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Photobioreactors based on Microalgae-Bacteria and Purple Phototrophic Bacteria Consortia: A promising technology to reduce the load of veterinary drugs from piggery wastewater

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ABSTRACT

Traditional swine manure treatments are not fully effective in the removal of veterinary drugs. Moreover, they are costly and entail a significant carbon footprint in many cases. Innovative biological approaches based on phototrophic microorganisms have recently emerged as promising alternatives to overcome those limitations. This work evaluated the removal of 19 veterinary drugs (i.e., 16 antibiotics, 1 analgesic, 1 anti-parasitic and 1 hormone) from piggery wastewater (PWW) in two open photobioreactors (PBR) operated with a consortium of microalgae-bacteria (AB-PBR) and purple photosynthetic bacteria (PPB-PBR). Multiple hydraulic retention times (HRT), in particular 11, 8 and 4 days, were tested during stage I, II and III, respectively. Ten out of 19 target compounds were detected with inlet drug concentrations ranging from 'non-detected' (n.d.) to almost 23,000 ng L^{-1} for the antibiotic oxytetracycline. Moreover, three of the antibiotics (i.e., enrofloxacin, sulfadiazine and oxytetracycline) were found at concentrations above the analytical linearity range in some or all of the samples under study. AB-PBR supported higher removal efficiencies (REs) than PPB-PBR, except for danofloxacin. Overall, REs progressively decreased when decreasing the HRT. The highest REs (>90%) were observed for doxycycline (95±3%) and oxytetracycline (93±3%) in AB-PBR during stage I. The other drugs, except sulfadimidine that was the most recalcitrant, showed REs above 70% during stage I in the same photobioreactor. In contrast, no removal was observed for danofloxacin in AB-PBR during stage III, sulfadimidine in PPB-PBR during stage III or marbofloxacin in PPB-PBR during the entire experiment.

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1. Introduction

The most straightforward strategy to manage piggery wastewater (PWW) is by directly spreading it onto agricultural land as fertilizer (Khatri and Tyagi, 2015). However, the European Directive 91/676/EEC, concerning the protection of waters against pollution caused by nitrates from agricultural sources, has established a limit of discharge of 170 Kg ha^{-1} y^{-1} for total nitrogen. Pig manure is rich in ammonium, which could undertake biological oxidation into nitrate once it reaches the soil. In addition, the presence of antibiotics and metals in piggery wastewater hinders land spreading in some countries. Therefore, alternative management strategies often need to be sought. Traditional swine manure treatments have consisted of drying, composting, anaerobic digestion, activated sludge and aerobic/anaerobic lagoons (Van Epps and Blaney, 2016). Of them, anaerobic digestion has been likely the most popular technology due to its energy recovery potential (Almeida Streitwieser, 2017). However, compared to other easily-biodegradable substrates such as food waste, PWW contains a low carbon to nitrogen (C:N) ratio that limits nutrients recovery (Kafle and Kim, 2013; Liu et al., 2017; Yenigün and Demirel, 2013). In this context, new approaches based on phototrophic microorganisms have recently emerged as a cost-effective and environmentally friendly alternative to recover nutrients from piggery wastewaters (García et al., 2019). More specifically, algal-bacterial

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consortia (AB) have shown efficient removal of organic matter and nutrients, and even heavy metals and pathogens, as a result of their dual autotrophic and heterotrophic metabolism (Rittmann and McCarty, 2012). In addition, this symbiosis entails a low energy consumption and carbon footprint since the carbon dioxide (CO_2) generated during organic matter oxidation is photosynthetically fixed (Cheah et al., 2016; Dassey and Theegala, 2013). On the other hand, purple phototrophic bacteria (PPB), which use the infra-red spectrum of solar radiation as energy source, can also support high rates of organic matter and nutrients assimilation and exhibit a high tolerance towards wastewater toxicity (Hülsen et al., 2016a; Hülsen et al., 2016b). Furthermore, some authors have claimed that PPB possess a more versatile metabolism than microalgae (Hülsen et al., 2014).

Nonetheless, organic matter and nutrients are not the only environmental concern that pig manure entails. Despite the fact that sub-therapeutic use of antimicrobial growth promoters is prohibited in the European Union (European Commission, 1998), their use as disease control is still widespread. Fluoroquinolone, sulfonamide and tetracycline classes are the most commonly utilized veterinary drugs (Van Epps and Blaney, 2016). Although antimicrobial loads in swine manure vary with each operation, concentrations mostly range between 0.01 and 100 mg Kg^{-1} (or mg L^{-1}) (Van Epps and Blaney, 2016). Occurrence of these pollutants in environmental continental waters (surface and ground- water) commonly reaches hundreds of ng L^{-1} in Europe and even thousands of ng L^{-1} in some areas of Asia (Gothwal and Shashidhar, 2015). In this regard, the connection between antibiotic residues and antibiotic resistance in pathogenic bacteria has been consistently

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reported in recent years, especially with respect to antibiotic use in animal production (Peeples, 2015). In addition to this, the presence of veterinary pharmaceuticals in environmental compartments opens their entry into biota. These drugs can be taken up by vegetables, crops, aquatic plants, and animals (Li et al., 2013; Na et al., 2013). In fact, their presence in vegetables and fishes has challenged the standards of food safety (Gothwal and Shashidhar, 2015).

