experiments were used for OH-BTH due to its high removal efficiency during the first aerobic stage of tested systems (>70%) and the low remaining concentrations at the influents of the second stage (Table S3).

As it can be seen in Figure 4, the increase of applied organic loading enhanced the removal efficiency of three of target micropollutants for the range of OLR values applied in these studies (0.05 to 0.64 kg COD m^{-3} d^{-1}). The correlation was stronger for CBTR and XTR and weaker for BTR. No correlation was observed for OH-BTH and 5TTR. OH-BTH is an easily biodegradable compound that is removed significantly during biological wastewater treatment [43]. As a result, its removal seems to be independent of the applied OLR. On the other side, the removal of 5TTR seems not to be affected by the OLR but from other factors that were not studied in the current article (e.g. characteristics of biomass). Previous articles have also reported the slow and partial removal of 5TTR under different experimental conditions and the positive effect of biomass acclimatization on its biodegradation [40, 42, 13].

As OLR value is affected by COD concentration in influent wastewater as well as by HRT value, the role of influent COD on CBTR, XTR and BTR removal was studied separately using data from 7 out of 10 systems where similar HRT values had been applied (10.8 to 13 hours, see also Table S3). According to the results shown in Figure 5, the increase of COD concentration in influent wastewater enhanced these micropollutants' removal. The positive role of organic substrate on several micropollutants' removal has also been reported in MBBR and other biological wastewater treatment systems and is due to co-metabolic phenomena [44, 12, 15, 16, 18].

According to Dalton and Stirling [45], co-metabolism is defined as "the transformation of a non-growth substrate in the obligate presence of a growth substrate or another transformable compound". Non-growth substrate refers to compounds that cannot support cell replication due to their low concentrations (e.g.

micropollutants in wastewater). Cometabolic processes depend on the nature and the amount of the growth substrate and it is considered that the higher the substrate concentration, the faster the degradation of the micropollutant. Casas et al. [12] investigated the removal of different pharmaceuticals in a staged MBBR system and reported that their biodegradation occurred in parallel with the removal of COD and nitrogen. Tang et al. [16] observed that the biodegradable pharmaceuticals degraded faster in the presence of higher humic acid concentrations and the average increase of their first-order degradation rate was 5% per mg DOC. Torresi et al. [19] reported the cometabolic activity of phosphorus accumulating organisms towards the removal of several micropollutants that stopped when the primary substrate (phosphorus) and/or the internal stored polyhydroxyalkanoates were no longer available. Concerning the compounds investigated in the current study, previous batch experiments with activated sludge and attached biomass have also shown that the biodegradation kinetics of BTR, CBTR and XTR were increased with increase of organic substrate [13].

3.4. Biodegradation kinetics and prediction of the removal

As described in Session 2.5, batch experiments were conducted at the end of Phase C to estimate first-order biodegradation rate constants (*k*) in different reactors and to calculate predicted removal using Equation 3. Biodegradation rate constants values reported in Table S4 indicate that the attached biomass developed in both aerobic MBBR had the ability to biodegrade all target compounds. In aerobic reactors the lowest *k* values were estimated for 5TTR (AeMBBR1: 0.491 d^{-1}) and the highest for OH-BTH (AeMBBR1: 8.254 d^{-1}). These values are similar (for BTR, CBTR, OH-

BTH) or higher (for CBTR, XTR) than those reported in a previous study for pure aerobic MBBR systems [13] (Table S4). For the AnMBBR, *k* values ranged between 0.068 d^{-1} (BTR) and 1.104 d^{-1} (5TTR). It should be mentioned that it is the first time that biodegradation kinetics are estimated for the target compounds in a strictly anaerobic, methanogenic biological wastewater treatment system.

According to Figure 6, the predicted removal in the system including a methanogenic and two aerobic MBBR was close to the measured removal for 4 out of the 5 target compounds (BTR, CBTR, XTR and OH-BTH). On the other side, the model seems to underestimate system's performance for 5TTR.

4. Conclusions

The tested lab-scale system was able to biodegrade target polar micropollutants under all OLR values applied. Total removal efficiencies ranged from 33 to 60% for BTR, 31 to 86% for 5TTR, 37 to 56% for CBTR, 43 to 91% for XTR and 80 to 97% for OH-BTH. The contribution of the strictly anaerobic, methanogenic bioreactor was important for 5TTR, CBTR, XTR and COD, while the use of the first aerobic bioreactor resulted to important increase of target micropollutants' removal and it was exclusively responsible for the removal of OH-BTH, BTR and NH_4-N. The experimental results show that for municipal wastewater containing target micropollutants, there is no important benefit from the addition of a second aerobic step in anaerobic-aerobic MBBR system. The increase of OLR and COD concentrations in influent wastewater enhanced biodegradation of most target microcontaminants, indicating the important role of co-metabolism on their elimination.

In summary, the operation of the current attached growth biomass wastewater treatment system achieved efficient removal COD and NH_4-N removal, negligible P removal, biogas production and partial polar micropollutants' elimination. These results encourage the future use of this system as the required biological treatment step for the production of recovered water capable for agricultural irrigation with simultaneously energy recovery and low biomass production. Further research is needed for identifying the biotransformation products of polar micropollutants in such systems as well as for studying their performance when an anoxic step has been added for full nitrogen removal.

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Table 1

Table 1. Experimental conditions and removal of major pollutants in anaerobic and aerobic moving bed biofilm reactors during different Experimental Phases.

Anaerobic Moving Bed Biofilm Reactor (AnMBBR)									
Phase	**OLR (Kg COD m^{-3} d^{-1})**	**T (˚C)**	**pH**	**Alkalinity (mgL^{-1} $CaCO_3$)**	**VFA-COD (mg L^{-1})**	**Biogas Production (mL $L^{-1}_{reactor}$ d^{-1})**	**COD_{dis} Removal (%)**	**NH_4-N Removal (%)**	**TP Removal (%)**
Phase A	0.82 (± 0.4)	34.1 (± 0.1)	7.67 (± 0.2)	554 (± 1)	46.4	53.3 (± 20.9)	45 (± 24)	< 5%	< 5%
Phase B	0.20 (± 0.1)	34.2 (± 0.4)	7.90 (± 0.1)	445 (± 26)	9.0	24.3 (± 5.6)	57 (± 26)	< 5%	< 5%
Phase C	2.10 (± 0.2)	32.5 (± 1.1)	7.47 (± 0.4)	670 (± 120)	193.7	172.7 (± 25.3)	69 (± 5)	< 5%	< 5%
Aerobic Moving Bed Biofilm Reactor 1 (AeMBBR1)									
	OLR (kg COD m^{-3} d^{-1})	**T (˚C)**	**pH**	**Alkalinity (mg L^{-1} $CaCO_3$)**	**Attached Biomass (mg L^{-1})**		**Total[1] COD_{dis} Removal (%)**	**Total[1] NH_4-N Removal (%)**	**Total[1] TP Removal (%)**
Phase A	0.33 (± 0.20)	24.3 (± 4.3)	6.90 (± 0.5)	42 (± 20)	885		89 (± 6)	99 (±1)	< 5%
Phase B	0.10 (± 0.04)	27.9 (± 0.3)	7.90 (± 0.1)	136 (± 26)	952		84 (±10)	99 (±1)	< 5%
Phase C	0.47 (± 0.30)	24.8 (± 1.7)	6.85 (± 0.5)	132 (± 101)	3007		96 (± 2)	96 (± 7)	< 5%