Currently, pharmaceuticals in wastewaters are typically degraded using costly physical-chemical technologies such as photocatalysis or ozonation (Kanakaraju et al., 2018; Yap et al., 2019). In the present work, the performance of two photobioreactors (PBRs) based on AB and PPB, respectively, operated under multiple hydraulic retention times (HRT) was assessed in terms of their ability to remove 19 veterinary drugs (including 16 antibiotics) from PWW. To the best of our knowledge, this study is the first one to report on the potential of PPB-PBRs to remove veterinary drugs in real wastewater samples. In addition, the elimination of most of the target pharmaceuticals has never been assessed in photobioreactors before.

Up-to-date there is not available data reporting occurrence of veterinary drugs in the environmental compartments surrounding the sampled farm. However, the information obtained in this study could be useful as an estimation of the impact both the PWW and the proposed treatment effluents would have if they were employed as fertilizers.

2. Materials and methods

2.1 Chemicals and reagents

Nineteen veterinary drugs were selected as target analytes. The selection was based on high consumption according to the 2011 report by the Spanish Agency of Medicines and Medical Devices (AEMPS) about sales of veterinary drugs in Spain ((AEMPS), 2011). The standards for the drugs (**Supplementary data 5**) were of high purity grade (>95%). Tylosin and florfenicol were obtained from LGC Standards (Barcelona, Spain). The other veterinary drugs were purchased from Sigma-Aldrich (Tres Cantos, Madrid, Spain). All of them were acquired as neutral non-solvated molecules, except for penicillin G (potassium salt), amoxicillin (trihydrate), doxycycline (hyclate), tetracycline (hydrochloride), oxytetracycline (hydrochloride), apramycin (sulfate salt), tiamulin (fumarate) and tylosin (tartrate). The isotopically labelled internal standard, enrofloxacin-d_5, was also supplied by Sigma-Aldrich. Individual stock solutions at 1 g L^{-1} for all the standards were prepared on a weight basis in methanol (MeOH), except for amoxicillin and danofloxacin (which were dissolved in a H_2O/MeOH mixture (1:1)), ciprofloxacin (which was dissolved in H_2O/MeOH (1:1) containing 0.2% v/v hydrochloric acid (HCl)) and apramycin (which was dissolved in H_2O) due to their low solubility in pure MeOH. Mixture stock solutions, were subsequently prepared from them and stored at -80 ˚C in darkness to avoid uncontrolled degradation, until they were employed during method validation protocols and calibration curve building for sample quantification.

Ultrapure water was in-house generated by a Milli-Q (MQ) Advantage Ultrapure Water purification system and filtered through a 0.22 μm Millipak Express membrane and an LC-

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Pak polishing unit by Merk Millipore (Billercia, MA, USA). MeOH, acetonitrile (ACN) and formic acid (FA) of high analytical grade were acquired from Sigma-Aldrich (Stockholm, Sweden). HCl (37%), ammonium hydroxide (>28%) and ethylenediaminetetraacetic acid disodium salt dehydrate (Na2EDTA) were obtained from Sigma-Aldrich (Tres Cantos, Madrid, Spain).

2.2 Inocula and wastewater

A *Chlorella vulgaris* culture obtained from an outdoors high rate algal pond (HRAP) treating centrate was used as inoculum in the AB-PBR, while the inoculum for the PPB-PBR was obtained through a batch enrichment using diluted PWW (17%) under continuous infrared light (IR) illumination at 50 W m^{-2}.

Fresh PWW was collected just before every new stage from a nearby swine farm at Cantalejo (Spain) and stored at 4 ˚C. The PWW was centrifuged for 10 min at 10,000 rpm before it was diluted by a factor of 20 using tap water to reduce the concentration of total suspended solids (TSS) and the NH$_3$ inhibition to the microbial photosynthetic communities. The resulting solution (for details, see **Supplementary data 1**) was used as the influent to the studied PBRs.

2.3 Experimental set-up

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The experimental set-up consisted of two 3-L open PBRs (0.15 m deep and 0.02 m^2 of cross sectional illuminated area) under continuous operation. The AB-PBR was illuminated at 1390 ± 30 µmol $m^{-2}s^{-1}$ for 12 h d^{-1} (4 am to 4 pm) using visible light-emitting diode (LED) lamps, which were arranged 60 cm above the surface of the PBR in a horizontal configuration (**Figure 1**). During the same period, the PPB-PBR was illuminated at 48±4 W m^{-2} by IR LED lamps, which were also horizontally arranged at 20 cm above the surface of the PBR (**Figure 1**). The AB-PBR was jacketed and connected to a cooling water bath to maintain both PBRs at similar temperatures. Two water immersion pumps were used to mix continuously the cultivation broths of AB- and PPB-PBRs. Both PBRs were initially filled with tap water, inoculated with fresh biomass at 275 mg L^{-1} TSS and fed with diluted PWW using a 205U7CA multi-channel cassette pump (Watson-Marlow, UK) at HRTs of 11, 8 and 4 days in stage I (August – October 2017), stage II (October – December 2017) and stage III (January – March 2018), respectively (**Supplementary data 1**). An inherent biological activity is expected in the PWW, which would require PWW sterilization by autoclaving if an abiotic control test would be performed. PWW sterilization is expected to impact on the fate of veterinary drugs. Therefore, the implementation of a control reactor to assess the abiotic removal potential of the system was disregarded as it would not provide with relevant information.

Samples from the influent and effluents of AB and PPB were collected weekly during the whole experimental period to determine the concentrations of total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN), ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), total phosphorus (TP) and TSS. In addition, a quadruplicate of influent and each effluent

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samples was collected during the steady state of every stage to determine the concentration of the veterinary drugs. Dissolved oxygen concentration (DO) and pH in the cultivation broth of the PBRs were daily measured. In addition, the flow rates of the influent and effluents were daily recorded to monitor water evaporation losses. The process was considered under steady state when the TSS concentrations in the PBRs remained constant for at least four consecutive samplings.

For the validation of the analytical method of veterinary drugs, influent and effluents samples were spiked at two levels of concentration, i.e., 500 ng L^{-1} and 2000 ng L^{-1}, respectively. Those levels were chosen as typical low and high concentrations for most of the target compounds in those types of matrixes.

Additionally, the removal efficiencies (REs) of the veterinary drugs were calculated according to Eq. (1):

$$RE\ (\%) = \frac{([Influent] \times QInfluent) - ([Effluent] \times QEffluent)}{([Influent] \times QInfluent)} \tag{1}$$

where *[Influent]* and *[Effluent]* represent the concentrations of each analyte in the influent and effluent, respectively, while *QInfluent* and *QEffluent* represent the influent and effluent flow rates, respectively. The results here provided correspond to the average ± standard deviation from quadruplicate measurements drawn weekly along one month of steady state.

2.4 Analytical methods

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A CellOX® 325 oximeter was used to measure the dissolved oxygen and temperature (WTW, Germany). To measure the pH, a 510 pH meter (EUTECH Instrument, The Netherlands) was utilized. The photosynthetically active radiation (PAR) was measured using a LI-250A light meter (LI-COR Biosciences, Germany), while the intensity of IR radiation was determined with a PASPort light meter (PASCO airlink®, California. USA). TOC, IC and TN concentrations were analyzed by using a TOC-V CSH analyzer equipped with a TNM-1 module (Shimadzu, Japan). In addition, NO_2^- and NO_3^- concentrations were determined by liquid chromatography (Waters 515 HPLC pump) coupled to ionic conductivity detection (Waters 432 IC) equipped with an IC-Pak Anion HC (150 mm × 4.6 mm) Waters column (García et al., 2017a). TP and TSS concentrations were determined according to standard methods as described elsewhere (APHA, 2005).

The quantitative analyses of the veterinary drugs were based on (López-Serna et al., 2011) and (Kantiani et al., 2010) with further optimization to fully adapt the methodology to the matrixes and analytes of the present study. Method development and validation are described in **Supplementary data 2 and 3**, respectively. In brief, 100 mL of 0.45-μm-filtered samples (n=4) were spiked at 0.1% Na2EDTA and 1,000 ng L^{-1} of internal standard (enrofloxacin-d_5) before solid phase extraction (SPE) using Oasis® HLB cartridges (60 mg, 3 cc; Waters Chromatography, Barcelona, Spain). Then, cartridges were eluted with 6 mL of ACN, and the resulting organic solutions were subsequently evaporated and reconstituted in 1 mL of 0.1% FA in a mixture H_2O/MeOH (95:5). Finally, the extracts were analyzed by ultra-high performance liquid chromatography (UHPLC) – tandem mass spectrometry (MS/MS) in selected reaction monitoring (SRM) mode. More specifically, chromatographic

separation was carried out by a Thermo Scientific DIONEX Ultimate 3000 UHPLC (Waltham, MA, USA) and a Waters Chromatography reversed-phase column BEH C18 (100 mm × 2.1 i.d., 1.7 μm particle size; Manchester, UK), making use of H_2O- and MeOH-based mobile phases containing 0.1% FA as modifier. Mass detection was performed by the triple quadrupole TSQ Quantiva from Thermo Scientific (Waltham, MA, USA). The full list of SRMs and instrumental conditions are given in **Supplementary data 4**.

2.5 Statistical processing

Average, standard deviation and relative standard deviation (%RSD) were calculated for the concentration of each veterinary drug in every set of four samples (n=4) taken from the influent and both photobioreactor effluents during the steady state of all stages.