Aerobic Moving Bed Biofilm Reactor 2 (AeMBBR2)								
	OLR (kg COD m^{-3} d^{-1})	**T (°C)**	**pH**	**Alkalinity (mg L^{-1} $CaCO_3$)**	**Attached Biomass (mg L^{-1})**	**Total[2] COD_{dis} Removal (%)**	**Total[2] NH_4-N Removal (%)**	**Total[2] TP Removal (%)**
Phase C	0.06 (± 0.03)	22.2 (± 1.8)	6.24 (± 0.9)	57.8 (± 18.1)	1079	<1%	5 (± 5.7)	< 5%

[1]Total removal: total removal of major pollutants in AnMBBR + AeMBBR1; [2]Total removal: total removal of major pollutants in AnMBBR + AeMBBR1+ AeMBBR2

Figure 1

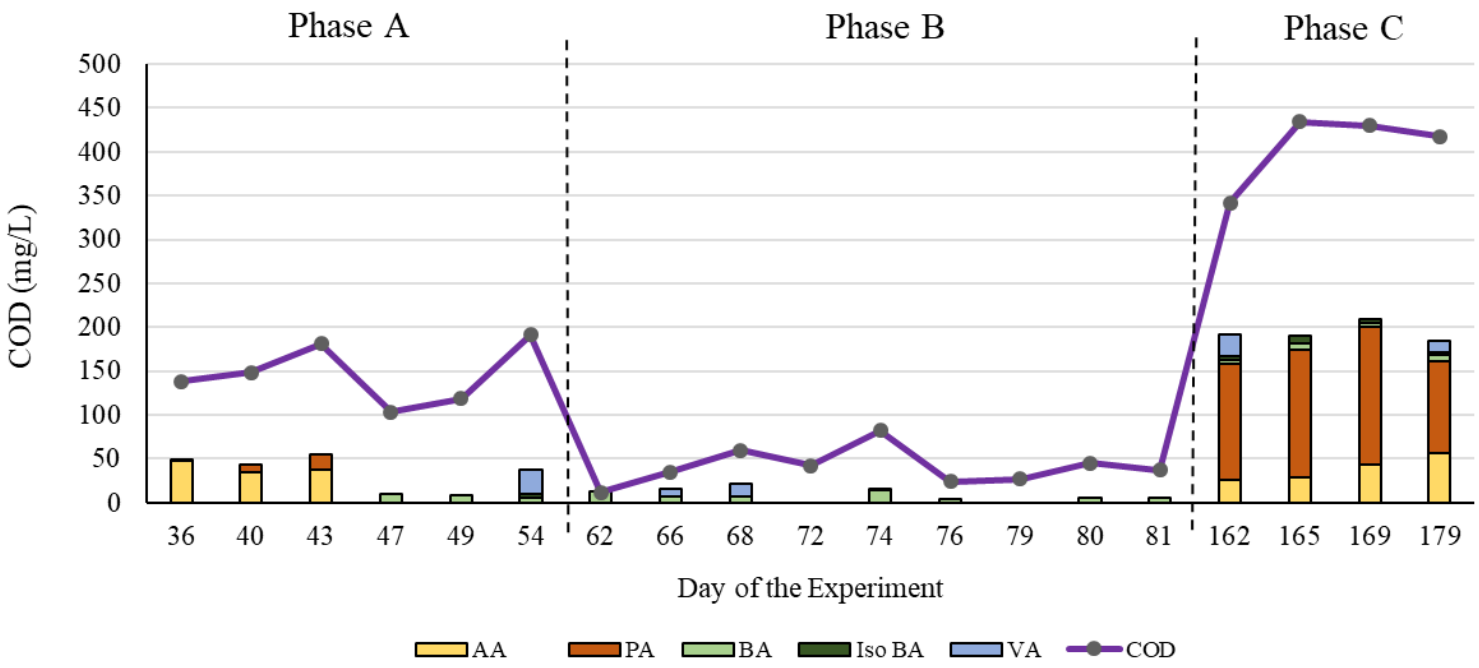


Figure 1. Volatile fatty acid production in Experimental Phases A, B and C (acetic acid, AA; propionic acid, PA; butyric acid, BA; isobutyric acid, Iso BA; valeric acid, VA; isovaleric acid, Iso VA).