3. Results and discussion

3.1. Bioremediation performance of the AB-PBR and PPB-PBR

The HRT influenced carbon and nitrogen removal in both PBRs. The steady state removal efficiencies of TOC in AB-PBR averaged 84±4%, 79±3% and 66±3% during stage I, II and III, respectively, and 87±4%, 84±3% and 77±5% in PPB-PBRs, respectively (García et al., 2019). On the other hand, the steady state removal efficiencies of TN in AB-PBR averaged 87±2%, 69±3% and 47±1% during stage I, II and III, respectively. Similar results were found in PPB-

PBR, where the removal efficiencies of TN amounted 83±2, 65±6 and 48±3%, in stage I, II and III, respectively (García et al., 2019). Finally, the steady state removal efficiencies of TP were 91±3%, 84±4% and 83±3% in AB-PBR, and 89±3, 81±1 and 82±9% in PPB-PBR during stage I, II and III, respectively. *Chlorella vulgaris* and *Chlorella Kessieri* were present along the whole experiment in AB-PBR. Likewise, *Scenedesmus acutus* and *Tetradesmus obliquus* were also frequently observed in AB-PBR, and *Chlorella minutissima* was occasionally detected during stage III. On the other hand, no microalgae was found in PPB-PBR during stage I, while *Chlorella vulgaris* and *Chlorococcum sp.* were occasionally present along stage II at very low concentrations. In stage III, a total of 6 microalgae species, including *Chlorella vulgaris*, *Chlorella kessieri*, *Chlorella minutissima* and *Aphanothece saxicola* were identified in PPB-PBR-PPB, although at a very low concentration.

3.1 Occurrence of veterinary drugs in PWW

Out of 19 monitored compounds, 12 were found in the PWWs including the anti-parasitic fenbendazol and 11 antibiotics, namely trimethoprim, the β-lactam penicillin G, the tetracyclines oxytetracycline and doxycycline, the fluoroquinolones marbofloxacin, enrofloxacin and danofloxacin, the sulfonamides sulfadiazine, sulfathiazole and sulfadimidine and the pleuromutilin tiamulin. The chemical concentrations for each compound in raw PWW are compiled in **Table 1**. Average concentrations were generally in the tens hundreds of μg L^{-1} level. The highest concentrations were observed for enrofloxacin, especially during stages II and III, surpassing 1,000 μg L^{-1}. More moderate

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concentrations (<120 μg L^{-1}) were observed in pig manure samples from Germany (Wohde et al., 2016). Doxycycline was found at low hundreds of μg L^{-1} level in all stages in this study, while oxytetracycline was a bit more concentrated than doxycycline, but its concentration dropped during stage III. This decrease was observed for other veterinary drugs such as penicillin G and sulfadimidine. These seasonal fluctuations could be due to the use of a larger amount of water during pig farm cleaning, and/or a change in the medication routine. Penicillin G, marbofloxacin and sulfadimidine were generally quantified in the tens of μg L^{-1} level. Slightly lower concentrations of penicillin G (a few μg L^{-1}) were found in US swine manure (Campagnolo et al., 2002). In contrast, much higher concentrations of sulfadimidine were determined in Swiss and German farms, with maximum concentrations above thousands of mg L^{-1} (Burkhardt et al., 2005; Wohde et al., 2016). Levels of a few μg L^{-1} were determined for danofloxacin, while tiamulin and fenbendazol were detected at hundreds of ng L^{-1} levels. Despite sulfathiazole and trimethoprim were detected in the PWW samples, they could not be reliably quantified because they presented a signal below their limit of quantification (10 and 6 ng L^{-1}, respectively). Finally, amoxicillin, ciprofloxacin, sulfamethizole, sulfamethoxazole, apramycin, dexamethasone and progesterone were never detected in any of the PWW used along the three stages. In contrast, ciprofloxacin has been reported in pig manure with concentrations of up to 28 μg L^{-1} in farms in Germany (Wohde et al., 2016). Overall, the differences between the concentrations determined in the present study and comparable studies elsewhere may be explained by differences in the cleaning

procedures, and in the protocols of pharmaceutical administration and their seasonally variability (piglets growing, sow farrows, pig fattening, epidemic breakouts, etc.).

3.3 Removal of veterinary drugs in AB-PBR and PPB-PBR

The average concentrations of the target veterinary pharmaceuticals in the PBR influent and AB-PBR and PPB-PBR effluents during stage I, II and III, along with their corresponding REs, are listed in **Supplementary data 6.** This data is also depicted in **Figures 2A-H** for a selection of the detected compounds.

No large differences in concentration for the target compounds in the PBR influents were observed during stage I and II. In contrast, a substantial decrease in the concentration of several pharmaceuticals (i.e. oxytetracycline, sulfadimidine and penicillin G among others) was observed in the influent samples during stage III (**Figures 2A**, **C** and **D**, respectively). This event represented a realistic scenario, as variations in the pig slurry composition within a farm are expected depending on the dilution effect after pigpen cleaning and seasonal medical routines (Van Epps and Blaney, 2016). The concentrations of pharmaceuticals detected in the influents revealed dramatic differences among the different target analytes. Hereby, some antibiotics such as the fluoroquinolone enrofloxacin, the tetracycline oxytetracycline and the sulfonamide sulfadiazine were present at concentrations in the μg L^{-1} level. In contrast, seven veterinary drugs (amoxicillin, ciprofloxacin, sulfamethizole, sulfamethoxazole, apramycin, dexamethasone and progesterone) were not detected in the influent samples during the whole