Figure 2

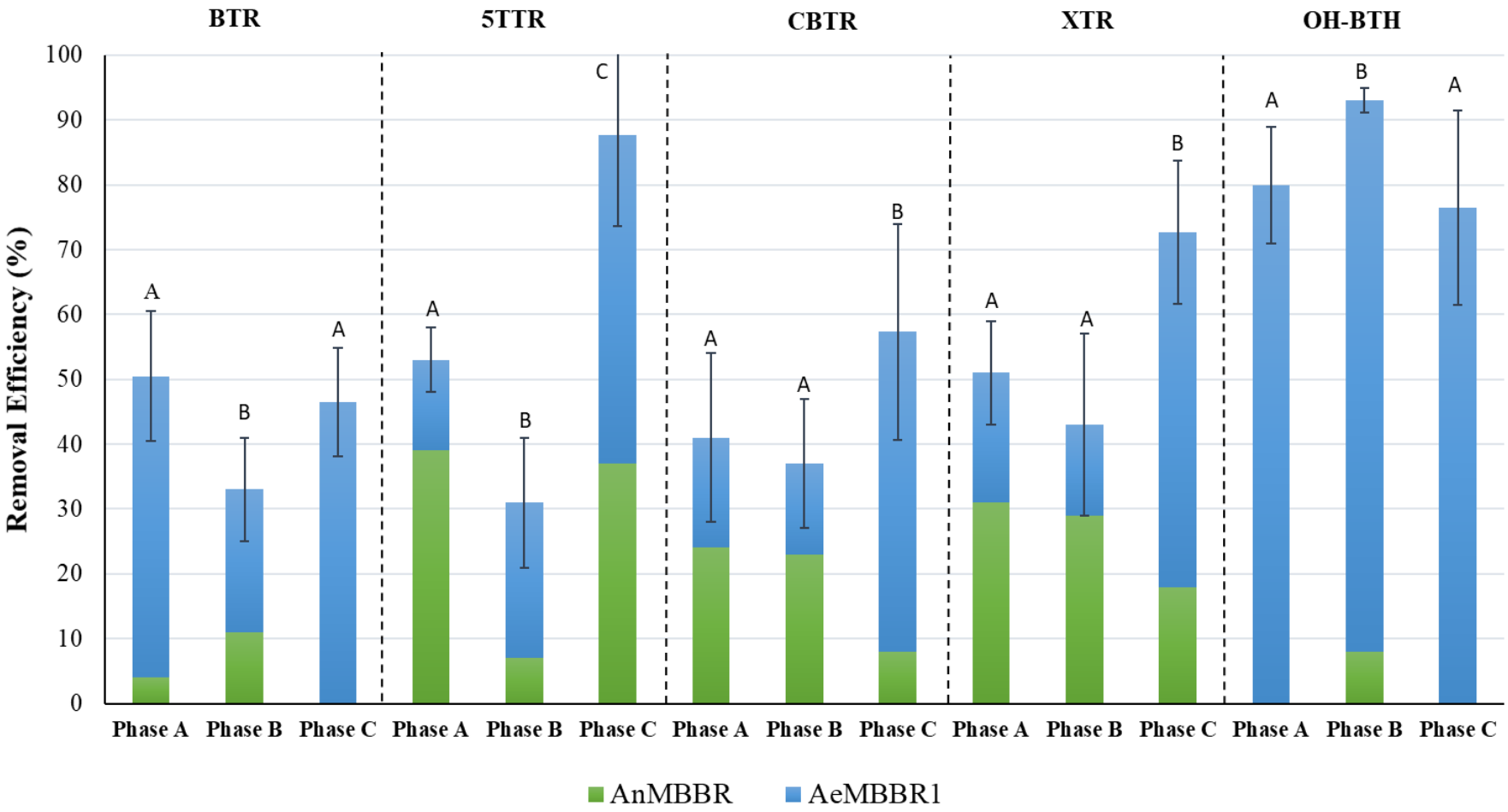


Figure 2: Removal efficiency of target compounds in tested system consisting of AnMBBR and AeMBBR1 during different Experimental Phases. The different letters above the t-bars indicate statistical difference in the removal rate for each Phase.

Figure 3

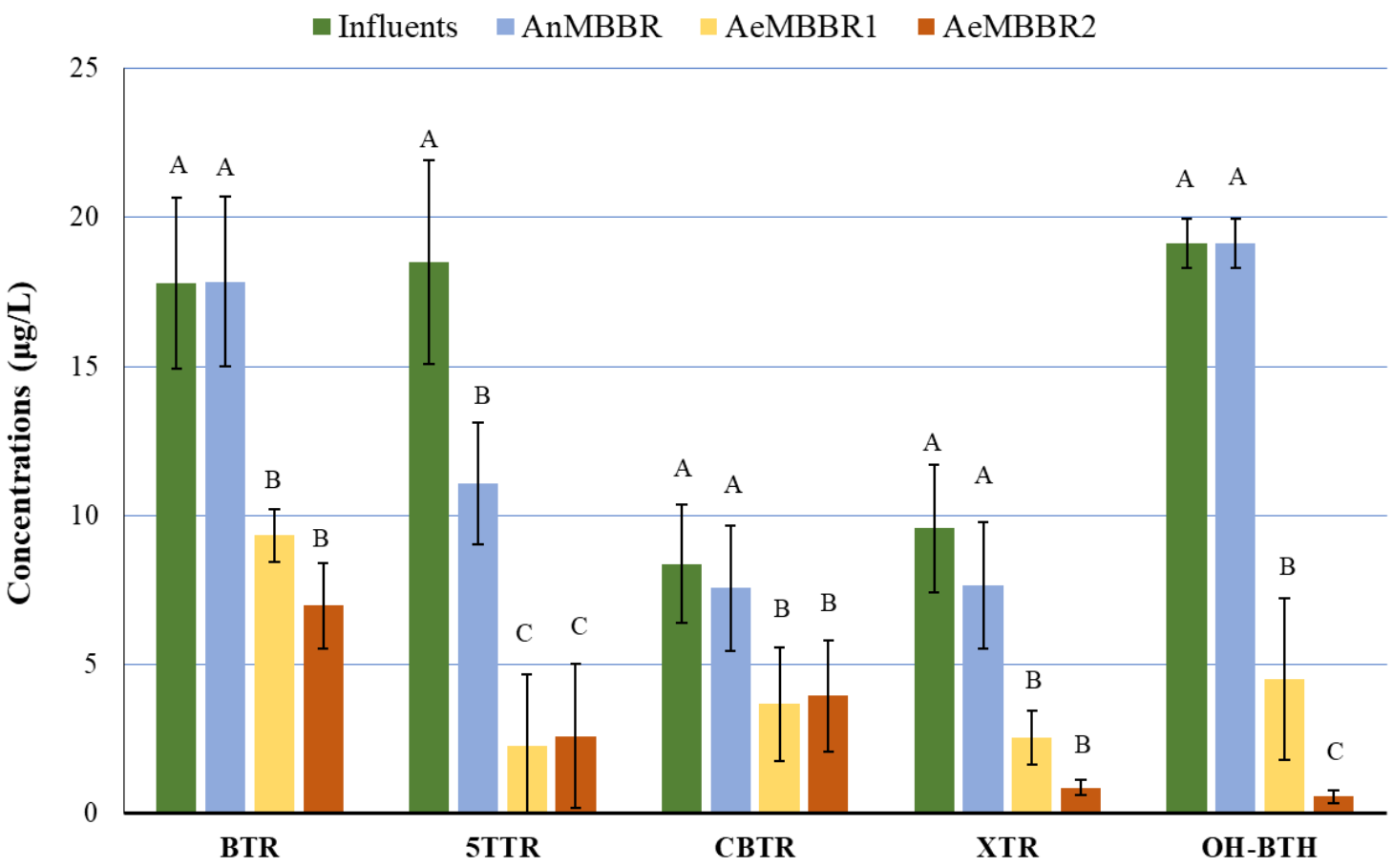
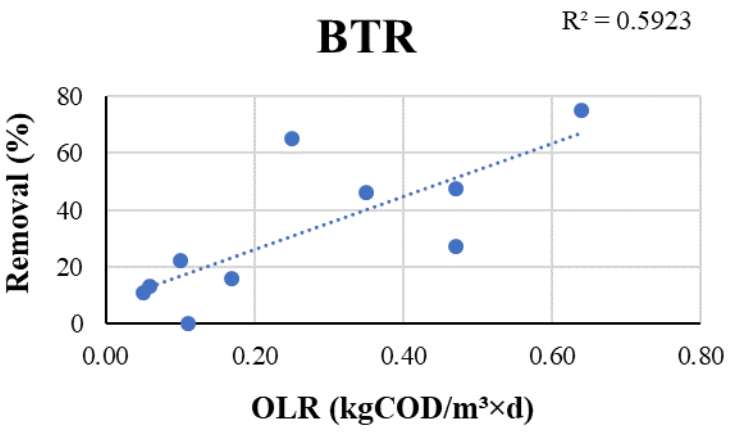
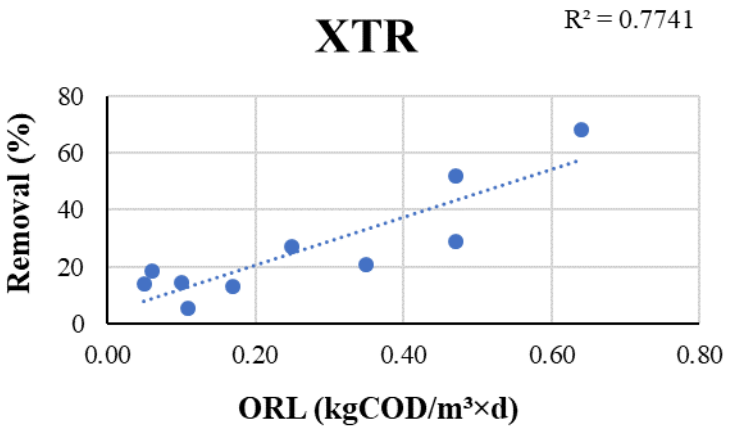
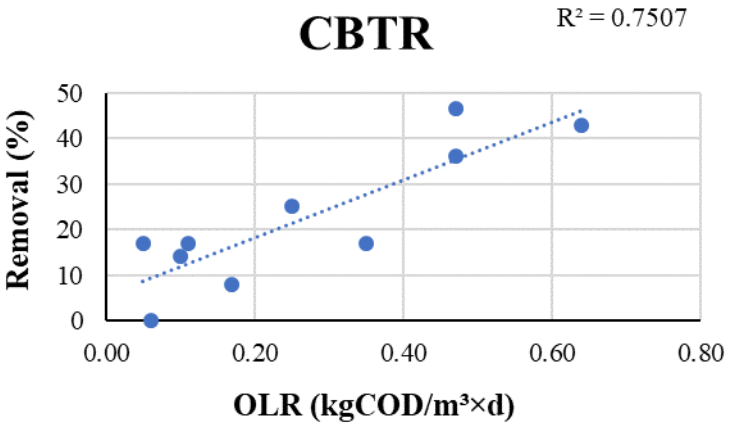