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experiment. The other target veterinary drugs were quantified at concentrations ranging from 14 ng L^{-1} for fenbendazol to 8,500 ng L^{-1} (**Figure 2H**) for doxycycline (**Figure 2B**). It should be noticed that a low excretion rate (high metabolic biotransformation) could result in low parent pharmaceutical concentrations in the PWW despite a high consumption of veterinary drugs in the farm exists (Zhang et al., 2018). In addition, certain drugs are typically supplied at constantly high doses in the farm medical treatments. In contrast, certain medicines might be only used seasonally or are not considered at all in the studied farm. In any case, some compounds like tiamulin and progesterone present a significant lipophilia, with an octanol-water partition coefficient (log P) of 4.4 and 3.8, respectively (**Supplementary data 5**), and are expected to be predominantly present in the solid phase of the pig slurry. Overall, several target antibiotic residues entered the PBR through the influent, some of them at high concentrations. The presence of the target non-antibiotic drugs was only limited to fenbendazol, as the analgesic dexamethasone and the hormone progesterone were not detected in any of the influent samples. These concentrations entail typical concentrations among the so-called contaminants of emerging concern in conventional domestic wastewater treatment plants (González et al., 2016; López-Serna et al., 2019). However, the pattern of compounds in PWW might not necessary coincide with those in domestic wastewater (Verlicchi et al., 2012).

UV photodegradation was not considered as a potential mechanism in the removal of the target veterinary pharmaceuticals in this study as the visible LED and IR lamps in the PBRs only comprised the 400 nm – 700 nm and 700 nm – 1 mm wavelength range, respectively. Similarly, volatilization was unlikely to be a significant removal mechanism based on the

fact that the vapor pressures of the 19 target compounds remained below 3.5 10^{-8} Pa (**Supplementary data 5**) at the range of temperatures prevailing in the experimental set-up along the experiment. As observed for the macrocontaminants (TOC, IC, TN and TP) in section 3.1, the removal efficiencies for the target veterinary drugs in AB-PBR decreased when the HRT decreased from 11 to 4 days. This deterioration in the removal of veterinary drugs in microalgae-based PBRs treating real domestic wastewater has been reported elsewhere (Hom-Diaz et al., 2017; López-Serna et al., 2019; Matamoros et al., 2015). This tendency was not as evident in the PPB-PBR, where the REs of the target veterinary drugs decreased from stage I to stage II and then stabilized in stage III. REs for enrofloxacin and sulfadiazine might not be considered accurate in all cases, as the levels were above the analytical method linearity range.

High REs were observed for most of the quantified target analytes during stage I in AB-PBR. The tetracyclines oxy- (**Figure 2A**) and doxycycline (**Figure 2B**) showed the highest REs (93 and 95%, respectively). Only danofloxacin (**Figure 2C**) and sulfadimidine (**Figure 2F**) exhibited removal efficiencies below 70% in AB-PBR. High elimination percentages, mainly attributed to photodegradation and sorption, were recently observed for tetracycline (Norvill et al., 2017). As photodegradation was not relevant in the present study, most of the removed oxy- and doxycycline was likely adsorbed onto the biomass (assuming the occurrence of similar mechanisms than those observed for tetracycline). A low to moderate elimination (31-60%) was also observed for other two sulfonamide antibiotics (sulfamethoxazole and sulfamethazine) in a recent batch study with *Scenedesmus obliquus* (Xiong et al., 2019), where biodegradation was pointed out as the

main removal mechanism. Similarly, low-moderate REs have been consistently reported for sulfonamide antibiotics in conventional domestic wastewater treatment plants based on activated sludge (Gros et al., 2007; Verlicchi et al., 2012). On the other hand, moderate to high REs (> 68%) for most of the target veterinary drugs were also recorded during stage II in AB-PBR. In particular, oxytetracycline, doxycycline and penicillin G removal efficiencies averaged 78% (**Figure 2A**, **C** and **D**, respectively), and tiamulin (**Figure 2G**) and fenbendazol (**Figure 2H**) REs accounted for 68% in AB-PBR. To the best of our knowledge, there is no previous study in literature reporting the behavior of β-lactams or pleuromutilins antibiotics or anti-parasitics in biological reactors treating wastewater. During stage III, the AB-PBR provided moderate to low REs for most of the target micropollutants. Only doxycycline (**Figure 2B**) and fenbendazol (**Figure 2H**) exhibited removal efficiencies similar to those recorded in stage II (above 70% and 60%, respectively). The rest of the analyzed veterinary drugs were eliminated with efficiencies below 50 %, while no elimination occurred for sulfadimidine (**Figure 2C**) and danofloxacin (**Figure 2F**). In contrast, moderate to high removal percentages (generally between 48-68%) were observed for fluoroquinolones such as ciprofloxacin and ofloxacin by (Hom-Diaz et al., 2017) in a high algal pond operated with domestic wastewater at HRTs of 8 and 12 d. Nonetheless, very variable elimination efficiencies (from 30 to 100%) have been reported for fluoroquinolones in conventional treatments based on activated sludge (Gros et al., 2007; Verlicchi et al., 2012).