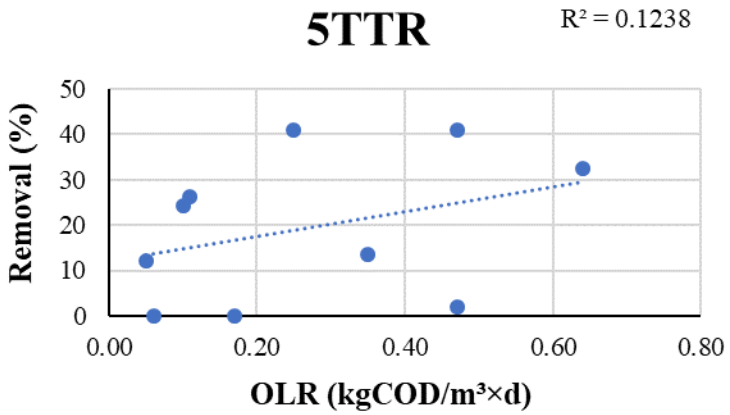
Figure 3: Concentrations (µg L^{-1}) of the target compounds in influents of the system and effluents of AnMBBR, AeMBBR1 and AeMBBR2 during Phase C. The different letters above the t-bars indicate statistical difference in concentrations for sampling point.

Figure 4









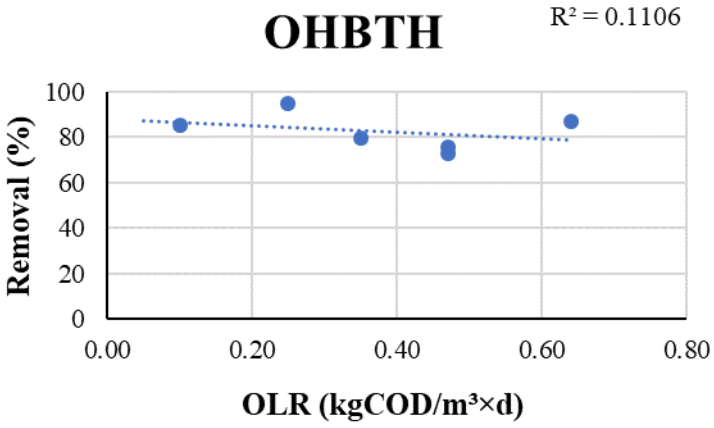


Figure 4. Effect of organic loading (OLR) on target micropollutants' removal. Data originate from the current study and previous studies (Mazioti et al., 2015a; Mazioti et al., 2017).

Figure 5

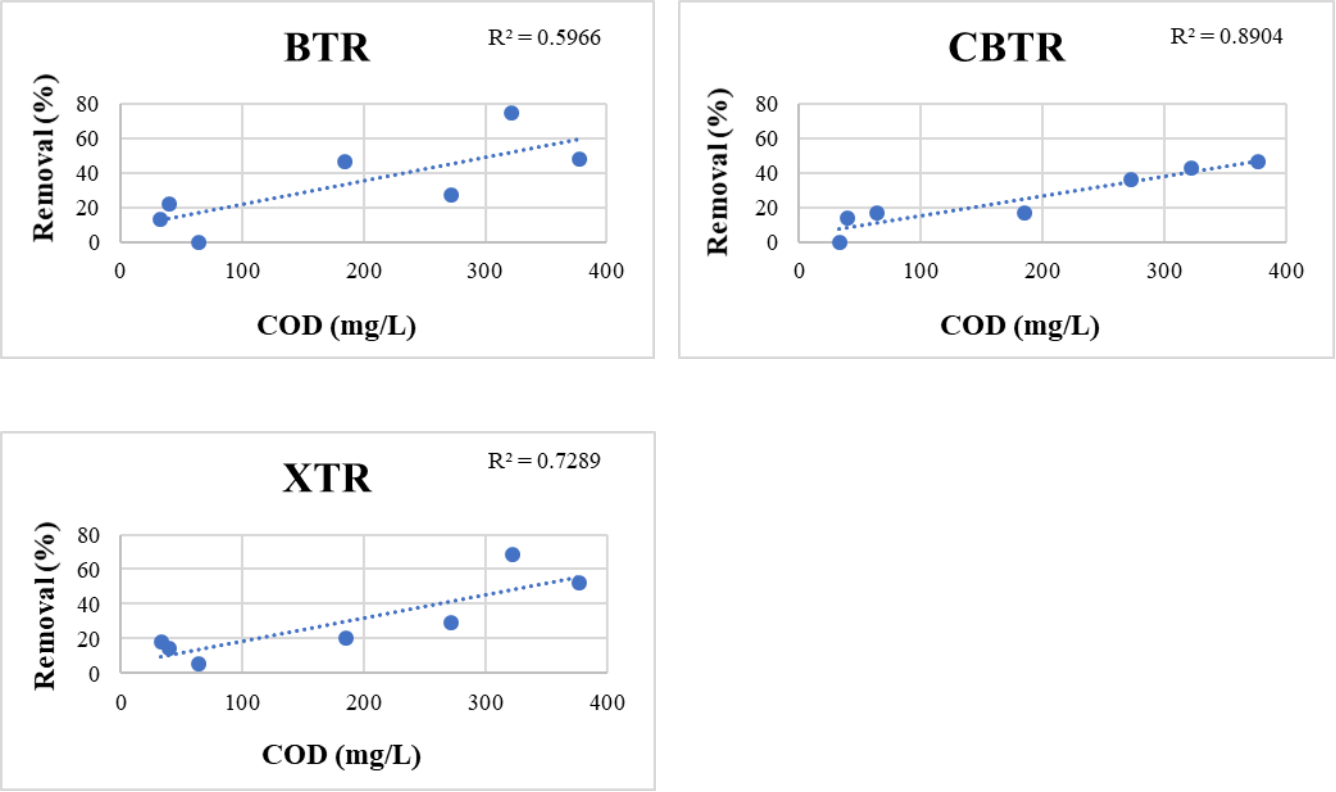


Figure 5. Effect of influent COD concentration on target micropollutants' removal. Data originate from the current study and previous studies (Mazioti et al., 2015 [13]; Mazioti et al., 2017 [26]).

Figure 6

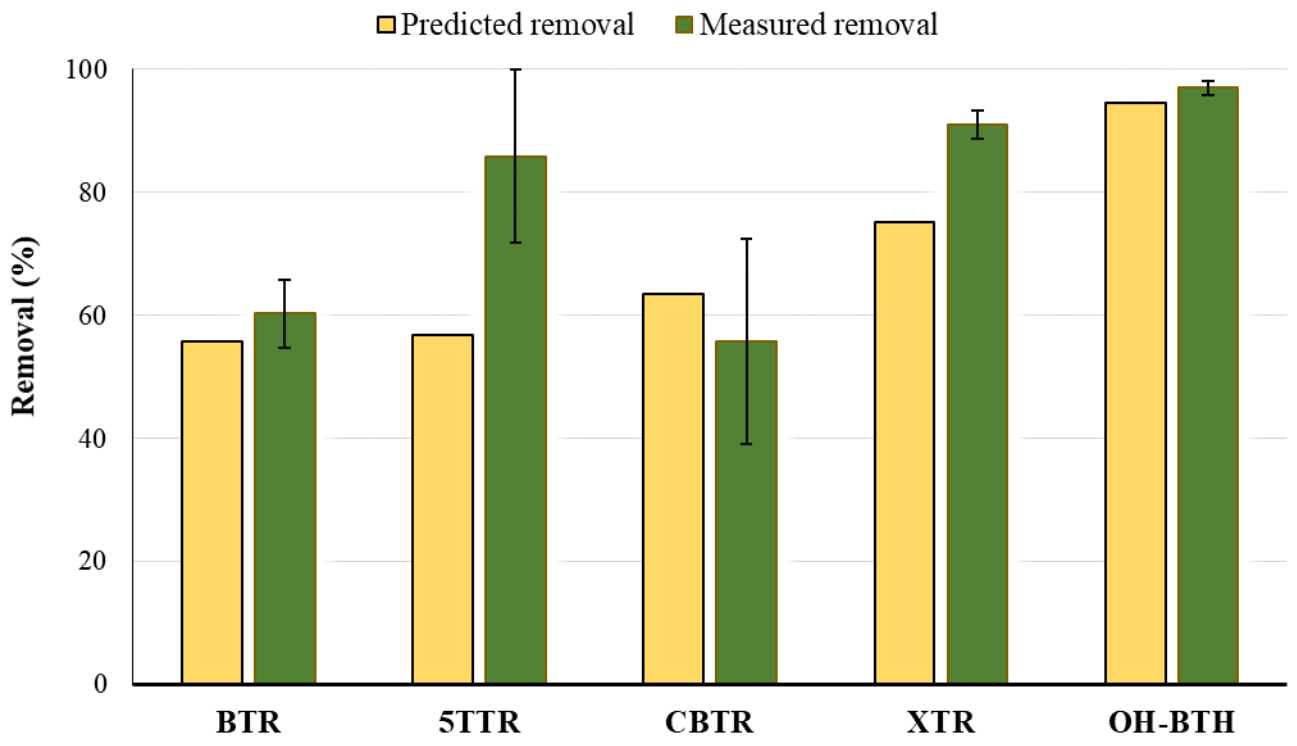


Figure 6. Comparison of predicted and measured micropollutants' removal in the lab-scale AnMBBR-AeMBBR1-AeMBBR2 system used in the current study.