The fate of the target veterinary drugs in the PPB-PBR was less effective than in the AB-PBR, except for danofloxacin, which exhibited a moderate-low elimination in AB-PBR

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(even during stage I where it was removed at 48%) compared to the moderate-high removals in PPB-PBR (80% during stage I) (**Figure 2F**). Hence, the PPB-PBR supported REs ranging from 80% for danofloxacin (**Figure 2F**) to negligible removals for sulfadimidine (**Figure 2C**) and marbofloxacin (**Figure 2E**) in stage I. In stage II, the highest eliminations were observed again for danofloxacin (72%) and the lowest for sulfadimidine (11%) and marbofloxacin (no removal). In stage III, the maximum removal corresponded to penicillin G (67%) (**Figure 2D**), while oxytetracycline, sulfadimidine and marbofloxacin exhibited no elimination (**Figures 2A**, **C** and **E**, respectively). Comparison with literature was not possible as this is the first study assessing the performance of a continuous PPB-PBR for the treatment of contaminants of emerging concern despite the potential of purple photosynthetic bacteria for the treatment of pharmaceutical wastewaters was identified by (Madukasi et al., 2010) almost ten years ago.

Regarding the concentrations of the target veterinary drugs in the resulting effluents of both PBRs, enrofloxacin and sulfadiazine remained in the μg L^{-1} level during the whole experimentation. For the rest of compounds, concentrations dropped below 500 ng L^{-1} after AB-PBR treatment during stage I, at a HRT of 11 d. Oxytetracycline and sulfadimidine accounted for the only exceptions, with concentrations above 1,500 and 2,000 ng L^{-1}, respectively. The lower bioremediation performance of PPB-PBR mediated higher concentrations of the target veterinary drugs in the PPB effluents compared to AB-PBR regardless of the operational stage. Hence, doxycycline and marbofloxacin were present at concentrations above 500 ng L^{-1} in the PPB effluent during stage I, and sulfadimidine surpassed 5,000 ng L^{-1} along the same stage. During stage II, at a HRT of 8 d, in addition to

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enrofloxacin and sulfadiazine, oxytetracycline, doxycycline and sulfadimidine were present in the AB-PBR effluent at concentrations over 500 ng L^{-1}, with oxytetracycline exhibiting the highest concentration (>4,400 ng L^{-1}). In the PPB-PBR effluent during the same stage, concentrations were widely higher in most of cases as a result of the less effective treatment. Hence, seven pharmaceuticals were determined above 500 ng L^{-1}, with oxytetracycline, doxytetracycline, enrofloxacin, sulfadiazine and sulfadimidine present at concentrations >4,000 ng L^{-1}, and oxytetracycline exhibiting a maximum concentration over 16,500 ng L^{-1}. During stage III, influent concentrations were generally lower than in stage I and II, which entailed that effluent concentrations were more moderate than in stage II, despite the decrease in the HRT to 4 d. Hence, doxycycline, as well as enrofloxacin and sulfadiazine, were the only target compounds present above 500 ng L^{-1} in the AB effluent. Again, the situation aggravated in the PPB effluent during the same stage, because of the diminished effectiveness observed for that PBR. Surprisingly, some concentrations in the effluents were found to be above the ones in the inlets for a few compounds, especially after PPB treatment during stage I. This could be attributed to the application of an inaccurate evaporation rate correction factor, analyte desorption phenomena from the suspended solids and/or deconjugation process (deglucuronidation, deacetylation, desulphation, etc.) (Lopez-Serna et al., 2012). Regardless, in comparison to the concentration in the effluents of conventional domestic wastewater treatment plants based on activated sludge, the levels here reported were higher than the ones for antibiotics but similar to the ones for analgesics and anti-inflammatories (Gros et al., 2007; Verlicchi et al., 2012), which highlights the differences in the consumption patterns

of this kind of pharmaceuticals in human and porcine. The authors want to point out the risk these levels would entail if these effluents were not managed appropriately, as they could exceed the ecotoxic effect trigger value set by the Steering Committee of Veterinary International Committee on Harmonization (Du and Liu, 2012).

4. **Conclusions**

The present work monitored for the first time the fate of 19 veterinary drugs in continuous PBRs operated with a microalgae-bacteria consortium and purple photosynthetic bacteria during the treatment of real piggery wastewater. In this context, the removal of the anti-parasitic fenbendazol, as well as eight antibiotics, namely amoxicillin, penicillin G, oxy- and doxycycline, marbo- and danofloxacin, sulfadimidine and tiamulin had never been reported before in these emerging photosynthetic treatment technologies. The main conclusions are as follows:

1) Out of the 19 target veterinary drugs, amoxicillin, ciprofloxacin, sulfamethizole, sulfamethoxazole, apramycin, dexamethasone and progesterone were not present in the PWW. In contrast, oxytetracycline, doxytetracycline, enrofloxacin and sulfadizine were present in the PWW at concentrations in the hundreds μg L^{-1} level. The rest of the drugs were present at levels between hundreds of ng L^{-1} (i.e. tiamulin and fenbendazol) and tens of μg L^{-1} (i.e. penicillin G and sufadimidine).

2) AB-PBR was more effective than PPB-PBR in the removal of the detected drugs, except for danofloxacin.

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3) The decrease in HRT caused a deterioration in the bioremediation performance of the PBRs, which was more severe in AB-PBR.

4) Tetracyclines exhibited the highest removal percentages along the whole experiment, with maximum values above 90% at HRT of 11 d in AB-PBR. In contrast, sulfadimidine was identified as the most recalcitrant compound in both PBRs.

5) Despite the good removal efficiencies observed for many of the target compounds, effluent concentrations remained above 500 ng L^{-1} and even in the low μg L^{-1} level for antibiotics like tetracyclines due to their high inlet concentrations.