Figure 1 gray scale

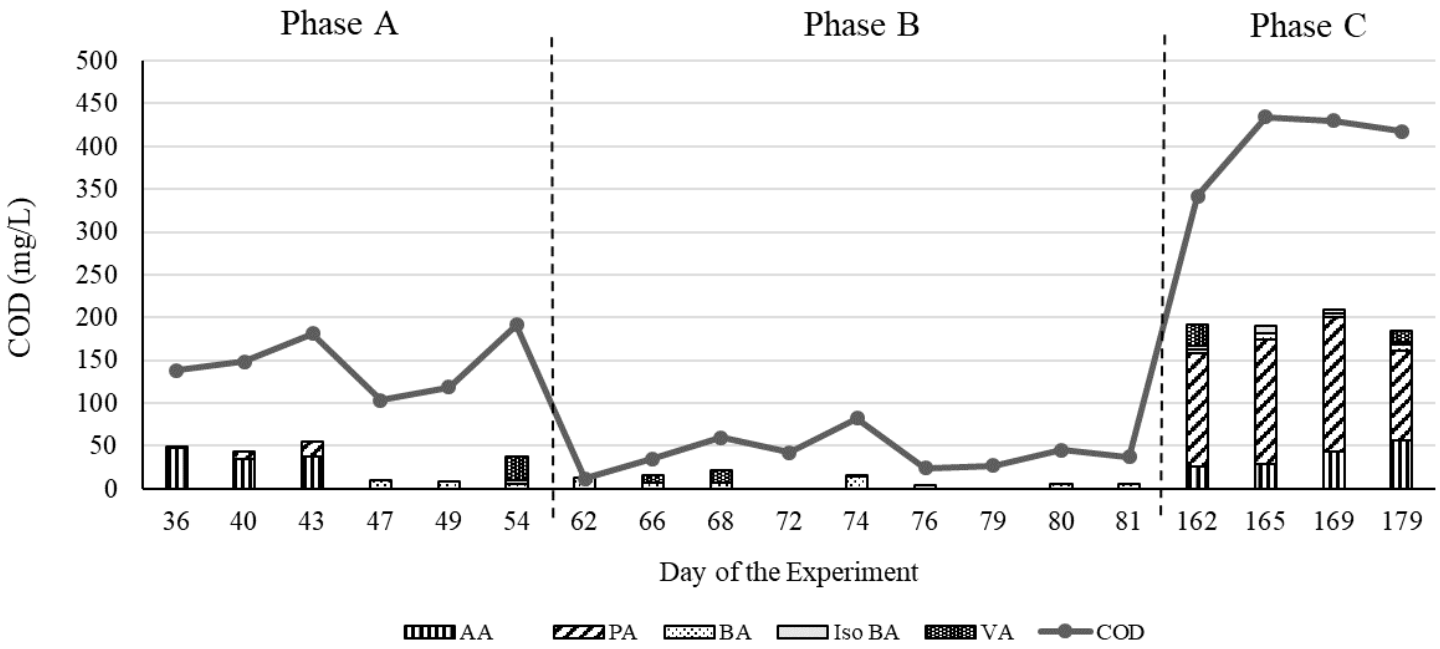


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Figure 2 - grey scale

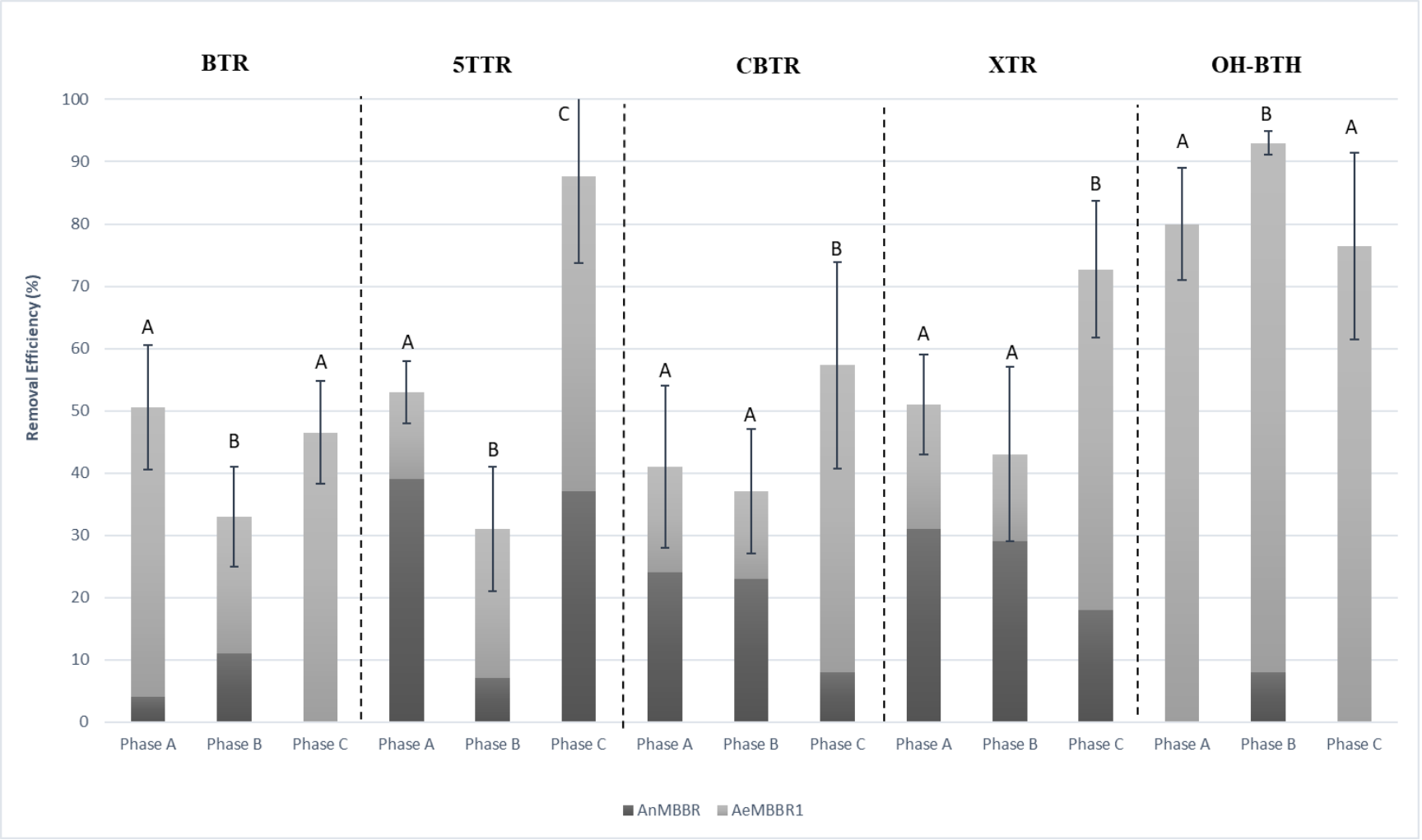

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Figure 3 - grey scale