In light to these results, biological treatments with phototrophic microorganisms, especially those based on microalgae-bacteria consortia, represent a promising eco-friendly and low cost alternative that should be considered in future pig manure management projects.

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Figure 1: Schematic of the AB and PPB photobioreactors (PWW: piggery wastewater; IR: infrared; LED: light-emitting diode; PPB: purple photosynthetic bacteria; PBR: photobioreactor; AB: microalgae-bacteria)

Figure 2: Average concentrations (n=4) found in the PBR influent and AB and PPB effluents during stage I, II and III, for a selection of detected veterinary pharmaceuticals. Error bars represent the standard deviation.

Table 1: Concentrations (%RSD)[a] of veterinary drugs found in the studied PWW[b]

			PWW concentration (ng L^{-1})		
			Stage I	**Stage II**	**Stage III**
Antibiotics	**β-lactams**	**Amoxicillin**	n.d.	n.d.	n.d.
		Penicillin G	38,300 (10)	34,900 (16)	19,300 (6)
	Tetracyclines	**Oxytetracycline**	447,000 (0)	401,000 (31)	9,790 (11)
		Doxycycline	171,000 (43)	202,000 (33)	108,000 (13)
	Fluroquinolones	**Marbofloxacin**	14,300 (40)	16,000 (10)	15,500 (6)
		Ciprofloxacin	n.d.	n.d.	n.d.
		Enrofloxacin	371,000 (0)	2,970,000 (22)	1,620,000 (14)
		Danofloxacin	5,080 (4)	6,450 (16)	6,720 (5)
	Sulfonamides	**Sulfadiazine**	> 780,000	> 780,000	> 780,000
		Sulfathiazole	< MQL	< MQL	< MQL
		Sulfamethizole	n.d.	n.d.	n.d.
		Sulfadimidine	81,800 (6)	90,000 (19)	377 (25)
		Sulfamethoxazole	n.d.	n.d.	n.d.
	Pleuromutilins	**Tiamulin**	692 (46)	1,110 (18)	< MQL
	Aminoglycosides	**Apramycin**	n.d.	n.d.	n.d.
	Others	**Trimethoprim**	< MQL	< MQL	n.d.
Anti-parasitics		**Fenbendazol**	286 (22)	447 (7)	456 (30)
Analgesics /Anti-inflammatory drugs		**Dexamethasone**	n.d.	n.d.	n.d.
Hormones		**Progesterone**	n.d.	n.d.	n.d.

[a] %RSD: Relative standard deviation

[b] n.d.: not detected; < MQL = below method quantification limit

Highlights

- This work assessed the removal of veterinary drugs by phototrophic microorganisms
- AB-PBR was more effective than PPB-PBR in the removal, except for danofloxacin
- Tetracyclines exhibited the highest removal with up to 90% at HRT of 11 d in AB-PBR
- Sulfadimidine was as the most recalcitrant compound in both PBRs
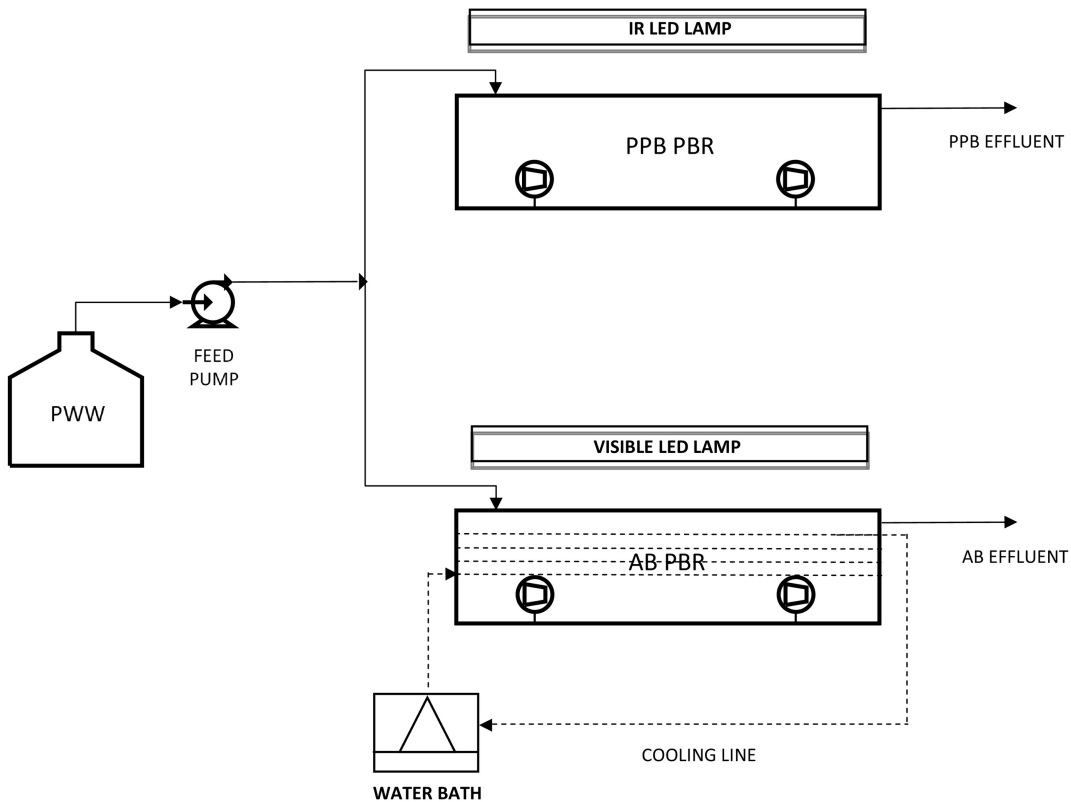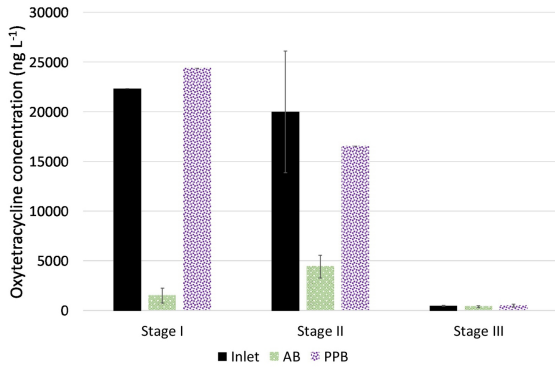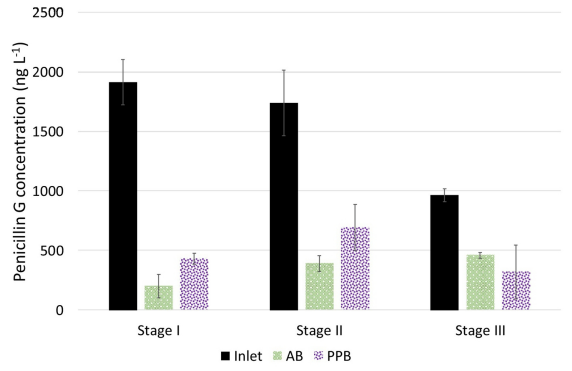- Tetracyclines in effluent were > 500 ng L^{-1} due to their high inlet concentrations

Figure 1

A) Oxytetracycline

B) Doxycycline

C) Sulfadimidine

D) Penicillin G
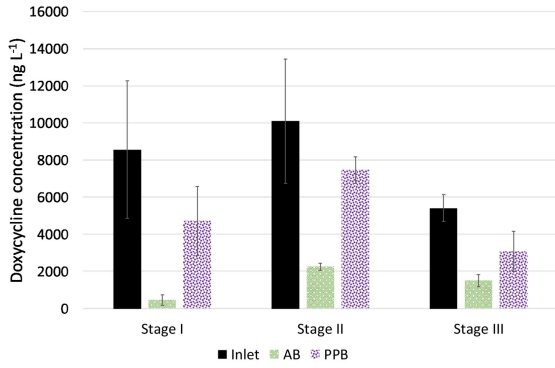
E) Marbofloxacin
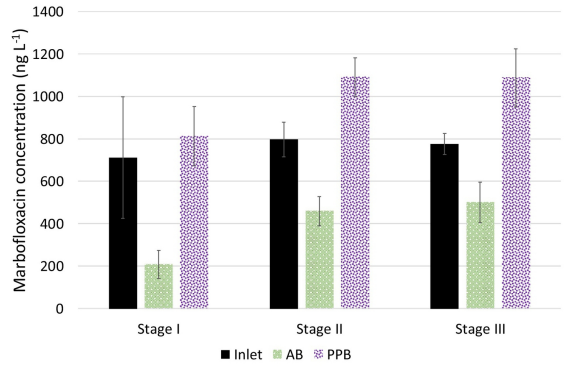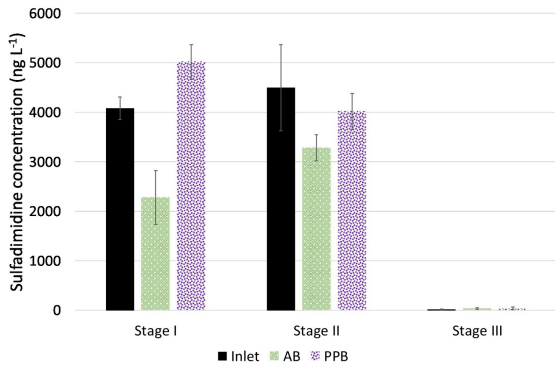
F) Danofloxacin
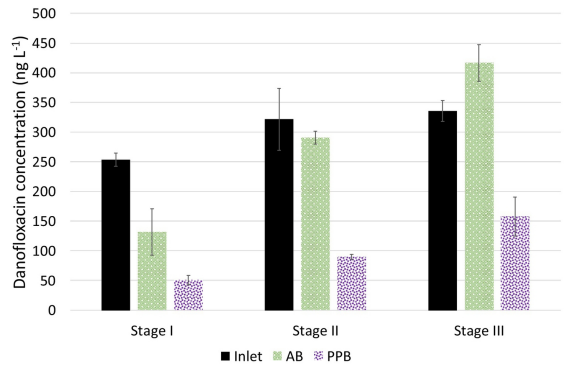
G) Tiamulin

H) Fenbendazole

Figure 2