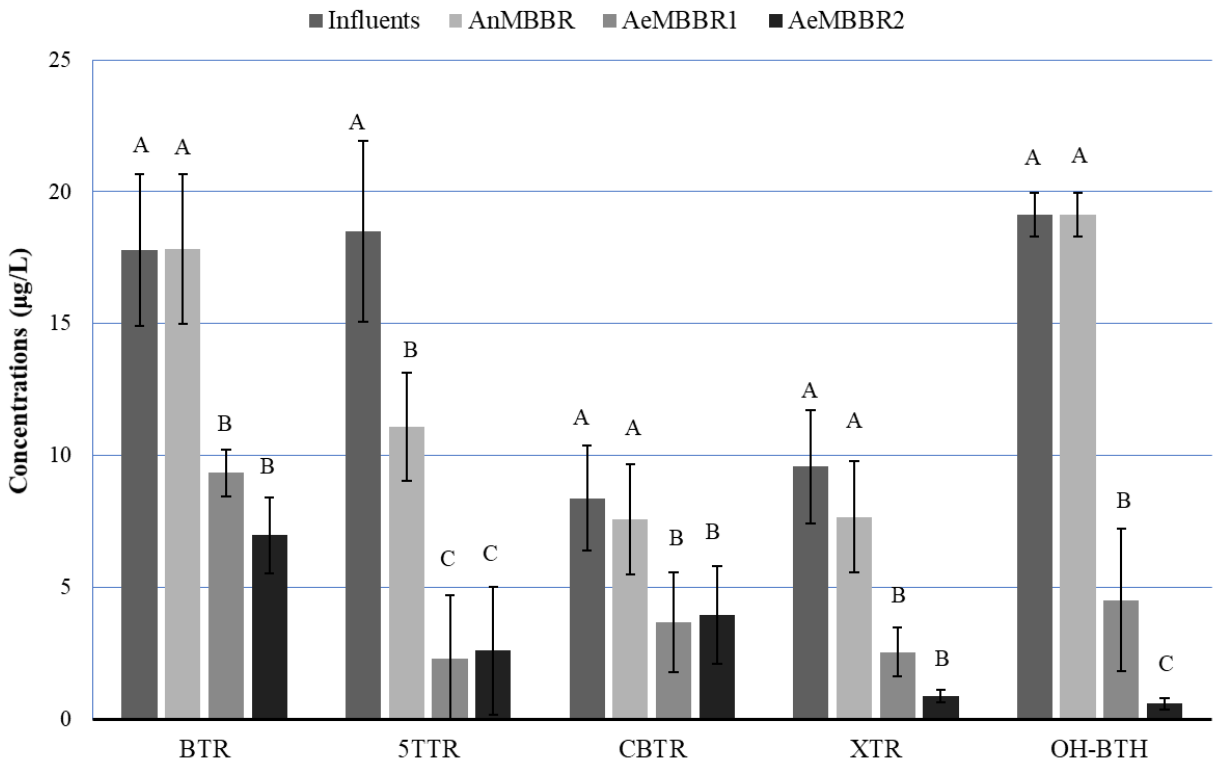

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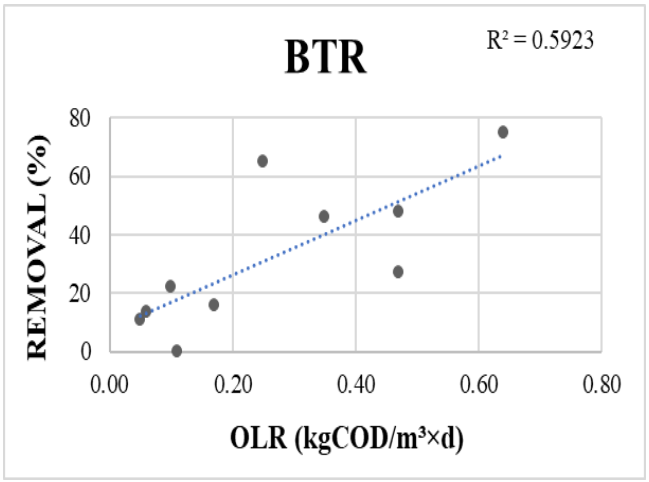
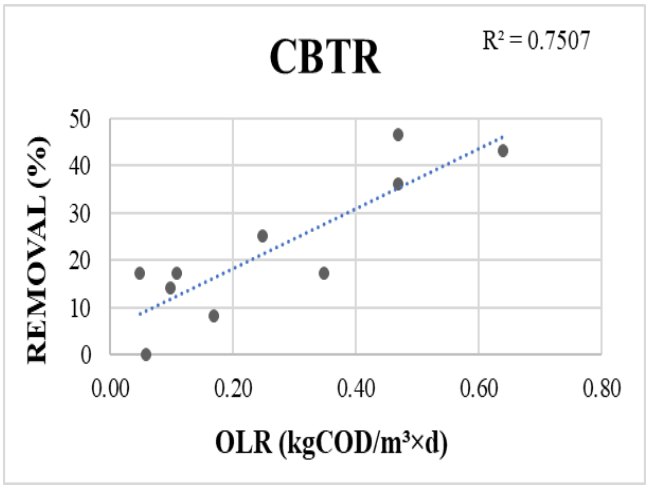
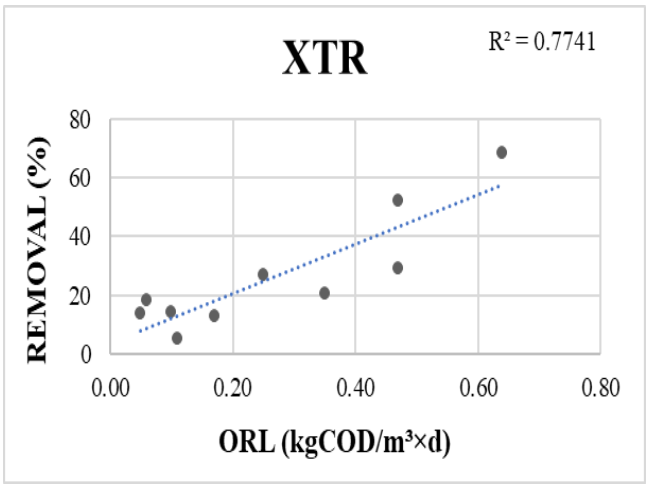
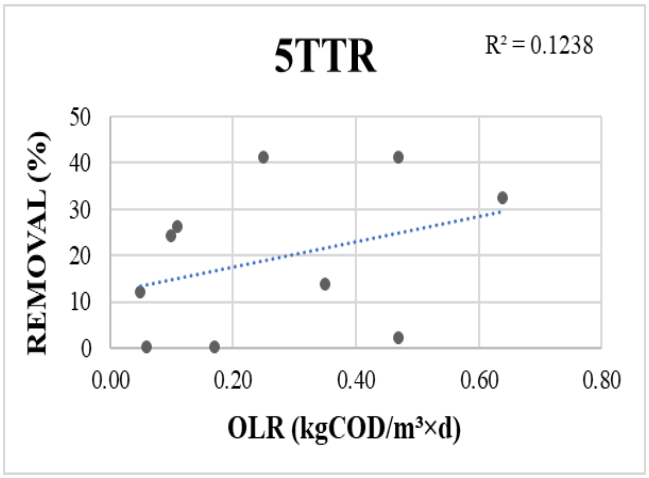
Figure 4 - grey scale









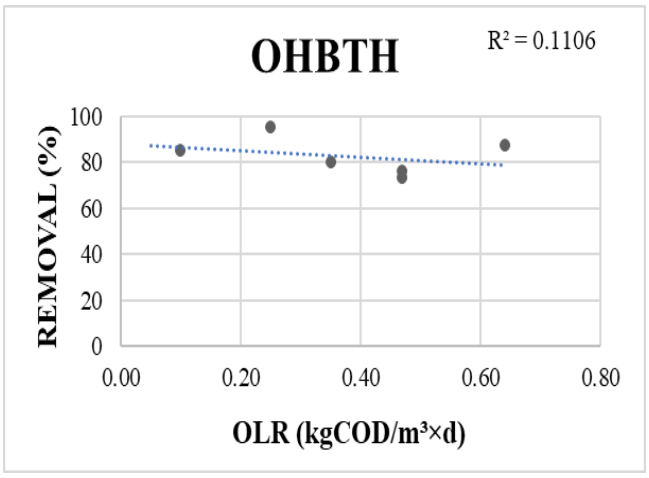


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Figure 5 - grey scale

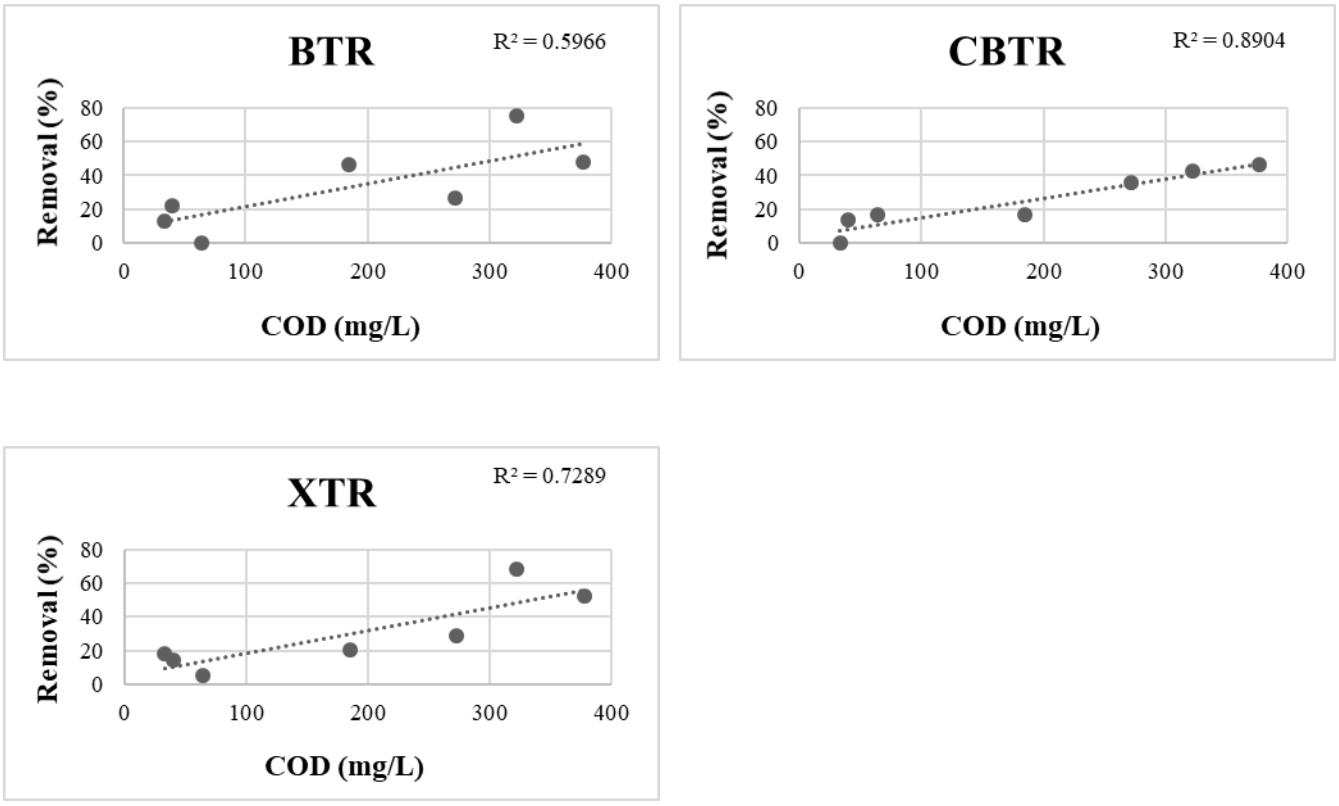

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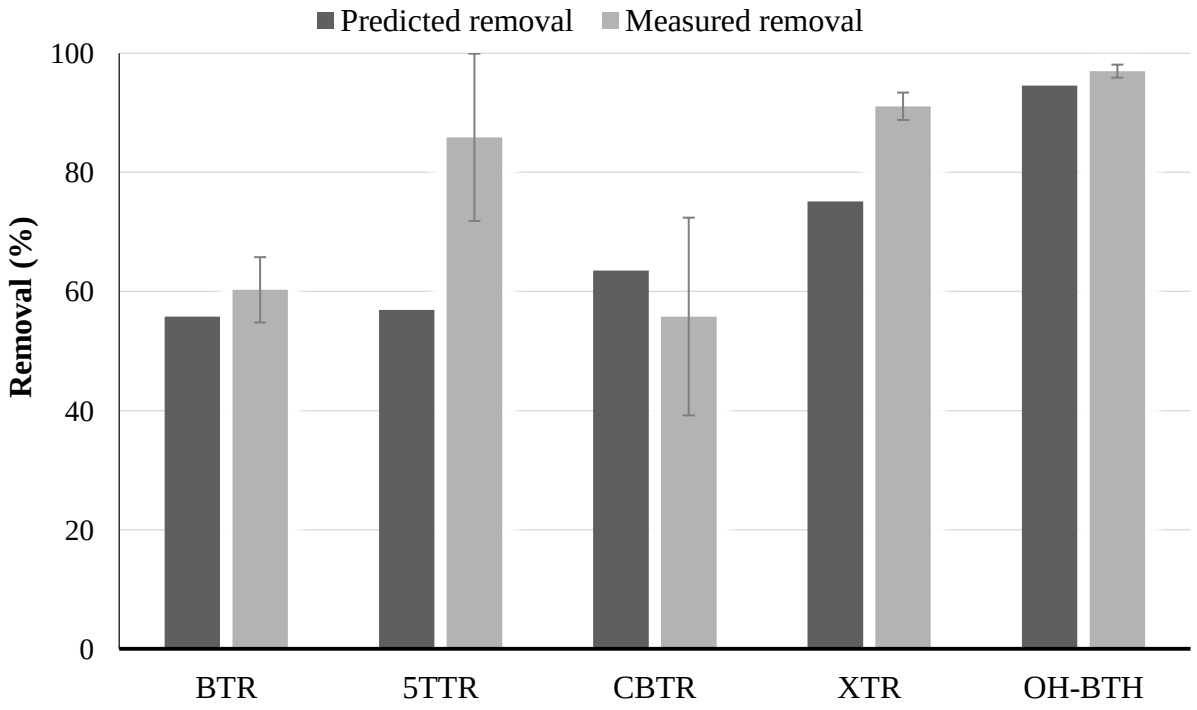
Figure 6 - grey scale



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Supplementary Material

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***Declaration of Interest Statement**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: